Chemical Engineering Journal 159 (2010) 257–263

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

# Synthesis of thermosensitive micelles based on poly(N-isopropylacrylamide) and poly(L-alanine) for controlled release of adriamycin

Pan Qiao, Qinsu Niu, Zhibin Wang, Dapeng Cao*

*Key Lab for Nanomaterials, Ministry of Education, College of Chemical Engineering, Beijing University of Chemical Technology, Bei-San-Huan East Road 15#, Beijing 100029, PR China*

ARTICLE INFO

*Article history:*
Received 17 November 2009
Received in revised form 3 February 2010
Accepted 17 February 2010

*Keywords:*
Drug delivery and release
PNIPAAm-b-PAla copolymer
Drug carrier
Adriamycin
Radical polymerization

ABSTRACT

A drug delivery system is synthesized by using poly(N-isopropylacrylamide)-b-poly(L-alanine) (PNIPAAm-b-PAla) block copolymers with different molecular weights, in which the molecular weight can be controlled by adjusting concentration ratio of the PNIPAAm-$NH_2$ and L-Ala-NCA. The lower critical solution temperature (LCST) of the copolymer is a little lower than that of the pure PNIPAAm. Furthermore, with the increase of chain length of the poly(L-alanine) block, the LCST shifts to much lower value. The FT-IR, DSC and TEM characterizations of the products indicated that the amphiphilic PNIPAAm-b-PAla block copolymer can self-assemble into a spherical core–shell micelle structure. This micelle carrier exhibits a reversible phase transition within a very narrow temperature range (about 4 °C). This drug delivery system not only shows good dissolution and hydrophilicity, but also is biodegradable. As a drug carrier of adriamycin (ADR), the PNIPAAm-b-PAla and ADR can form the core–shell micelle easily without precipitation, which prolongs the release time (it is more than 20 h here) of the ADR. Accordingly, this PNIPAAm-b-PAla copolymer is an excellent carrier for controlling drug delivery and release.



## 1. Introduction

Micelles self-assembled by amphiphilic copolymers have attracted much attention for their intrinsic scientific interest or biomedical applications in the past decades [1–3]. They possess unique features highlighted with capacity of transporting hydrophilic as well as hydrophobic species such as anticancer drugs [4]. Amphiphilic copolymers are made up of hydrophilic and hydrophobic segments, and can self-assemble into polymeric micelles with a hydrophobic core stabilized by a hydrophilic shell in an aqueous solution. In this core–shell structure, the hydrophilic shell protects the interior hydrophobic core from avoiding the interaction of the core with the external environment, which provides the polymer micelles with the potential as vehicles for drug delivery.

Recently, much interest has been paid to smart polymer materials that respond to the changes of internal or external stimulus, such as pH value, temperature, light, and magnetic field either reversibly or non-reversibly. The most commonly studied stimuli-responsive polymers are thermoresponsive [5] and pH-responsive [6–9] ones. In particular, the thermoresponsive polymers, which are usually formed by amphiphilic copolymers, have attracted growing interest due to their tremendous potential applications in medicine.

Poly(N-isopropylacrylamide) (PNIPAAm) is a typical thermosensitive polymeric material, which exhibits a reversible thermoresponsive phase transition in an aqueous solution and around the lower critical solution temperature (LCST) (it is 32 °C for pure PNIPAAm) [10–12]. PNIPAAm is water-soluble and hydrophilic at the environmental temperature below its LCST, but after undergoing a phase transition it becomes an insoluble and hydrophobic aggregate as the temperature exceeds the LCST. This phase transition is reversible and occurs within a narrow temperature range [13,14]. Based on the LCST behavior, several PNIPAAm-containing block copolymers have been used as a novel drug carrier in the field of drug target [14–16]. This copolymer consisting of a hydrophilic outer shell of hydrated PNIPAAm segments and a hydrophobic inner core can form a core–shell micelle structure [14,17–25]. The inner core can load hydrophobic drugs, while the PNIPAAm outer shell acts as the role of aqueous solubility and temperature response. When PNIPAAm forms the hydrophilic shell of the micelle, the drug is released and exhibits its bioactivity for a time period upon local heating. The simplicity of the micelle formed by self-assembly of amphiphilic block copolymers and the drug encapsulations by physical mixing are extremely attractive features of polymeric micelles.

Adriamycin (Doxorubicin, ADR) is an active medicine against many cancers. It is most commonly used in the treatment of neoplastic diseases, but the unmodified ADR rapidly releases from the liposome upon intravenous injection while too many doses could cause side effects [26,27]. Several PNIPAAm-containing polymeric

* Corresponding author. Tel.: +86 10 64443254; fax: +86 10 64427616.
*E-mail addresses:* caodp@mail.buct.edu.cn, cao_dp@hotmail.com (D. Cao).

1385-8947/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.cej.2010.02.035

micelles have been adopted [14,27–34] to reduce adverse drug reactions. However, the hydrophobic blocks, such as PMMA and PBMA, could not be degraded easily. Furthermore, the time of the release reaching the maximum is very short. Therefore, it is necessary to develop the safe and efficient drug carriers that can deliver ADR exclusively without provoking adverse reactions in disease therapy.

Poly(amino acid) is a kind of biodegradable macromolecule material with low toxicity, excellent biocompatibility, biodegradability and bioabsorbability *in vivo* [34–39]. The peptide bond existing in the poly(amino acid) main chains can be degraded to non-toxic short peptide and amino acid monomer under the microorganism and enzymes action. So, the biocompatibility and biodegradability of the poly(amino acid) could not only enhance the dissolution and stability of drugs, but also increase the controlled release properties. Copolymers synthesized by amino acid and another component could be improved in dissolution, stability, mechanical properties, and controlled release properties [26,27].

In this work, we used poly(L-alanine), a kind of typical hydrophobic poly(amino acid), as the hydrophobic block and poly(N-isopropylacrylamide) as the hydrophilic block to synthesize a temperature-responsive amphiphilic block copolymer of PNIPAAm-b-PAla. As a novel drug carrier, the delivery properties of ADR in the polymeric micelles were investigated.

## 2. Materials and methods

### 2.1. Materials

N-isopropylacrylamide (ACROS Organics, MorrisPlains, NJ) was purified by recrystallization in hexane and dried in vacuum at room temperature before using. 2,2′-azoisobutyronitrile (AIBN) was recrystallized from ethanol. 2-Amino ethanethiol hydrochloride (AET-HCl) was purchased from Alfa Aesar without further purification. L-alanine (Shanghai, China) and triphosgene (Beijing, China) were dried in vacuum at room temperature. N,N′-dimethylformamide (DMF) from Beijing Chemical Works was purified under reduced pressure distillation prior to the use. Tetrahydrofuran (THF) and petroleum ether (30–60 °C) from Beijing Chemical Works were dehydrated by 4A zeolite (Beijing, China). Unless specially stated, otherwise, all reagents and solvents were of commercial grade and were dried just before using.

### 2.2. Synthesis of PNIPAAm-$NH_2$

The amino-terminated PNIPAAm (PNIPAAm-$NH_2$) was prepared by radical polymerization using AET-HCl as a chain transfer agent and AIBN as an initiating agent [34]. NIPAAm ($8 \times 10^{-3}$ mol), AIBN ($1.6 \times 10^{-5}$ mol) and AET-HCl ($1.2 \times 10^{-4}$ mol) were dissolved in 10 mL DMF. The solution was degassed by bubbling nitrogen for 1 h. The reaction mixture was refluxed at 70 °C for 10 h. After the polymerization, the solution was condensed by the reduced pressure distillation to evaporate most DMF. The product was precipitated by the addition of diethyl ether, and then was dried in vacuum. An excess of triethanolamine (TEA) was added dropwise to this polymer solution in tetrahydrofuran (THF) at room temperature in order to convert PNIPAAm-$NH_2$-HCl into PNIPAAm-$NH_2$. The obtained polymer was purified by the repeated precipitation in an excess of diethyl ether, and then was filtered using a 0.22 μm pore-sized percolation film. Finally, the product was dried in vacuum at 30 °C.

### 2.3. Synthesis of L-Ala-NCA

L-Alanine-N-carboxyanhydride (L-Ala-NCA) was synthesized by triphosgene method [40]. To prepare L-Ala-NCA, excess of triphosgene (11 g) was put into the suspension of L-alanine (7 g) in purified anhydrous tetrahydrofuran (THF, 100 mL) when the temperature was raised to 50 °C. To get rid of phosgene, the solution was bubbled by nitrogen for 30 min after the suspension growing clarity. The L-Ala-NCA was precipitated by pouring the solution in the excess petroleum ether (30–60 °C). Finally, the product was dried in vacuum at 30 °C.

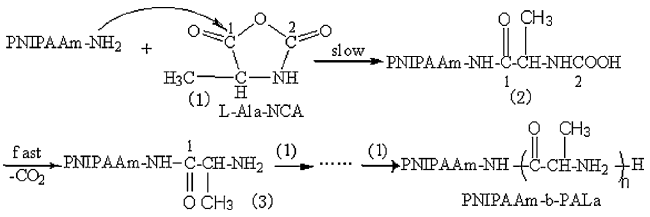


**Fig. 1.** Synthesis of PNIPAAm-b-PAla diblock copolymers.

### 2.4. Preparation of PNIPAAm-b-PAla copolymers and micelles

PNIPAAm-b-PAla was prepared by thermal ring-opening polymerization of L-Ala-NCA [40,41]. The synthesis scheme is shown in Fig. 1. In the procedure, PNIPAAm-$NH_2$ was dissolved in the purified anhydrous DMF. The solution was degassed by bubbling nitrogen for 30 min. Then, L-Ala-NCA was added into the solution. All procedures were carried out at room temperature for 72 h. The obtained polymer was purified by a repeated precipitation in excessive diethyl ether. Finally, the product was dried in vacuum at 30 °C. PNIPAAm-b-PAla with different poly(L-alanine) molecular weights were obtained by changing the concentration ratio of PNIPAAm-$NH_2$ and L-Ala-NCA.

The dialysis method was used to prepare the PNIPAAm-b-PAla polymeric micelle. In the procedure, the PNIPAAm-b-PAla copolymer was dissolved (50 mg) in 10 mL DMF. The solution was put into a dialysis bag (molecular weight cut-off: 3500) and dialyzed against water at 10 °C for 24 h. PNIPAAm-b-PAla micelles were purified by a 0.45 μm pore size filtration membrane, and lyophilized to leave a white powder of micelles.

### 2.5. Characterization of polymers

The molecular weights and polydispersity index of PNIPAAm-$NH_2$, and PNIPAAm-b-PAla were determined by gel permeation chromatography (GPC) system equipped with Waters 515 HPLC Pump and Waters 2410 refractive index detector. THF was used as the solvent at a flow rate of 1.0 mL/min at 30 °C with narrow dispersive polystyrene as calibration standards.

Fourier-transform infrared (FT-IR) spectra of PNIPAAm-$NH_2$, L-Ala-NCA and PNIPAAm-b-PAla were measured in the range of 4000–500 $cm^{-1}$ with a Nexus 8700 (Thermofisher Co.) using KBr pellets. Proton nuclear magnetic resonance ($^1$H NMR) spectroscopy (600 MHz) measurements were performed on Bruker AV 600 spectrometer in $CDCl_3$ at 45 °C.

Transmission electron microscopy (TEM) images were obtained by a JEM 3010 instrument operated at an accelerating voltage of 150 keV. TEM sample was prepared by dipping a drop of micelle dispersion on a copper grid with carbon film and staining with 2% (w/v) phosphotungstic acid aqueous solution. The hydrodynamic particle sizes and size distribution of the micelles were measured by quasi-elastic light scattering on a model ZETA-SIZER 3000HSA nanoinstrument.

Temperature-responsive behaviors of PNIPAAm-$NH_2$ and amphiphilic block copolymer micelles were measured by a turbidity method and differential scanning calorimeter (DSC). The aqueous polymer solutions (500 mg/L of PNIPAAm-$NH_2$ and 300 mg/L of PNIPAAm-b-PAla(2)) at various temperatures were

measured at 500 nm and 542 nm, respectively, by a UV-vis spectrometer (UV-vis 2501 PC, Shimadzu). In order to measure the LCST, the samples were thermostated in a refrigerated circulator bath at a series of temperatures from 22 to 44 °C. The values for the LCSTs of polymer solutions were determined at the temperature showing the onset of turbidity. A DSC204F1 differential scanning calorimeter (Netasch Instruments, Germany) was used to analyze hydrogel discs. The samples were prepared by allowing the hydrogel discs to swell for 24 h in distilled water maintained at room temperature. The discs were then removed from water and the excessive water on the surfaces was blotted by the filter paper. Finally, 3 mg sample of hydrogel was put into pans. The thermal analysis was carried out with a closed pan system in a nitrogen gas flow. The thermal analysis programme used raised the temperature from −10 to 50 °C at a rate of 2 °C/min.

### 2.6. Drug loading and release

Low-aqueous solubility doxorubicin was used as a model drug to investigate the loading and release properties of drugs in the polymer carrier [15,34]. The dry copolymers (15 mg) and doxorubicin (here it is PNIPAm-b-PAla(2), 15 mg) were dissolved in 3 mL DMF and stirred for 3 h. Water is added at a slow rate, and the solution was then dialyzed against 900 mL distilled water in a dialysis tube (molecular weight cut-off: 3500 g/mol). The obtained red solution was ultrafiltered after dialysis. The dialysis tube was directly immersed into 300 mL distilled water. 3 mL of solution was taken from the solution at various time intervals to measure the drug concentration which is the amount of unloaded drugs. The volume of the solution keeps constant by adding 3 mL distilled water after each sampling. The amount of the adriamycin acetate released from micelles was measured at different temperatures by using Shimadzu UV-vis spectrophotometer (based on standard curve: $C$ (μg/mL) = $A$/0.0184, where $A$ is the UV absorbance at 479 nm). The cumulative drug release of doxorubicin was calculated from the following equation:

$$\text{cumulative drug release } (\%) = \left(\frac{W_t}{W_0}\right) \times 100 \quad (1)$$

where $W_t$ is the weight of released doxorubicin at time $t$, and $W_0$ is the total doxorubicin absorbed in the polymeric micelle structure. $W_0$ (here it is 1.44 mg) was calculated by the free drug amount (here it is 15 mg) minus the amount of unloaded drugs. The amount of unloaded drugs was analyzed by measuring the absorbance of dialyzate at 479 nm after drug loading. The amount of the doxorubicin loaded into the copolymer micelles was calculated from the following relationship:

$$\text{loaded drug } (\%) = \left(\frac{W_0}{W_F}\right) \times 100 \quad (2)$$

where $W_F$ is the free drug amount (here it is 15 mg).

## 3. Results and discussion

### 3.1. Synthesis of PNIPAAm-b-PAla

Tables 1 and 2 list the parameters of PNIPAAm-$NH_2$ prepared by the radical polymerization in different concentrations of AET-HCl and AINB respectively, where the PNIPAAm-$NH_2$ molecular weight is in the range of 4500–25,000, depending on the amount of the AET-HCl and AIBN. Apparently, the chain length of the polymers becomes short as the amount of AIBN increases, because the density of the free-radical increases as the AIBN amount increases, which causes excessive partial concentrated instantaneously in the reaction system. Less stability and high termination rate result in the decrease of the average molecular weight. Polymer molecular weight also decreases as the amount of AET-HCl increases in the range of the ratio studied, as listed in Table 2. It is found that the chain length of the PNIPAAm-$NH_2$ can be controlled by adjusting the ratio of AET and AIBN to the monomer. Small homogeneous ($M_n$ < 20,000 g/mol, polydispersity < 2.0) molecules were synthesized through the method of introducing a clearly defined ligand-coupling site in terminal position. In addition, The GPC data for the PNIPAAm-$NH_2$ and PNIPAAm-b-PAla are given in Table 3. Apparently, the polydispersity index basically keeps less than 2, which is acceptable. It is noted that we used the PNIPAAm-$NH_2$ with molecular weight of 12,269 to synthesize the copolymer due to its longer chain and good symmetry of GPC trace, because the longer hydrophilic chain is more helpful for loading drugs [42]. Copolymers of different poly(L-alanine) molecular weights were obtained by changing the concentration ratio of L-Ala-NCA. The increment in molecular weight of the copolymer, compared to the PNIPAAm-$NH_2$, is approximately equal to that of the PAla block, which is equal to molecular weight of the L-Ala-NCA multiplying N, as listed in Table 3.

**Table 1**
The effect of initiator AIBN on the molecular weight of PNIPAAm-$NH_2$.

| $[n_i]/[n]$[a] | $M_n$[b] | $M_w$ | $M_w/M_n$ |
|---|---|---|---|
| 0.05% | 14,888 | 19,363 | 2.64 |
| 0.10% | 14,524 | 19,889 | 1.37 |
| 0.15% | 16,714 | 24,428 | 1.46 |
| 0.50% | 9220 | 15,952 | 1.73 |
| 1.00% | 4689 | 11,361 | 2.42 |
| 1.50% | 5028 | 8426 | 1.68 |
| 2.00% | 5050 | 8455 | 1.67 |

[a] Molar ratio of chain transfer agent [$n_i$] and monomer [$n$] (NIPAAm).
[b] The molecular number ($M_n$) and molecular weight ($M_w$) of the polymer were determined by GPC.

### 3.2. Structure of the block copolymer and micellar formation

Fig. 2 shows the FT-IR spectra of the homopolymers of PNIPAAm-$NH_2$ and L-Ala-NCA and the resultant block copolymer. In the spectrum of the PNIPAAm-$NH_2$, the peaks at 3311 and 1543 $cm^{-1}$ were assigned to the vibration of the N–H bond, and the obvious peak at 1649 $cm^{-1}$ was attributed to the vibration of amide carbonyl group [34]. In the spectrum of the L-Ala-NCA, the peak at 3340 $cm^{-1}$ was assigned to the symmetrical stretching vibration of the N–H bond. The infrared spectrum shows the C=O stretching bands of the acid anhydride at 1860 and 1770 $cm^{-1}$ [41,42]. The peaks at 1368 and 1286 $cm^{-1}$ were assigned to the twisting vibration of the–$CH_3$. As for the PNIPAAm-b-PAla, the peaks

**Table 2**
The effect of chain transfer agent AET-HCl on the molecular weight of PNIPAAm-$NH_2$.

| $[n_{t]}]/[n]$[c] | $M_n$ | $M_w$ | $M_w/M_n$ | |
|---|---|---|---|---|
| 2.3% | 17,516 | 31,544 | 1.8 | Multimodal |
| 3.0% | 12,269 | 20,249 | 1.65 | |
| 4.0% | 10,573 | 15,217 | 1.44 | |
| 5.0% | 6949 | 11,425 | 1.64 | |
| 6.0% | 3078 | 4573 | 1.49 | Multimodal |

[c] Molar ratio of chain initiating agent [$n_t$] and monomer [$n$] (NIPAAm).

**Table 3**
GPC Data of PNIPAm-$NH_2$ and Block Copolymer of PNIPAm-b-PAla.

| Materials | $M_n$ | $M_w$ | $M_w/M_n$ |
|---|---|---|---|
| PNIPAAm-$NH_2$ | 12,269 | 20,249 | 1.65 |
| PNIPAm-b-PAla(1) ($N$ = 10) | 13,001 | 24,803 | 1.89 |
| PNIPAm-b-PAla(2) ($N$ = 30) | 14,524 | 27,158 | 1.87 |
| PNIPAm-b-PAla(3) ($N$ = 50) | 15,320 | 29,262 | 1.91 |

$N$: molar ratio of L-Ala-NCA and PNIPAAm-$NH_2$.

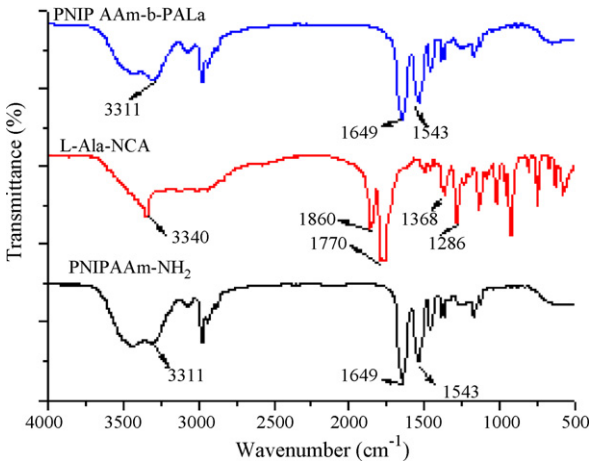


**Fig. 2.** FT-IR spectra of PNIPAAm-$NH_2$, L-Ala-NCA, and PNIPAAm-b-PAla.

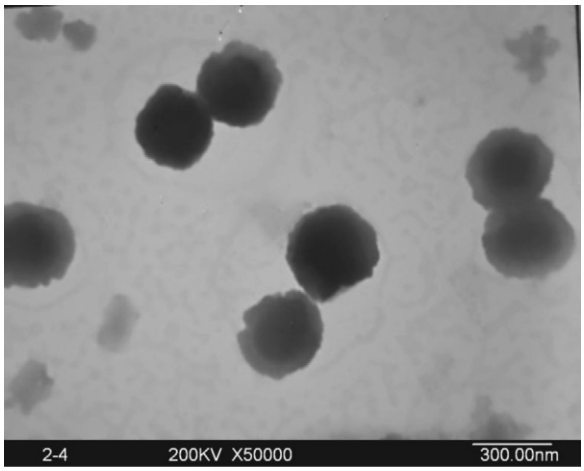


**Fig. 4.** TEM image of the block copolymer of PNIPAAm-b-PAla.

at 1860 and 1770 $cm^{-1}$ disappear, suggesting that the acid anhydride was broken. The peak at 1650 $cm^{-1}$ was assigned to the C=O band. The peak at 1539 $cm^{-1}$ was the absorption peak of amide II bands which is combined by the twisting vibration of N–H bond and stretching vibration of the C–N bond. To testify the occurrence of the copolymerization, $^1$H NMR was also employed to characterize the structure of the copolymer. The peak at 4.1 ppm is assigned to the methine proton in the PNIPAAm and the one at 4.2 ppm is assigned to the methine proton in the PAla. All $^1$H NMR resonances attributed to the PNIPAAm and PAla units were detected, as shown in Fig. 3.

Morphology of resulting polymeric micelles was investigated by TEM. The PNIPAAm homopolymer is water-soluble, while the poly(L-alanine) is water-insoluble. So, in an aqueous solution, the PNIPAAm-b-PAla block copolymers often self-assemble into the core–shell micelles, with the PNIPAAm as the shell and poly(L-alanine) block as the core. As shown in Fig. 4, the PNIPAAm-b-PAla micelles mainly form spherical core–shell structure with an average diameter of about 200 nm. It is evident that the micelles are dispersed as individual nanoparticles with well-defined spherical shape. This indicates that hydrophobic terminals of the copolymers self-assemble into a phase-separated inner core due to the hydrophobic effect. The hydrated out-shell isolates the aggregated hydrophobic inner core from the surrounding water.

The hydrodynamic diameters (geometrical diameter for hard spheres) of the particles in water were measured by the quasi-elastic light scattering, and the size distribution is shown in Fig. 5. The PNIPAAm-b-PAla micelles exhibit a narrow size distribution with an average diameter of around 280 nm at 20 °C. The hydrodynamic particle size is larger than 200 nm measured by TEM, because such particles are highly swollen in water. Fig. 6 shows the size distribution of micelles at 40 °C, where the average size increases to 500 nm. It is significantly greater than that at 20 °C, which is a sign of intermicellar aggregations when the temperature increases from 20 to 40 °C. The intermicellar aggregates are unsymmetrical, which leads to the broad distributions.

### 3.3. Thermoresponsive structural changes of PNIPAAm-b-PAla micelles

As the temperature exceeds the LCST, the PNIPAAm becomes insoluble and aggregates for precipitation. This phase transition is reversible and occurs within a narrow temperature range. To determine whether the PNIPAAm-b-PAla micelles show a similar thermal sensitivity as the amino-terminated PNIPAAm, we

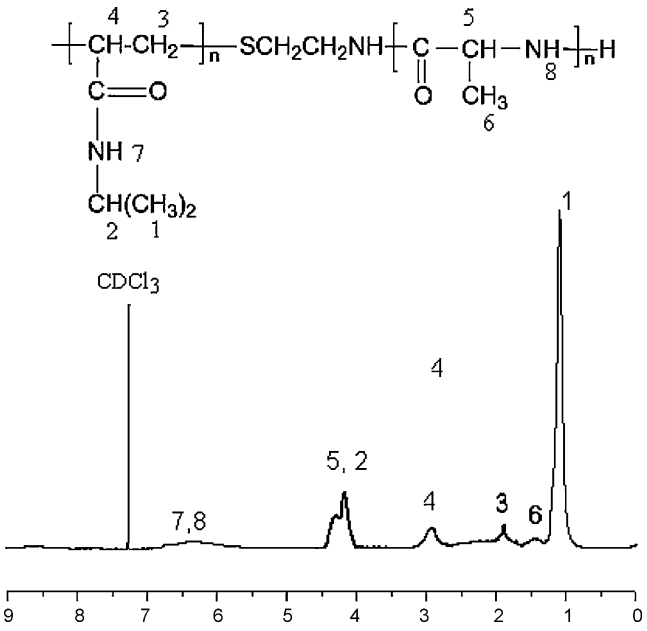


**Fig. 3.** $^1$H NMR spectra of PNIPAAm-b-PAla in $CDCl_3$ and peak assignment of the synthesized copolymer.

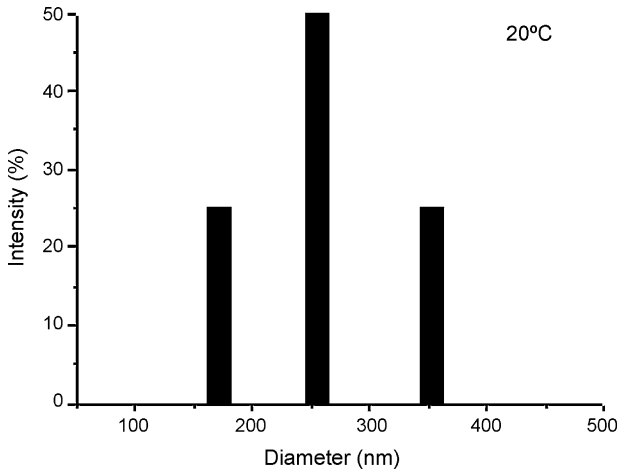


**Fig. 5.** Size distribution of PNIPAAm-b-PAla micelles in aqueous media at 20 °C.

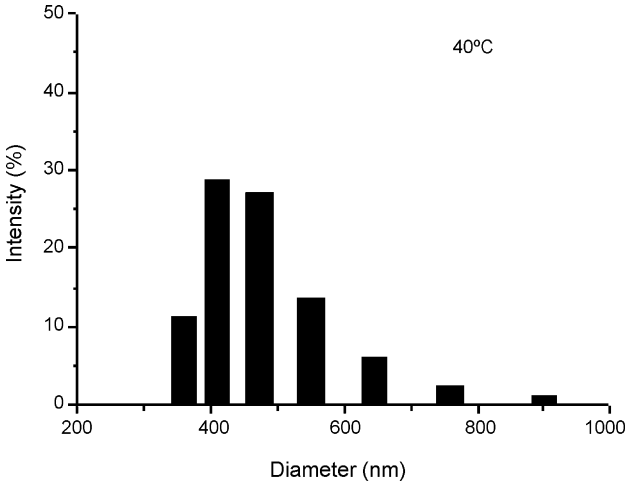


**Fig. 6.** Size distribution of PNIPAAm-b-PAla.

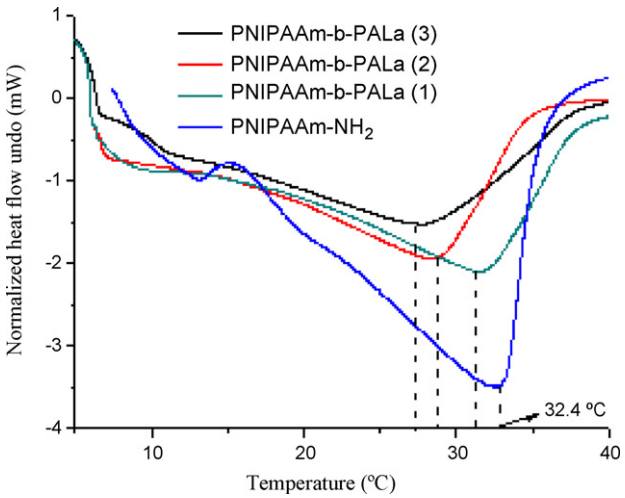


**Fig. 8.** DSC scan plot of microgel dispersions.

examined the optical absorbance of the polymeric micelle aqueous solution as a function of external temperature. As shown in Fig. 7, the LCST of the PNIPAAm-b-PAla copolymer is slightly smaller than that of the pure PNIPAAm [10,14]. When the temperature increases, the copolymer exhibits a sharp phase transition, and a complete transition occurs within a very narrow temperature range (about 4 °C).

The differential scanning calorimetry (DSC) method was also used to determine the LCST, and shown in Fig. 8. It is found that the LCST of the PNIPAAm-$NH_2$ homopolymer is about 32.4 °C. The LCST of the PNIPAAm-b-PAla shifts to a lower temperature than that of pure PNIPAAm. With the increase of the composition of the poly(L-alanine) block, the LCST decreases, because the incorporation of the hydrophobic block facilitates the chain aggregation. That is to say, the longer the poly(L-alanine) block, the lower the LCST. However, in Fig. 7, the LCST change of the copolymer is slight, almost the same as that of pure PNIPAAm. It supports the fact that the block copolymers form completely phase-separated core–shell micelle structures, which minimizes the contact of hydrophobic chains with water. Another possible explanation is that the LCST mainly results from the uninterrupted chain lengths of the PNIPAAm. The length of the PNIPAAm chain is longer than that of the PAla chain in the PNIPAAm-b-PAla copolymer. The long PNIPAAm block in the copolymer is expected to have the similar LCST with the pure PNIPAAm, especially when the PAla segment is buried in the micelle.

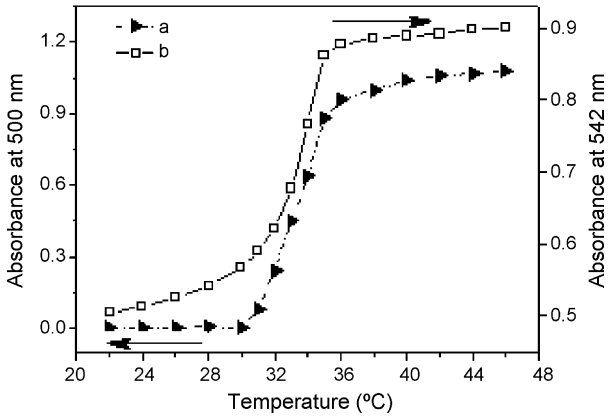


**Fig. 7.** LCST determination of (a) PNIPAAm-$NH_2$ and (b) PNIPAAm-b-PAla in aqueous solution at 500 nm and 542 nm.

### 3.4. ADR incorporation into PNIPAAm-b-PAla

PNIPAAm-b-PAla block copolymers would self-assemble into the micellar structure in water by self-association of the hydrophobic poly(L-alanine) block. It was effective to prepare the stable and monodisperse micelle by dialysis against water. Therefore, hydrophobic drugs can be physically loaded and stabilized in the inner hydrophobic cores comprising poly(L-alanine) chains by hydrophobic interaction. The drug delivery system formed by the physical method is simpler than the chemical conjugations. The formation of the PNIPAAm-b-PAla micelle and the drug loading were affected significantly by the solution temperature and the interactions of the solvents with polymers and drugs. In the gradual replacement of the organic solvent by water during dialysis, self-assembly of hydrophobic blocks provides a driving force for the micelle formation and the drug loading in the micelle cores. Therefore, the interactions of drugs with both the hydrophobic block and solvents determine the drug loading amount [16,17]. Chung et al. [21] have prepared a polymeric micelle of PNIPAAm-PBMA block copolymers, where the solvent must be N-ethylacetamide and the temperature must be controlled at 20 °C. Otherwise, other conditions will cause the precipitation. However, in this work, we can use N,N-dimethylformaldehyde as a solvent to obtain the micelle-ADR solution at room temperature. Furthermore, the optimum hydrophobic interactions to form the PNIPAAm-b-PAla micelles containing ADR drug can be controlled by adjusting the hydrophilic/hydrophobic block lengths of the polymer and the concentrations of both the copolymer and ADR drug in the dialysis bag. Here, PNIPAm-b-PAla(2) exhibits best performance for both micelle formation and drug (ADR) loading, where the loaded drug amount reaches 9.6 wt.%. The semipermeable membrane prevents the micelle diffusion out of the dialysis tube but allows removal of unloaded free drugs. We got the copolymer micelles loading with ADR using the loading method mentioned earlier.

Fig. 9 shows the ADR release profile from polymeric micelles in distilled water, which is a normal thermosensitive drug release behavior. When the temperature is 25 °C (below the LCST), only a small amount (40%) of drug is released from the micelles during the 50 h study. One of the most attractive features of PNIPAAm-based micelles as drug carriers is their intelligent property to external temperature changes. When the temperature is raised to 37 °C (above LCST), the total ADR release increases to 70% due to the temperature-induced structural changes of the polymeric micelles. The PNIPAAm shell becomes hydrophobic above the LCST, which leads to the outer shell disappeared. The deformation of the

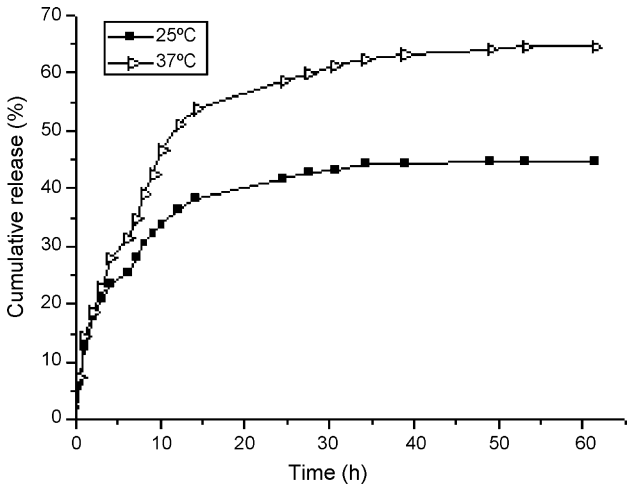


**Fig. 9.** ADR release from PNIPAAm-b-PAla micelles at different temperature.

core–shell nanoparticles causes the release of the enclosed drug. Fig. 9 indicates that the drug release does not reach 100%. The reason might be that partial drugs were entrapped in the collapsed micelles for the interactions between carriers and drugs.

Many drug delivery systems were developed in order to control the release of ADR, but in some drug delivery systems formed by the PNIPAAm-based diblock copolymer micelles, the time reaching the maximum release amount is less than 10 h [15]. Interestingly, the PNIPAAm-b-PAla copolymers synthesized by amino acid and PNIPAAm blocks get a significant improvement in dissolution and controlled release properties. Impressively, the novel block copolymer PNIPAAm-b-PAla as a drug carrier prolongs the release time up to 20 h and the release amount can also be controlled by changing the temperature. However, it is not quite long, compared to the poly(organophosphazene) hydrogels synthesized by Kang et al. [43], in which the ADR release duration of the poly(organophosphazene) hydrogels reaches one month. As well known, one of the main barriers applying thermoresponsive polymers to the fields of biomedicine is their poor degradability by organism. The peptide bond existing in the poly(L-alanine) main chains can be degraded to non-toxic short peptide and amino acid monomer in the microorganism and enzymes action. So, the biocompatibility and biodegradability of the poly(L-alanine) could improve the degradability of the copolymer. Therefore, the PNIPAAm-b-PAla copolymer is very promising drug carrier for the delivery and controlled release application.

## 4. Conclusions

A new thermoresponsive polymeric micelle was constructed by using block copolymers (PIPAAm-PAla). The molecular weights of the copolymer can be controlled by changing the concentration ratio of the PNIPAAm-$NH_2$ and L-Ala-NCA. The micellar inner core formed by self-assembly of the poly(L-alanine) segments can load ADR (an anticancer drug) by optimizing the hydrophobic interactions between ADR and poly(L-alanine) segments. The PNIPAAm chains formed the outer shell of the core–shell structure. The PNIPAAm chains played a key role in stabilization of the carrier, because the PNIPAAm chains exhibit hydrophilicity below the LCST. The release of the drug loaded in the polymeric micelles upon heating above the LCST is due to the PNIPAAm structural deformation. Therefore, the release amount of ADR could be controlled by the temperature of the aqueous solution. Furthermore, this drug delivery system exhibits an improved dissolution. More importantly, it is biodegradable. These properties indicate that the PNIPAAm-b-PAla copolymer is an excellent carrier for controlling drug delivery and release.

It should be mentioned that if the thermoresponsive phase transition is not used for drug loading and release, the PNIPAAm-b-PAla carrier has not significant difference from the PEG-b-PAla copolymer. However, if the carrier is used as the target therapy, the thermoresponsive nature of the carrier plays an important role, because the target therapy often involves in the local heating, which is closely related to the thermoresponsive nature of the carriers. Due to the potential application in the target therapy, it is expected that the PNIPAAm-b-PAla copolymer would become novel multifunctional drug delivery carrier.

## Acknowledgements

This work is supported by NSF of China (20874005 and 20736002), Beijing Novel Program (2006B17), the NCET Program (NCET-06-0095) and ROCS Foundation (LX2007-02) and Novel Team (IRT0807) from MOE, Chemical Grid Program and Excellent Talent of BUCT.

## References

[1] L.F. Zhang, A. Eisenberg, Multiple morphologies of crew-cut aggregates of polystyrene-b-poly(acrylic acid) block-copolymers, Science 268 (1995) 1728–1731.
[2] B.M. Discher, Y.Y. Won, D.S. Ege, J.C.M. Lee, F.S. Bates, D.E. Discher, D.A. Hammer, Polymersomes: tough vesicles made from diblock copolymers, Science 284 (1999) 1143–1146.
[3] D.N.T. Hay, P.G. Rickert, S. Seifert, M.A. Firestone, Thermoresponsive nanostructures by self-assembly of a poly(N-isopropylacrylamide)-lipid conjugate, J. Am. Chem. Soc. 126 (2004) 2290–2291.
[4] Y. Kakizawa, K. Kataoka, Block copolymer self-assembly into monodispersive nanoparticles with hybrid core of antisense DNA and calcium phosphate, Langmuir 18 (2002) 4539–4543.
[5] T. Aoki, M. Kawashima, H. Katono, K. Sanui, N. Ogata, T. Okano, Y. Sakurai, Temperature-responsive interpenetrating polymer networks constructed with poly(acrylic acid) and poly(N,N-dimethylacrylamide), Macromolecules 27 (1994) 947–952.
[6] E. Schacht, A. Gevaert, E.R. Kenawy, K. Molly, W. Verstraete, P. Adriaensens, R. Carleer, J. Gelan, Polymers for colon specific drug delivery, J. Controlled Release 39 (1996) 327–338.
[7] K. Dayananda, B.S. Pi, B.S. Kim, T.G. Park, D.S. Lee, Synthesis and characterization of pH/temperature-sensitive block copolymers via atom transfer radical polymerization, Polymer 48 (2007) 758–762.
[8] E.R. Gillies, T.B. Jonsson, J.M.J. Frechet, Stimuli-responsive supramolecular assemblies of linear-dendritic copolymers, J. Am. Chem. Soc. 126 (2004) 11936–11943.
[9] D.P. Huynh, M.K. Nguyen, B.S. Pi, M.S. Kim, S.Y. Chae, C.L. Kang, S.K. Bong, S.W. Kim, D.S. Lee, Functionalized injectable hydrogels for controlled insulin delivery, Biomaterials 29 (2008) 2527–2534.
[10] M. Heskins, J. Guillet, Solution properties of poly(N-isopropylacrylamide), J. Macromol. Sci., Part A: Pure Appl. Chem. 2 (1968) 1441–1455.
[11] Y. Hirokawa, T. Tanaka, Volume phase transition in a non-ionic gel, J. Chem. Phys. (1984) 6379–6380.
[12] K. Otake, H. Inomata, M. Konno, S. Saito, Thermal-analysis of the volume phase-transition with N-isopropylacrylamide gels, Macromolecules 23 (1990) 283–289.
[13] X.Z. Zhang, R.X. Zhuo, Dynamic properties of temperature-sensitive poly(N-isopropylacrylamide) gel cross-linked through siloxane linkage, Langmuir 17 (2001) 12–16.
[14] J.E. Chung, M. Yokoyama, T. Aoyagi, Y. Sakurai, T. Okano, Effect of molecular architecture of hydrophobically modified poly(N-isopropylacrylamide) on the formation of thermoresponsive core–shell micellar drug carriers, J. Controlled Release 53 (1998) 119–130.
[15] A.R. Quesada, R. Munoz-Chapuli, M.A. Medina, Anti-angiogenic drugs: from bench to clinical trials, Med. Res. Rev. 26 (2006) 483–530.
[16] H. Wei, S.X. Chen, X.Z. Zhang, R.X. zhou, Thermosensitive polymeric micelles based on poly(N-isopropylacrylamide) as drug carriers, Prog. Polym. Sci. 34 (2009) 893–910.
[17] H. Wei, X.Z. Zhang, C. Cheng, S.X. Cheng, R.X. Zhuo, Self-assembled, thermosensitive micelles of a star block copolymer based on PMMA and PNIPAAm for controlled drug delivery, Biomaterials 28 (2007) 99–107.
[18] H. Wei, X.Z. Zhang, Y. Zhou, S.X. Cheng, R.X. Zhou, Self-assembled thermoresponsive micelles of poly(/iN/-isopropylacrylamide-/ib/-methyl methacrylate), Biomaterials 27 (2006) 2028–2034.
[19] X.M. Liu, L.S. Wang, L. Wang, J.C. Huang, C.B. He, The effect of salt and pH on the phase-transition behaviors of temperature-sensitive copolymers based on N-isopropylacrylamide, Biomaterials 25 (2004) 5659–5666.

[20] J. Lehto, K. Vaaramaa, E. Vesterinen, H. Tenhu, Uptake of zinc, nickel, and chromium by N-isopropylacrylamide polymer gels, J. Appl. Polym. Sci. 68 (1998) 355–362.
[21] J.E. Chung, M. Yokoyama, M. Yamato, T. Aoyagi, Y. Sakurai, T. Okano, Thermo-responsive drug delivery from polymeric micelles constructed using block copolymers of poly(N-isopropylacrylamide) and poly(butylmethacrylate), J. Controlled Release 62 (1999) 115–127.
[22] J.E. Chung, M. Yokoyama, T. Okano, Inner core segment design for drug delivery control of thermo-responsive polymeric micelles, J. Controlled Release 65 (2000) 93–103.
[23] Y. Liu, X. Cao, M. Luo, Z. Le, W. Xu, Self-assembled micellar nanoparticles of a novel star copolymer for thermo and pH dual-responsive drug release, J. Colloid Interface Sci. 329 (2009) 244–252.
[24] M. Nakayama, T. Okano, T. Miyazaki, F. Kohori, K. Sakai, M. Yokoyama, Molecular design of biodegradable polymeric micelles for temperature-responsive drug release, J. Controlled Release 115 (2006) 46–56.
[25] K.S. Kim, T.K. Kim, N.B. Graham, Controlled release behavior of prodrugs based on the biodegradable poly(L-glutamic acid) microspheres, Polym. J. 31 (1999) 813–816.
[26] F.M. Veronese, G. Ceriotti, P. Caliceti, S. Lora, M. Carenza, Slow release of narciclasine from matrices obtained by radiation-induced polymerization, J. Controlled Release 16 (1991) 291–298.
[27] K.S. Soppimath, D.C.W. Tan, Y.Y. Yang, pH-triggered thermally responsive polymer core–shell nanoparticles for drug delivery, Adv. Mater. 17 (2005) 318–323.
[28] F. Kohori, K. Sakai, T. Aoyagi, M. Yokoyama, M. Yamato, Y. Sakurai, T. Okano, Control of adriamycin cytotoxic activity using thermally responsive polymeric micelles composed of poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide)-b-poly(D,L-lactide), Colloids Surf. B 16 (1999) 195–205.
[29] S.Q. Liu, Y.W. Tong, Y.Y. Yang, Incorporation and in vitro release of doxorubicin in thermally sensitive micelles made from poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide)-b-poly(D,L-lactide-co-glycolide) with varying compositions, Biomaterials 26 (2005) 5064–5074.
[30] S.Q. Liu, N. Wiradharma, S.J. Gao, Y.W. Tong, Y.Y. Yang, Bio-functional micelles self-assembled from a folate-conjugated block copolymer for targeted intracellular delivery of anticancer drugs, Biomaterials 28 (2007) 1423–1433.
[31] M. Yang, Y.T. Ding, L.Y. Zhang, X.P. Qian, X.Q. Jiang, B.R. Liu, Novel thermosensitive polymeric micelles for docetaxel delivery, J. Biomed. Mater. Res., Part A (2007) 847–857.
[32] G.Y. Li, S. Song, L. Guo, S.M. Ma, Self-assembly of thermo- and pH-responsive poly(acrylic acid)-b-poly(N-isopropylacrylamide) micelles for drug delivery, J. Polym. Sci., Part A: Polym. Chem. 46 (2008) 5028–5035.
[33] W. Li, W.X. Tu, D.P. Cao, Synthesis of thermo-responsive polymeric micelles of PNIPAAm-b-OMMA as a drug carrier for loading and controlled release of prednisolone, J. Appl. Polym. Sci. 111 (2008) 701–702.
[34] H. Iwata, S. Matsuda, K. Mitsuhashi, E. Itoh, Y. Ikada, A novel surgical glue composed of gelatin and N-hydroxysuccinimide activated poly(L-glutamic acid): Part 1. Synthesis of activated poly(L-glutamic acid) and its gelation with gelatin, Biomaterials 19 (1998) 1869–1876.
[35] V. Skarda, F. Rypacek, M. Ilavsky, Biodegradable hydrogel for controlled release of biologically-active macromolecules, J. Bioact. Compat. Polym. 8 (1993) 24–40.
[36] G. Giammona, G. Pitarresi, V. Tomarchio, G. Cavallaro, M. Mineo, Crosslinked alpha,beta-polyasparthydrazide hydrogels: effect of crosslinking degree and loading method on cytarabine release rate, J. Controlled Release 41 (1996) 195–203.
[37] N. Tsubokawa, Y. Nagano, Y. Sone, Grafting of poly-beta-alanine onto carbon-black—the hydrogen transfer polymerization of acrylamide catalyzed by normal-butyllithium in the presence of carbon-black, J. Appl. Polym. Sci. 29 (1984) 985–993.
[38] G. Giammona, G. Cavallaro, G. Fontana, G. Pitarresi, B. Carlisi, Coupling of the antiviral agent zidovudine to polyaspartamide and in vitro drug release studies, J. Controlled Release 54 (1998) 321–331.
[39] S. Cammas, K. Kataoka, Functional poly(ethylene oxide)-co-(beta-benzyl-L-aspartate) polymeric micelles—block-copolymer synthesis and micelles formation, Macromol. Chem. Phys. 196 (1995) 1899–1905.
[40] K. Aoi, K. Tsutsumiuchi, M. Okada, Glycopeptide synthesis by an alpha-amino-acid N-carboxyanhydride (NCA) method—ring-opening polymerization of a sugar-substituted NCA, Macromolecules 27 (1994) 875–877.
[41] L. Xing, W.L. Mattice, Strong solubilization of small molecules by triblock-copolymer micelles in selective solvents, Macromolecules 30 (1997) 1711–1717.
[42] W.D. Fuller, M.S. Verlander, M. Goodman, Procedure for facile synthesis of amino-acid N-carboxyanhydrides, Biopolymers 15 (1976) 1869–1871.
[43] G.D. Kang, S.H. Cheon, S.C. Song, Controlled release of doxorubicin from thermosensitive poly(organophosphazene) hydrogels, Int. J. Pharm. 319 (2006) 29–36.