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Spray-dried microparticles composed of cinnamoyl poly(*N*-isopropylacrylamide-*co*-hydroxyethylacrylate) and gold nanoparticle

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ABSTRACT: Temperature- and NIR irradiation-responsive microparticles composed of cinnamoyl poly(*N*-isopropylacrylamide-*co*-hydroxyethylacrylate) [CinP(NIPAM-HEA)] and gold nanoparticle (GNP) were prepared by a spray-drying method. According to the cloud points determined by an optical method, the HEA content in P(NIPAM-HEA) had no marked effect on the lower critical solution temperature (LCST). However, the cinnamoyl group content in CinP(NIPAM-HEA) had a significant effect on the LCST. The LCSTs determined by a calorimetric method was in agreement with those determined by an optical method. The hydrodynamic mean diameter of gold nanoparticle (GNP) prepared by reducing gold ions was about 30 nm and it seemed to be a nanosphere on TEM photo. Spray-dried CinP(NIPAM-HEA) microparticles containing GNP was 1.5 μm to 12 μm in diameter on SEM photo. Gold was detected on the energy-dispersive X-ray spectrum of the microparticles. The amount of FITC-dextran released for 12 h from the microparticles was much higher at temperatures above the LCST (at 37 °C and 45 °C) than below the LCST (at 20 °C and 25 °C). The cumulative release amount in 12 h was only about 3% without NIR irradiation, whereas the value was about 26.5% when NIR was irradiated to the microparticle suspension. The photothermal energy generated by GNP was believed to render the thermosensitive copolymers de-swollen and hydrophobic, allowing for the active release of dye from the microparticles. The NIR irradiation-responsive GNP-loaded microparticles could be applied to the development of NIR-responsive drug carriers which release their contents in response to an external stimulus (i.e., NIR irradiation). © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 44141.

KEYWORDS: drug delivery systems; irradiation; nanoparticles; nanowires and nanocrystals; stimuli-sensitive polymers

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INTRODUCTION

Polymers exhibiting lower critical solution temperature (LCST) or upper critical solution temperature (UCST) have been exploited in developing thermoresponsive drug carriers. Poly(*N*-isopropylacrylamide) (PNIAPM) is one of the polymers showing LCST behavior in aqueous phase. The polymer chains are hydrated and expanded when the solution temperature are below LCST and they become dehydrated and collapse into condensed form due to the intramolecular hydrophobic interaction when the solution is heated to a temperature above LCST.[1–3] PNIPAM microgels were reported to control the release of their content by skin formation or by squeezing-out mechanism, when the medium temperature increased across LCST.[4,5] The thermosensitive polymer was immobilized on the surface of liposome, and the thermal contraction of the polymer chains was claimed to render liposomal membrane disordered, allowing for the temperature-triggered release.[6,7] PNIPAM was immobilized in the water channel of monoolein cubic phase and it was reported to act as a thermal valve to control the release in response to temperature change.[8] For the same purpose, the thermosensitive polymer was included in alginate bead.[9] Recently, in order to develop NIR irradiation-responsive drug carrier, gold nanoparticles (GNP) were included in thermoresponsive drug carriers. Liposome bearing PNIPAM showed NIR irradiation-triggered release in the presence of GNP.[10] Under NIR irradiation, photothermal energy is generated by the surface plasmon resonance of GNP[11] and it could cause not only the liposomal membrane but also the thermosensitive polymer to undergo their phase transition, leading to NIR irradiation-triggered release. Monoolein cubic phase incorporating GNP exhibited NIR irradiation-responsive release due to the phase transition of the bilayers.[12] In the

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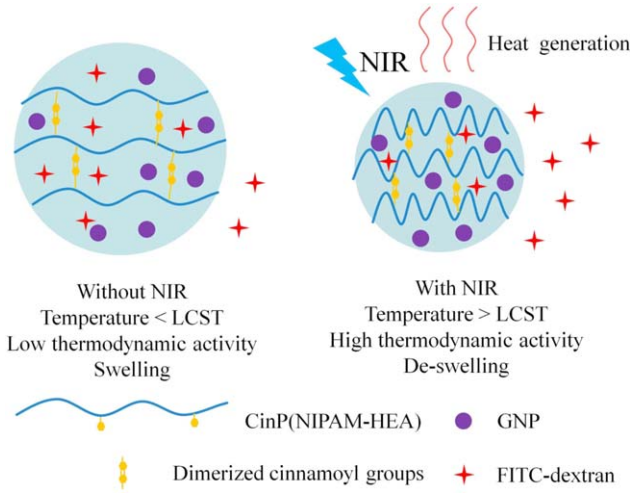

Scheme 1. Schematic representation of NIR irradiation-responsive microparticles composed of cinnamoyl poly(*N*-isopropylacrylamide-*co*-hydroxyethylacrylate) and gold nanoparticle. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

present study, NIR irradiation-responsive microparticles composed of cinnamoyl poly(*N*-isopropylacrylamide-*co*-hydroxyethylacrylate) [CinP(NIPAM-HEA)] and GNP were prepared by spray-drying the mixture solution of CinP(NIPAM-HEA) and GNP. UV light was irradiated to the microparticles to dimerize the cinnamoyl groups and crosslink the copolymer [CinP(NIPAM-HEA)] chains. Under NIR irradiation, the microparticle suspension could be heated up across the LCST due to the surface plasmon resonance of GNP included in the microparticles. Accordingly, the copolymer chains in the microparticles would become dehydrated, de-swollen and hydrophobic. In this circumstance, a water-soluble compound would be squeezed out of the microparticles by the thermally induced deswelling of the cross-linked copolymer chains or to be expelled from the microparticles by the thermally induced increase in the thermodynamic activity of the compound (Scheme 1). If NIR is externally irradiated to a specific tissue site where GNP-loaded microparticles exist, the light can penetrate deeply into the tissue[13] and reach GNP. As a result, heat can be generated by GNP and the microparticles would exhibit a promoted release at the specific tissue site to which NIR is irradiated. The NIR irradiation-responsive release mechanism of GNP-loaded microparticles could be applied to the development of NIR-responsive drug carriers which release their contents in response to an external stimulus (i.e. NIR irradiation).

EXPERIMENTAL

Materials

N-isopropylacrylamide (NIPAM), hydroxyethyl acrylate (HEA), triethylacetate, tetrahydrofuran, cinnamoyl chloride, phosphorous pentoxide, trisodium citrate dihydrate ($Na_3C_6H_5O_7{\cdot}2H_2O$), fluorescein isothiocyanate–dextran (FITC-dextran, MW 10,000), cetrimonium bromide (CTAB) were purchased from Sigma–Aldrich Co. (St. Louis, MO). Hydrogen tetrachloroaurate (III) tetrahydrate ($HAuCl_4{\cdot}H_2O$) was purchased from Wako (Osaka, Japan). Diethylether was purchased from Dae Jung Chemical Co. α,α′-Azobis(isobutyronitrile) (AIBN) was purchased from Junse Chemical Co.

Preparations of Poly(*N*-isopropylacrylamide-*co*-hydroxyethyl acrylate)

Poly(*N*-isopropylacrylamide-*co*-hydroxyethyl acrylate) [P(NIPAM-HEA)] was prepared by a free radical reaction.[14] NIPAM and HEA were co-dissolved in 200 mL of dioxane contained in a 250 mL three-neck round bottom flask so that NIPAM to HEA molar ratio was 9:1, 8:2, and 7:3 while the total concentration of monomers being kept constant [5.67% (w/v)]. AIBN [82 mg (0.5 mmol)] was added to the mixture solution and degassed using nitrogen stream for 30 min. The mixture solution was heated to 70 °C and was stirred at the same temperature for 24 h with reflux. P(NIPAM-HEA) was precipitated in diethylether, filtered, and dried in a dry oven at 40 °C. P(NIPAM-HEA) prepared from the reaction mixture whose NIPAM to HEA molar ratio was 9:1, 8:2, and 7:3 was termed as P(NIPAM-HEA) (9:1), P(NIPAM-HEA) (8:2), and P(NIPAM-HEA) (7:3), respectively.

Preparations of Cinnamoyl Poly(*N*-isopropylacrylamide-*co*-hydroxyethyl acrylate)

Cinnamoyl poly(*N*-isopropylacrylamide-*co*-hydroxyethyl acrylate) [CinP(NIPAM-HEA)] was prepared by condensation between the hydroxyl group of HEA of P(NIPAM-HEA) and the acid chloride of cinnamoyl chloride. Triethylacetate (0.89 mL (6.37 mol)) and either P(NIPAM-HEA) (9:1) (5.16 g), P(NIPAM-HEA) (8:2) (3.37 g), or P(NIPAM-HEA) (7:3) (2.33 g) was dissolved in 40 mL of tetrahydrofuran contained in a 100 mL round bottom flask, and the solution was degassed with nitrogen stream for 30 min. The flask was immersed in an ice bath to cool the solution down to 5 to 6 °C. Cinnamoyl chloride (1.06 g (6.37 mmol)) was dissolved in 5 mL of tetrahydrofuran and it was dropped to P(NIPAM-HEA) solution. The reaction mixture was stirred at 5 to 6 °C for 12 h then stirred at 20 °C for 24 h. CinP(NIPAM-HEA) was precipitated in diethyl ether, filtered, washed with diethyl ether, then dried in a dry oven (40 °C). CinP(NIPAM-HEA) prepared from P(NIPAM-HEA) (9:1), P(NIPAM-HEA) (8:2), and P(NIPAM-HEA) (7:3) was termed as CinP(NIPAM-HEA) (9:1), CinP(NIPAM-HEA) (8:2), and CinP(NIPAM-HEA) (7:3), respectively.

^{1}H NMR Spectroscopy

P(NIPAM-HEA) and CinP(NIPAM-HEA) were dried overnight with phosphorous pentoxide in a vacuum oven at 45 °C. The copolymers were dissolved in D_2O, and the ^{1}H NMR spectra were taken on a Bruker Avance 400 MHz spectrometer (Karlsruhe, Germany, located in the Central Laboratory Center of Kangwon National University). The spectrometer parameters were relaxation time = 2 s, sweep width = 8223 Hz, acquisition time = 1.99 s, and number of scan = 64.

Determination of Cloud Point of P(NIPAM-HEA) Solution and CinP(NIPAM-HEA) Solution

Each of P(NIPAM-HEA) and CinP(NIPAM-HEA) was dissolved in distilled water so that the concentration was 0.3% (w/v). 2.5 mL of copolymer solution was put in a 3 mL cuvette and the optical density at 600 nm was determined on a UV spectrophotometer (6505 UV/Vis. Spectrophotometer, JENWAY, UK)

equipped with a Peltier temperature controller in the temperature range of 20 °C to 50 °C at heating rate of 3 °C min^{-1}.

Differential Scanning Calorimetry of P(NIPAM-HEA) Solution and CinP(NIPAM-HEA) Solution

Each of P(NIPAM-HEA) and CinP(NIPAM-HEA) was dissolved in distilled water so that the concentration was 15% (w/v) except for CinP(NIPAM-HEA) (7:3). Since CinP(NIPAM-HEA) (7:3) was poorly soluble in water due to the higher content of cinnamoyl group, the concentration used for calorimetric study was only about 2%. The copolymer solutions were put in aluminum pans (T zero pan), and they were capped and tightly sealed using a press (TA Instruments). The pans containing copolymer solutions were scanned on a differential scanning calorimetry (DSC Q2000, TA Instruments, installed in the Central Laboratory Center of Kangwon National University) in the temperature range of 20 °C to 50 °C at the heating rate of 2 °C min^{-1}. Temperature and enthalpy were calibrated using indium as a standard. An empty pan was used as reference.

Preparation of Gold Nanoparticles

Gold nanoparticle (GNP) was prepared by a method described elsewhere.[12] Hydrogen tetrachloroaurate (III) tetrahydrate ($HAuCl_4.H_2O$, 123 mg (0.3 mmol)) was dissolved in 120 mL of distilled water and the gold ion solution was heated to 80 °C. Trisodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$, 342 mg (1.16 mmol)) was dissolved in 30 mL of distilled water and the solution was heated to the same temperature. 30 mL of trisodium citrate solution was added to 120 mL of gold ion solution, then the mixture was stirred at 80 °C for 10 min to reduce gold ion to gold nanoparticles. The GNP solution (2 m*M*) was cooled to room temperature and it was stored in a refrigerator (4 °C).

Characterization of Gold Nanoparticles

GNP was characterized by methods described in a previous report.[12] The hydrodynamic diameter of GNP was measured by a dynamic light scattering technique. GNP solution was diluted with distilled water so that the light scattering intensity on a dynamic light scattering equipment (Plus 90; Brookhaven, Holtsville, NY) fell within 50 to 200 kilo counts per second. In parallel, the zeta potential of GNP was determined under the same condition as used for the determination of the hydrodynamic diameter. The shape of GNP was investigated by transmission electron microscopy. A drop of GNP suspension was put on a formvar/copper-coated grid (200 mesh) and it was air-dried at room temperature. The shape of GNP was observed through the TEM photo taken on a Transmission Electron Microscope (LEO 912AB OMEGA, Germany, at KBSI, Chuncheon, Korea). The photothermal conversion property of GNP was investigated by measuring the temperature of GNP solution under NIR irradiation. 3 mL of GNP solution (1 m*M* and 2 m*M*) contained in a 1 mL-glass vial was subjected to the irradiation of NIR laser (808 nm, 2 W, Power Technology, AR) for 60 min. The surface of GNP solution was 10 cm distant from the source of laser. While subjected to NIR irradiation, the temperature was measured using a thermo couple every 5 or 10 min. As a control, distilled water was subjected to NIR irradiation and the temperature was measured under the same condition.

Preparation of Cin P(NIPAM-HEA) Microparticles Containing GNP by Spray-Drying

One gram of CinP(NIPAM-HEA) (8:2), 5 mg of FITC-dextran, and 0.2 g of CTAB were co-dissolved in 200 mL of GNP solution (1 m*M*). CinP(NIPAM-HEA) microparticles containing GNP were prepared by spray-drying the mixture solution in a spray drier (Buchi-B290 Mini-Spray Drier) operating at the pumping rate of 20%, the inlet temperature of 160 °C, and the aspiration of 90%. UV light (254 nm, 6 W) was irradiated to the spray-dried microsphere to dimerize the cinnamoyl group of CinP(NIPAM-HEA) and cross-link the copolymer chains.

Scanning Electron Microscopy and Energy Dispersive Spectroscopy

CinP(NIPAM-HEA) microparticles containing GNP were mounted on metal stubs, and coated with gold using sputter coating. The SEM photo was taken on a scanning electron microscope (Jeol JSM-840A, located in the Central laboratory of Kangwon National University). The surface composition of CinP(NIPAM-HEA) microparticles was examined by taking the energy dispersive spectroscopy of gold-uncoated microparticles on a UHR-SEM equipped with energy dispersive X-ray spectrometer.

Temperature- and NIR Irradiation-Responsive Release of FITC-Dextran from CinP(NIPAM-HEA) Microparticles Containing GNP

Thirty-six milligrams of CinP(NIPAM-HEA) microparticles was put in 0.5 mL of distilled water contained in a dialysis bag (MWCO 10,000), then it was soaked in 2.5 mL of distilled water contained in a 5 mL glass vial. The temperature of release medium was adjusted to 20 °C, 25 °C, 37 °C, and 45 °C. 0.5 mL of release medium was taken at a given time for 12 h and the fluorescence intensity at 492 nm was determined on a fluorescence spectrophotometer (F-2500, HITACHI, Tokyo, Japan) with the excitation wavelength of 515 nm. After measuring the fluorescence intensity, the release medium was put back to the dialysis medium. In order to investigate the effect of NIR irradiation on the release, NIR light (808 nm, 2 W, Power Technology, AR) was irradiated to the suspension of CinP(NIPAM-HEA) microparticles (0.5 mL) contained in a dialysis bag which was immersed in 2.5 mL of distilled water contained in a 5 mL glass vial. 0.5 mL of release medium was taken at a given time for 12 h and the fluorescence intensity was determined under the same condition as mentioned above. During the NIR irradiation, the temperature of release medium was measured using a thermo couple at a given time for 12 h. The cumulative release amount at a given time was defined as the percent of the amount of dye released with respect to the initial amount of dye loaded in the microparticles.

RESULTS AND DISCUSSION

^{1}H NMR Spectroscopy

Figure 1 shows the ^{1}H NMR spectrum of P(NIPAM-HEA) (9:1) and CinP(NIPAM-HEA) (9:1). In the spectrum of P(NIPAM-HEA) (9:1), the methyl group of NIPAM was found at 1.0 ppm, the methylene group next to the hydroxyl group of HEA was found at 3.8 ppm, the methylene group next to the ester bond of HEA was found at 4.2 ppm, the vinyl methylene group was

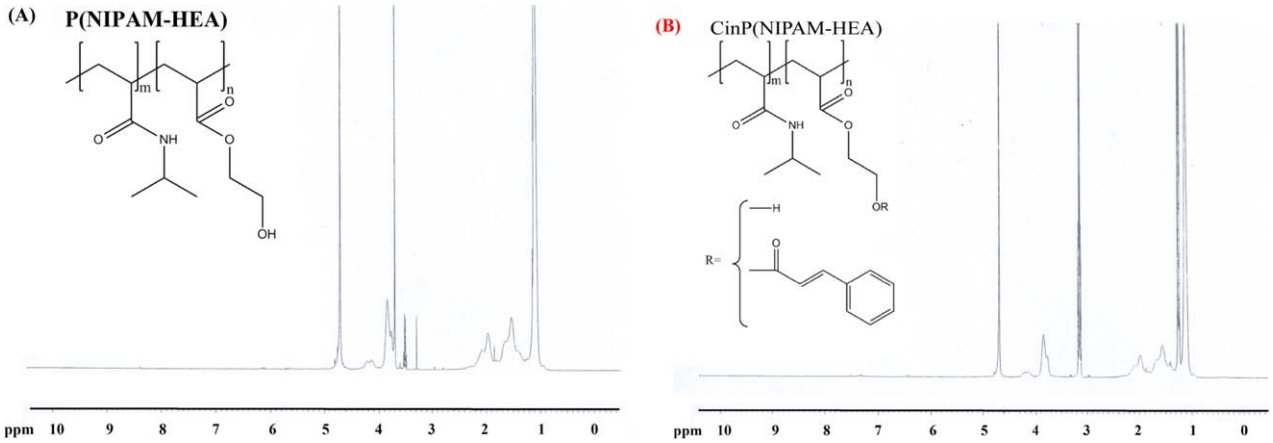

Figure 1. ^{1}H NMR spectrum of P(NIPAM-HEA) (9:1) (A) and CinP(NIPAM-HEA) (9:1) (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

found at 1.5 ppm, and the vinyl methine group was found at 1.9 ppm.[14] Using the peak area of the methyl group of NIPAM and the peak area of the methylene group next to the ester bond of HEA, the NIPAM to HEA molar ratio was calculated to be 86:14. Using the peak areas on the ^{1}H NMR spectrum of P(NIPAM-HEA) (8:2) [Supporting Information Figure 1(A)] and that of P(NIPAM-HEA) (7:3) [Supporting Information Figure 1(B)], the NIPAM to HEA molar ratio of P(NIPAM-HEA) (8:2) and that of P(NIPAM-HEA) (7:3) were determined to be 78.5:21.5 and 68.8:31.2, respectively. In the spectrum of CinP(-NIPAM-HEA) (9:1), cinnamoyl group was found in 7.2 ppm to 7.7 ppm, together with the characteristic signals of NIPAM and HEA.[14,15] Using the peak area of cinnamoyl group and the peak area of the methyl group of NIPAM, the NIPAM to cinnamoyl group molar ratio was calculated to be about 86:5. Thus, the NIPAM/HEA/cinnamoyl group molar ratio of CinP(NIPAM-HEA) (9:1) was estimated to be about 86:14:5. In the same way, the NIPAM/HEA/cinnamoyl group molar ratio of CinP(NIPAM-HEA) (8:2) and that of CinP(NIPAM-HEA) (7:3) were determined to be about 78.5:21.5:7.5 and 68.8:31.2:17, respectively.

Determination of Cloud Point of P(NIPAM-HEA) Solution and CinP(NIPAM-HEA) Solution

Figure 2(A) shows the temperature-dependent optical density of P(NIPAM-HEA) (9:1), P(NIPAM-HEA) (8:2), P(NIPAM-HEA) (7:3) solution. The optical density of P(NIPAM-HEA) (9:1) solution was almost 0 in the temperature range of 20 °C to 30 °C and it began to increase rapidly at 31 °C. It is well known that the homopolymer (poly(*N*-isopropylacrylamide), PNIPAM) shows lower critical solution temperature (LCST) behavior in water and its LCST is around 32 °C.[16,17] Below the phase transition temperature, PNIPAM chains are hydrated and solubilized in water and they take an expanded form. The polymer chains become hydrophobic and dehydrated as the solution temperature increases, and they begin to collapse into a condensed form at a certain temperature (LCST) due to intramolecular hydrophobic interaction.[17,18] P(NIPAM-HEA) (9:1) was believed to

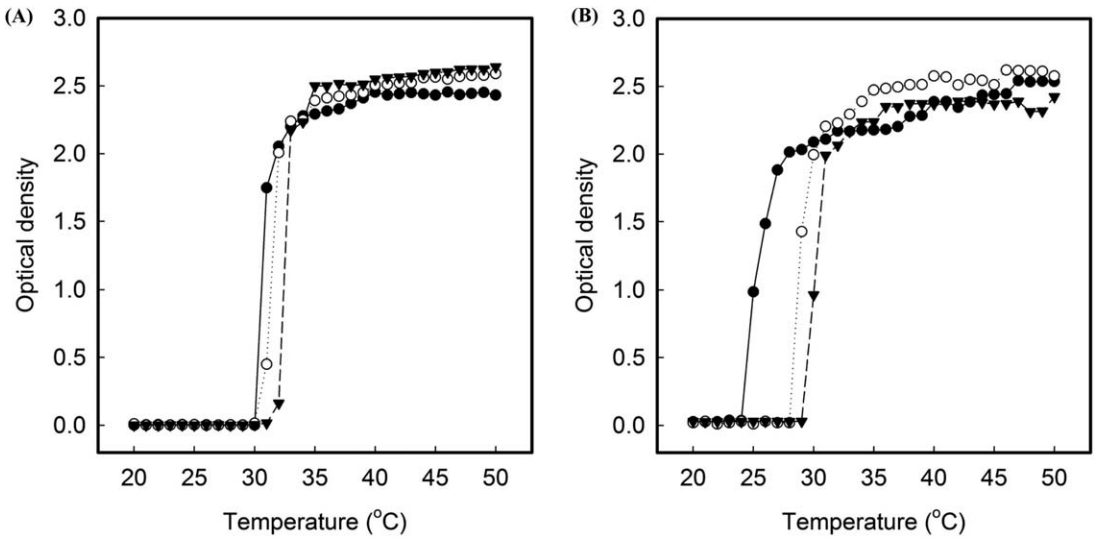

Figure 2. Temperature-dependent optical density of P(NIPAM-HEA) (9:1) (▼), P(NIPAM-HEA) (8:2) (○), P(NIPAM-HEA) (7:3) (●) solution (A). Temperature-dependent optical density of CinP(NIPAM-HEA) (9:1) (▼), CinP(NIPAM-HEA) (8:2) (○), and CinP(NIPAM-HEA) (7:3) (●) solution (B).

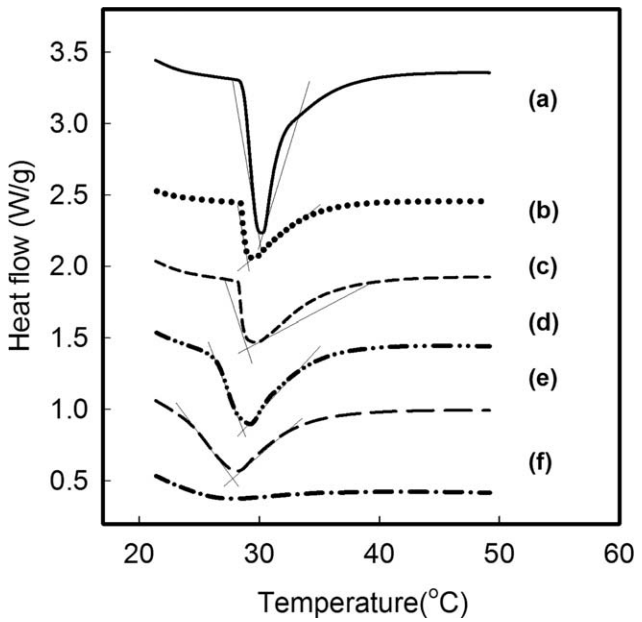

Figure 3. Thermogram of P(NIPAM-HEA) (9:1) (A), P(NIPAM-HEA) (8:2) (B), P(NIPAM-HEA) (7:3) (C), CinP(NIPAM-HEA) (9:1) (D), CinP(NIPAM-HEA) (8:2) (E), and CinP(NIPAM-HEA) (7:3) (F) solution.

be solubilized in water in the temperature range of 20 °C to 30 °C because its solution was optically transparent. The copolymer chains seemed to collapse at 31 °C because the optical density began to increase at the temperature, thus the LCST of P(NIPAM-HEA) (9:1) was believed to be around 31 °C. The optical density profile of P(NIPAM-HEA) (8:2) solution and that of P(NIPAM-HEA) (7:3) solution were similar to the optical density profile of P(NIPAM-HEA) (9:1) solution. According to the starting point of optical density increase, P(NIPAM-HEA) (8:2) and P(NIPAM-HEA) (7:3), both of them, seemed to exhibit their LCST around 30 °C. The copolymerization of NIPAM with a hydrophilic monomer leads to an increase in the LCST, and the copolymerization with a hydrophobic monomer results in a decrease in the LCST.[1] When butyl methacrylate (BMA) was copolymerized with NIPAM so that the molar content of BMA in the copolymer was 2.3%, the LCST was found to be about 7 °C lower than that of homo NIPAM polymer.[10] Since BMA is a hydrophobic monomer, it could facilitate the thermal collapse of the thermosensitive polymer chains. Despite the HEA content was significant in the copolymers (14% in P(NIPAM-HEA) (9:1), 21.5% in P(NIPAM-HEA) (8:2), 31.2% in P(NIPAM-HEA) (7:3)), HEA had no marked effect on the LCST of the thermosensitive polymer. HEA is a kind of hydrophilic monomer but it is thought not to be hydrophilic enough to affect the LCST. Figure 2(B) shows the temperature-dependent optical density of CinP(NIPAM-HEA) (9:1), CinP(NIPAM-HEA) (8:2), and CinP(NIPAM-HEA) (7:3) solution. The optical density of CinP(NIPAM-HEA) (9:1) CinP(NIPAM-HEA) (8:2), and CinP(NIPAM-HEA) (7:3) solution began to increase rapidly at 30 °C, 28 °C, and 24 °C, respectively. Since the content of cinnamoyl group in CinP(NIPAM-HEA) (9:1), CinP(NIPAM-HEA) (8:2), and CinP(NIPAM-HEA) (7:3) was 4.76%, 6.98%, and 14.53%, respectively, it can be said that LCST decreased with increasing the content of cinnamoyl group. Cinnamoyl group is hydrophobic due to its phenyl group so it can promote the thermal collapse of the copolymer chains.

Differential Scanning Calorimetry of P(NIPAM-HEA) Solution and CinP(NIPAM-HEA) Solution

Figure 3 shows the thermogram of P(NIPAM-HEA) (9:1), P(NIPAM-HEA) (8:2), P(NIPAM-HEA) (7:3), CinP(NIPAM-HEA) (9:1), CinP(NIPAM-HEA) (8:2), and CinP(NIPAM-HEA) (7:3) solution. All the copolymer solutions exhibited an endothermic peak except for CinP(NIPAM-HEA) (7:3) solution. As described previously, NIPAM homopolymer and its copolymers exhibit LCST behavior in an aqueous phase.[18–21] The thermosensitive polymer chain undergoes the configurational change from expanded to collapsed form when the polymer solution is heated across its LCST. Since heat input is required for the configurational change, the endothermic peaks could be ascribed to the configurational change and the temperatures where the endothermic peaks appeared were believed to be their LCST. The endothermic peak of P(NIPAM-HEA) (9:1), P(NIPAM-HEA) (8:2), P(NIPAM-HEA) (7:3), CinP(NIPAM-HEA) (9:1),

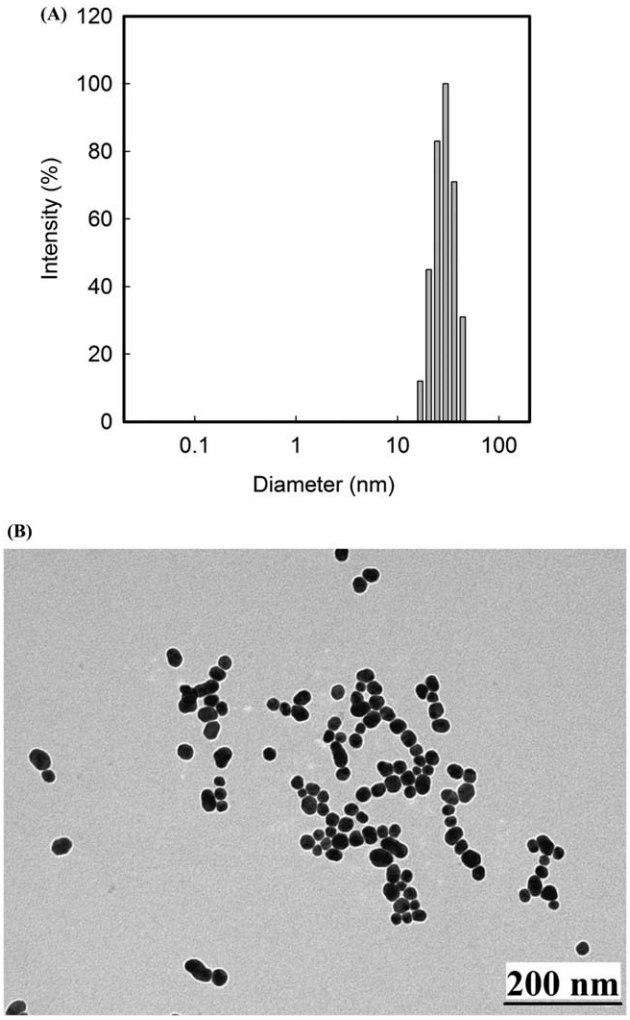

Figure 4. Distribution of hydrodynamic diameter of GNP (A). TEM photo of GNP (B).

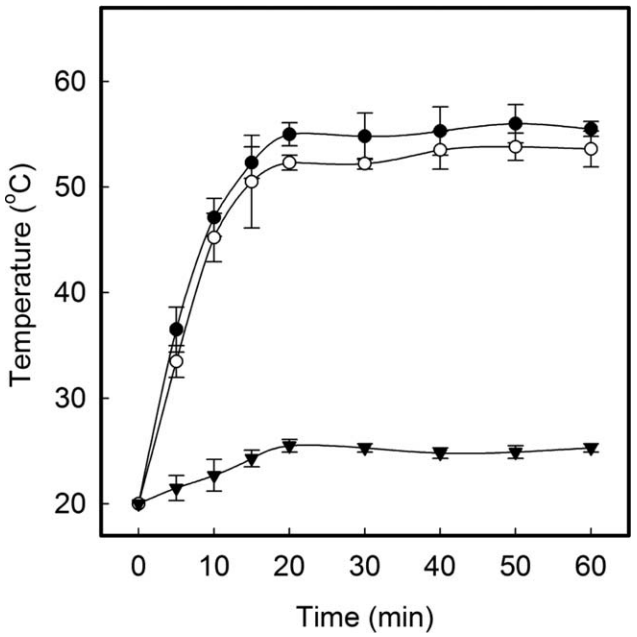

Figure 5. Temperature profiles of distilled water (▼) and GNP solution [1 m*M* (○), 2 m*M* (●)] under NIR irradiation.

and CinP(NIPAM-HEA) (8:2) was found at 30.2 °C, 29.5 °C, 29.7 °C, 29.2 °C, and 28.2 °C, respectively. The temperatures where the endothermic peaks were found did not markedly deviate from the cloud points. No endothermic peak was found with CinP(NIPAM-HEA) (7:3) solution. The copolymer were poorly soluble in water due to the higher content of cinnamoyl group, thus the diluted solution (2% (w/v)) was subjected to the thermal scan, resulting in no detection of heat flow change at its LCST.

Characterization of Gold Nanoparticles

Figure 4(A) shows the distribution of hydrodynamic diameter of GNP. The diameter of the first population ranged from 15 nm to 50 nm and the mean diameter was about 30 nm. The zeta potential of GNP was −43.5 mV, not markedly different from the result reported in a previous work.[10] The TEM photo of GNP was shown in Figure 4(B). GNP seemed to be sphere and the diameter was 10 to 30 nm on the TEM photo. Figure 5 shows the temperature profile of distilled water and GNP solution under the irradiation of NIR. The temperature of distilled water increased from 20 °C to 24 °C for 60 min, possibly due to the dissipation of NIR irradiation energy. The temperature of GNP solution (1 m*M*) increased from 20 °C to 52.5 °C for the first 20 min then no marked increase was observed during the rest period of irradiation. If GNP absorbs NIR light, electrons are excited due to the surface plasmon resonance and they collide with the lattice of GNP to generate heat.[11,22] No marked increase in later stage can be ascribed to equilibrium between heat generation and heat loss. The temperature profile of GNP solution (2 m*M*) resembled that of GNO solution (1 m*M*). Despite of two times higher concentration, the maximum temperature of GNP solution (2 m*M*) was only about 3 °C higher than that of GNP solution (1 m*M*).

Scanning Electron Microscopy and Energy Dispersive Spectroscopy

Figure 6(A) shows the SEM photo of spray-dried CinP(NIPAM-HEA) microparticles. Sphere-like particles were observed together with flat ball-like particles, and the diameter ranged from 1.5 μm to 12 μm. Figure 6(B) shows the energy dispersive X-ray spectrum of CinP(NIPAM-HEA) microparticles. The signal of carbon, nitrogen, oxygen, and gold was found in the spectrum. Carbon, nitrogen, and oxygen were those of CinP(NIAPM-HEA) and gold was originated from GNP. Thus, the microparticles were believed to contain GNP.

Temperature- and NIR Irradiation-Responsive Release of FITC-Dextran from CinP(NIPAM-HEA) Microparticles Containing GNP

Figure 7 shows the release profiles of FITC-dextran from CinP (NIPAM-HEA) microparticles containing GNP at 20 °C, 25 °C, 37 °C, and 45 °C. When the temperature of release medium was 20 °C and 25 °C, no marked release was observed. Since the LCST of CinP(NIPAM-HEA) (8:2) was about 28 °C, the copolymer chains constituting microparticles would be hydrated and swollen in the release medium at those temperatures. The dye release would take place by the diffusion through the mesh of crosslinked copolymer chains. The NIPAM/HEA/CA molar ratio of CinP(NIPAM-HEA) (8:2) was 78.5:21.5:7.5. This indicated that one cinnamoyl group was attached to every 13.3 of vinyl

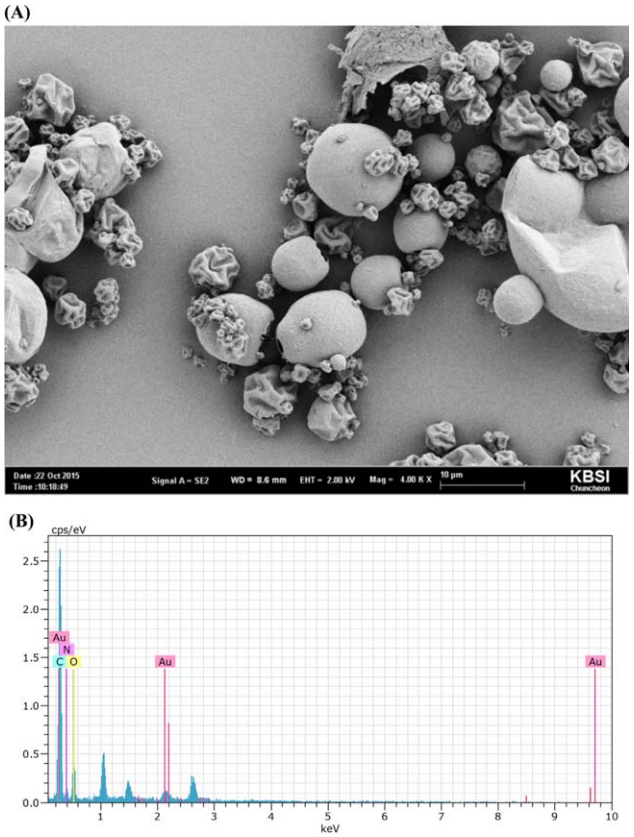

Figure 6. SEM photo of spray-dried CinP(NIPAM-HEA) microparticles (A). Energy dispersive X-ray spectrum of CinP(NIPAM-HEA) microparticles (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

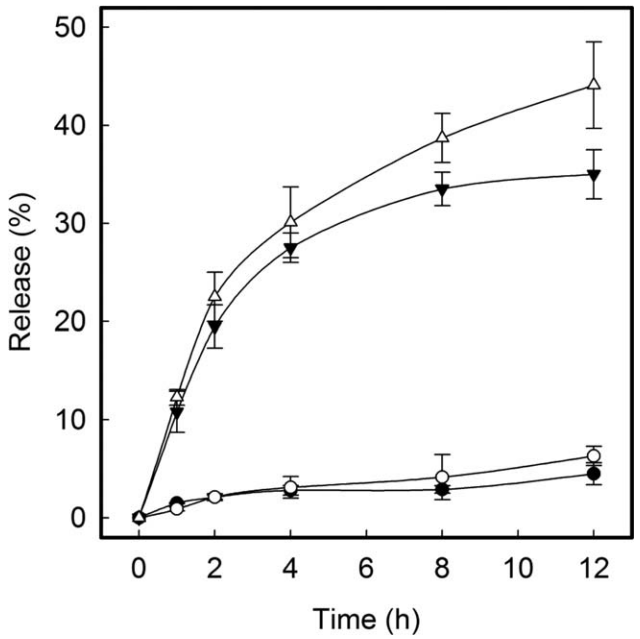

Figure 7. Release profiles of FITC-dextran from CinP(NIPAM-HEA) microparticles containing GNP at 20 °C (●), 25 °C (○), 37 °C (▼), and 45 °C (△).

units. Assuming that the bond length of C-C in the copolymer chain was 2.54 Å, the average distance between two adjacent cinnamoyl groups was estimated to be about 66 Å. If all the cinnamoyl groups participated in the photodimerization, it can be said that the mesh size of photo cross-linked copolymer chains was about 66 Å. The molecular weight of FITC-dextran used in the present study was 10,000, corresponding to approximately the two-dimensional metric length of 15 Å. The size of the dye was estimated to be in the same order as the mesh size of cross-linked copolymer chains, so the mean free path of the dye would be comparable to the mesh size of cross-linked copolymer chains. This could account for why the cumulative release amount in 12 h was not marked at the lower temperatures (20 °C and 25 °C). When the temperature of release medium was 37 °C, a significant release was observed. For example, the cumulative release amount in 12 h was about 30%. The CinP(NIPAM-HEA) chains of microparticles would be dehydrated and deswollen when the temperature of release medium was 37 °C, because the temperature was higher than the LCST of the copolymer chains (about 28 °C). In this circumstance, the dye could be forced to release out of the microparticles by squeezing-out mechanism.[23,24] Another reason for the significant release at 37 °C could be explained by the temperature-dependent thermodynamic activity of the FITC-dextran in the microparticles. The thermodynamic activity of the dye in the microparticles would be relatively low when the temperature was lower than the LCST of the copolymer, because the dye is polar and hydrophilic, and it was in the hydrophilic copolymer matrix of microparticles. Accordingly, the dye would tend to stay in the microparticles and it could hardly escape from them. Whereas, the thermodynamic activity in the microparticles would be relatively high above the LCST, because the hydrophilic dye was in the hydrophobic matrix of microparticles. In this circumstance, the dye would be likely to escape from the microparticles. This may be another reason why a significant release was observed at 37 °C. The cumulative release amount at 45 °C was somewhat higher than that obtained at 37 °C. The squeezing-out mechanism would be also applicable to the release at 45 °C, because the temperature was higher than the LCST. The degree of dehydration and deswelling would be higher at a higher temperature thus the dye could be more forced to release at 45 °C. In addition, the thermodynamic activity of the dye in the microparticles at 45 °C would be higher than the activity at 37 °C. Figure 8(A) shows the temperature profile of the suspension of CinP(NIPAM-HEA) microparticles

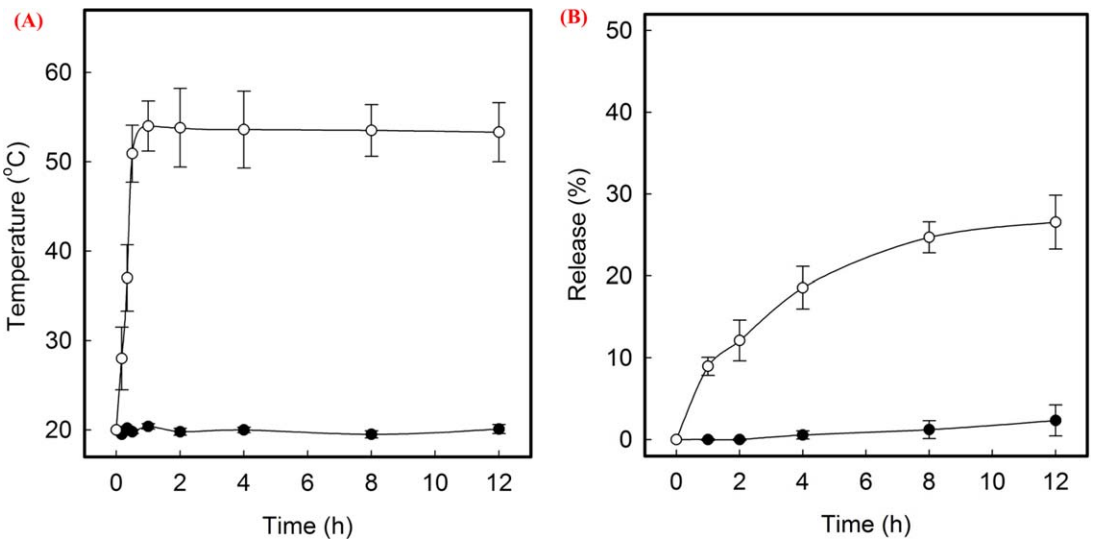

Figure 8. Temperature profiles of suspension of CinP(NIPAM-HEA) microparticles containing GNP without (●) and with (○) NIR irradiation (A). Release profiles of FITC-dextran from CinP(NIPAM-HEA) microparticles containing GNP without (●) and with (○) NIR irradiation (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

containing GNP without and with NIR irradiation. The temperature of the microparticle suspension was almost constant (18.5–20 °C) for 12 h without NIR irradiation. When the microparticle suspension was subjected to the NIR irradiation, the temperature increased from 20 °C to 55 °C for the first 1 h then no more increase in the temperature was observed during the rest period of irradiation. The temperature increase in the early stage of irradiation can be ascribed to the surface plasmon resonance of GNP loaded in the microparticles. Some of GNPs would be on the surface of microparticles but most of them might be in the inside of microparticles. Nevertheless, the photothermal conversion efficiency of GNP loaded in microparticles was as high as that of free GNP, possibly because of the high penetration property of NIR. Figure 8(B) shows the release profile of FITC-dextran from CinP(NIPAM-HEA) microparticles containing GNP without and with NIR irradiation. The cumulative release amount in 12 h was only about 3% without NIR irradiation. The release would be dominated by simple diffusion. As described previously, the mesh size of cross-linked copolymer chains in the microparticles was estimated to be in the same order as the size of the diffusate (dye), thus the mean free path in the matrix of the microparticles would be comparable to the mesh size (approximately 66 Å). This could be a reason why the cumulative release amount in 12 h was only about 3%. When NIR was irradiated to the microparticle suspension, the cumulative release amount increase in a saturation manner and the value in 12 h was about 26.5%. Under NIR irradiation, the temperature of microparticle suspension increased to a temperature above the LCST of CinP(NIPAM-HEA) (8:2) [Figure 8(A)], thus the copolymer chains in the microparticles would become dehydrated, deswollen, and hydrophobic. In this circumstance, the dye would be likely to be squeezed out by the thermally induced de-swelling of the microparticles or to be expelled from the microparticles by the thermally induced increase in the thermodynamic activity of the dye. Upon 1 h-NIR irradiation, the temperature of microparticle suspension increased to 55 °C (a temperature far above the LCST of CinP(NIPAM-HEA) (8:2) [Figure 8(A)] and the cumulative release amount became significant (about 8%) [Figure 8(B)], indicating that the release was photo-thermally triggered. Recently, monoolein (MO) cubic phase containing GNP was prepared as a NIR irradiation-responsive drug carrier.[12] The release of FITC-dextran loaded in the cubic phase was promoted by NIR irradiation. For example, the cumulative release amount in 60 min of the dye with NIR irradiation was about three times higher than the release amount without NIR irradiation. The local phase transition of the cubic phase caused by the photothermal energy was claimed to be responsible for the promoted release under NIR irradiation.

CONCLUSIONS

Temperature-and NIR irradiation-responsive microparticles composed of CinP(NIPAM-HEA) and GNP were prepared by a spray-drying method. The LCST of P(NIPAM-HEA) was little affected by the HEA content, but the LCST of CinP(NIPAM-HEA) was significantly affected by the cinnamoyl group content. GNP prepared by reducing gold ions in aqueous solution was about 30 nm in the hydrodynamic mean diameter, it was almost spherical on TEM photo, and it exhibited photothermal effect under NIR irradiation. On SEM photo, CinP(NIPAM-HEA) microparticles containing GNP were sphere-like or flat ball-like particles, and the diameter ranged from 1.5 μm to 12 μm. Gold was found on the energy-dispersive X-ray spectrum of the microparticles, indicating that it was contained in the microparticles. The release of FITC-dextran loaded in the microparticles was triggered when the temperature of release medium was above the LCST. NIR irradiation caused the microspheres to release the dye as much as 26.5% in 12 h. CinP(NIPAM-HEA) would become deswollen and hydrophobic due to the photothermal energy from GNP, causing the triggered release. If NIR is irradiated to a specific tissue site where GNP-loaded microparticles exits, NIR can penetrate deeply into the tissue and cause GNP to generate heat. Accordingly, the microparticles would show a triggered release at the specific site to which NIR is irradiated. The NIR irradiation-responsive release mechanism of GNP-loaded microparticles would be exploited for the development of NIR-responsive drug carriers which release their contents in response to an external stimulus (i.e. NIR irradiation).

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