ELSEVIER

International Journal of Pharmaceutics 211 (2000) 1–8

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Core-shell type polymeric nanoparticles composed of poly(L-lactic acid) and poly(*N*-isopropylacrylamide)

In-Sook Kim [a], Young-Il Jeong [b], Chong-Su Cho [c], Sung-Ho Kim [a,*]

[a] *College of Pharmacy, Chosun University, # 375 Seosuk-dong, Dong-gu, Kwangju501-759, South Korea*
[b] *Department of Pharmacokinetics, Kyoto Pharmaceutical University, Kyoto607-8414, Japan*
[c] *School of Biological Resources and Biomaterials Engineering, Seoul National University, Suwon441-744, South Korea*

Received 12 June 2000; received in revised form 21 August 2000; accepted 29 August 2000

Abstract

Poly(L-lactic acid)/poly(*N*-isopropylacrylamide) (abbreviated as LN) block copolymers were synthesized and the LN nanoparticles were prepared by simple diafiltration method. The thermal transition of the LN nanoparticles was at 32.3°C, the lower critical solution temperature (LCST) of the polymer. The fluorescence spectroscopy data showed that LN was self-assembled in water to form core-shell structure nanoparticles, and the critical association concentration (CAC) value was estimated as 1.3×10^{-2} g/l. From the transmission electron microscope observations, the LN nanoparticles were spherically shaped and ranged in size between 30 and 50 nm below the LCST. The hydrated size was measured by photon correlation spectroscopy, and reversible size changes were investigated by the factor of temperature. The release of indomethacin from the LN nanoparticles was thermo-sensitive due to the unique characteristic of poly(*N*-isopropylacrylamide).

Keywords: Core-shell structure; Polymeric nanoparticle; Poly(L-lactic acid); Poly(*N*-isopropylacrylamide)

1. Introduction

Various drug delivery systems have been proposed for effectively targeting drugs to a specific organ of the body (e.g. the polymeric micelles, surface-modified particles, liposomes and nanoparticles) (Bador et al., 1984; Kopecek, 1990; Lasic, 1992; Blume et al., 1993; Davis et al., 1993; Gref et al., 1994; Leroux et al., 1996). However, many problems still exist including the biodistribution of drugs, drug solubility, undesirable side effects, rapid clearance by the reticuloendothelial system (RES), thermal instability, short blood circulation time, structural fragility and lower loading efficiency.

Ringsdorf et al. first reported that the polymeric micelles could be used to achieve the sustained release of a drug from the polymer-drug conjugates (Bador et al., 1984). Recently, diblock copolymer was used to prepare the polymeric micelles by Kataoka coworkers. (Yokoyama et al., 1990; Kataoka et al., 1993; Cammas and

* Corresponding author. Tel.: +82-62-2306379; fax: +82-62-2225414.
E-mail address: shkim@mail.chosun.ac.kr (S.-H. Kim).

0378-5173/00/$ - see front matter
PII: S0378-5173(00)00592-5

Kataoka, 1995). Also, Cho et al. (1997) reported that poly(γ-benzyl L-glutamate) and poly(*N*-isopropylacrylamide) block copolymers can form a core-shell structure in water. Diblock copolymers generally exhibit surfactant behavior (Brown et al., 1991) and form core-shell structures due to their amphiphilicity. Hydrophobic segments form the inner core of the structure, which acts as the incorporation site of hydrophobic drugs. Hydrophobic drugs can be easily entrapped within the inner core of the polymeric carriers by hydrophobic interaction (Kwon et al., 1995). Hydrophilic blocks form the hydrated outer shell, which surrounds the hydrophobic core to prevent the nanoparticles from being quickly taken up by the RES.

Poly(*N*-isopropylacrylamide) (PNIPAAm) exhibits a lower critical solution temperature (LCST) at 31–32°C. Hydrophobically modified PNIPAAm shows thermo-responsive water-solubility and can form heterogeneous structures composed of hydrophilic microdomains of PNIPAAm chains and hydrophobic segments in aqueous solution. The solution properties of hydrophobically modified PNIPAAm have been reported with fluorescence measurements in cases where the hydrophobic moieties were randomly incorporated along the main chain and the long hydrophobic segments were conjugated at one end of the main chain (Schild and Tirrell, 1991; Chung et al., 1997). Forming a heterogeneous structure is clearer in the latter case than the former case. A heterogeneous structure is formed by the aggregate forces of the hydrophobic segments against the intramolecular hydrophilicity. Therefore, it is the intramolecular hydrophilic/hydrophobic balance that is closely related to the heterogeneous separation of the microdomains, thermodynamic stability of the structure and physical properties of the microdomains. In order to design and facilitate a reversibly thermo-responsive nanoparticle for a drug delivery system, it is essential to elucidate the mechanism and the effective factors that form a stable core-shell structure.

In this study, we synthesized AB type diblock copolymers of poly(L-lactic acid) (PLA) as the A component and PNIPAAm as the B component. PLA is one of the poly (a-hydroxy esters) with bioerodible characteristics, and it degrades into naturally occurring substances (Kulkarni et al., 1966; Heller, 1984; Langer, 1990). We expected PLA to have more hydrophobic properties than PNIPAAm. The core-shell type nanoparticles were prepared by a diafiltration method (Lasic, 1992; La et al., 1996; Cho et al., 1997). Also, indomethacin (IN) was incorporated into the nanoparticles as a model drug, since the hydrophobic PLA core may serve as a microcontainer for drugs that are segregated from the outer environment by a palisade-like hydrophilic PNIPAAm segment, which has the ability to release drugs in a controlled manner according to changes in temperature.

2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPAAm, Tokyo Kasei, Tokyo, Japan) was purified by recrystallization in *n*-hexane and dried in a vacuum at room temperature. The PLA with a molecular weight of 2000 and 2,2′-azobisisobutyronitrile (AIBN) were obtained from Polysciences. (Warrington, PA). The 2-aminoethanethiol hydrochloride (AET·HCl), KOH·methanol (potassium hydroxide volumetric standard, 1.003 M solution in methyl alcohol), and *N*,*N*′-dicyclohexyl carbodiimide (DCC) were obtained from Aldrich Chemical Company (Milwaukee, WI). The IN was purchased from Sigma Chemical (St. Louis, MO). The *N*,*N*′-dimethylformamide (DMF), dimethylsulfoxide (DMSO) and all other chemicals were of reagent grade and used without further purification.

2.2. Synthesis of amine-terminated PNIPAAm

Amine-terminated PNIPAAm (ATPNIPAAm) was prepared according to the method reported by Chen and Hoffman (1995). It was synthesized by the polymerization of NIPAAm (50 mmol) in 20 ml of methanol at 60°C for 22 h using AIBN (0.5 mmol) and AET·HCl (1.0 mmol) as the initiator and chain transfer reagent, respectively.

KOH·methanol was added to remove the HCl from the AET·HCl salt. The semitelechelic PNIPAAm with an amino end group was obtained by precipitating the reaction solution into diethyl ether. The reaction procedure is shown in Fig. 1. The number-average molecular weight of ATPNIPAAm was determined by GPC.

2.3. Synthesis of LN diblock copolymer

The LN diblock copolymer was prepared by a coupling reaction of PLA with ATPNIPAAm using DCC as a coupling agent as shown in Fig. 2. PLA (0.2 mmol), DCC (0.4 mmol), and ATPNIPAAm (0.2 mmol) were separately dissolved in DMSO. The DCC solution was added to the PLA solution, and was stirred for 30 min to activate the carboxyl group of PLA. The ATPNIPAAm solution was added dropwise into the activated

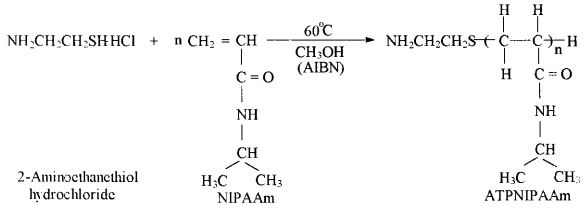

Fig. 1. Synthetic scheme of the ATPNIPAAm.

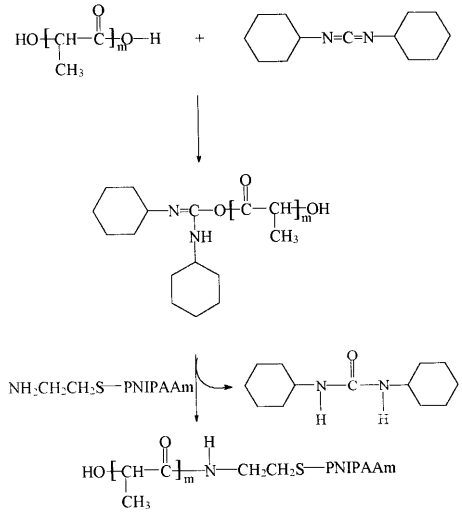

Fig. 2. Synthesis of LN diblock copolymer.

PLA solution, and the reaction was carried out at room temperature for 10 days (Li, 1991). The reaction solution was filtered to remove the precipitated dicyclohexylurea (DCU), and then was put into a dialysis tube (molecular weight cutoff (MWCO) 2000 g/mol), where it was dialyzed in deionized distilled water for 7 days. The resultant solution was freeze-dried.

2.4. Transmittance measurement

The optical transmittance of the polymer in aqueous solutions (concentration: 1 g/l) at various temperatures was measured at 500 nm with a UV spectrophotometer (Shimadzu UV-1201, Japan). The temperature was gradually increased with a maximum heating rate of 0.5°C/min, and controlled with a circular system. The LCST of the polymer solution was determined at a temperature showing an optical transmittance of 50% (Chung et al., 1997).

2.5. Preparation of LN nanoparticles and drug loading

The core-shell type nanoparticles were formed using the diafiltration method (Yokoyama et al., 1994; Kwon et al., 1995; Cho et al., 1997; Jeong et al., 1998). 20 mg of LN diblock copolymer was dissolved in 10 ml of DMF. The solute was entirely solubilized with stirring at room temperature. To form the core-shell type nanoparticles, the solution was dialyzed using a MWCO 2000 g/mol dialysis tube against distilled water. The medium was replaced every 30 min for the first 3 h and every 1 h for 21 h. Then, the solution was analyzed or freeze-dried.

For drug loading, 20 mg of LN block copolymer was dissolved in 4 ml of DMF, after which 20 mg of IN in 1 ml DMF was added. The solution was stirred at room temperature for 30 min, and the solution was dialyzed using MWCO 2000 g/mol dialysis tube against distilled water for 24 h as mentioned above. The dialyzed solution in the dialysis tube was freeze-dried and the prepared nanoparticles were kept in a refrigerator at 4°C until use. In order to measure the drug loading content, a freeze-dried sample of IN-loaded LN

nanoparticles was suspended in methanol, vigorously stirred for 2 h and sonicated for 15 min. The resultant solution was centrifuged at 3000 rpm for 20 min, and the supernatant was taken to measure the drug concentration using a UV spectrophotometer (Shimadzu UV-1201, Japan) at 312 nm.

2.6. Measurement of fluorescence spectroscopy

To investigate the fluorescence spectroscopy characteristics, the LN block copolymer solution without the drug was prepared as described above. After dialysis, resultant solution was adjusted to various concentrations of block copolymers. The critical association concentration (CAC) of the LN block copolymers was estimated to prove the potential for forming core-shell type nanoparticles. This was done by the measurement of the fluorescence spectroscopy with spectrofluorophotometer (Shimadzu RF-5301 PC, Japan) using pyrene (Kalyanasundaram and Thomas, 1977; Wilhelm et al., 1991) as a hydrophobic probe. To obtain the sample solutions, a known amount of pyrene in acetone was added to each of a series of 20 ml vials, and then the acetone was evaporated. The final concentration of the pyrene was 6.0×10^{-7} M. 10 ml of various concentrations of block copolymer solutions was added to each vial, which was then heated at 65°C for 3 h to equilibrate the pyrene and the nanoparticles, and left to cool overnight at room temperature. Emission wavelength was 390 nm for excitation spectra. The excitation and emission bandwidths were 1.5 and 1.5 nm, respectively.

2.7. Transmission electron microscope measurement

The morphology of the LN polymeric nanoparticle was observed using a JEM-2000 FX II (Jeol, Japan) at 80 kV. A drop of polymeric nanoparticle in aqueous solution was placed on a copper grid coated with carbon film and dried at 20°C. The specimen on the copper grid was negatively stained with 0.01% phosphotungstic acid.

2.8. Photon correlation spectroscopy measurement

The PCS was measured with a Zetasizer 3000 (Malvern Instruments, England) with a He-Ne laser beam at a wavelength of 633 nm at 20°C (scattering angle of 90°). The nanoparticle solution prepared by the diafiltration method was used for measuring the particle size (concentration: 1 g/l), which was performed without filtration.

2.9. In vitro drug release studies

The release experiment was carried out using a dialysis tube. The 5 mg of IN-loaded nanoparticles and 1 ml of phosphate buffer solution (PBS, 0.1 M and pH 7.4) were put into a dialysis tube (MWCO 2000 g/mol). The dialysis tube was then introduced into a vial with 10 ml PBS. The medium was stirred at 100 rpm at desired temperature. At specific time intervals, the entire medium was removed and replaced with the same amount of fresh PBS. The amount of IN released from the nanoparticles was measured with an UV spectrophotometer (Shimadzu UV-1201, Japan).

3. Results and discussion

3.1. Analysis of LN nanoparticles

The number-average molecular weight of ATP-NIPAAm was 7880 and the molecular weight distribution of the obtained polymer was 1.74. Fig. 3 shows the optical transmittance of the aqueous solutions of LN at various temperatures. The results indicated that the transition point of the LN was 32.3°C, which was almost similar to the LCST of the PNIPAAm homopolymer.

The micelle formation of polystyrene (PS)/poly(ethylene oxide) (PEO) di- or triblock copolymers in water was already reported by the fluorescence technique using pyrene as a hydrophobic probe, and the CMC was identified from the fluorescence emission and excitation spectra as pyrene partitions between the aqueous and nanoparticle environments (Wilhelm et al., 1991). This method was also used by Jeong et al. (1998)

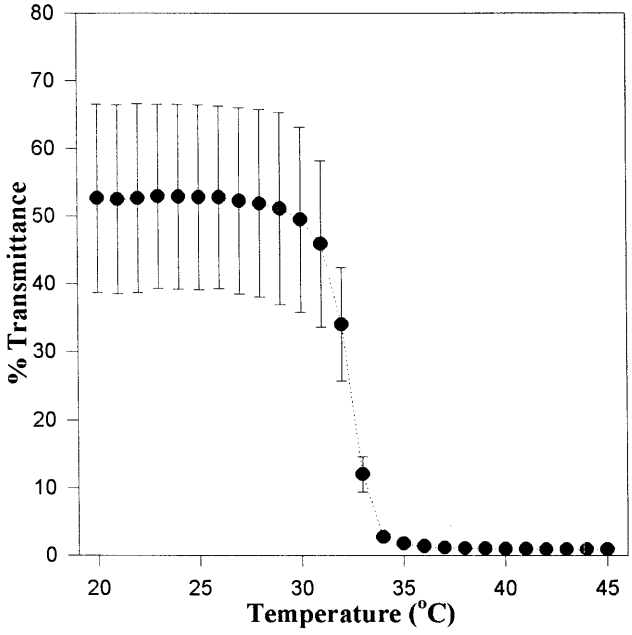

Fig. 3. Optical transmittance change of LN aqueous solution against temperature (concentration: 1 g/l, absorbance at 500 nm).

to confirm the polymeric micelle formation of poly(γ-benzyl L-glutamate) (PBLG) and PEO block copolymer in water. The behavior of the nanoparticle formation was investigated by the fluorescence spectroscopy, and the CAC was determined.

Fig. 4 shows the fluorescence emission spectra of pyrene, at a fixed excitation wavelength of 339 nm, against various concentrations of LN. The fluorescence intensity increased along with the concentration of LN, which indicated the formation of self-assembled polymeric nanoparticles of LN in water, such as other block copolymeric micelles (Wilhelm et al., 1991). It is thought that pyrene was preferentially solubilized into the nanoparticles composed of the core-shell structure when pyrene was introduced into the core domain from a good solvent (Zhao et al., 1990; Xu et al., 1991).

Fig. 5(a) shows the excitation spectra of pyrene in the various concentrations of LN. A red shift of pyrene in the excitation spectra was observed with increasing concentrations of LN, with a similar tendency of the PBLG/PEO block copolymers (Jeong et al., 1998) and PS/PEO block copolymers

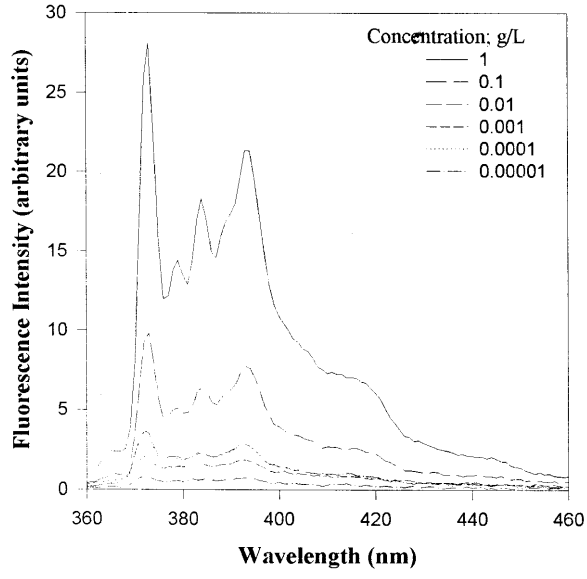

Fig. 4. Fluorescence emission spectra of pyrene (6.0×10^{-7} M) against LN concentration in distilled water (excitation wavelength: 339 nm).

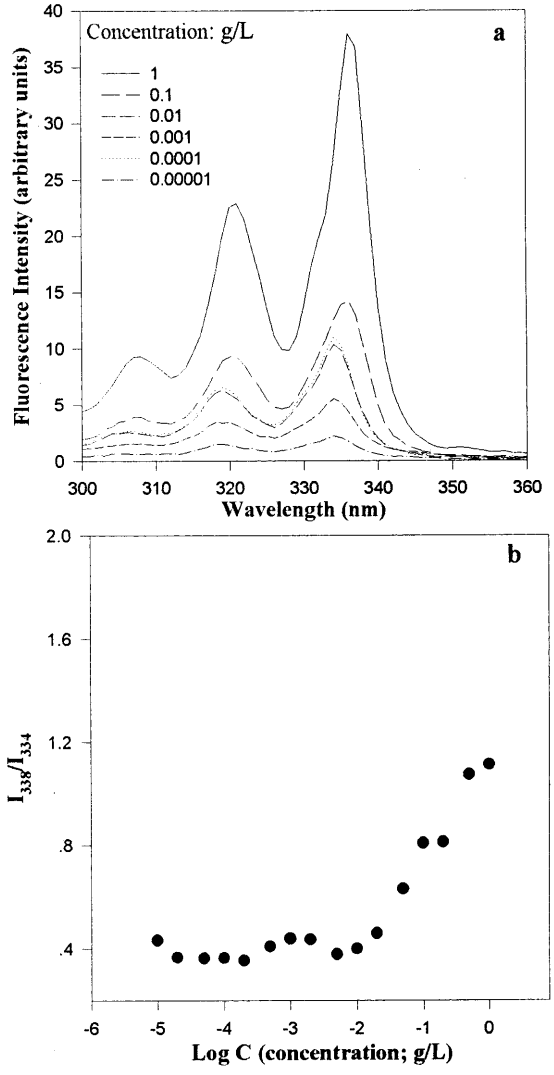

Fig. 5. Fluorescence excitation spectra of pyrene (6.0×10^{-7} M) against LN concentration in distilled water (emission wavelength: 390 nm) (a) and plots of the intensity ratio I_{338}/I_{334} from pyrene excitation spectra vs. log C of the LN in distilled water (b).

(Wilhelm et al., 1991). The intensity ratio of I_{338}/I_{334} vs. log C of LN in the pyrene excitation spectra was plotted in Fig. 5(b). This shows that the ratio was almost flat at lower concentrations, and rapidly increased at higher concentrations. The CAC was taken from the intersection of the tangent to the curve at the inflection with the horizontal tangent through the points at low concentrations. The estimated CAC value was 1.3×10^{-2} g/l. From the study of the fluorescence probe measurements, it can be said that the LN can form core-shell type nanoparticles in water upon critical concentrations (i.e. CAC) and has an amphiphilic nature like that of other block copolymer micelles.

Fig. 6 shows the transmission electron micrograph of the LN nanoparticles dried at 20°C. The shapes of the LN nanoparticles were observed as approximately spherical, and the diameter of these nanoparticles was estimated to be about $30 \sim 50$ nm as in a dehydrated state. The sizes in a hydrated state were reversibly changed by temperature fluctuations, as shown in Fig. 7. This result may be caused by the thermal sensitivity of the PNIPAAm outer shell of the LN nanoparticles. It is thought that, when the temperature is low (below the LCST), PNIPAAm chains of the outer shell exist in an expanded form, and

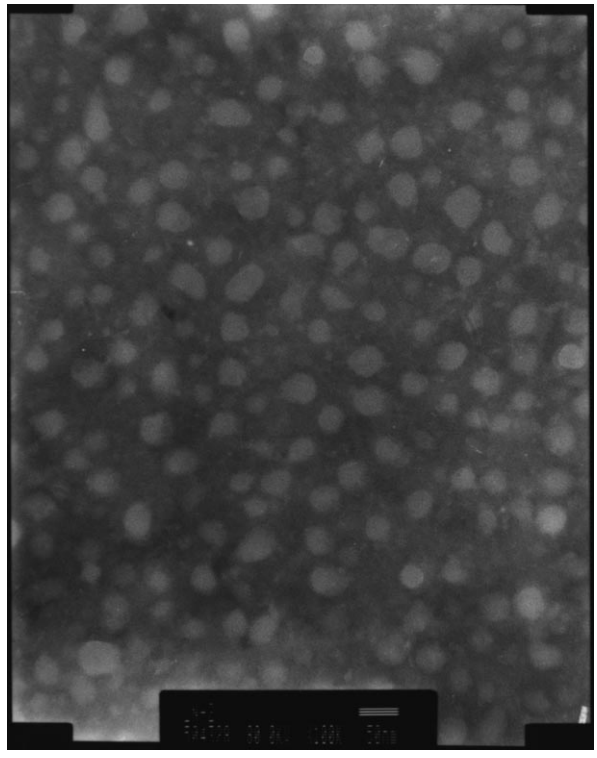

Fig. 6. Transmission electron micrograph of LN nanoparticles dried at 20°C. Samples were negatively stained with 0.01% phosphotungstic acid.

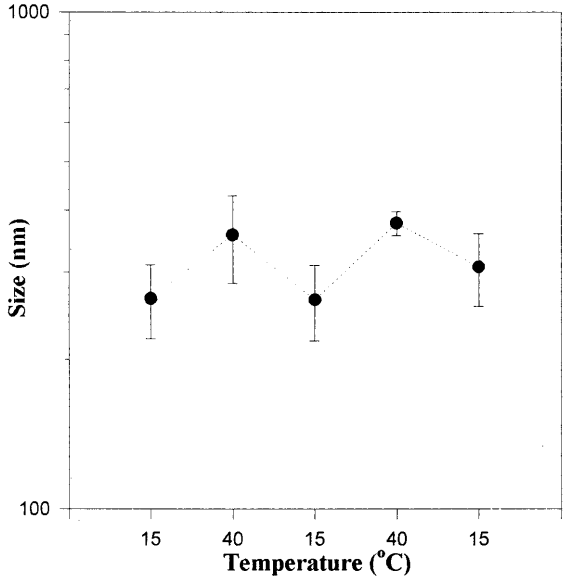

Fig. 7. Particle size changes by reversible temperature fluctuation.

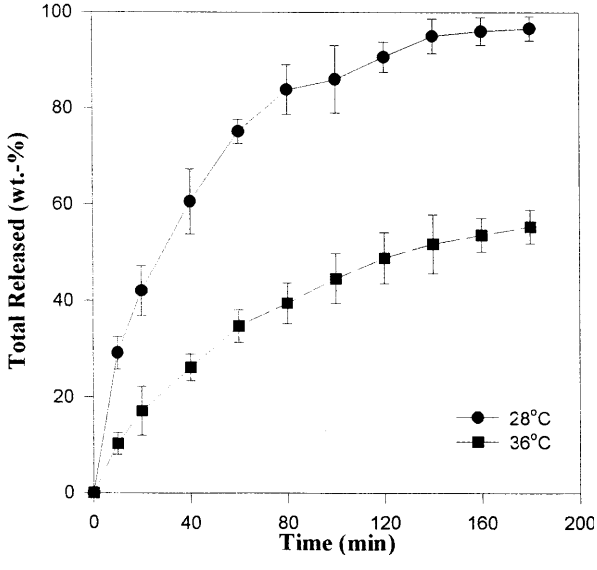

Fig. 8. Release of IN from nanoparticles of LN in PBS (0.1 M, pH 7.4) at 28°C and 36°C ($n = 3$).

shrink to a more compact form at high temperature (above the LCST). However, the particle sizes were unexpectedly higher above the LCST. The result indicated that the secondary aggregates formed upon heating and then the aggregated nanoparticles reversibly redispersed to the initial structure upon cooling below the LCST.

3.2. Drug release study

The calculated IN loading content was 25.6 wt.%. To study the drug release behavior, IN-loaded nanoparticles of LN were simply redispersed in PBS (0.1 M, pH 7.4) without surfactant. Fig. 8 shows the release kinetics of IN from the nanoparticles as a function of temperature. The IN was slowly released with increasing temperatures. This result may have close relationship with the thermal sensitivity of the LN nanoparticles. It is thought that, when the temperature is above the LCST of LN, the expanded form of PNIPAAm in the shell part is changed into the compact one. Furthermore, the diffusion of the IN from core may be restricted by the shrunken structure of the shell. As a result, it can be expected that a drug release from the thermo-sensitive nanoparticles will be applied to the site-specific drug delivery system by modulating the temperature of the target site.

Acknowledgements

This study was supported by the 2000 research grant from Chosun University.

References

Bador, H., Ringsdorf, H., Schmidt, B., 1984. Water soluble polymers in medicine. Angew. Makromol. Chem. 123/124, 457–485.

Blume, G., Cevc, G., Crommelin, M.D.J.A., Bakker-Woudenberg, I.A.J.M., Kluft, C., Storm, G., 1993. Specific targeting with poly(ethylene glycol)-modified liposomes: coupling of homing devices to the ends of the polymeric chains combines effective target binding with long circulation times. Biochem. Biophys. Acta. 1149, 180–184.

Brown, W., Schillen, K., Almgren, M., Hvidt, S., Bahadur, P., 1991. Micelle and gel formation in a poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) triblock copolymer in water solution. Dynamic and static light scattering and oscillatory shear measurements. J. Phys. Chem. 95, 1850–1858.

Cammas, S., Kataoka, K., 1995. Functional poly[(ethylene oxide)-co-(β-benzyl-L-aspartate)] polymeric micelles formation. Macromol. Chem. Phys. 196, 1899–1905.

Chen, G., Hoffman, A.S., 1995. Graft copolymers that exhibit temperature-induced phase transitions over a wide range of pH. Nature 373, 49–52.

Cho, C.S., Cheon, J.B., Jeong, Y.I., Kim, I.S., Kim, S.H., Akaike, T., 1997. Novel core-shell type thermo-sensitive nanoparticles composed of poly(γ-benzyl L-glutamate) as the core and poly(*N*-isopropylacrylamide) as the shell. Macromol. Rapid Commun. 18, 361–369.

Chung, J.E., Yokoyama, M., Suzuki, K., Aoyagi, T., Sakurai, Y., Okano, T., 1997. Reversibly thermo-responsive alkyl-terminated poly(*N*-isopropylacrylamide) core-shell micellar structures. Coll. Surf. B: Biointerfaces 9, 37–48.

Davis, S.S., Illum, L., Moghimi, S.M., Davies, M.C., Porter, C.J.H., Muir, I.S., Brindley, A., Christy, N.M., Norman, M.E., Williams, P., Dunn, S.E., 1993. Microspheres fort-argeting drugs to specific body sites. J. Control. Rel. 24, 157–163.

Gref, R., Minamitake, Y., Peracchia, M.T., Trubetskoy, V., Torchilin, V., Langer, R., 1994. Biodegradable long-circulating polymeric nanospheres. Science 263, 1600–1603.

Heller, J., 1984. Bioerodible systems. In: Langer, R., Wise, D.L. (Eds.), Medical Applications of Controlled Release, vol. 1. CRC Press, Boca Raton, p. 70.

Jeong, Y.I., Cheon, J.B., Kim, S.H., Nah, J.W., Lee, Y.M., Sung, Y.K., Akaike, T., Cho, C.S., 1998. Clonazepam release from core-shell type nanoparticles in vitro. J. Control. Rel. 51, 169–178.

Kalyanasundaram, K., Thomas, J.K., 1977. Environmental effects on vibronic band intensities in pyrene monomer fluorescence and their application in studies in micellar systems. J. Am. Chem. Soc. 99, 2039–2044.

Kataoka, K., Kwon, G.S., Yokoyama, M., Okano, T., Sakurai, Y., 1993. Block copolymer micelles as vehicles for drug delivery. J. Control. Rel. 24, 119–132.

Kopecek, J., 1990. The potential of water-soluble polymeric carriers in targeted and site-specific drug delivery. J. Control. Rel. 11, 279–290.

Kulkarni, R.K., Pani, K.C., Neuman, C., Leonard, F., 1966. Polylactic acid for surgical implants. Arch. Surg. 93, 839–843.

Kwon, G.S., Naito, M., Yokoyama, M., Okano, T., Sakurai, Y., Kataoka, K., 1995. Physical entrapment of adriamycin in AB block copolymer micelles. Pharm. Res. 12, 192–195.

La, S.B., Okano, T., Kataoka, K., 1996. Preparation and characterization of the micelle-forming polymeric drug indomethacin-incorporated poly(ethylene oxide)-poly(β-benzyl L-aspartate) block copolymer micelles. J. Pharm. Sci. 85, 85–90.

Langer, R., 1990. New methods of drug delivery. Science 249, 1527–1533.

Lasic, D.D., 1992. Mixed micelles in drug delivery. Nature 355, 279–280.

Leroux, J.C., Allemann, E., Jaeghere, F.D., Doelker, E., Gurny, R., 1996. Biodegradable nanoparticles — from sustained release formulations to improved site specific drug delivery. J. Control. Rel. 39, 339–350.

Li, X., 1991. Biodegradable polymeric prodrugs of antihypertensive agents. Ph. D Thesis, The University of Utah, USA.

Schild, H.G., Tirrell, D.A., 1991. Microheterogeneous solution of amphiphilic copolymers of *N*-isopropylacrylamide. An investigation via fluorescence methods. Langmuir 7, 1319–1324.

Wilhelm, M., Zhao, C.L., Wang, Y., Xu, R., Winnik, M.A., Mura, J.L., Riess, G., Croucher, M.D., 1991. Poly(styrene-ethylene oxide) block copolymer micelle formation in water: a fluorescence probe study. Macromolecules 24, 1033–1040.

Xu, R., Winnik, M.A., Hallett, F.R., Riess, G., Croucher, M.O., 1991. Light scattering study of the association behavior of styrene-ethylene oxide block copolymers in aqueous solution. Macromolecules 24, 87–93.

Yokoyama, M., Miyauchi, M., Yamada, N., Okano, T., Sakurai, Y., Kataoka, K., Inoue, S., 1990. Polymer micelles as novel drug carrier: adriamycin-conjugated poly(ethylene glycol)-poly(aspartic acid) block copolymer. J. Control. Rel. 11, 269–278.

Yokoyama, M., Okano, T., Sakurai, Y., Kataoka, K., 1994. Improved synthesis of adriamycin-conjugated poly(ethylene oxide)-poly(aspartic acid) block copolymer and formation of unimodal micellar structure with controlled amount of physically entrapped adriamycin. J. Control. Rel. 32, 269–277.

Zhao, C.L., Winnik, M.A., Riess, G., Croucher, M.D., 1990. Fluorescence probe techniques used to study micelle formation in water-soluble block copolymers. Langmuir 6, 514–516.