Poly(*N*-isopropylacrylamide-*co*-hydroxyethyl methacrylate) Graft Copolymers and Their Application as Carriers for Drug Delivery System

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ABSTRACT: Poly(*N*-isopropylacrylamide-*co*-hydroxyethyl methacrylate) [P(NIPAM-*co*-HEMA)] copolymer was synthesized by controlled radical polymerization from respective *N*-isopropylacrylamide (NIPAM) and hydroxyethyl methacrylate (HEMA) monomers with a predetermined ratio. To prepare the thermosensitive and biodegradable nanoparticles, new thermosensitive graft copolymer, poly(L-lactide)-*graft*-poly(*N*-isoporylacrylamide-*co*-hydroxyethyl methacrylate) [PLLA-*g*-P(NIPAM-*co*-HEMA)], with the lower critical solution temperature (LCST) near the normal body temperature, was synthesized by ring opening polymerization of L-lactide in the presence of P(NIPAM-*co*-HEMA). The amphiphilic property of the graft copolymers was formed by the grafting of the PLLA hydrophobic chains onto the PNIPAM based hydrophilic backbone. Therefore, the graft copolymers can self-assemble into uniformly spherical micelles ò about 150–240 nm in diameter as observed by the field emission scanning electron microscope and dynamic light scattering. Dexamethasone can be loaded into these nanostructures during dialysis with a relative high loading capacity and its *in vitro* release depends on temperature. Above the LCST, most of the drugs were released from the drug-loaded micelles, whereas a large amount of drugs still remains in the micelles after 48 h below the LCST. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 2368–2376, 2012

Key words: poly(*N*-isopropylacrylamide-*co*-hydroxyethyl methacrylate); poly(L-lactide); graft copolymer; thermosensitivity micelle

INTRODUCTION

Recently, amphiphilic polymers have attracted much attention as carriers for drug delivery systems. Because they contain both hydrophilic and hydrophobic segments, the amphiphilic polymers can self-assemble into various ordered structures such as spherical micelles, cylindrical micelles, and vesicles in selective solvents.[1–3] During the process, different small molecules such as proteins, genes, or drugs can be loaded into and then slowly released from these structures.

Poly(*N*-isopropylacrylamide) (PNIPAM), an amphiphilic polymer with lower critical solution temperature (LCST) at about 32°C, has been intensively studied due to its potential applications in biomedicine.[3–7] This LCST of PNIPAM can be modulated by copolymerization of with other hydrophilic or hydrophobic monomers.[6–8] Hydroxyethyl methacrylate (HEMA) is a hydrophilic monomer, which can be applied to increase the LCST of PNIPAM-based materials. Poly (HEMA) is an excellent biocompatible polymer that was successfully applied in biomedicine,[9] and its block copolymers have proven to be suitable for grafting of poly(caprolactone) (PCL) and poly(lactide) (PLA) onto their primary hydroxyl groups.[10]

PLA is a biopolymer, which have been extensively studied and used as a biomaterial,[11] therefore, the copolymers of PNIPAM and this biodegradable polymer are promising candidates for the drugs delivery systems due to their *in vivo* degradability. Block and graft copolymers based on PLA and PNIPAM will gain both biodegradability and thermosensitivity, which are very favorable for controlling of the drug release. In the last decades, some polymeric micelles obtained from the block copolymers have been published.[12–15] However, a few studies on the graft copolymers have been reported, due to the difficulties in preparation of the micelles, resulting in these useful graft copolymers have not been studied as much as respective block copolymers.[16–19]

In the previous works, we obtained different graft copolymers by the radiation polymerization concurrent with direct radiation grafting of PNIPAM onto a PLLA backbone.[20] However, the presence of

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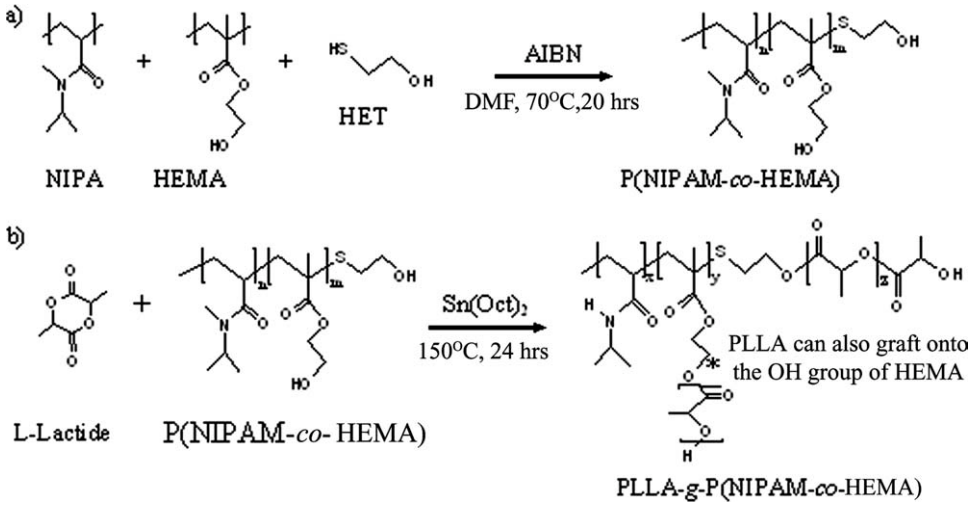

Scheme 1 Polymerization of P(NIPAM-*co*-HEMA) (a) and PLLA-*g*-P(NIPAM-*co*-HEMA) (b).

hydrophobic PLLA segments has slightly reduced the LCST of the graft polymers. For the effective delivery systems, it is required that they should have the LCST higher than normal body temperature but lower than that routinely used in clinical hyperthermia.[21] The copolymers formed by the grafting of several hydrophobic PLLA chains onto the hydrophilic backbones composed of PNIPAM and PHEMA, are amphiphilic, and their balance of hydrophilic and hydrophobic domains can be easily adjusted by changing the relative length of constituent segments as well as the grafting density.[19] The present study is aiming to prepare P(NIPAM-*co*-HEMA)-OH copolymers, and then its relative graft copolymers, PLLA-*g*-P(NIPAM-*co*-HEMA), with the LCST near the body temperature. The graft copolymer is also allowed to self-assemble into the thermosensitive and biodegradable micelles, which can be used as a new drug carrier for dexamethasone (DEX).

These micellar nanoparticles may not be suitable for carrying all kinds of drugs, especially those drugs that are less potent because the higher dose of the drug would make the amount of the drugs much larger, which would be difficult to administer.[15] DEX is a glucocorticoid that is used clinically as an effectively anti-inflammatory agent,[22] but the conventional prolonged administration of DEX usually causes the undesired side effects.[23] DEX is also applied in chemotherapy to cancer patients. Aiming to use the amphiphilic and biodegradable copolymers as effective drug carriers, which can be applied for cancer therapy, DEX was loaded into the polymeric micelles as a drug model and its *in vitro* release from the drug-load micelles was investigated with different incubation time at the temperatures above and below the LCST.

EXPERIMENTAL

Materials

N-isopropylacrylamide (NIPA), 2,2′-azobis(isobutyronitrile) (AIBN) were purchased from Wako Pure and Tokyo Chemical Industries, respectively. NIPA monomer was recrystallized from *n*-hexane followed by vacuum drying for 48 h before use. 2-Hydroxylethanethiol (HET), 2-hydroxylethyl methacrylate (HEMA), L-lactide, and Tin (II) 2-ethylhexanoate were purchased from ς-Aldrich. HEMA was purified under reduced pressure prior to polymerization. DEX, D_2O, DMSO-d_6, and other solvents were bought from Wako Pure Chemical Industries (Tokyo, Japan) and used as received.

Synthesis of P(NIPAM-*co*-HEMA)-OH

Hydroxyl terminated poly(*N*-isopropylacrylamide-*co*-hydroxyethyl methacrylate) copolymer [P(NIPAM-*co*-HEMA)-OH] was prepared by the controlled radical polymerization. NIPAM (5.65 g; 50 mM), HEMA (0.65 g; 5 mM), HET, transfer agent, (78 mg) and 22 mg of AIBN (initiator) were dissolved in 30 mL DMF. The solution was bubbled with N_2 gas for 20 min, and then polymerization reaction was carried out at 70°C under nitrogen. The synthetic process was described in Scheme 1(a). After 20 h, the reaction was terminated and the products were precipitated with diethyl ether. P(NIPAM-*co*-HEMA)-OH was purified by repeated precipitation in diethyl ether from DMF, followed by vacuum-dried for 24 h.

Synthesis of PLLA-*g*-P(NIPAM-*co*-HEMA)

Graft copolymer was prepared by ring opening polymerization of L-lactide (5 g) in the presence of the same amount of P(NIPAM-*co*-HEMA)-OH using

tin (II) 2-ethylhexanoate as a catalyst. The reactants were dissolved in 30 mL xylene, and then the solution was bubbled with N_2 for 30 min. Polymerization was performed at 150°C under nitrogen for 24 h. After termination, reaction solution was precipitated in the mixture of 90% diethyl ether and 10% chloroform to remove the PLLA homopolymer. The obtained PLLA-*g*-P(NIPAM-*co*-HEMA) graft copolymers was further purified by dialysis using a dialysis membrane with molecular weight cut-off (MWCO) of 12–14,000 in the mixture of methanol and chloroform (50 : 50) to remove low molecular weight molecules, dried under vacuum for 24 h and kept in refrigerator.

Characterization

Molecular properties of P(NIPAM-*co*-HEMA) and PLLA-*g*-P(NIPAM-*co*-HEMA) copolymers were determined by gel permeation chromatography (GPC, Jasco, Japan) with polystyrene as the standards. The constituents of these copolymers were determined by NMR spectrometer (500 MHz, JNM-λ500, JEOL, Japan). The samples were obtained by dissolving the respective polymer in D_2O or DMSO-d_6 containing 0.05% tetramethylsilane (TMS) as the internal standard and ^{1}H-NMR spectra were recorded at 20°C. Fourier transform infrared (FTIR) spectrophotometer (Nicolet, Magna 560, Japan) was also used to characterize the molecular structure of the obtained copolymers.

Ratios of the constituent polymers in the graft copolymers also evaluated by themogravimetric analysis (TGA) using a TGA-50 thermal analyzer (Shimadzu, Japan) under nitrogen atmosphere at a flow rate of 50 mL/min. About 3 mg of each copolymer was placed on an aluminum pan for sampling. The sample was heated from room temperature (RT) to 500°C with a heating rate of 10°C/min and its weight loss was recorded with temperature.

Aqueous solutions (1 mg/mL, 0.1%) of corresponding copolymers were used to investigate their phase transition behaviors. The solution was thermostated by a cell holder and its optical transmittances were measured from 30 to 45°C at 500 nm using a UV–vis spectrometer (Shimadzu, Japan). At each temperature, the sample was kept at least 5 min before measurement for reaching the stable state. The solution was thickened by heating and the LCST value was defined as the temperature showing a 50% reduction of the optical transmittance.

Micelle formation and drug loading

Fifty milligram of PLLA-*g*-P(NIPAM-*co*-HEMA) was dissolved in 10 mL DMF. The solution was dialyzed against 1000 mL distilled water using a dialysis membrane of 12–14 kDa MWCO (Viskase Companies, Japan). The water was renewed every 3 h for first 12 h, then renewed every 12–48 h. After the dialysis, the micelles were purified by filtration with a 0.8 μm filter membrane (Advantec, Japan), freeze-dried for 48 h and kept in a refrigerator.

For drug loading, 20 mg of the graft copolymer and 10 mg of DEX were dissolved in 5 mL DMF. The solution was vigorously stirred at RT for 30 min, and then dialyzed against distilled water as mentioned above. The suspension was filtered with 0.8 μm filter membrane, and subjected to ultracentrifugation (Hitachi 18PR-5 Centrifuge) at 12,000 rpm for 30 min to remove the drugs, which may be entangled in the hydrophilic outer shell of the micelles during loading process. The supernatant containing the free drug was discarded; the drug-loaded micellar nanoparticles (DEX-loaded micelles) were collected, freeze-dried for 48 h, and kept in refrigerator.

To determine the content of DEX that has been loaded into the micelles, 1 mg of freeze-dried DEX-loaded micelles was suspended in 10 mL methanol, vigorously stirred for 2 h, and sonicated for 20 min. The solution was centrifuged at 3,000 rpm for 20 min, the supernatant was taken and its absorbance was measured by the UV spectrometer at 242 nm.[22] The drug content was calculated from the calibration curve for DEX. In this experiment, the loaded DEX was determined as $\sim$ 16 wt %.

Morphology and dimension of micellar nanoparticles

A field emission scanning electron microscope, Fe-SEM, (JSM-6330F, JEOL, Japan) working at 15 kV was used to observe the morphologies of both free and DEX-loaded micelles. Before observing, the micellar nanoparticles were attached to the sample stage, coated by Pt with a currency of 10 mA under argon.

The dried micelles were dispersed in distilled water with a concentration of 0.5 g/mL. The aqueous solution was filtered through a 0.8-μm membrane filter (Advantec, Japan), then the hydrodynamic diameters of these micellar nanoparticles were determined by a dynamic light scattering (DLS, Zetasizer ZS-3600, Malvern) equipped with a He-Ne laser source (4 mW at 633 nm) and a digital autocorrelator. The scattering angle was kept at 173° and the result was reported as the average of three independent measurements.

Biodegradability

Enzymatic degradation of the graft copolymers and their micelles were investigated with proteinase K, one kind of enzyme can digest PLLA completely. One milligram of the graft copolymer was dissolved into 5 mL phosphate buffer solution (PBS, pH 7.4)

TABLE I
Syntheses of Copolymers and Their Molecular Characterization

Copolymers	Yield[a] of Polymerization	LA/NIPA/HEMA[b] Feed	LA/NIPA/HEMA[b] Obtained[c]	M_n	M_w/M_n
P(NIPAM-*co*-HEMA)	83.3	0 : 5 : 1	0 : 13 : 2	5300	1.51
PLLA-*g*-P(NIPAM-*co*-HEMA)	82.5	5 : 5 : 1	15 : 13 : 2	10,300	1.67

[a] Determined by ^{1}H-NMR analysis.
[b] Weight ratio of LA and P(NIPAM-*co*-HEMA)-OH.
[c] Molar ratios were calculated from the integrals of the peaks representative of each component in ^{1}H-NMR spectra of corresponding copolymers in DMSO.

containing a small amount of NaN_3 to prevent microorganisms growth, incubated with proteinase K at 37°C. After certain periods, the samples were filtered through 1.2 μL membrane filter and transferred to sample cells for DLS measurements. All sample cells were densely sealed with a stopper and DLS measurements were carried out at 25°C as mentioned above.

In vitro drug release studies

The release experiments were carried out using a dialysis sack with MWCO of 2,000. Briefly, 10 mg DEX-loaded micelles was suspended in 1 mL phosphate buffer solution (PBS 0.05*M*, pH 7.4). The solution was put in the dialysis sack. This dialysis sack was immersed in 200 mL PBS containing 0.02 wt % NaN_3 at different temperatures. After predetermined time, 3 mL of the solution was withdrawn and measured by the UV spectrometer at 242 nm as mentioned above. The amount of DEX released from the micelles was calculated using the calibration curve for DEX.

RESULTS AND DISCUSSIONS

Syntheses of copolymers

Thiol compounds are the chain transfer agents that can limit the size of the obtained polymeric chains during the polymerization of NIPAM monomer,[24] where their hydroxyl group linked to one terminal of the polymer. When HEMA is used as a comonomer of NIPAM, it can modulate the LCST of the resulting P(NIPAM-*co*-HEMA) copolymer. In this process, the free hydroxyl group of HET also enhanced the hydrophilicity of the copolymer. After that, PLLA chains can graft onto both hydroxyl groups of HEMA and HET during the synthesis of PLLA-*g*-P(NIPAM-*co*-HEMA) as described in Scheme 1(b). Consequently, the thiol compounds, which are usually highly toxic have not to use during the synthesis of P(NIPAM-*co*-HEMA) copolymers, and the obtained graft copolymers are more suitable for the drug carriers.[25]

Table I summarizes the molecular properties of the resulting copolymers. Relatively high yields have been obtained for both copolymerization reactions. The comonomer ratios feeding to the reaction as well as determining from ^{1}H-NMR spectra of the obtained copolymers are showed in Table I. The different ratios mean that homopolymers also formed during the radical polymerization. These copolymers are soluble in water at room temperature and the optical transmittances of respective solutions are determined with temperature. Figure 1 shows the phase transition behaviors of both copolymers in aqueous solutions. The lower transmittance at low temperatures and higher transmittance at high temperatures could be attributed to aggregation of the copolymers. The LCST values of these solutions determined from the temperature at 50% reduction of transmittance are much higher than that of PNIPAM due to the presence of the hydrophilic groups of HEMA and HET components. The LCST of P(NIPAM-*co*-HEMA) copolymers are about 38.2°C, higher than 34.9°C, which has been reported by Li et al.[19] It is because the P(NIPAM-*co*-HEMA) copolymer in their study was prepared from a smaller ratio of HEMA.

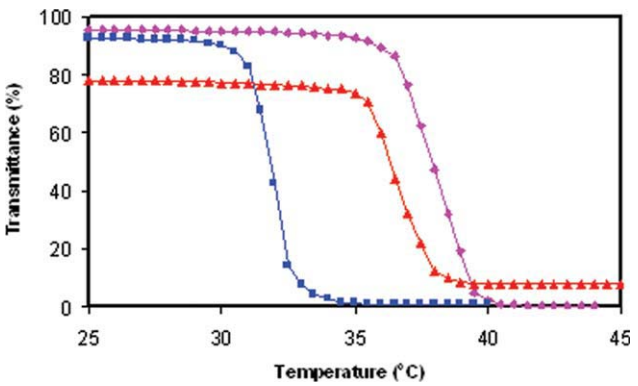

Figure 1 Phase transition behaviors of different copolymers (■PNIPAM; ◆(NIPAM-*co*-HEMA) and ▲ PLLA-*g*-P(NIPAM-*co*-HEMA) in distilled water. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

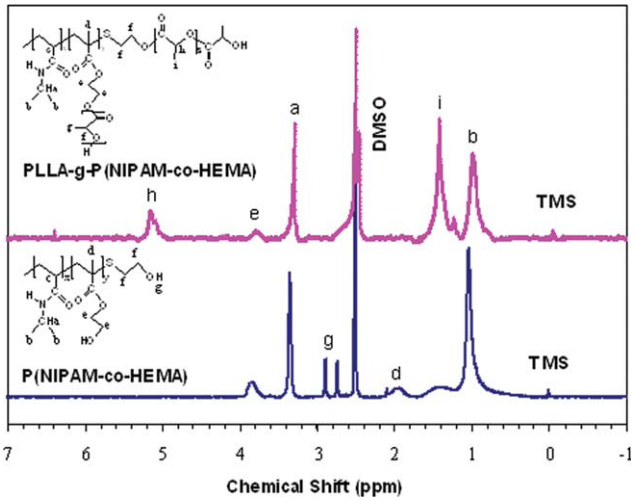

Figure 2 H-NMR spectrum of P(NIPAM-*co*-HEMA) (a) and PLLA-*g*-P(NIPAM-*co*-HEMA) (b) copolymers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

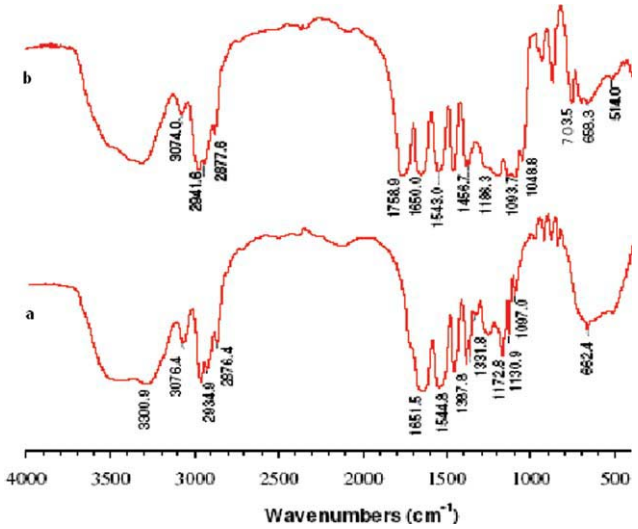

Figure 3 FTIR spectrum of P(NIPAM-*co*-HEMA) (a) and PLLA-*g*-P(NIPAM-*co*-HEMA) (b) copolymers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The LCST of PLLA-*g*-P(NIPAM-*co*-HEMA) is lower than that of P(NIPAM-*co*-HEMA). The grafting of hydrophobic PLLA chains onto the P(NIPAM-*co*-HEMA) backbone, modified the hydroxyl groups in HEMA and/or HET into hydrophobic ester bonds, as illustrated in Scheme 1. As a result, the graft copolymers become less hydrophilic. The phase transition temperature of these graft copolymers are about 37°C, the normal body temperature. Therefore, they can be used as effective drug carriers because their circulation in the body.

To determine chemical structures of the resultant copolymers, P(NIPAM-*co*-HEMA) and PLLA-*g*-P(NIPAM-*co*-HEMA) were subjected to NMR measurements. Figure 2 shows the ^{1}H-NMR spectra of both copolymers in DMSO-d_6. The peaks at around 1.0 and 3.4 ppm are ascribed to methyl (CH_3) and methine (C*H*) protons of PNIPAM segments. The peaks at 1.9 and 3.8 ppm are attributed to methyl (CH_3) and methylene (CH_2) protons of HEMA segments. Besides that, the peak representative of HET component is also observed at about 2.8–2.9 ppm. The spectrum of the graft copolymer reveals the peaks at 1.4 and 5.2 ppm corresponding to methyl (CH_3) and methine (C*H*) protons of PLLA segment, respectively.

IR measurements also confirm the PLLA chains grafted onto the P(NIPAM-*co*-HEMA) backbone. As indicated in Figure 3, IR spectra of both copolymers show an absorbent peak of amide carbonyl groups (C=O) in PNIPAM domain at 1650 cm^{-1}, while the peaks at 1545 and 1457 cm^{-1} are assigned to the characteristic absorptions of its C—N groups.[18] The IR spectrum of PLLA-*g*-P(NIPAM-*co*-HEMA) reveals an apparent peak at 1759 cm^{-1} caused by the stretching vibration of C=O groups in PLLA domain and a new absorbance at 1049 cm^{-1} representative for the absorptions of C—O—CO stretching,[26] which were formed by the grafting of PLLA chain onto P(NIPAM-*co*-HEMA) backbone as described in Scheme 1.

The weight changes of both copolymers during heating are recorded by thermogravity analyses. As presented in Figure 4, the weight loss of P(NIPAM-*co*-HEMA) starts at around 215°C, but keeps the weight up to 330°C because of the differences in thermal stabilities of HEMA and PNIPAM constituents. The thermal stability of PLLA-*g*-P(NIPAM-*co*-HEMA) is much reduced by the grafting of PLLA chains onto the P(NIPAM-*co*-HEMA) backbone. It is explained by the lower thermal stability of PLLA compared to PNIPAM as indicated in our previous

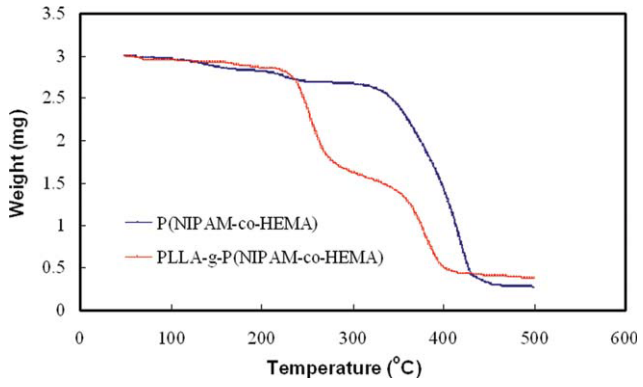

Figure 4 TGA heating curves of the copolymers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

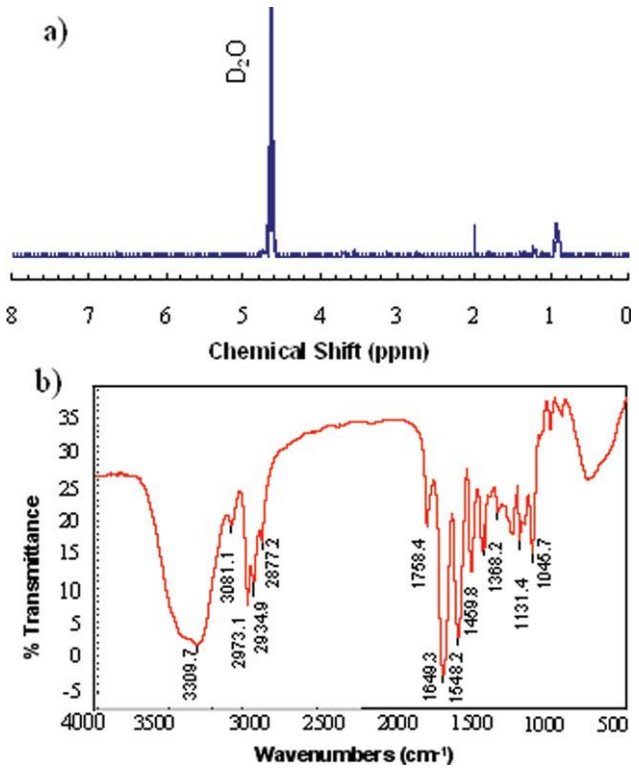

Figure 5 Characterization of the micelles from PLLA-*g*-P(NIPAM-*co*-HEMA) graft copolymers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

study.[20] The PLLA grafted chains are degraded at about 220°C, which is lower than the temperature where the PLLA backbones in PNIPAM-*g*-PLLA graft copolymers started to be degraded. It may be due to the PLLA side chains in this study are short ones, revealed by a lower thermal stability. The ratio of constituent polymers of the graft copolymer can be also estimated from the figure by their corresponding pyrolysis.

Micellar formation

Free and DEX-loaded micelles were obtained by self-assemblies of the graft copolymers and those with the drug in aqueous solution. By hydrophobic interactions among PLLA chains as well as PLLA and DEX, the resultant micelles will be spherical nanostructures. The formation of those structures can be confirmed by NMR or FTIR measurements.[14,19] Figure 5 shows the corresponding constituents of these micellar structures. ^{1}H-NMR spectrum of the micelles in D_2O (a) reveals characteristic peaks of PNIPAM segments at 0.93 and 3.68 ppm, the smaller peak at 3.6 ppm representing for CH_2OH hydrophilic groups of P(HEMA), whereas no other peaks can be observed. It is explained by the hydrophobic domains composed of PLLA side chains formed in D_2O was isolated from the hydrophilic domains composed of P(NIPAM-*co*-HEMA). Figure 5(b) shows more evidence for micellization of the graft copolymers. The peak caused by C=O stretching vibration in the PLLA domain of the micelles at 1758 cm^{-1} become smaller compared to that of the graft copolymer (Fig. 3). It is explained by the vibration of C=O groups in the hydrophobic PLLA core was suppressed by the well-ordered structure of the micelles.

Scanning electron microscopy has been proved to be very useful for the structural research of nanoparticles. Morphology and size of the obtained micellar nanoparticles were investigated by FE-SEM observation and DLS measurement. Figure 6 shows the SEM images of the free and DEX-loaded micelles. It is evident that the micelles are spherical nanoparticles with a narrow size distribution. There is no significant difference in the dimension between free and DEX-loaded micelles.

The average diameters of both free and DEX-loaded micellar nanoparticles are also obtained by DLS and reported in the Table II. Figure 7 shows the

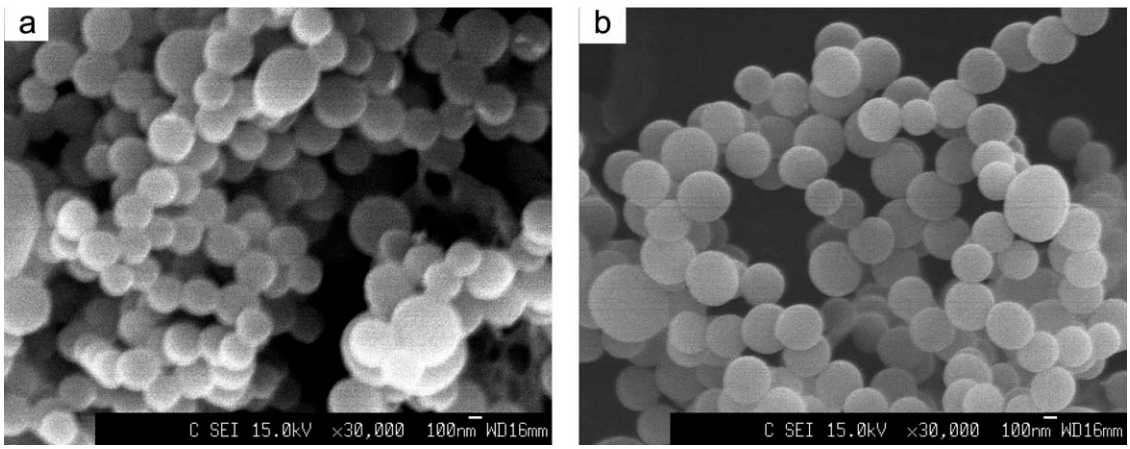

Figure 6 SEM images of free (a) and DEX-loaded (b) micelles.

TABLE II
Size Distribution and LCST of the Micellar Nanoparticles

Micelles	Diameter[a] (nm)	Z-Ave[b] (nm)	PdI	LCST
Free	150–250	180	0.65	36.9
DEX-loaded	160–280	200	0.78	36.8

[a] Determined by microscope (SEM images).
[b] Average diameter and polydispersity index of micelles measured by DLS at room temperature.

size distributions of these nanoparticles. The particulate diameters determined from DLS were completely consistent with those calculated from the SEM images, though small particles with diameter ranging from 5 to 30 nm were not be observed by SEM as indicated in the Figure 7.

Table II also shows the similar phase transition behaviors of both micelles. The results suggest that DEX has no significant influence on the phase transition behavior of micelles. DEX is a hydrophobic drug, which was loaded and stabilized in the hydrophobic part of the micelles by its hydrophobic interaction with PLLA segments in the micelle, but the length of hydrophilic and hydrophobic segments remains constant. Therefore, the drugs have insignificant effect on its hydrophilic and hydrophobic balance of the micelles.

DLS also indicates the scattering intensities and sizes of the micelles quickly reduced after enzymatic degradation as presented in the Figure 8. It is explained by the attack of proteinase K on PLLA domains after their penetration into the micelles and the enzyme can cleave the ester bond in PLLA domains, leaving the hydrophilic PNIPAM-HEMA chains and shorter degraded PLLA chains.

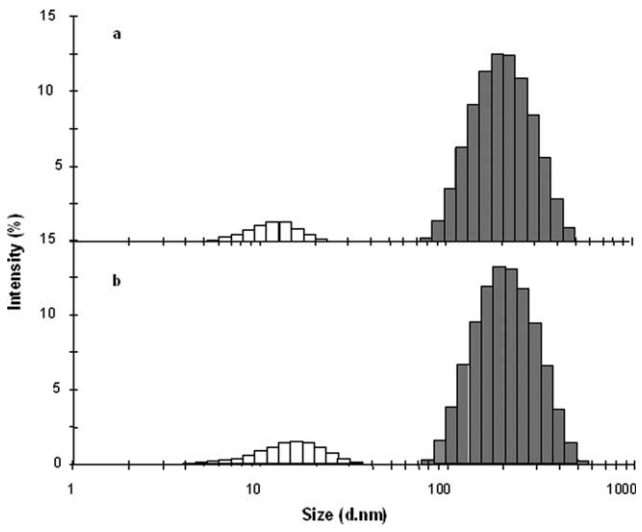

Figure 7 Size distribution by intensity of free (a) and DEX-loaded (b) micellar nanoparticles in distilled water.

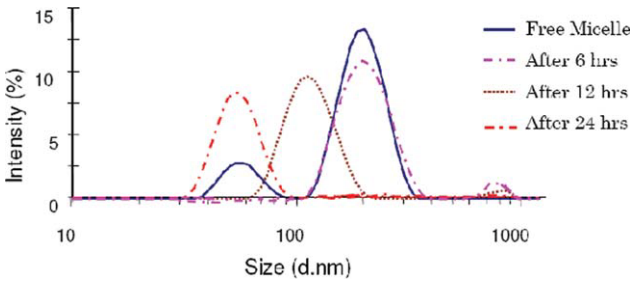

Figure 8 Size-intensity curves of the degraded micellar nanoparticles with incubation times. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Therefore, intensity of the micelles is reduced with incubation time. Furthermore, changes in morphology of the polymeric micelles can also affect scattering intensity. After incubation, the micelles are significantly degraded but the enzymatic degradation is not completed. Some micelles can keep the particulate structure though they are partly degraded. And other smaller micelles can be also formed from the degraded graft copolymers as observation in the Figure 9. As a result, the size of micelles was much reduced.

Thermoresponsive drug release *in vitro*

Through the hydrophobic interaction with the micellar core, different hydrophobic drugs can be loaded into the micelles. In this study, about 16% w/w DEX was loaded into the micelles after dialysis process. Because of the thermosensitivity of the micelles, the drug release is temperature-dependent. When temperature increases up to the LCST, the hydrophilic shell becomes hydrophobic, resulting in aggregation of several adjacent micelles as observed in Figure 10(a). However, these aggregated states of the DEX-loaded micelles are reversibly changed by raising and lowering temperature quickly [Fig. 10(b)].

Figure 11 shows the drug release behaviors of the DEX-loaded micelles as functions of time above and below the LCST. At room temperature, 15% DEX was released after 1 h, and then the release rate is gradually reduced with time. About 60% of the drug was released from the particles after 12 h, whereas a large amount of DEX still remains in the micelles because of stable well-ordered assembly of the micelles under the LCST. Based on these stable structures, they can escape from the nonselective reticuloendothelial system (RES), then passive target to the specific site. Active targeting can be also modulated with controlling the interaction of the micelles with the target sites, where the drugs are released and become effectual by deformation or degradation

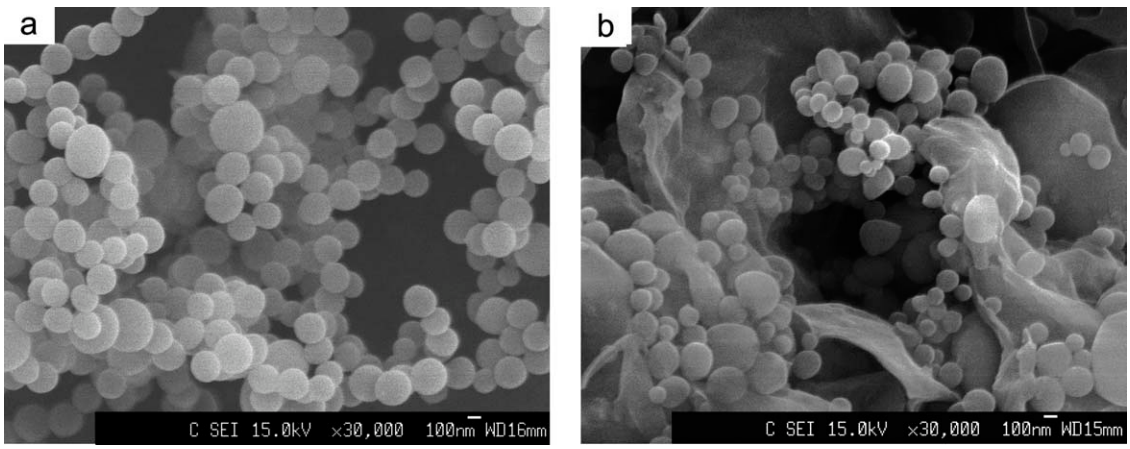

Figure 9 The SEM image of the grafted micelles before (a) and after (b) enzymatic degradation.

of the micelles through the changes of physiological conditions or attacks by enzymes.[27]

At the normal body temperature, the drug release was accelerated and about 96% of DEX was released from the micelles during experiment. It may be due to the changes of the micellar structure at the LCST. At 40°C, above the LCST, the amount and the rate of drug release further increased and the drugs continue to be released up to 48 h. The hydrophilic outer shell layer consisting of P(NIPAM-*co*-HEMA) chains stabilized the structure of DEX-loaded micelles below the LCST, where the drug release mainly occurred by diffusion.[28] When the temperature is raised over the LCST, the hydrogen bonding interaction between water and amide groups of P(NIPAM-*co*-HEMA) segments are broken down and exposed the hydrophobic inner core composed of drugs and PLLA segments. As a result, the drugs are rapidly diffused from the micelles in an early state. At the same time, more hydrophobic micelles start to aggregate and a thermoinduced structural deformation, which much increases the drugs release, may occur. Thus, these thermosensitive micellar nanoparticles can be utilized as the effective carriers for delivery of DEX.

CONCLUSIONS

New graft copolymers of PLLA-*g*-P(NIPAM-*co*-HEMA) were obtained from biodegradable PLLA side chains and amphiphilic copolymers of PNIPAM with HEMA as the backbone chains. The results proved that the micelles from the graft copolymer are thermosensitive with the LCST near the normal body temperature. In aqueous solutions, they self-assemble into regular spherical micelles in the presence or absence of DEX. The micelles are enzymatic degraded by incubation with proteinase K. Both free and DEX-loaded micellar nanoparticles are uniform with narrow size distribution, but still there are several unimers and small particles have been also observed. In PBS solution, DEX was slowly released by diffusion and a large amount of the drugs remained in the DEX-loaded micelles below the LCST, but most of the drugs are released from the micelles with accelerated rate above the LCST.

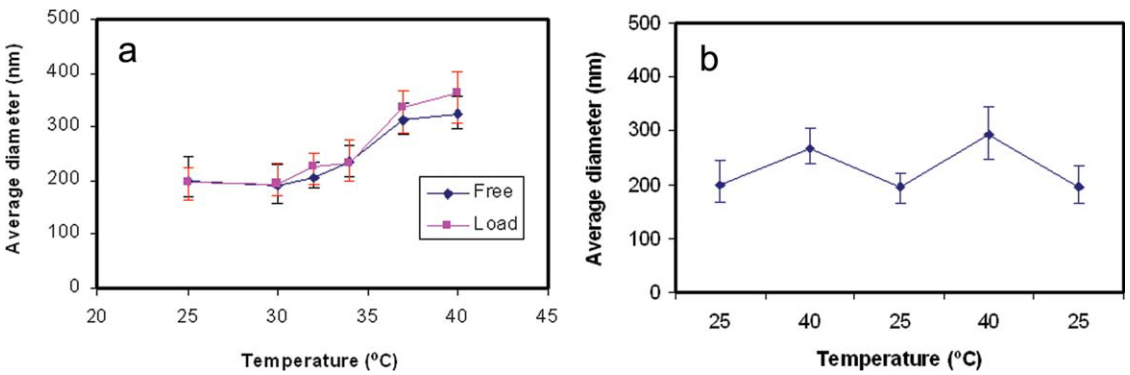

Figure 10 Temperature-dependences of particle size (▲ free; and ■ DEX-loaded micelles) in distilled water. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

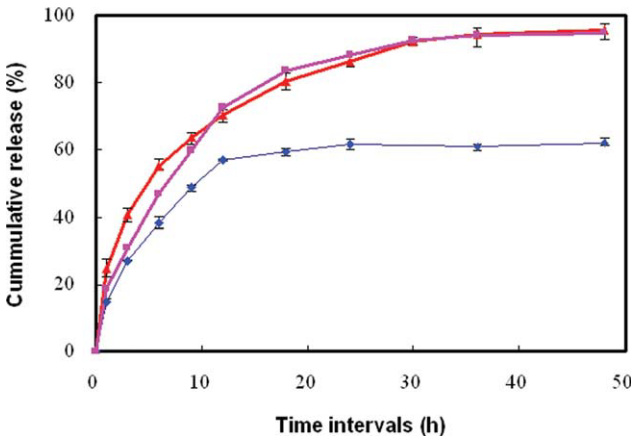

Figure 11 Drug release from DEX-loaded micellar nanoparticles in PBS (0.05*M*, pH 7.4) at 25°C (◆), 37°C (■) and 40°C (▲). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

These thermosensitive and biodegradable micelles are expected to be an effective drug delivery system for not only DEX but also other poorly-water soluble drugs via the hydrophobic interaction between the drugs and PLLA segments. The obtained micellar nanoparticles may be also applied to other site-specific drug delivery systems.

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