JOURNAL OF Applied Polymer SCIENCE

Epoxidized poly(*N*-isopropyl acrylamide)-*b*-*epo*HTPB-*b*-poly(*N*-isopropyl acrylamide) triblock copolymer micelle nanoparticles for 10-hydroxycamptothecin drug release

Yan-Ling Luo, Jun Zhang, Fang-Jie Han, Feng Xu, Ya-Shao Chen, Ru Liu

Key Laboratory of Macromolecular Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an 710062, People's Republic of China

Correspondence to: Y.-L. Luo (E-mail: luoyanl@snnu.edu.cn) and F. Xu (E-mail: fengxu@snnu.edu.cn)

ABSTRACT: Thermoresponsive poly(*N*-isopropyl acrylamide) (PNIPAM)-*block*-hydroxy-terminated polybutadine-*block*-PNIPAM triblock copolymers were synthesized by atom transfer radical polymerization; this was followed by the *in situ* epoxidation reaction of peracetic acid. The copolymers were characterized by ^{1}H-NMR, Fourier transform infrared spectroscopy, and size exclusion chromatography measurements, and their physicochemical properties in aqueous solution were investigated by surface tension measurement, fluorescent spectrometry, ultraviolet–visible transmittance, transmission electron microscopy observations, dynamic light scattering, and so on. The experimental results indicate that the epoxidized copolymer micelle aggregates retained a spherical core–shell micelle structure similar to the control sample. However, they possessed a decreased critical aggregate concentration (CAC), increased hydrodynamic diameters, and a high aggregation number and cloud point because of the incorporation of epoxy groups and so on. In particular, the epoxidized copolymer micelles assumed an improved loading capacity and entrapment efficiency of the drug, a preferable drug-release profiles without an initial burst release, and a low cytotoxicity. Therefore, they were more suitable for the loading and delivery of the hydrophobic drug as a controlled release drug carrier. J. Appl. Polym. Sci. **2015**, *132*, 41877.

KEYWORDS: biomaterials; copolymers; drug-delivery systems; self-assembly; stimuli-sensitive polymers

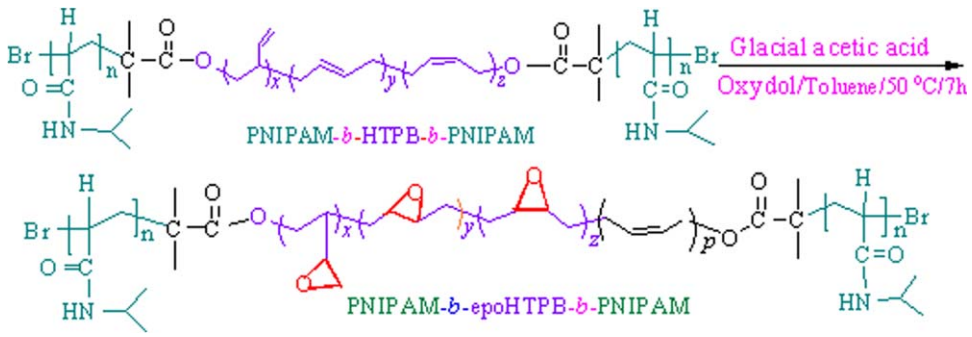
Received 22 September 2014; accepted 11 December 2014

DOI: 10.1002/app.41877

INTRODUCTION

In the past 10 years, amphiphilic block copolymers have extensively been used as controlled drug-release carriers because of their unique physicochemical properties in aqueous solutions.[1,2] Their hydrophobic inner cores can entrap water-insoluble drugs, and this improves the solubility, stability, and bioavailability of the drugs, whereas the hydrophilic shells provide the stabilization of the micelle aggregates. In most cases, drug encapsulation is achieved via hydrophobic interactions between the drug and the core of the copolymer micelles. Thus, the drug-loading capacity (LC) and encapsulation efficiency (EE) are generally low, and an obvious burst release emerges at the onset of release. There are several factors that affect the physicochemical properties of copolymer micelles and the loading and release profiles of drug, for example, the molecular architecture and block length of copolymers, the block proportion and properties of hydrophobic cores, the crystalline behavior, and the glass-transition temperature (T_g).[3–6] The crystalline and T_g effects are supposed to be correlated with the formation of a phase-separated structure, the degree of compatibility, or interactions between the polymers and the drug; these, in turn, influence the micelle stability, EE, and drug-release kinetics.[3–8] A proper T_g value, for example, in the range of the body temperature of mammalians, including humans, may lead to a change in the segmental mobility or relaxation of the (drug-loaded) copolymer micelles after intravenous injection into the blood compartment.[5] The deformation of the polymer tablets above T_g may cause a burst release of the incorporated drugs.[3,5] Shuai *et al.*[8] reported that the level of doxorubicin encapsulated inside poly(ethylene glycol)-*block*-poly(ε-caprolactone) was highly dependent on the poly(ε-caprolactone) crystallinity in addition to the polymer–drug hydrophobic and hydrogen bonding interactions. With these descriptions, it is interesting to engineer and exploit amphiphilic block copolymers with specific functionalities in their hydrophobic segments. In this way, hydrophobic drugs can be entrapped by additional hydrogen bonds or complexation

Additional Supporting Information may be found in the online version of this article.



Scheme 1. Synthesis scheme of the epoxidized PNIPAM-*b*-*epo*HTPB-*b*-PNIPAM triblock copolymer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

interactions between the functional groups of drugs and the hydrophobic cores of copolymer micelles on the basis of hydrophobic encapsulations. It is anticipated that this new kind of copolymer micelle drug carriers will possess improved stability, high drug LC and EE, and preferable drug-release profiles.

10-Hydroxycamptothecin (HCPT) is an indole alkaloid isolated from a Chinese tree and also a natural camptothecin (CPT) analogue; it contains hydroxyl, lactone, and lactam rings in its structure. It has a broad spectrum of antitumor activity against (non)small-cell lung carcinoma, colon carcinoma; ovarian, breast, pancreatic and stomach cancers; Kaposi's sarcoma; acute lymphoblastic leukemia; non-Hodgkin lymphomas; Hodgkin's lymphoma; multiple myeloma; and other hormone-sensitive tumors etc. in clinical practice.[9,10] Its therapy efficiency is more potent, and its toxicity is less than that of CPT.[11] A large number of references have reported that amphiphilic block copolymer micelles have been adopted as drug-loading and drug-release carriers to improve solubility in both water and physiological media and the *in vitro* and *in vivo* instability of the drug,[12,13] to minimize the adverse drug reaction, to modify the drug-transport properties across biological membranes, to enhance the bioavailability of drug,[9,14] and finally to develop the extensive clinical practical use of HCPT.

In our previous article, we reported the synthesis and self-assembly of thermotriggered poly(*N*-isopropyl acrylamide) (PNIPAM)-*block*-hydroxyl-terminated polybutadiene (HTPB)-*block*-PNIPAM triblock copolymers.[15] The copolymer micelles had a lower critical solution temperature (LCST), or cloud point (CP), of about 34–35°C and a critical aggregate concentration (CAC) of about 45–57 mg/L. The double bonds in the copolymers had a certain reactivity, and thus, various functional groups, such as epoxy groups and carboxyl, hydroxyl, and ester bonds in addition to the residual double bonds and so on, especially the epoxy and carboxyl groups, can be introduced by epoxidization in hydrophobic polybutadiene blocks. These groups can endow the hydrophobic blocks of the block copolymers with unique physicochemical properties and drug-loading and drug-release characteristics. Because most anticancer drug molecules contain carboxyl, hydroxyl, and carbonyl or amido groups and so on, the hydrophobic drug molecules will be captured in the hydrophobic cores not only through hydrophobic interactions with hydrophobic cores but also via nonbonded interactions, such as intermolecular hydrogen bonds, or complexation with polar functional groups introduced in the micellar cores. On the other hand, when the physiological environment, such as the temperature and pH, changes at specific targeted areas, the previous effect will abate, and drug molecules will slowly be delivered, and the goal of controlled release will be achieved. The cytotoxicity or biocompatibility is of crucial importance for biomedical applications of newly developed materials. Ding *et al.*[16] found that the epoxidized methyl oleate–graphite oxide/poly(L-lactide) electrospun hybrid fibrous scaffolds presented good biocompatibility for tissue engineering applications. Hu *et al.*[17] produced epoxidized amphiphilic upconverting rare-earth nanophosphors with the low cytotoxicity and good cell membrane permeability as biological labels. Lotter *et al.*[18] reported that the introduction of hydroxyl groups could increase the biocompatibility. Inspired by these works, the block copolymers containing the epoxidation hydrophobic chain structure were, therefore, expected to obtain good biocompatibility and further reduce the cytotoxicity of drugs. Consequently, with the aid of the characteristic reactivity, the molecular self-assembly of block copolymers in the solution and related biomedical performances can be altered or mediated.

In this study, our aim was to investigate effect of epoxidation on the micellization, drug loading, and drug-release behavior of the block copolymers in aqueous solutions. We expected that the epoxidized copolymers could form more stable micelle aggregates with higher CP in aqueous solution by intermolecular hydrogen-bonding interactions. At the same time, the drug-loading and drug-release profiles could be improved on the basis of the intermolecular hydrogen-bonding interactions between the drug molecules and modified micellar cores. Finally, block copolymer micelle aggregates with better biocompatibility were achieved for practical applications.

EXPERIMENTAL

Materials and Reagents

HTPB, a reactive polybutadiene diol, was kindly supplied by the Zibo Qilong Chemical Corp. (Shandong, China) and dried in a vacuum oven at 110°C for 24 h before use; it had a molecular weight (MW) of 2300 and hydroxyl values of 1.08×10^{-3} mol/g of oligomer. The 1,2-, *trans*−1,4-, and *cis*-1,4- contents were about 20, 60, and 20%, respectively, as supplied by the

manufacturer, and hence, the *x*, *y*, and *z* values in the HTPB structure (Scheme 1) were approximately 8, 25, and 8, respectively. *N*,*N*,*N*′,*N*′,*N*″-Pentamethyldiethylenetriamine (98%) and 2-bromoisobutyryl bromide (97%) were supplied by the Aladdin Corp. and were used as received. *N*-Isopropyl acrylamide (NIPAM; 98%, TCI, Japan) was recrystallized three times from a toluene/*n*-hexane (1:1 v/v) mixture before use. Cuprous bromide (CuBr; 98%) was purchased from Aldrich Corp. and was treated in line with ref. [19]. Toluene and xylene (analytically pure, Sinopharm Chemical Reagent Corp., China) and triethylamine (analytically pure, Tianjin FuChen Chemical Reagent Factory, China) were used after dewatering via refluxing over CaH_2 for 6 h and distilling over a few Na filaments. Neutral Al_2O_3, analytically pure, was obtained from the Tianjin Kermel Chem Reagent Co., Ltd., China. Hydrogen peroxide aqueous solution (30%, density = 1.1 g/mL), glacial acetic acid (GAA; analytically pure, density = 1.05 g/mL), and *para*-toluene sulfonic acid (PTSA; analytically pure) were provided by the Sinopharm Chem. Reagent Co., Ltd. (China), and were used without further purification.

Synthesis Procedures

The synthesis of the epoxidized PNIPAM-*b*-HTPB-*b*-PNIPAM triblock copolymers was achieved via a four-step reaction process. The PNIPAM-*b*-HTPB-*b*-PNIPAM triblock copolymers were first synthesized according to our previous work.[15] The detailed synthesis procedure is described in the Supporting Information, and the schematic illustration of a representative synthesis route is shown in Figure S1 in the Supporting Information, and the prepared PNIPAM-*b*-HTPB-*b*-PNIPAM was named Polym A and had a mean yield of 33%.

The epoxidized PNIPAM-*b*-HTPB-*b*-PNIPAM triblock copolymers were then attained by an *in situ* peroxide acetic acid avenue at a molar ratio of H_2O_2/PTSA/GAA/—C=C— double bonds of 20.5:0.25:4.2:1,[20,21] as shown in Scheme 1. Specifically, 0.32 g of PNIPAM-*b*-HTPB-*b*-PNIPAM and 0.048 g of PTSA as a catalyst were added into a three-necked flask and followed by 5 mL of toluene. After complete dissolution, 0.2745 mL of GAA was introduced into the mixture solution. As the mixture solution was heated to 50°C, 0.73 mL of a 30% H_2O_2 solution was gradually added into the previous three-necked flask. The epoxidization reaction proceeded for 7 h with stirring. The resulting product was cooled and filtered to remove the catalyst residues. The filtrate was allowed to sit overnight for stratification, and then, the organic phases were washed with distilled water until the neutrality was reached. The organic layers were evaporated at 65°C for 3 h by rotary evaporators to remove toluene and a small amount of water. Finally, a watery, white viscous product was obtained (mean yield = 89%), and it was named Polym B or PNIPAM-*b*-epoxidized hydroxyl-terminated polybutadiene or epoxidized HTPB (*epo*HTPB)-*b*-PNIPAM.

The epoxide numbers or epoxy values were determined according to method reported elsewhere.[22,23] In detail, 0.2 g of epoxidized PNIPAM-*b*-*epo*HTPB-*b*-PNIPAM triblock copolymer sample was placed in a 100-mL conical flask; then, 20 mL of a hydrochloride acid/isopropyl alcohol solution (volume ratio = 1:40) was added to dissolve the samples. The reaction was allowed to proceed for 10 min, and then, three drops of 0.1% methyl red dissolved in isopropyl alcohol as an indicator were added. Sodium hydroxide with a certain concentration was adopted to titrate the previous reaction solution until yellow was reached as the terminal point with magnetic stirring. The same method was used to determine the epoxide number of the control. The epoxide numbers (mol/100 g) and the epoxidation percentage (EP; %) were calculated according to the following equations:

$$\text{Epoxide number} = (V_1 - V_2)c/10m \quad (1)$$

$$\text{EP (\%)} = (\text{Number of epoxy groups after epoxidation}/\text{Number of double bonds before epoxidation}) \times 100\%$$

where V_1 and V_2 are the volumes of the NaOH titrants consumed for the tested samples and the control samples (mL), respectively; m is the mass of the samples (g); and c is the concentration of the NaOH titrants (0.21 mol/L).

Micelle Formation

A dialysis method was used to prepare copolymer micelles. Briefly, 30 mg of copolymer was dissolved in 10 mL of tetrahydrofuran (THF), and then placed in a dialysis bag with a MW cutoff of 8000–14000 and dialyzed against 1000 mL of distilled water for 24 h at room temperature to remove THF. The water was replaced hourly for the first 3 h and then once every 7 h. After dialysis, the aqueous solution in the dialysis bag was collected in a 50-mL volumetric flask for measurements, and the micelle concentration was 600 mg/mL.

Structural Characterization and Measurements

Fourier transform infrared (FTIR) spectra were recorded on an Avatar 360 ESP FTIR spectrometer (Nicolet) with KBr pellets. ^{1}H-NMR spectra were recorded on a Varian Unity 300-MHz NMR spectrometer (Bruker Avance, Germany) with $CDCl_3$ as solvents at 25°C and tetramethylsilane or $(CH_3)_4Si$ (TMS) as an internal standard. The MW and polydispersity index (PDI) were determined by size exclusion chromatography (SEC; EcoSEC, Tosoh Corp., Japan) with THF as an eluent at a flow rate of 1.0 mL/min at a column temperature of 35°C.

Physicochemical Characterization and Determination

A surface tension technique was used to examine the self-assembly micellization and to determine CAC of the block copolymers in aqueous solutions. The surface tension measurements were carried out on a DCAT 21 surface/interface tensiometer (DataPhysics Corp., Germany) with the Wilhelmy plate method. Before measurements, sample solutions with concentrations from 5×10^{-1} to 5×10^{-5} mg/mL were prepared by the dilution of the previously dialyzed copolymer micelle solution with a concentration of 600 mg/L.

The aggregation number of the copolymer micelles (N_{ma}) was detected by a static fluorescence quenching method with pyrene as a probe and benzophenone as a quencher according to ref. [24]:

$$\ln(I^0/I) = c_Q/[\mathrm{M}] = [N_{ma}c_Q/(c - \mathrm{CAC})] \quad (3)$$

where I^0 and I are the emission intensities before and after the addition of a quencher, respectively; c_Q is the concentration of quencher in the micelles; [M] is the concentration of micelles; and c is the total concentration of the copolymers. CAC was determined by fluorescence spectrophotometry according to our previous

method[25] and showed values of about 49.4 and 10.8 mg/L for the micelles before and after epoxidation, respectively.

The preparation of the copolymer micelle solutions containing a pyrene concentration of 6.0×10^{-7} mol/L was conducted according to our previous work,[25] and the detailed procedure is described in the Supporting Information. To determine N_{ma}, six polymer concentrations of 5–10 times the CAC values and seven benzophenone c_Q values ranging from 2×10^{-5} to 20×10^{-5} mol/L were prepared with acetone and absolute methanol, respectively. The excitation wavelength of the fluorophotometer was 339 nm, and the fluorescence intensity was read at an emission wavelength of 338 nm on a PerkinElmer LS55 fluorescence spectrophotometer.

The thermo-induced phase transition behavior and LCST or CP of the polymer solutions were investigated by optical transmittance measurements at 500 nm on an ultraviolet–visible (UV–vis) spectrometer (TU-1901, Beijing Purkinje General Instrument Co., China). The copolymer solution had a concentration of 250 mg/L. LCST or CP was defined as the temperature producing a 50% decrease in the optical transmittance.[26]

A dynamic light scattering (DLS) instrument (BI-90Plus) equipped with an argon-ion laser operating at $\lambda = 660$ nm was adopted to measure CP and the hydrodynamic diameters (D_hs) of the copolymer micelle aggregates, and the sample concentration was 250 mg/L. The measurements were carried out at a deflection angle of 90° with an output power of 15 mW at room temperature. For CP measurements, the detection temperature was from 20 to 60°C to investigate the D_h change with temperature.

A laser-particle-size ζ-potential analyzer (Delsa Nano C, Beckman Coulter) was adopted to measure the ζ potentials of the micelle aggregates. The measurements were performed at 25°C and at a fixed scattering angle of 90°.

Transmission electron microscopy (TEM) images of all of the samples were obtained at an accelerating voltage of 200 keV with a JEM-2100 TEM instrument (JEOL, Japan). The samples were prepared by the placement of a drop of micelle solution onto a copper grid with carbon films, drying at room temperature, and staining with a 2 wt % phosphotungstic acid aqueous solution before the measurements.

Drug Loading and *In Vitro* Drug Release

Amounts of 35 mg of epoxidized PNIPAM-*b*-*epo*HTPB-*b*-PNIPAM and 10 mg of HCPT were dissolved in 5 mL of DMF, and then, the mixed solution was transferred into a dialysis bag (MW cutoff = 2000 Da) to dialyze for 24 h. The dialysate was dried by lyophilization and weighed. An amount of 5 mg of the HCPT-loaded sample was then dissolved in DMF to measure the LC and EE according to the following equations:

$$\text{LC (\%)} = (\text{Mass of drug in the micelles}/\text{Mass of polymer in the formulation}) \times 100\% \quad (4)$$

$$\text{EE (\%)} = (\text{Mass of drug in the micelles}/\text{Mass of drug in the initial solution}) \times 100\% \quad (5)$$

The concentration of HCPT was determined with a UV–vis spectrometer (U-3900/3900H, Hitachi Corp., Japan), and the detection wavelength was 391 nm.

Meanwhile, another 5 mg of the HCPT-loaded sample was dissolved in 5 mL of PBS solution and subsequently diverted into dialysis tubes. The tubes were immersed in 300 mL of PBS to study the drug release at 25 and 40°C. Aliquots of 4 mL of dialysate were taken out once an hour, and 4 mL of PBS was placed into the beaker after sampling to maintain a constant volume of the solution. The UV absorption of dialysate at different times was measured by a UV–vis spectrophotometer at 391 nm. The cumulative drug release (wt %) was calculated by the following formula:

$$\text{Cumulative drug release (wt \%)} = M_t/M_0 \times 100 \quad (6)$$

where M_t is the amount of drug released at time t and M_0 is the amount of HCPT loaded in the copolymer nanoscale micelles.

In the same way, HCPT was loaded into the blank PNIPAM-*b*-HTPB-*b*-PNIPAM and then released for comparison.

MTT Assay

The cytotoxicity of the copolymer micelles before and after epoxidation was assessed by MTT assay with L929 mouse embryonic fibroblasts. The cells were seeded into a 96-well plate at a density of 1×10^4 cells/well and incubated in complete Dulbecco's modified Eagle's medium (DMEM) containing 10% hyclone fetal bovine serum (high-glucose DMEM) at 37°C in a 5% CO_2 atmosphere for 24 h before the assay. To determine the cytotoxicity, the previous micelle solutions with concentrations ranging from 1 to 500 mg/L in complete DMEM were used to replace the culture medium, and the cells were cultured further for 24 h. After 72 h of culturing, the medium was replaced by 100 μL of fresh DMEM; this was followed by the addition of 20 μL of MTT stock solution (5 mg/mL) to the fibroblasts. After incubation for an additional 4 h, the supernatant was discarded, and then, 150 μL of DMSO was added, and the mixture was shaken for 10 min at room temperature. At the same time, cells were seeded in a fresh culture medium (negative control) under the same conditions for reference purposes. The optical density (OD) was monitored at 490 nm by a 96-well universal microplate reader (model 680, Bio-Rad Laboratories, Ltd., United Kingdom), and the relative cell viability was calculated with the following equation:

$$\text{Cell relative viability (\%)} = (\text{OD}_{\text{sample}}/\text{OD}_{\text{control}}) \times 100\% \quad (7)$$

$\text{OD}_{\text{control}}$ and $\text{OD}_{\text{sample}}$ were obtained in the absence and presence of the copolymer micelles, respectively. The Student's t test was used to determine the significance of any pairs of observed differences. Differences were considered statistically significant when p was less than 0.05. All of the quantitative results are reported as mean values plus or minus the standard deviation from data obtained from at least three separate experiments.

RESULTS AND DISCUSSION

Structural Characterization and Determination of the Epoxidized Block Copolymers

The synthesis of PNIPAM-*b*-HTPB-*b*-PNIPAM triblock copolymers was reported in our previous article.[15] The epoxidized PNIPAM-*b*-*epo*HTPB-*b*-PNIPAM triblock copolymers were synthesized by an *in situ* epoxidation reaction process in which

peroxy acid was formed *in situ* through the reaction between organic acids and hydrogen peroxide, and then, the newly generated peroxy acid in the nascent state directly reacted with PNIPAM-*b*-HTPB-*b*-PNIPAM to produce the epoxidation reaction. Namely, the formation of peroxy acids and the epoxidation reaction took place simultaneously.[20] The epoxide number of the resulting copolymers was estimated to be about 0.0958 mol/100 g, and the EP was approximately 27.4%.

To further acquire information about the epoxidization, ^{1}H-NMR and FTIR analyses were performed, as illustrated in Figures 1 and 2. Figure 1(A) displays the ^{1}H-NMR spectrum of the PNIPAM-*b*-HTPB-*b*-PNIPAM block copolymer. It was clear that the chemical shifts at 1.15 ppm [d, signal a, —NHCH(*CH*3)2] were attributable to the methyl proton signals. Those at 4.01 ppm [m, signal b, —NH*CH*$(CH_3)_2$] were assigned to the methane proton signals. Those at 6.10–6.90 ppm [m, signal c, —*NH*CH$(CH_3)_2$] belonged to the methylene signals, and those at 1.88 ppm (br, signal d) and 1.63 ppm (t, signal e) were ascribed to —CH_2—*CH* and —CH—*CH*2— from the PNIPAM skeletons. The characteristic chemical shift signals of polybutadiene appeared at 5.58 ppm (8H, signal *f*, —CH_2—CH—*CH*=CH_2), 4.97 ppm (16H, signal g, —CH_2—CH—CH=*CH*2), 5.37–5.41 ppm (68H, signals h, i, j, and k, —CH_2—*CH*=*CH*—CH_2—), 2.03 ppm (signals l and n, —*CH*2—CH=CH—*CH*2— and —CH_2—*CH*—CH=CH_2), and 0.91 ppm (16H, signal m, —*CH*2—CH—CH=CH_2). After epoxidization, the characteristic hydrogen shift signals from the double bonds of polybutadiene at 4.97–5.58 ppm almost disappeared or were significantly weakened, as demonstrated in Figure 1(B). In addition, the proton signal m′ appeared at 2.06–2.16 ppm, and the signals l′; and n′ were produced at 2.36–2.46 ppm. In particular, new chemical shift signals related to epoxy groups emerged at 3.02 ppm corresponded to signals f′ to k′. These results confirmed that the epoxidized PNIPAM-*b*-*epo*HTPB-*b*-PNIPAM triblock copolymers were successfully synthesized. Because the NIPAM chain units in the copolymer structure were not epoxidized, the degree of unsaturation of butadiene units before epoxidation were obtained from ^{1}H-NMR by the integral ratio of the chemical shifts at $\delta = 1.15$ or 4.01 ppm to the proton resonance signals in 1,4- and 1,2-polybutadiene at 4.97–5.58 ppm. This was estimated to be about 63.6%, and the unit ratio of butadiene to NIPAM was 42/26. After epoxidization, the degree of unsaturation of butadiene units was 40.9%, and the unit ratio of butadiene to NIPAM was 27:26. Therefore, about 15 butadiene units were epoxidized. EP was calculated by the following formula:

$$\mathrm{EP}\ (\%) = [(d_b - d_a)/d_b] \times 100\% \qquad (8)$$

where d_a and d_b are the degrees of unsaturation before and after epoxidation, respectively. The EP value was calculated to be about 35.8%; this was slightly higher than that determined previously. Because of the obvious decrease in the shift signals at 4.97–5.58 ppm, the low EP value was possibly ascribed to some side effects, including the ring-opening reaction of epoxy groups and the formation of hydroxyl, carboxyl, and ester groups on the side chains of the copolymers.

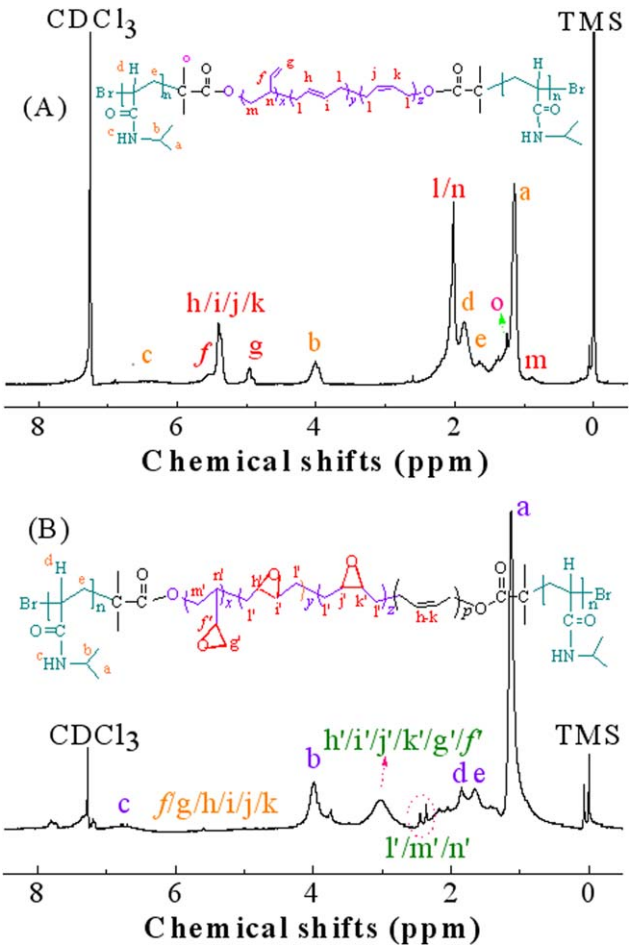


Figure 1. ^{1}H-NMR spectra of the PNIPAM-*b*-HTPB-*b*-PNIPAM triblock copolymers (a) before and (b) after epoxidation (solvent: $CDCl_3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FTIR spectroscopy was used further to characterize the structure of the epoxidized PNIPAM-*b*-*epo*HTPB-*b*-PNIPAM triblock copolymers, as depicted in Figure 2. Before epoxidization, the

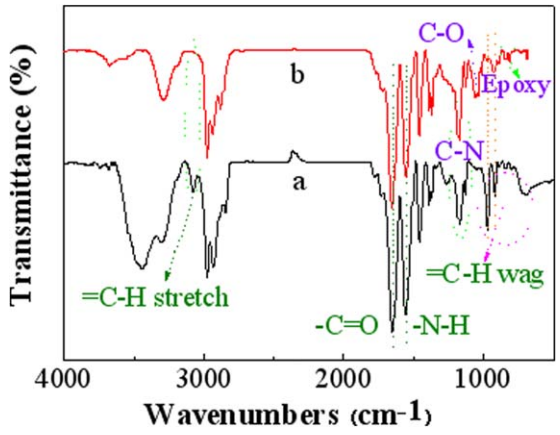


Figure 2. FTIR spectra of the PNIPAM-*b*-HTPB-*b*-PNIPAM triblock copolymers (a) before and (b) after epoxidation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Codes and SEC Data of the PNIPAM-*b*-HTPB-*b*-PNIPAM Triblock Copolymers Before and Ffter Epoxidization

Polymer sample	Code	M_n	M_w	PDI	Yield (%)
PNIPAM-*b*-HTPB-*b*-PNIPAM	Polym A	11650	12930	1.11	33
PNIPAM-*b*-epoHTPB-*b*-PNIPAM	Polym B	12530	14410	1.15	89

M_n, number-average molecular weight; M_w, weight-average molecular weight. The molar ratio of the macroinitiator to NIPAM was 1:200.

PNIPAM-*b*-HTPB-*b*-PNIPAM copolymers exhibited characteristic vibration bands at 1727 cm^{-1} (ester carbonyl C═O stretching), 1652 cm^{-1} (C═O, amide I and —C═C— stretching overlapping), 1550 cm^{-1} (—NH—, amide II), 1173 cm^{-1} (C—N stretching), 1370–1386 cm^{-1} [—$CH(CH_3)_2$], 3295–3445 cm^{-1} (—NH— stretching), and 2847–2974 cm^{-1} (—C—H stretching). In particular, characteristic spectra of double bonds appeared at 3075 cm^{-1}; this was attributable to ═C—H stretching vibrations. Peaks at 968, 900, and 700 cm^{-1} were assigned to the *trans*−1,4, 1,2-, and *cis*−1,4 ═C—H wagging vibrations, respectively. After epoxidization, the resulting copolymer sample produced different FTIR spectra, as displayed in Figure 2(b). We clearly noticed that the C═C stretching, ═C—H stretching, and wagging characteristic peaks at 3075 and 968–700 cm^{-1} almost disappeared, whereas new peaks appeared at 1036–1063 cm^{-1}, assigned to C—O stretching, and 820–850 cm^{-1}, which were attributable to the epoxy group characteristic modes. Other vibration modes related to the PNIPAM blocks remained the same; this signified that the epoxidation reaction of the double bonds took place in the PNIPAM-*b*-HTPB-*b*-PNIPAM triblock copolymers. The apparent MWs of the resulting copolymers were measured by SEC, and the results are summarized in Table I. We observed that the epoxidation resulted in a slight increase in the MW and PDIs within an experimental error. We concluded that the double bonds were epoxidized by the *in situ* epoxidation reaction of peracetic acid. The SEC curves of the block copolymers before and after epoxidization showed that the two copolymers exhibited monomodal and almost symmetric distribution, with no shoulder peak and no tailing in both low- and high-MW regions (Figure S2, Supporting Information); this signified good control over the polymerization reaction and effective purification.

Effect of Epoxidation on the Physicochemical Properties of the Copolymers

The physicochemical properties of the PNIPAM-*b*-*epo*HTPB-*b*-PNIPAM copolymers in aqueous solution were expected to change because the unsaturated double bonds in their structure turned into epoxy groups. Figure 3 compares the micellization behavior and CAC values of PNIPAM-*b*-HTPB-*b*-PNIPAM with those of the PNIPAM-*b*-epoHTPB-*b*-PNIPAM block copolymers in aqueous solution. It was clear that the CAC value was 43.71 mg/L before epoxidation, whereas it became about 7.68 mg/L after epoxidation. The decreased CAC may have been related to the incorporation of epoxy groups and a small amount of hydroxyl, carboxyl, and ester groups in the structure of the block copolymers. This led to the formation of intramolecular hydrogen-bonding interactions in the epoxidized hydrophobic HTPB chains and made the HTPB blocks move away from the aqueous phase; this allowed the formation of copolymer micelle aggregates, especially the micelle cores, which produced more stable micelle aggregates with a lower CAC value. Although intermolecular H-bonding was possible between the water molecules and epoxidized HTPB chains, only a small number of residual epoxy groups participated in the combination with water molecules at the interfaces of the hydrophobic cores and hydrophilic PNIPAM shells. Herein, the hydrophobic and intramolecular hydrogen-bonding interactions remained a prevailing control factor in the epoxidized hydrophobic HTPB chains. Only after the micelles were formed by the previous driving force was the intermolecular hydrogen-bonding interaction possible. Therefore, water molecules could not enter the core of the micelles. They could only interact with the possible residual epoxy groups at the core–shell interfaces. In fact, they had stronger interactions with hydrophilic PNIPAM chains. The CAC value after epoxidation was also lower than that of the thermosensitive $PNIPAM_2$-*b*-poly(lactic acid)-*b*-$PNIPAM_2$ and PNIPAM-*b*-HTPB-*b*-PNIPAM block copolymer micelle counterparts reported in our previous work,[15,27] and was equivalent to that found in our other studies.[28–30] Low CAC values are valuable for attaining micellar stability; this can prevent early leakage of drugs that is due to the destruction of the micelles when the drug-loaded micelles enter into human microenvironments full of a large volume of body fluids.

N_{ma} is a characteristic parameter that describes the micellar structure of the surfactants or copolymers and bears significant meaning for understanding the micellar structure and properties, even the drug loading and drug release.[31] There are many methods capable of measuring N_{ma} of the copolymer micelles, such as the static light scattering and DLS methods, small-angle neutron scattering, NMR, electron spin resonance, and steady-

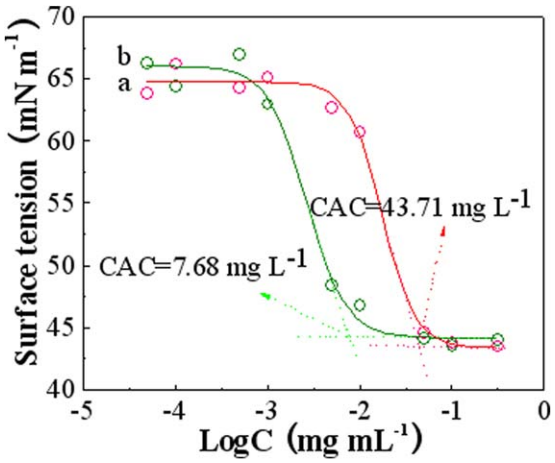


Figure 3. Representative plots of the static surface tension versus the logarithm of the PNIPAM-*b*-HTPB-*b*-PNIPAM triblock copolymer concentrations in aqueous solution (a) before and (b) after epoxidation. C, concentrations. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

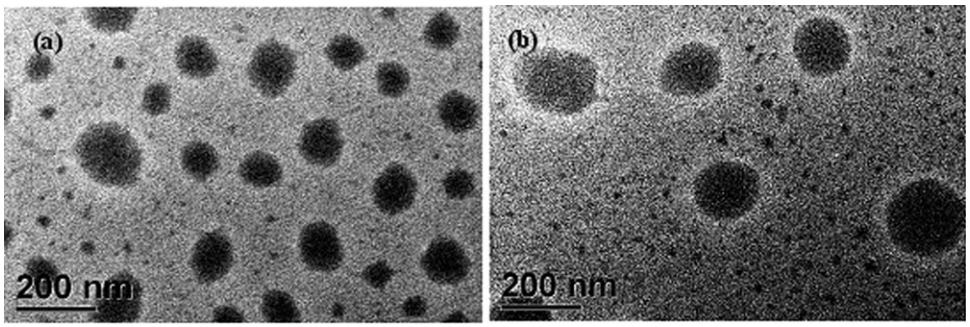


Figure 4. TEM photographs of the PNIPAM-*b*-HTPB-*b*-PNIPAM triblock copolymer micelles: (a) before and (b) after epoxidation (copolymer micelle concentration = 250 mg/L).

state fluorescence quenching. In this study, the N_{ma} values for the target copolymers, including the critical N_{ma} (the aggregation number at CAC), were determined by the static fluorescence quenching method to preliminarily disclose their effects on drug loading and drug release. Detailed measurements and calculation are given in the Supporting Information. The N_{ma}–c plots of the PNIPAM-*b*-HTPB-*b*-PNIPAM copolymer micelles in aqueous solutions before and after epoxidation are depicted in Figure S3 in the Supporting Information, and the regression equations of N_{ma} versus the copolymer concentration relation and the aggregation number are shown in Table III. As shown in Table III, the $[N_{ma}]$ values of the Polym A and Polym B micelles formed in aqueous solution were calculated to be about 120.5 and 132.4, respectively. N_{ma} of the epoxidized copolymer micelle was higher than that of the control, and this was in agreement with the increase in D_h. The increased N_{ma} value was due to a difference in the core structure, where intramolecular hydrogen-bonding interactions were formed in the epoxidized hydrophobic HTPB chains, and more polymer chains could aggregate together.

Figure 4 further compares the TEM morphologies of the PNIPAM-*b*-HTPB-*b*-PNIPAM triblock copolymer micelles before and after epoxidization. Both copolymers spontaneously assembled into almost spherical core–shell nanoscale micelle aggregates by means of hydrophobic driving forces. However, the TEM particle size showed a slight difference. For the epoxidized sample, the diameter range was from 92 to 185 nm, with an average size of about 138 nm. However, in the case of the control sample, the micelle aggregates possessed a wide TEM diameter distribution, with sizes ranging from 77 to 170 nm and a mean diameter of about 108 nm. These differences were possibly due to the existing hydrogen-bonding interactions between the water molecules and epoxy groups in the hydrophobic HTPB at the interfaces of hydrophobic cores and hydrophilic PNIPAM shells after epoxidization, which required more polymer chains for hydrophobic aggregation and led to a slight increase in the micelle size. This was also in agreement with the obtained N_{ma} data.

As a major contribution of this study, we expected to attain a suitable CP or LCST value so that hydrophobic drugs encapsulated in copolymer micelle aggregates could be preferably delivered to targeted sites without leakage during normal blood circulation. PNIPAM is a well-known and widely studied thermosensitive polymer; it has an LCST of about 32°C, which is lower than the human body temperature of 37°C. To enhance the phase-transition temperature, the copolymerization routes of NIPAM and hydrophilic monomers are generally adopted. In our previous studies, we once synthesized brushlike and AB_4-like block copolymers,[15,27–29] and their self-assembled micelles either had LCST values below 37°C or did not show an

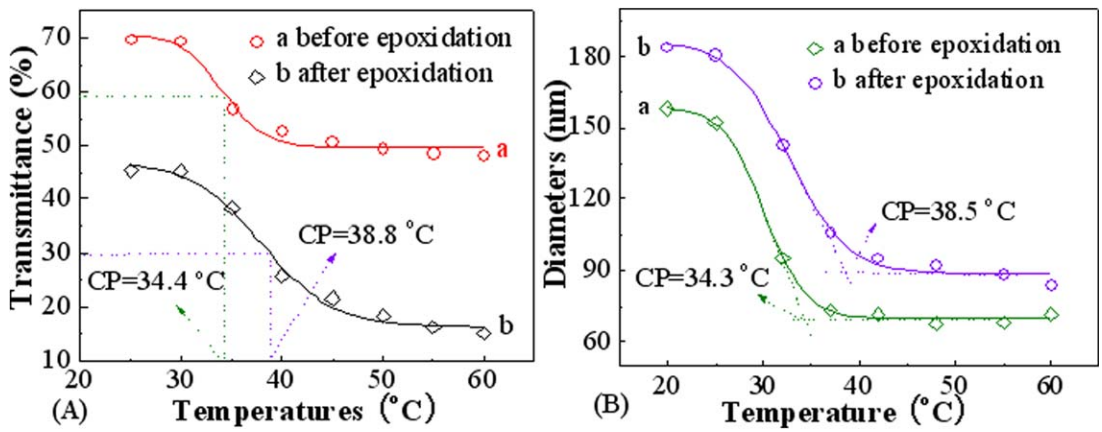


Figure 5. (A) Light transmittance at 500 nm and (B) apparent D_h dependence on the temperature change of the PNIPAM-*b*-HTPB-*b*-PNIPAM triblock copolymer micelles (a) before and (b) after epoxidation (polymer micelle concentrations = 250 mg/L). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Physicochemical Parameters and LC and EE Values of the PNIPAM-*b*-HTPB-*b*-PNIPAM Copolymer Micelle Aggregates Before and After Epoxidation at Room Temperature

	D_h (nm)[a]	PDI[a]	ζ (mV)[a]	LC (%)	EE (%)
Polym A	155.12 ± 4.23	0.2757 ± 0.0220	−2.75 ± 0.59	7.95 ± 0.03	35.78 ± 0.08
Polym B	183.10 ± 1.87	0.2510 ± 0.0148	−1.85 ± 0.11	15.79 ± 0.02	65.76 ± 0.05

[a] The copolymer micelle concentration: 250 mg/L.

environmental temperature response; this is adverse for positive targeted applications. In this study, we attempted to realize the previous objective through an epoxidization method of the double bonds in hydrophobic blocks, and the experimental results are depicted in Figure 5. As shown in Figure 5(A), we noticed that the CP value of the PNIPAM-*b*-HTPB-*b*-PNIPAM triblock copolymer micelle before epoxidization was 34.4°C. After epoxidation, this value increased up to 38.8°C, which was comparable to the CP values found in our previous work[30] and slightly higher than the normal temperature of the human body. This represents a step forward in improving the thermosensitive phase transition and further increasing the targeting of drug release. The reason that CP increased was because partial water molecules probably existed at the interfaces of the hydrophobic cores and hydrophilic PNIPAM shells via hydrogen-bonding interactions of the epoxy and carbonyl groups. At a relatively low temperature, this interaction did not change significantly. Only when the medium temperature was elevated up to a higher value were the water molecules completely expelled from the interfaces and shell layers; this led to the contraction of the PNIPAM chains and a decrease in the transmittance at higher temperatures and gave a higher CP value. It is worth noting that the transmittance value after epoxidation was much lower than that before epoxidation. A possible reason was that hydrophobic interactions of the HTPB chains and additional hydrogen-bonding interactions or complexation existed inside the epoxidized hydrophobic cores, which more readily formed stable micelle aggregates below CP, existed, and this resulted in a lower transmittance. Conversely, only hydrophobic interaction existed during the formation of the micelle aggregates in the case of the copolymer sample before epoxidization, and the transmittance was higher.

DLS analysis was carried out to investigate the effects of epoxidation on D_h, as tabulated in Table II. The epoxidized copolymer micelle aggregate at room temperature produced an increased D_h (183.10 ± 1.87 nm) and decreased the particle size distribution (PDI = 0.2510 ± 0.0148) in comparison with the control sample (D_h = 155.12 ± 4.23 nm and PDI = 0.2757 ± 0.0220). The micelle size, however, was below 200 nm, which was less than the D_h values reported in our previous work at identical concentrations.[28,30] This kind of nanoscale polymer micelles not only reduces the nonspecific uptake of reticuloendothelial systems but also extends the circulation time of the copolymer micelles in the human body; this, in turn, would make the drug accumulate at lesion sites and achieve a positive effect of disease treatment when they are used as drug-release carriers. The increased D_h may have been due to the greater availability of polymer chains for hydrophobic aggregation on the basis of the improved hydrophilicity of the hydrophobic HTPB skeletons and increased N_{ma} values after epoxidation, as stated previously.

The D_h values were found to be dependent on temperature. Figure 5(B) demonstrates the D_h change of the PNIPAM-*b*-HTPB-*b*-PNIPAM block copolymer micelle aggregates with temperature before and after epoxidization. It was clear that the D_hs of both of the copolymer micelle aggregates decreased when the medium temperature was increased. In particular, the D_h values sharply descend in a narrow temperature range from 27 to 35°C in the presence of the control sample, and from 29 to 39°C in the presence of the epoxidized sample. After that, the D_h values tended to be stable and were kept to approximately 70 and 88 nm, respectively. This change in D_h was attributable to the chain contraction of thermosensitive PNIPAM blocks with temperature. From the change in D_h, the phase-transition temperature was estimated to be 34.3 and 38.5°C; this was in good agreement with the results of the UV–vis transmittance measurements. The high phase-transition temperature could be correlated with the water incorporated

Table III. Regression Equations and N_{ma} of the PNIPAM-*b*-HTPB -*b*-PNIPAM Copolymer Micelles Before and After Epoxidation at Room Temperature

				N_{ma}		
Sample	CAC (mg/L)	Regression equation	R^2	$[N_{ma}]$[a]	N_{ma}[b]	N_{ma}[c]
Polym A	49.4	$y = 0.0289x + 119.12$	0.9539	120.5	126.3	148.0
Polym B	10.8	$y = 0.0491x + 131.85$	0.9448	132.4	134.5	181.0

CAC was measured by fluorescence spectrophotometry.
[a] $[N_{ma}]$
[b] and
[c] N_{ma} were obtained under the following conditions of copolymer concentration: CAC, 5 × CAC, and 1000 mg/L, respectively.

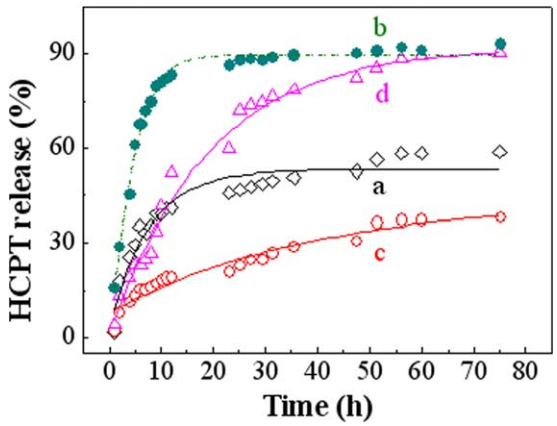


Figure 6. HCPT release profiles from the PNIPAM-*b*-HTPB-*b*-PNIPAM triblock copolymer micelles in PBS of pH 7.4 at (a) 25 and (b) 40°C before epoxidation and (c) 25 and (d) 40°C after epoxidation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

by hydrogen bonds between epoxy groups and water molecules; this caused the water molecules to be completely discharged at higher temperatures.

ζ potentials are significant for understanding the stability of colloidal dispersions. As shown in Table II, the ζ potentials of the two micelle aggregates were small negative values in the aqueous medium; this suggested that they were negatively charged more or less. After epoxidization, the ζ value of the PNIPAM-*b*-*epo*HTPB-*b*-PNIPAM copolymer micelle aggregates slightly increased up to −1.85 mV from −2.75 mV before epoxidation. The possible reason was that the partial —$CONH_2$ functional groups were located on the surface of the micelle nanoparticles.[28,32] The reaction byproduct residues from the polymerization initiators, GAAs, and so on absorbed on the surface of the micelle nanoparticles were also responsible for the negative ζ potentials (e.g., 2-bromoisobutyric acid and acetic acids). In addition, contact potentials may exist between the two materials with different permittivities, of which the material with the lower dielectric constant generally carries the negative charges.[28,33]

Loading and *In Vitro* Delivery of HCPT

The important contribution of this study was to exploit an appropriate delivery system for HCPT to increase LC and EE and improve drug-release profiles on basis of the stability improvement of its lactone form.[34]

To investigate the effect of epoxidation on the LC and release performances of hydrophobic HCPT, loading and release experiments were made, and the results are shown in Table II and Figure 6. Compared with the control sample, the epoxidized micelle aggregates possessed enhanced LC and EE values (Table II), presumably because the entrapment of drug was dependent on not only the hydrophobic interactions between the drug and cores of the micelles but also on the possible hydrogen-bonding interactions between hydroxyl, lactone, and lactam rings in the HCPT and epoxy groups. Because of the high aggregation number after epoxidization, the increased LC and EE values were also correlated with the N_{ma} values, in that more hydrophobic segments could accommodate more hydrophobic drug. The N_{ma} values for the target Polym A and Polym B at a concentration of 1000 mg/L were 148.0 and 181.0, respectively, as shown in Table III, which fully validated and revealed the relation between the N_{ma} and LC of the drug. These EE values were also determined be higher than those reported in our and other CPT-related systems;[27,30,35] this is important for desired drug formulations.

In particular, as shown in Figure 6, the burst release at the onset was significantly decreased, and the sustained and controlled HCPT drug release was achieved on the basis of the hydrophobic and hydrogen-bonding interactions. Below CP, the epoxidized sample produced a low release amount of about 19% within 12 h because of the previous double interactions, whereas the control sample bore a high release amount of about 41% because of the only hydrophobic interaction. After 72 h, the cumulative HCPT release amount reached 38 and 59% for the epoxidized and control samples, respectively. This hinted that the epoxidized copolymer micelle could keep the drug inside the core of the micelles without leakage better than the control sample. Above CP, the control sample produced burst release at the early stage, and the HCPT release amount was approximately 84% within 12 h. The epoxidized sample possessed preferable release profiles without initial burst release, and within the same release period, the release amount was only 52%. After 72 h, the two samples attained almost the same cumulative HCPT release amount of about 90%. Therefore, the epoxidized copolymer micelle aggregates had desired drug-release profiles and could more easily avoid the early leakage of HCPT than the control sample in normal physiological environments and more effectively deliver the drug at a specific tumor site. In contrast, several copolymer micelles prepared previously[27,28] produced leakage of more drug during normal circulation at 37°C and physiological environments and thus did harm to normal cells. Particularly, the amphiphilic block copolymers consisting of hydroxyl-functionalized carboxyl-terminated poly(butadiene-*co*-acrylonitrile) and methoxy poly(ethylene glycol) actually did not exhibit the thermo-induced response.[29] Drug release was, therefore, achieved only by diffusion, and the release doses were small. This kind of

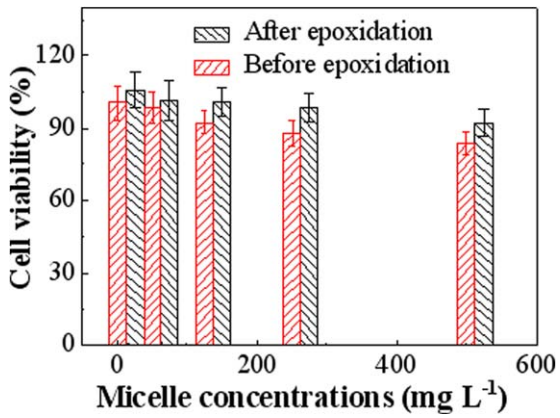


Figure 7. Effect of the epoxidation on the cytotoxicity of L929 cells of the PNIPAM-*b*-HTPB-*b*-PNIPAM copolymer micelles after 48 h of incubation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

drug formulations is only suitable for the passive targeting release of drugs and cannot deliver the drug actively and precisely to a targeted site.

Cytotoxicity Studies

As a high-performance drug-delivery system, low cytotoxicity, even nontoxicity, is one of the major goals we are seeking for *in vitro* and *in vivo* applications. To assess the effect of epoxidation on the cytotoxicity of the exploited drug carrier, an MTT assay was carried out with the control and epoxidized copolymer micelles with various concentrations by the determination of the viability of L929 cells, as shown in Figure 7. It is clear that the control sample exhibited quite a low cytotoxic effect on L929 cells in the micelle concentration range from 1 to 500 mg/L, with a cell viability above 85% ($p < 0.05$); this indicated that the prepared PNIPAM-*b*-HTPB-*b*-PNIPAM copolymer micelles were nontoxic to the L929 cells. After epoxidation, the cytotoxicity of the PNIPAM-*b*-HTPB-*b*-PNIPAM copolymer micelle nanoparticles to the L929 cells decreased remarkably as expected, and the cell viability was even above 94% after 48 h of incubation. Therefore, we inferred that the epoxidation of the unsaturated double bonds could improve the biocompatibility of the drug-delivery systems.

On the whole, in the case of the existing polymer micelles reported previously, either some single performance was appropriate when they were used as drug-release carriers or the overall performance did not meet the needs of drug-release applications. The findings in this study demonstrated improvements in the comprehensive performances, such as low CAC, a CP above 37°C, D_h below 200 nm, high LC and EE, and desired drug-release profiles, compared with materials in earlier studies. Particularly, the micelle-based drug formulation in this approach produced an EE value reached up to 65.76% in comparison with the drug carriers used for release of HCPT and CPT-related drugs.

CONCLUSIONS

In summary, the epoxidation reaction of the PNIPAM-*b*-HTPB-*b*-PNIPAM copolymers was successfully implemented, as confirmed by ^{1}H-NMR, FTIR spectroscopy, and SEC measurements. The epoxidized triblock copolymers could spontaneously assemble into nanoscale spherical core–shell micelle aggregates, and the physicochemical properties were influenced remarkably by the epoxidation because of the introduction of polar epoxy groups. The epoxidation led to a decrease in CAC and increases in D_h, N_{ma}, CP, and ζ potentials. Particularly, the phase-transition temperature above 37°C after epoxidation should allow us to achieve active targeted release of the drug at lesion sites. The epoxidation also allowed drug encapsulation and controlled release, the entrapment efficiency after epoxidation was obviously higher than that before epoxidation, and the release profile was more suitable for HCPT targeted release.

ACKNOWLEDGMENTS

This work was supported by the Natural Science Foundation of China (contract grant numbers NSFC21072124 and NSFC21273142), Natural Science Foundation of Shaanxi Province (contract grant number 2012JM6009), and College Students' Innovative Entrepreneurial Training Project of the Ministry of Education of China (contract grant number 201410718008).

REFERENCES

1. Yan, J. L.; Ye, Z. Y.; Chen, M.; Liu, Z. Z.; Xiao, Y.; Zhang, Y.; Zhou, Y.; Tan, W. S.; Lang, M. D. *Biomacromolecules* **2011**, *12*, 2562.
2. Wei, H.; Zhang, X. Z.; Chen, W. Q.; Cheng, S. X.; Zhuo, R. X. *J. Biomed. Mater. Res. Part A* **2007**, *83*, 980.
3. Yamamoto, Y.; Yasugi, K.; Harada, A.; Nagasaki, Y.; Kataoka, K. *J. Controlled Release* **2002**, *82*, 359.
4. Moughton, A. O.; Hillmyer, M. A.; Lodge, T. P. *Macromolecules* **2012**, *45*, 2.
5. Steendam, R.; van Steenbergen, M. J.; Hennink, W. E.; Frijlink, H. W.; Lerk, C. F. *J. Controlled Release* **2001**, *70*, 71.
6. Li, F.; Danquah, M.; Mahato, R. I. *Biomacromolecules* **2010**, *11*, 2610.
7. Gaucher, G.; Dufresne, M. H.; Sant, V. P.; Kang, N.; Maysinger, D.; Leroux, J. C. *J. Controlled Release* **2005**, *109*, 169.
8. Shuai, X.; Ai, H.; Nasongkla, N.; Kim, S.; Gao, J. *J. Controlled Release* **2004**, *98*, 415.
9. Tang, D. L.; Song, F.; Chen, C.; Wang, X. L.; Wang, Y. Z. *Nanotechnology* **2013**, *24*, 145101.
10. Lu, J.; Liong, M.; Li, Z.; Zink, J. I.; Tamanoi, F. *Small* **2010**, *6*, 1794.
11. Zhao, X.; Zu, Y.; Jiang, R.; Wang, Y.; Li, Y.; Li, Q.; Zhao, D.; Zu, B.; Zhang, B.; Sun, Z.; Zhang, X. *Int. J. Mol. Sci.* **2011**, *12*, 2678.
12. Ping, Y. H.; Lee, H. C.; Lee, J. Y.; Wu, P. H.; Ho, L. K.; Chi, C. W.; Lu, M. F.; Wang, J. *J. Oncol. Rep.* **2006**, *15*, 1273.
13. Yang, L.; Cui, F.; Cun, D.; Tao, A.; Shi, K.; Lin, W. *Int. J. Pharm.* **2007**, *340*, 163.
14. Li, X.; Kong, X.; Zhang, J.; Wang, Y.; Wang, Y.; Shi, S.; Guo, G.; Luo, F.; Zhao, X.; Wei, Y.; Qian, Z. *J. Pharm. Sci.* **2011**, *100*, 232.
15. Luo, Y. L.; Yang, X. L.; Xu, F.; Chen, Y. S.; Zhang, B. *Colloids Surf. B* **2014**, *114*, 150.
16. Ding, Q.; Liao, J.; Yang, Y.; Qu, Y.; Peng, J.; Fan, M.; Luo, F.; Qian, Z. *Sci. Adv. Mater.* **2014**, *6*, 1769.
17. Hu, H.; Yu, M. X.; Li, F. Y.; Chen, Z. G.; Gao, X.; Xiong, L. Q.; Huang, C. H.; Li, F. Y. *Chem. Mater.* **2008**, *20*, 7003.
18. Lotter, J.; Botes, A. L.; van Dyk, M. S.; Breytenbach, J. C. *Biotechnol. Lett.* **2004**, *26*, 1191.
19. Yamamoto, S. I.; Pietrasik, J.; Matyjaszewski, K. *Macromolecules* **2008**, *41*, 7013.
20. Sun, J.; Zheng, Y. S.; Gao, G. X.; Wang, L.; Ling, Y. J.; Gao, G. X.; Tan, Y. B. *Modern Chem. Ind.* **2007**, *27*, 331.
21. Sun, J.; Zheng, Y. S.; Gao, G. X.; Wang, L.; Wang, J.; Liu, Y. *China Synth. Rubber Ind.* **2008**, *31*, 286.
22. Sun, M. L.; Wu, L. Y. Application Theory and Technologies of Epoxy Resins; China Machine Press: Peking, **2002**.
23. Liu, J.; Tang, L.; Li, Y. *Leather Chem.* **2012**, *29*, 26, 35.
24. Dai, Y.; Wu, F.; Li, M.; Wang, E. *Acta Polym. Sinica* **2006**, *50*, 500.

25. Luo, Y. L.; Yu, W.; L.; Xu, F. *Polym. Bull.* **2012**, *69*, 597.
26. Yuan, W. Z.; Li, X. F.; Gu, S. Y.; Cao, A.; Ren, J. *Polymer* **2011**, *52*, 658.
27. Luo, Y. L.; Yang, X. L.; Xu, F.; Chen, Y. S.; Ren-Ting, Z. M. *J. Appl. Polym. Sci.* **2013**, *130*, 4137.
28. Luo, Y. L.; Zhang, L. L.; Xu, F. *Chem. Eng. J.* **2012**, *189–190*, 431.
29. Luo, Y. L.; Chen, L. L.; Miao, Y.; Xu, F. *Ind. Eng. Chem. Res.* **2013**, *52*, 1571.
30. Xu, F. S.; Zheng, Z.; Luo, Y. L. *J. Polym. Sci. Part A: Polym. Chem.* **2013**, *51*, 4429.
31. Ahmad, Z.; Shah, A.; Siddiq, M.; Kraatz, H.-B. *RSC Adv.* **2014**, *4*, 17028.
32. Hou, X. M. Ph.D. dissertation, Nanjing Normal University, **2011**.
33. Tauer, K.; Gau, D.; Schulze, S.; Völkel, A.; Dimova, R. *Colloid Polym. Sci.* **2009**, *287*, 299.
34. Yang, X. L.; Luo, Y. L.; Xu, F.; Chen, Y. S. *Pharm. Res.* **2014**, *31*, 291.
35. Sun, X. T.; Wang, J. L.; Li, L. B. *J. Shandong Univ. (Heath Sci.)* **2009**, *47*, 128.