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International Journal of Pharmaceutics 200 (2000) 231–242

international journal of pharmaceutics

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Clonazepam release from core-shell type nanoparticles of poly(ε-caprolactone)/poly(ethylene glycol)/poly(ε-caprolactone) triblock copolymers

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Received 28 October 1999; received in revised form 3 March 2000; accepted 13 March 2000

Abstract

The triblock copolymer based on poly(ε-caprolactone) (PCL) as hydrophobic part and poly(ethylene glycol) (PEG) as hydrophilic one was synthesized and characterized. Core-shell type nanoparticles of poly(ε-caprolactone)/poly(ethylene glycol)/poly(ε-caprolactone) (CEC) block copolymer were prepared by a dialysis technique. According to the amphiphilic characters, CEC block copolymer can self-associate at certain concentration and their critical association concentration (CAC) was determined by fluorescence probe technique. CAC value of the CEC-2 block copolymer was evaluated as 0.0030 g/l. CAC values of CEC block copolymer decreased with the increase of PCL chain length, i.e. the shorter the PCL chain length, the higher the CAC values. From the observation of transmission electron microscopy (TEM), the morphologies of CEC-2 core-shell type nanoparticles were spherical shapes. Particle size of CEC-2 nanoparticles was 32.3 ± 17.3 nm as a monomodal and narrow distribution. Particle size, drug loading, and drug release rate of CEC-2 nanoparticles were changed by the initial solvents and the molecular weight of CEC. The degradation behavior of CEC-2 nanoparticles was observed by ^{1}H NMR spectroscopy. It was suggested that clonazepam (CNZ) release kinetics were dominantly governed by diffusion mechanism.

Keywords: Clonazepam release; Poly(ε-caprolactone); Poly(ethylene glycol); Poly(ε-caprolactone)

1. Introduction

Nanoparticles or colloidal carriers have been widely used for targeted drug delivery and other biomedical applications (Langer, 1995; Leroux et al., 1996; Jeong et al., 1999). Since these drug carrier systems can be used to intravenous injec-

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0378-5173/00/$ - see front matter
PII: S0378-5173(00)00392-6

tion of drugs, for site-specific drug delivery, nanoparticles or colloidal carriers have great potential in the therapy of several fatal diseases without unwanted side-effects (Couvreur et al., 1991). To achieve these objectives, a lot of series of nano-sized particles or colloidal carriers such as nanospheres (Couvreur et al., 1991; Jeong et al., 1999), polymeric micelles (Kataoka et al., 1993; Nah et al., 1998), liposomes (Lasic 1992; Vemuri and Rhodes, 1995), and surface-modified nanoparticles (Illum et al., 1986; Dunn et al., 1994) have been developed and suggested. However, the body distribution of drug and carriers, undesirable side effects, rapid clearance by macrophage, thermal unstability, structural fragility, and lower drug loading efficiency, etc., are not fully understood.

Amphipathic block copolymer exhibit surfactant behavior and then form core-shell type micelle structure due to their amphiphilic characters. Block copolymers consisting of a hydrophobic blocks and hydrophilic one form polymer micelles (Kataoka et al., 1993; Cho et al., 1995) or core-shell type nanoparticles (Jeong et al., 1998) in aqueous environment which has hydrophobic inner-core surrounded by hydrated outer-shell. In these structures, each segment of block copolymers has different functions. Hydrophobic blocks form the inner-core of the structure, which acts as a drug incorporation site, especially for hydrophobic drugs. Hydrophobic drugs may be easily physically entrapped within the inner-core of the structures by hydrophobic interactions (Kwon et al., 1995). Hydrophilic blocks form a hydrated outer-shell which may cloak the hydrophobic core to avoid its rapid uptake by the reticuloendothelial system (RES) and more active clearing organs such as liver, spleen, lung, and kidneys. Therefore, hydrated outer-shell increase the blood circulation times of nanoparticles. Predominent characteristics of this system have been reported such as reduced toxic side effects of antitumor agents, passive targeting to the specific sites, solubilization of hydrophobic drugs, stable storage of drugs, long blood circulation, favorable biodistribution, thermal stability, and lower interactions with RES (Kataoka et al., 1993).

Kataoka and collaborators (Kataoka et al., 1993) have extensively investigated polymeric micelles as a hydrophobic drug carriers such as anticancer agents, adriamycin. They have reported that diblock copolymers composed of poly(ε-benzyl L-aspartate) (PBLA) and poly(ethylene oxide) (PEO) form micelles through self-association in water and have a several tens of nanometer which is a similar size range with viruses. Also, they have reported enhanced tumor accumulation of antitumor agents, long blood circulation times, and effective treatment of solid tumors by micelle-forming block copolymer-adriamycin conjugates (Yokoyama et al., 1990). Gref et al. (1994) reported that core-shell type nanoparticles by poly(lactide-co-ethylene glycol) diblock or multiblock copolymer and poly(ε-caprolactone-co-ethylene glycol) diblock copolymers circulated for long period of time in blood and hydrophobic drug was released by sustained manner.

As described above, various drug carriers have been developed, but there are no reports of nano-sized drug carriers based on CEC triblock copolymers.

In this study, we have synthesized and characterized the CEC triblock copolymers. Poly(ε-caprolactone) (PCL) is a non-toxic biodegradable polymers and has hydrophobic characters. Poly(ethylene glycol) (PEG) is a non-immunogenic, non-toxic water soluble polymers and has an ability of prevention of protein adsorption and attack of RES (Lee et al., 1989). Clonazepam (CNZ) was used as a model hydrophobic drug for this study (White, 1995). Particle size, drug loading capacity, and their physicochemical properties of CEC core-shell type nanoparticles against various conditions were investigated in vitro.

2. Materials and methods

2.1. Materials

PEG (MW = 8000) was purchased from Sigma Chem. Co., USA. ε-Caprolactone was purchased from Aldrich Chem. Co. Inc., USA. CNZ was supplied from Roche Co., Switzerland. 1,4-Diox-

ane, acetone, dimethylformamide (DMF), dimethylsulfoxide (DMSO), dimethylacetamide (DMAc), tetrahydrofuran (THF), dichloromethane, methanol, and diethyl ether were used reagent grade without further purification.

2.2. Synthesis of triblock copolymers of CEC

CEC triblock copolymers were synthesized by a non-catalyzed ring opening polymerization of ε-caprolactone in the presence of PEG as shown in Fig. 1 (Cerrai et al., 1989). PEG and ε-caprolactone were mixed in a round-bottomed flask under vacuum condition. The mixture was cooled and degassed with vacuum pump. The round-bottomed flask was sealed off and placed in an oil bath at 185°C. After the polymerization was completed, the resultant product was cooled at room temperature and then dissolved in dichloromethane. The solution was precipitated into the excess amount of cold ethanol and filtered to remove the unreacted PEG homopolymers and ε-caprolactone monomers. The precipitates were washed with diethyl ether three times and then dried in vacuum oven for 3 days.

2.3. ^1H Nuclear magnetic resonance spectrometer (NMR) measurement

^1H NMR spectra of the copolymers were measured in $CDCl_3$ to estimate the copolymer compositions and the molecular weight of PCL blocks, using a 300 MHz NMR spectrometer (FT-NMR, Bruker AC-300F, 300 MHz). As the number-average molecular weight of PEG is known, one can estimate the number-average molecular weight of the PCL block, and the copolymer composition calculated from the peak intensities in the spectrum assigned to both polymers.

In order to determine the core-shell type structure of CEC block copolymer, ^1H NMR spectra measured in $CDCl_3$ and D_2O. The concentration of the CEC nanoparticles was 1.0 wt.% in $CDCl_3$ and 0.5 wt.% in D_2O.

2.4. Preparation of core-shell type nanoparticles

The core-shell type nanoparticles of CEC triblock copolymers were prepared by dialysis method as reported previously (Jeong et al., 1998). A total of 40 mg of CEC triblock copolymer was dissolved in 10 ml of 1,4-dioxane and then the solution was stirred at room temperature to solubilize entirely. To form core-shell type nanoparticles, the solution was dialyzed with molecular weight cut-off (MWCO) 12 000 g/mol dialysis tube against 1.0 l $\times$ 3 of distilled water for 3 h, and then distilled water was exchanged at intervals of 3–4 h during 24 h. Then, the solution was analyzed or freeze-dried.

To prepare the drug entrapped core-shell type nanoparticles, 40 mg of CEC triblock copolymer and 20–40 mg of CNZ were dissolved in organic solvents and then the solution was dialyzed as described above.

For measuring of drug loading content, the freeze-dried samples of CEC nanoparticles were suspended into ethanol and vigorously stirring for 12 h and sonicating for 1 h. Resulting solution was centrifuged with 12 000 g for 20 min and supernatant was taken for measurement of drug concentration using UV spectrophotometer (Shimadzu UV-1201) at 310 nm. Drug loading contents were calculated as following equation: (drug weight in the nanoparticles/weight of nanoparticles) $\times$ 100. Loading efficiency was calculated as: (residual drug amount in the nanoparticles/initial feeding amount of drug) $\times$ 100.

2.5. Measurement of fluorescence spectroscopy

To investigate the fluorescence spectroscopy characteristics, CEC triblock copolymer solutions without drug were prepared as the same method described above. Resultant solution was adjusted to the various concentrations of block copolymers.

To estimate the critical association concentrations (CAC) of block copolymers, pyrene (Kalyanasundaram and Thomas, 1977; Wilhelm et al., 1991; Marctic and Nair, 1994) was used as a hydrophobic probe. CAC of the CEC triblock copolymers were estimated to prove the potential of core-shell type nanoparticle formation by the measurement of fluorescence spectroscopy (Shimadzu F-7000 spectrofluorometer, Shimadzu Co.

Ltd., Tokyo, Japan) using pyrene as a probe. To obtain sample solutions, a known amount of pyrene in acetone was added to each of a series of 20 ml vial and the acetone evaporated. The final concentration of pyrene was 6.0×10^{-7} M. A volume of 10 ml of various concentrations of block copolymer solutions was added to each vial and then heated for 3 h at 65°C to equilibrate the pyrene and the block copolymer solution, and left to cool overnight at room temperature. Emission wavelength was 390 nm for excitation spectra. Excitation and emission bandwidths were 1.5 and 1.5 nm, respectively. Deoxygenation procedure was performed by nitrogen gas bubbling.

2.6. Transmission electron microscopy (TEM) measurements

A drop of nanoparticles suspension containing 0.1 wt.% phosphotungstic acids was placed on a carbon film coated on a copper grid. It was observed at 80 kV in a JEOL JEM-2000 FX II.

2.7. Photon correlation spectroscopy (PCS) measurements

PCS was measured with a Zetasizer 3000 (Malvern instruments, UK) with He–Ne laser beam at a wavelength of 633 nm at 25°C (scattering angle of 90°). A nanoparticle solution prepared by dialysis method was used for particle size measurement (concentration: 0.1 wt.%) and measured without filtering.

2.8. X-ray diffractometer measurement

X-ray powder diffractograms were obtained with a Rigaku D/Max-1200 (Rigaku) using Ni-filtered CuKα radiation (35 kV, 15 mA).

2.9. In vitro degradation test of CEC triblock copolymer nanoparticles

A total of 100 mg of CEC triblock copolymer was dissolved in 20 ml of 1,4-dioxane and then the solution was stirred at room temperature to solubilize entirely. The solution was dialyzed using MWCO 2000 or 12 000 g/mol dialysis tube and then dialyzed against phosphate buffered saline (PBS; 0.1 M, pH 7.4) for 2 days with exchange of fresh PBS at intervals of 3–6 h. The resultant solution was adjusted to 50 ml with PBS solution and then each 10 ml (i.e. 10 ml of aqueous solution contain 20 mg of CEC triblock copolymer nanoparticles) of them was injected into dialysis tube (MWCO 2000 g/mol). The dialysis tubes were introduced into 100 ml bottle with 50 ml PBS and incubated at 100 rpm at 37°C. The whole media was exchanged with fresh PBS at intervals of 2 days. At specific time intervals, dialysis tube samples were taken and dialyzed against distilled water for 6 h. The resultant solution was freeze-dried to analyze the change of molecular weight of PCL block by ^{1}H NMR as described above.

2.10. In vitro release studies

The release experiment in vitro was carried out according to the method reported previously (Jeong et al., 1998, 1999; Nah et al., 1998). A total of 7 mg of CNZ loaded CEC nanoparticles were suspended in 2 ml PBS by sonication for 10 s at 15 W using bar type sonicator (Ultrasonic homogenizer, UH-50, SMT Co. Ltd., Japan) and then put into a dialysis tubes (MWCO 12 000 g/mol). The dialysis tube was placed into a 100 ml bottle with 50 ml PBS and the media was stirred at 100 rpm at 37°C. At specific time intervals, whole medium (50 ml) was taken and replaced with same volume of fresh PBS. The released amount of CNZ was determined by UV spectrophotometer (Shimadzu UV-1201) at 310 nm.

3. Results and discussion

CEC triblock copolymers were prepared by ring-opening polymerization of ε-caprolactone monomer in the presence of PEG without any other catalysts as previous report (Cerrai et al., 1989). An active hydrogen atom at one end of PEG chains acts as an initiator and induces a

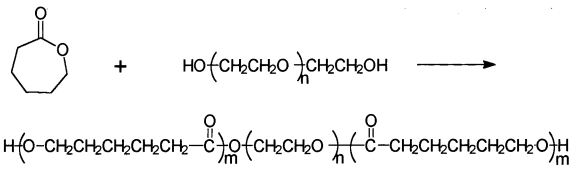


Fig. 1. Synthetic scheme of CEC triblock copolymers.

selective acyl-oxygen cleavage of ε-caprolactone (Fig. 1). PCL homopolymer is a semicrystalline polymer and has hydrophobic characteristics contributing to a long degradation time in vivo. The introduction of ε-caprolactone into PEG can be used to reduce the degradation time to short period. Better physicochemical properties and processibility can be resulted.

The CEC triblock copolymers with different molecular weight were prepared by changing the molar ratio of PEG homopolymer/ε-caprolactone monomer and their molecular weights and composition were determined by ^{1}H NMR spectroscopy. The unit ratio of PEG and ε-caprolactone was obtained from peak intensities of the methylene proton of the PEG chain and methylene proton in ε-caprolactone units, respectively. Since the signals at 3.7 and 4.13 ppm were assigned to these protons, calculated results of molecular weight and composition of CEC were summarized in Table 1.

The particle size of CEC-2 nanoparticles was measured using PCS and the result was shown in Fig. 2a. Particle size of CEC-2 was 32.3 ± 17.3 nm as a monomodal and narrow distribution. The morphologies of CEC-2 nanoparticles were observed by TEM as shown in Fig. 2b. It can be seen that CEC-2 core-shell type nanoparticles has spherical shapes and their size range was about 40–60 nm which was almost in accordance with the size of PCS measurement.

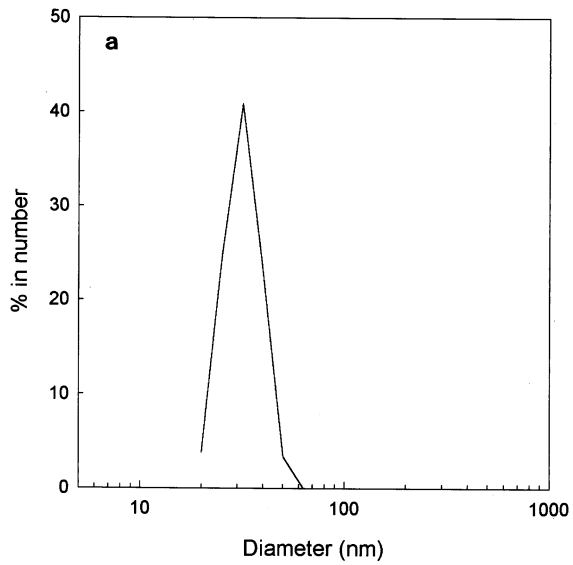
Table 1
Characterization of CEC triblock copolymer

Sample	$\overline{Mn}$ of PCL[a]	Total $\overline{Mn}$[b]	CMC (g/l)[c]
CEC-1	4810	12 810	0.0033
CEC-2	7500	15 550	0.0030
CEC-3	15 860	23 860	0.00195

[a] Number average molecular weight of PCL was estimated from the results of ^{1}H NMR.

[b] Calculated from the ^{1}H NMR results of PCL and PEG. MW of PEG was 8000 from Sigma Co. Ltd. USA.

[c] Evaluated from fluorescence spectroscopy measurement using pyrene as a hydrophobic probe.



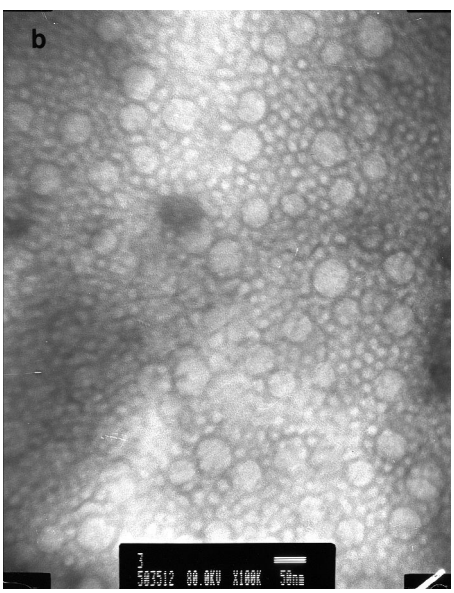


Fig. 2. Particle size distribution of CEC-2 core-shell type nanoparticles (concentration: 1 mg/ml) measured by PCS at 25°C (a) and morphology of CEC-2 core-shell type nanoparticles observed by TEM (b). Nanoparticles were negatively stained with 0.1 wt.% phosphotungstic acid.

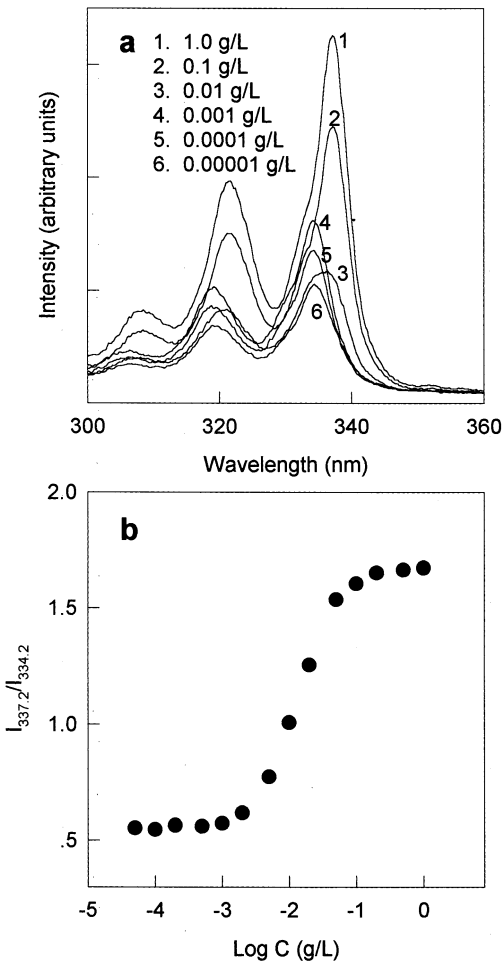


Fig. 3. Fluorescence excitation spectra of pyrene(6.0×10^{-7} M) against CEC concentration in distilled water (emission wavelength: 390 nm) and plots of the intensity ratio $I_{337.2}/I_{334.2}$ from pyrene excitation spectra versus log c (g/l) of the CEC in the distilled water (b).

The formation of core-shell type structures was confirmed by a fluorescence probe technique using pyrene as a hydrophobic probe. Fig. 3a shows the excitation spectra of pyrene (6.0×10^{-7} M) in the various concentrations of CEC-2 block copolymer. Pyrene will be preferentially partitioned into hydrophobic cores with a concurrent change of the photophysical properties of the molecules. In the excitation spectra, a red shift was observed with increasing concentration of CEC block copolymer as in the study of micelle formation of PS-PEO block copolymers (Wilhelm et al., 1991). The (0, 0) bands in the pyrene excitation spectra were examined and compared with the intensity ratio $I_{337.2}/I_{334.2}$. This ratio takes the value characteristic of pyrene in water at low concentrations and the value of pyrene entirely in the hydrophobic domain. A plot of $I_{337.2}/I_{334.2}$ versus log c is shown in Fig. 3b. A flat region in the low concentration extreme and sigmoidal region in the crossover region was noted. This result indicated that signal change in the region of 0.0030 g/l can be evaluated to the CAC value of CEC block copolymer. As shown in Table 1, CAC values of CEC block copolymer were decreased with the increase of PCL block chain length, i.e. the shorter the PCL chain length, the higher the CAC values. These results displayed a similar tendency to the previous reports (Jeong et al., 1998; Nah et al., 1998). Further evidence of core-shell type nanoparticles of CEC-2 block copolymer and limited mobility of the PCL chain in the core of the nanoparticles were obtained with ^{1}H NMR in $CDCl_3$ and D_2O as shown in Fig. 4. Since both of the PCL and PEO block are easily dissolved in $CDCl_3$ or DMSO (D-form) and exist in liquid-state (Fig. 4a), the core-shell structure formation is not expected. In $CDCl_3$ or DMSO (D-form), the characteristic peak of the methyl protons of the PCL segment was shown about 4.1 and 1–2.5 ppm, respectively. Also, in that solvent, protons of the ethylene oxide of the PEG segment was shown in 3.6–3.7 ppm (Fig. 4b). But, in D_2O, characteristic peaks of the PCL block were completely disappeared, whereas peculiar peaks of the PEG block were remained. These results indicated that protons of PCL block display restricted motions within the inner-core and PCL block has a rigid solid structure, whereas PEG blocks are existed as a liquid state in the aqueous environment. This behavior of CEC core-shell type nanoparticles is in contrast with low molecular amphiphiles and PEO–PPO–PEO block copolymers which typically exhibit liquid-like cores and relatively higher mobility. It was also reported that poly(ε-benzyl L-aspartate)(PBLA)/PEO diblock copolymer has a rigid PBLA core (Kwon et al., 1993). But, in their results, the peaks of 7.4 and 5.2 ppm were not completely disappeared and

this result suggested that PBLA /PEO diblock copolymer micelles may have a relatively less rigid core when compared with CEC core-shell type nanoparticles.

In the block copolymeric micelles, the selected solvent used to dissolve the block copolymer can affect the micellar properties due to the polymer solubility in the solvent, dissimilarity of diffusion rate of the solvent into the aqueous environment, the differences of each block of the copolymer in the solvent/water mixtures, and the solubility of the drug, etc. (La et al., 1996; Cheon et al., 1999). These parameters also can affect the particle size and drug loading contents of the CEC block copolymer nanoparticles.

Various water miscible solvents such as 1,4-dioxane, acetone, DMF, DMSO, DMAc, and THF were used to prepare the core-shell type nanoparticles of CEC-2 block copolymers by dialysis method, and the results were summarized in Table 2. When 1,4-dioxane and acetone were used as the initial solvent for the preparation of nanoparticles in water, the particle sizes were relatively smaller than those of other solvents. The use of THF, DMF, DMSO, and DMAc resulted in an increased particle size. Among them, 1,4-dioxane was resulted relatively high drug loading with small particle size. THF was resulted in highest drug loading content but their particle size was remarkably increased.

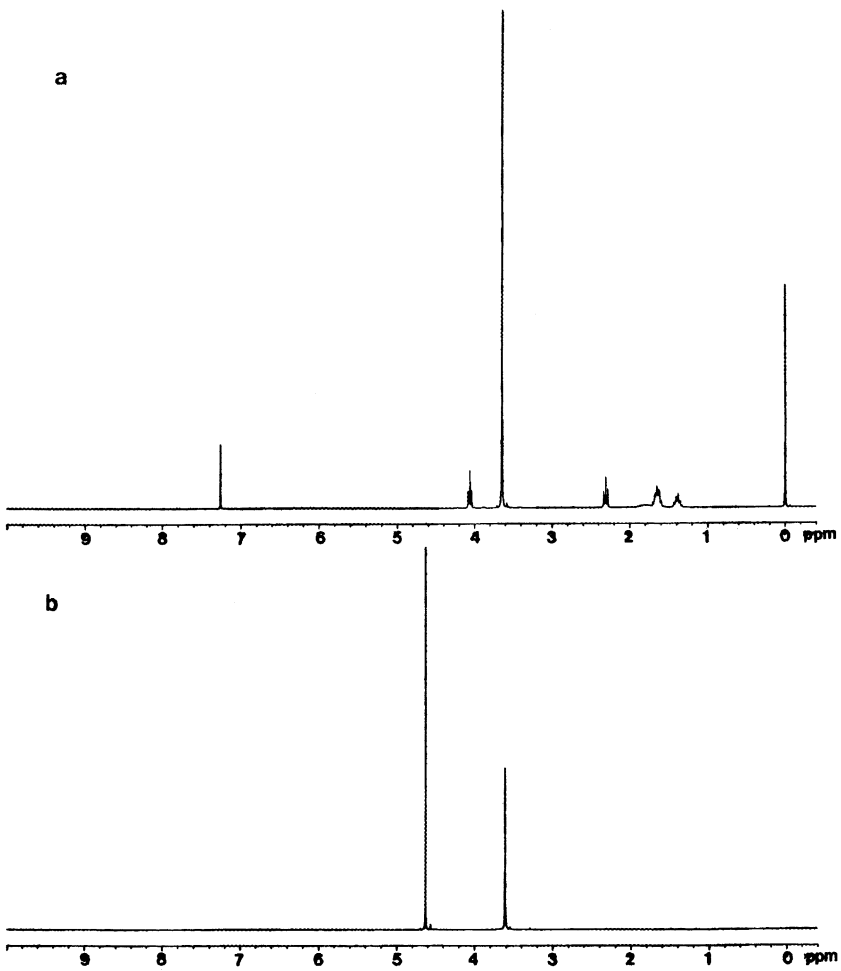


Fig. 4. ^{1}H NMR spectra of CEC-2 core-shell type nanoparticles dissolved in $CDCl_3$ (a) and redistributed in D_2O (b).

Table 2
Particle size of CEC-2 core-shell type nanoparticles against various initial solvent used

Solvent (ml)	Polymer (mg)	Drug (mg)	Particle size			Drug loading contents (wt.%)	Loading efficiency (wt.%)
			Intensity average	Volume average	Number average		
1,4-Dioxane	40	20	33.6 ± 6.3	33.3 ± 11.0	32.9 ± 11.3	8.3	18.1
Acetone	40	20	36.9 ± 10.9	36.3 ± 17.8	35.2 ± 16.9	7.0	15.1
DMF	40	20	1217.3 ± 443.9	1217.0 ± 668.7	1170.3 ± 655.6	8.9	19.5
DMSO	40	20	814.7 ± 325.2	759.9 ± 321.2	752.4 ± 310.6	7.7	16.7
DMAc	40	20	1160.8 ± 270.4	1176.7 ± 542.4	1175.4 ± 540.5	6.0	12.8
THF	40	20	863.8 ± 200.6	819.7 ± 330.3	804.3 ± 309.5	11.7	26.5

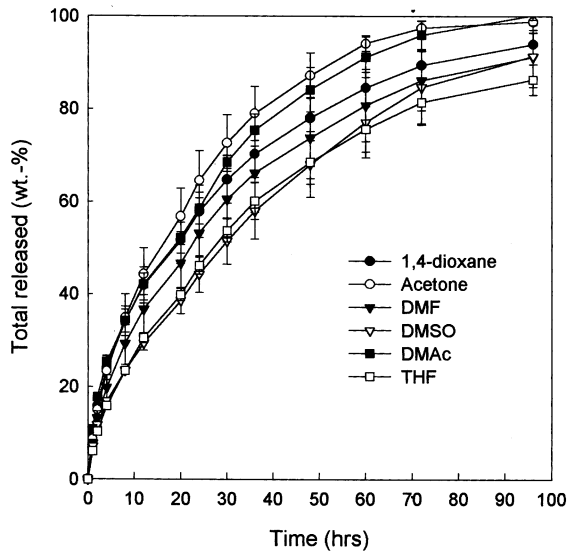


Fig. 5. CNZ release from CEC-2 nanoparticles against different initial solvent.

Fig. 5 shows the effect of initial solvent on the drug release from the CEC-2 core-shell type nanoparticles. Although the release rate was not significantly differenced against initial solvent used, the CNZ release rate in the case of 1,4-dioxane and acetone as an initial solvent, resulted in smaller particle size than that of other initial solvent, was faster than that of DMF, DMSO, and THF, indicating that the used initial solvents significantly affected particle size, drug loading contents, and physicochemical properties of nanoparticles. Also, these distinguished drug release rate might be due to the differences of drug loading contents and particle size of CEC core-shell type nanoparticles. In the case of DMAc, the CNZ release was relatively faster than that of other case in spite of their large particle size. It was thought that a low drug loading content of DMAc resulted in faster release rate of CNZ than that of other initial solvents.

Table 3 shows the particle size of CEC nanoparticles against the block copolymer composition and initial drug feeding ratio with 1,4-dioxane as an initial solvent. These results indicated that the particle sizes were dependent on the chain length and molecular weight of PCL block on the CEC block copolymer. Also, the drug loading content was increased with increased molecular weight of CEC block copolymer. These results might be due to the strong hydrophobic interaction between the longer hydrophobic PCL block chain and hydrophobic drug. Also, the loading efficiency was increased in accordance with the increased molecular weight of CEC block copolymer. When initial drug feeding amounts were differently supplied, the higher the feeding amount of the drug, the higher the drug loading contents, and the lower the loading efficiency of CEC-2 nanoparticles. The particle size of CEC-2 nanoparticles was slightly increased when the initial drug feeding amount was increased and, especially, secondary aggregates were appeared with highest amount of drug feeding although their fraction was about 5.0%.

Table 3
Particle size of CEC core-shell type nanoparticles against polymer composition and drug loading contents

Sample	Polymer (mg)	Drug (mg)	Particle size			Drug loading contents (wt.%)	Loading efficiency (wt.%)
			Intensity average	Volume average	Number average		
CEC-1	40	20	24.6 ± 2.2	25.6 ± 1.1	25.5 ± 1.2	7.7	16.7
CEC-2	40	20	33.4 ± 10.4	31.7 ± 13.7	30.8 ± 16.5	8.3	18.1
	40	40	33.6 ± 6.3	33.3 ± 11.0	32.9 ± 11.3	12.1	13.8
	40	80	34.3 ± 6.4 (210.9 ± 119.4)[a]	34.4 ± 5.9 (204.5 ± 128.8)[a]	34.1 ± 5.6	14.2	8.3
CEC-3	40	20	62.1 ± 4.7	62.0 ± 9.2	61.9 ± 9.1	11.7	26.5

[a] Secondary aggregation.

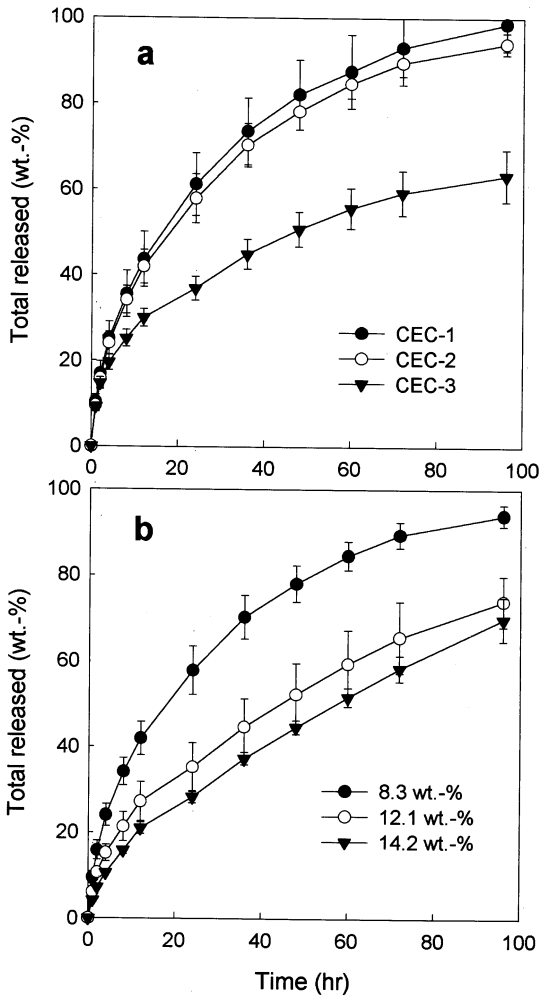


Fig. 6. CNZ release from CEC core-shell type nanoparticles as a function of polymer composition (a) and drug loading contents of CEC-2 block copolymer (b).

To study the drug release behavior, the CNZ-entrapped CEC core-shell type nanoparticles were simply redistributed in PBS (pH 7.4, 0.1 M) and drug release studies were performed in vitro. Fig. 6 shows the release kinetics of CNZ from the CEC nanoparticles against the molecular weight of block copolymer (a) and drug loading contents (b). In the results of Fig. 6a, CNZ was continually released in vitro over 3 days and the release pattern was revealed almost in pseudo zero-order kinetics. The drug release was shown that the higher the molecular weight of block copolymer, the slower the drug release kinetics. As shown in Fig. 6b, it was observed that the higher the drug loading contents, the slower the drug release. These phenomena were reported by several authors (Gref et al., 1994; Jeong et al., 1998; Nah et al., 1998). Gref et al. (1994) reported that crystallization of hydrophobic drug occurred inside the nanoparticles and, especially, at higher drug loading contents, a phase separation occurred, leading to the crystallization of drug in the nanoparticles. Then, hydrophobic drugs loaded into nanoparticles were released more slowly at higher drug loading contents differing from hydrophilic water-soluble drugs. Also, we observed that CNZ release had slower rate kinetics from the nanoparticles with higher drug loading contents. On the other hand, at low drug loading, CNZ existed as a molecular dispersion inside the nanoparticles (Jeong et al., 1998). The crystallized drug should be dissolved and diffused more slowly into the outer aqueous phase than in that of molecular dispersion. These characteristics of drug release behavior were supported by calorimetric analysis (data not shown) as previous report (Jeong et al., 1998). Also, because of the differences in the diffusivity of drug molecules to the outer aqueous phase, the drug release kinetics is affected not only by drug loading contents but also the size of nanoparticles. For the same drug loading contents, the drug release rates in large nanoparticles were slower than in those of small sized nanoparticles as reported elsewhere (Leroux et al., 1996). Resultantly, the control of the drug release kinetics can be achieved by optimizing the chemical nature of the used polymers, the drug loading contents, the used initial solvents, and the particle size of the nanoparticles.

To investigate the physicochemical characteristics of CNZ-entrapped CEC-2 core-shell type nanoparticles, X-ray powder diffraction was measured. Fig. 7 shows the X-ray diffraction scans of CNZ-loaded CEC-2 core-shell type nanoparticles and the corresponding physical blend. It can be observed that the X-ray diffraction patterns were showed sharp peaks in CNZ drug crystals and, in the physical blend, similar drug crystal peaks were appeared. However, when CNZ was entrapped into CEC-2 core-shell type nanoparticles, the spe-

cific drug crystal peaks were not observed in the X-ray diffraction patterns. It was thought that drug crystallines showed sharply their specific crystal peak when existed as a drug crystals but, after drug etrapped into the nanoparticles, the drug can be existed as a molecular dispersion in the nanoparticles (Gref et al., 1994). Also, these results showed that the drug was successfully entrapped into the nanoparticles as a molecular dispersions.

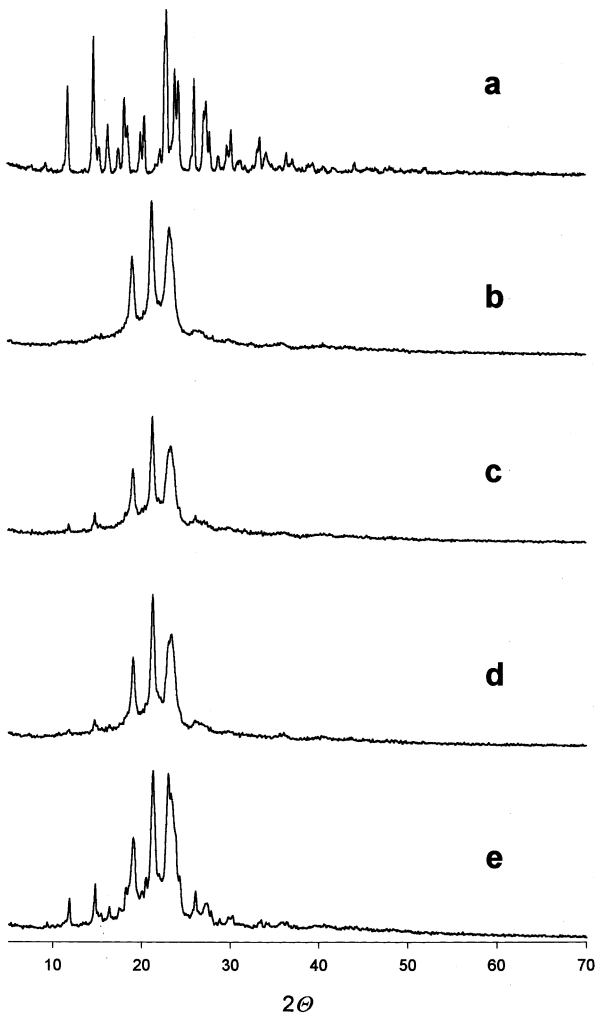


Fig. 7. X-ray diffraction patterns of CEC-2 nanoparticles. CNZ (a); empty nanoparticles (b); CNZ-entrapped CEC nanoparticles (drug contents: 8.3 wt.%) (c); CNZ-entrapped CEC nanoparticles (drug contents: 12.1 wt.%) (d); physical mixture (CEC empty nanoparticle: CNZ = 10:1) (e).

Table 4
Degradation of CEC-2 nanoparticles

Time (Day)	MW of PCL	Total MW	Degradation ratio (%)[a]
0	7550	15 550	0
5	7370	15 370	2.4
10	6980	14 980	7.55
20	6480	14 480	14.2
30	6260	14 260	17.1

[a] Calculated from following equation: [(Initial MW of PCL − PCL MW at time, T)/Initial MW of PCL] × 100.

To observe the degradation behavior of CEC-2 nanoparticles, the dialyzed CEC-2 nanoparticles were incubated in the PBS (0.1 M, pH 7.4) and the molecular weight was analyzed by ^{1}H NMR spectroscopy. Since the PEG block is not biodegradable whether the PCL block has biodegradability, the molecular weight of PEG is constantly same during the degradation test, but PCL block can be expected continuous degradation. The residual PCL block was calculated by ^{1}H NMR spectroscopy and their results were summarized in Table 4. As shown in Table 4, CEC-2 triblock copolymer core-shell type nanoparticles were degraded at a very slow rate, i.e. only 17.1% of PCL block was degraded for 30 days. From these results, it was suggested that the release kinetics of CNZ from the CEC core-shell type nanoparticles were dominantly controlled by the diffusion mechanism rather than the polymer degradation.

In conclusion, core-shell type nanoparticles of poly(ε-caprolactone)/poly(ethylene glycol)/poly(ε-caprolactone) triblock copolymer were prepared by a dialysis technique. Their size and morphology were confirmed using PCS and TEM, respectively. In the measurement of fluorescence spectroscopy using pyrene, CAC value of the CEC-2 block copolymer was evaluated as 0.0030 g/l. CAC values of CEC block copolymer decreased with the increase of PCL chain length, i.e. the shorter the PCL chain length, the higher the CAC values. Particle size, drug loading, and drug release rate of CEC-2 nanoparticles were changed by the initial solvents and the molecular weight of CEC. The degradation behavior of CEC-2

nanoparticles was observed by ^{1}H NMR spectroscopy. It was suggested that CNZ release kinetics were dominantly governed by diffusion mechanism.

Acknowledgements

This work was supported by the research grant from Chosun University, 1999.

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