Preparation of Core-shell Type Nanoparticles of Poly(ε-caprolactone) /Poly(ethylene glycol)/Poly(ε-caprolactone) Triblock Copolymers

Jae-Gon Ryu, Young-Il Jeong,[*] Young-Hoon Kim,[‡] In-Sook Kim, Do-Hoon Kim,[§] and Sung-Ho Kim[*]

College of Pharmacy, Chosun University, Kwangju 501-759, Korea
[*]Research Institute of Medical Science, Chonnam National University Medical School, Kwangju 501-746, Korea
[‡]Department of Chemistry, University of Massachusetts at Lowell, Lowell, Massachusetts 01854, USA
[§]Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
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A triblock copolymer based on poly(ε-caprolactone) (PCL) as the hydrophobic part and poly(ethylene glycol) (PEG) as the hydrophilic portion was synthesized by a ring-opening mechanism of ε-caprolactone with PEG containing a hydroxyl group at both ends as an initiator. The synthesized block copolymers of PCL/PEG/PCL (CEC) were confirmed and characterized using various analysis equipment such as ^{1}H NMR, DSC, FT-IR, and WAXD. Core-shell type nanoparticles of CEC triblock copolymers were prepared using a dialysis technique to estimate their potential as a colloidal drug carrier using a hydrophobic drug. From the results of particle size analysis and transmission electron microscopy, the particle size of CEC core-shell type nanoparticles was determined to be about 20-60 nm with a spherical shape. Since CEC block copolymer nanoparticles have a core-shell type micellar structure and small particle size similar to polymeric micelles, CEC block copolymer can self-associate at certain concentrations and the critical association concentration (CAC) was able to be determined by fluorescence probe techniques. The CAC values of the CEC block copolymers were dependent on the PCL block length. In addition, drug loading contents were dependent on the PCL block length: the larger the PCL block length, the higher the drug loading content. Drug release from CEC core-shell type nanoparticles showed an initial burst release for the first 12 hrs followed by pseudo-zero order release kinetics for 2 or 3 days. CEC-2 block copolymer core-shell type nanoparticles were degraded very slowly, suggesting that the drug release kinetics were governed by a diffusion mechanism rather than a degradation mechanism irrelevant to the CEC block copolymer composition.

Keywords : Triblock copolymer, Core-shell type nanoparticles, Biodegradation.

Introduction

Nanoparticulate drug carriers have been widely employed for drug targeting issues and biomedical applications.[1-7] Since drug carrier systems using nanoparticles are able to provide intravenous injection 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.[2] Many colloidal carriers such as nanospheres,[2-5] polymeric micelles,[8-10] and liposomes[11-14] have been developed and have been suggested to achieve these goals. Block copolymers exhibit surfactant behavior and easily form core-shell type nanoparticles or polymeric micelles by self assembling in an aqueous solution.[8-10] Block copolymers with hydrophobic block and hydrophilic block form polymeric micelles[10-18] or core-shell type nanoparticles,[4,17] respectively, in aqueous environment. Polymeric micelles have a hydrophobic inner-core surrounded by a hydrated outershell. Hydrophobic blocks form the inner-core of the polymeric micelle, which act as a drug incorporation site. Hydrophobic drugs can be physically entrapped in the innercore of core-shell type nanoparticles by hydrophobic interactions. Hydrophilic blocks form a hydrated outershell which may cloak the hydrophobic core preventing its quick uptake by the reticuloendothelial system (RES) and more active clearing organs such as the liver, spleen, lung, and kidneys. Therefore, the hydrated outershell can increase the blood circulation times of the nanoparticles. The predominant characteristics of this system have been reported to include reduced toxic side effects of antitumor agents, passive targeting to specific sites, solubilization of hydrophobic drugs, stable storage of drugs, longer blood circulation, favorable biodistribution, thermal stability, and lower interactions with the RES.[8]

Serious investigations have been performed to develop effective nanoparticles or colloidal drug carriers using various polymers, although to date core-shell type nanoparticles or polymeric micelles using triblock copolymers consisting of poly(ε-caprolactone)/poly(ethylene glycol)/poly(ε-caprolactone) (PCL/PEG/PCL, CEC) are scarcely reported in the literature. PCL is a biodegradable polyester and has attracted attention in controlled drug delivery due to its non-toxicity. However, the application of PCL as a drug delivery system has drawbacks such as a slow degradation rate *in vitro* and *in vivo* due to its high crystallinity and hydrophobicity. It has been reported that the biodegradability of PCL can be enhanced by copolymerization,[19] or by blending with other

*To whom correspondence should be addressed. Tel: +82-62-220-3745; Fax: +82-62-222-5414; e-mail: shkim@mail.chosun.ac.kr

polymers.[20] PEG is a non-immunogenic, non-toxic water soluble polymer and has an to prevent protein adsorption and attack of the RES.[18] It was thought that PEG could be used to increase the biodegradation rate and hydrophilicity.

In this study, we synthesized CEC triblock copolymers as hydrophobic drug carriers and performed physicochemical studies *in vitro*. Clonazepam (CNZ), a model hydrophobic drug, is an anticonvulsant benzodiazepine that is efficacious for the treatment of panic disorders and has considerable hydrophobic characteristics (water solubility (PBS, pH 7.4, 0.1 M at 37 °C): < 14.66 μg/mL). In particular, it exhibits a high interaction with proteins *in vivo* and can be easily cleared from the blood circulation.[21,22] Therefore, we expected to easily entrap CNZ into block copolymeric core-shell type nanoparticles. Additionally, CNZ need to extend the half-life by prevention of protein adsorption and rapid clearance by the unwanted organs or tissues, and also need to solve the drug solubility. Polymer characteristics, the particle size of CEC block copolymer, the drug loading capacity, and the physicochemical properties against various conditions were investigated *in vitro*.

Experimental Section

Materials. PEG (M.W.=8,000 g/gmole, PEG 8 K) was purchased from Sigma Chem. Co., USA. ε-caprolactone and PCL homopolymer (M.W.=10,000 g/gmole from Aldrich Chem. Co. Inc., USA) were purchased from Aldrich Chem. Co. Inc., USA. Clonazepam (CNZ) was kindly supplied by Roche Co., Switzerland. 1,4-dioxane, dichloromethane, ethanol, and diethyl ether were used at reagent grade without further purification.

Synthesis of Triblock Copolymers of CEC. Triblock copolymers based on PCL and PEG were prepared by ring-opening polymerization of ε-caprolactone monomer using dihydroxy PEG without catalysts as reported by Cerrai *et al.*,[23] where an active hydrogen atom at the end of chains of PEG homopolymers acted as an initiator and induced a selective acyl-oxygen cleavage of ε-caprolactone (see Figure 1). A weighed amount of PEG and ε-caprolactone was mixed in a round-bottomed flask under vacuum. The mixture was cooled and degassed with a vacuum pump. The round-bottomed flask was sealed off and placed in an oil bath at 185 °C. Following polymerization, the resultant product was cooled at room temperature and dissolved in dichloromethane. The solution was precipitated into an excess amount of cold ethanol and filtered to reject the non-reacted PEG homopolymers and ε-caprolactone monomers. The precipitates were washed with diethyl ether three times and then dried in vacuum oven for 3 days. The composition and molecular weights of the polymers were determined by ^{1}H NMR spectroscopy.

^{1}H Nuclear Magnetic Resonance Spectrometer (NMR) Measurement. In order to estimate the copolymer compositions and the molecular weights of the PCL blocks, the ^{1}H NMR spectra of the copolymers were measured in $CDCl_3$ using a 300 MHz NMR spectrometer (FT-NMR, Bruker AC-300F, 300 MHz). As the number-average molecular weight of PEG 8 K is known, one can estimate the number-average molecular weights of the PCL block and the copolymer composition as calculated from the peak intensities in the spectrum assigned to both polymers.

Fourier Transform-Infrared (FT-IR) Spectroscopy Measurement. FT-IR spectroscopy was used to confirm the synthesis and structure of the CEC triblock copolymer.

Wide Angle X-ray Diffractometer (WAXD) Measurement. X-ray diffractograms were obtained with a Rigaku D/Max-1200 (Rigaku) using Ni-filtered CuKa radiation (35 kV, 15 mA).

Preparation of Core-Shell Type Nanoparticles. The core-shell type nanoparticles of CEC triblock copolymers were prepared by a dialysis method as previously reported.[10] 40 mg of CEC triblock copolymer was dissolved in 10 mL of 1,4-dioxane and the solution was stirred at room temperature to solubilize entirely. To form core-shell type nanoparticles, the solution was dialyzed using a molecular cut-off 12,000 g/gmol dialysis tube against 1.0 L × 3 of distilled water for 3 hrs, followed by distilled water exchange at intervals of 3-4 hrs over 24 hrs. Afterward, 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 the solution was dialyzed as described above.

For measurement of the drug loading content, freeze-dried CEC nanoparticles were suspended in ethanol, vigorously stirred for 12 hrs and sonicated for 1 hr. The resulting solution was centrifuged with 12,000 g for 20 min and the supernatent was taken for the measurement of drug concentration using UV spectrophotometer (Shimadzu UV-1201) at 310 nm. The drug loading contents were calculated using the following equation: (drug weight in the nanoparticles/weight of nanoparticles)×100. Loading efficiency was calculated as: (Residual drug amount in the nanoparticles/initial feeding amount of drug)×100.

Measurement of Fluorescence Spectroscopy. CEC triblock copolymers were dialyzed against distilled water in order to prepare the core-shell type nanoparticles after being completely dissolved in 1,4-dioxane. Transparent aqueous solutions were observed after being dialyzed for 24 hrs. The association behavior of the CEC block copolymer nanoparticles in an aqueous environment was analyzed using fluorescence spectroscopy as same as polymeric micelle. CEC triblock copolymer solutions without drug were prepared as follows: 40 mg of CEC triblock copolymer was dissolved in 10 mL of 1,4-dioxane and dialyzed up to 24 hrs by the same method described above. The resultant solution was adjusted to the various concentrations of the block copolymers.

To estimate the critical micelle concentrations (CMC) of block copolymers, pyrene was used as a hydrophobic probe.[24-26] The critical micelle concentration (CMC) of the CEC triblock copolymers was estimated in order to prove the potential of the core-shell type micellar carrier 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 vials and the acetone evaporated. The final concentration of pyrene was 6.0×10^{-7} M. 10 mL of various concentrations of block copolymer solutions were added to each vial and heated for 3 hrs at 65 in order to equilibrate the pyrene and the block copolymer solution, and was left to cool overnight at room temperature. The emission wavelength was 390 nm for the excitation spectra. The excitation and emission bandwiths were 1.5 nm and 1.5 nm, respectively. The deoxygenation procedure was performed with nitrogen gas bubbling.

Transmission Electron Microscope (TEM) Measurements. A drop of nanoparticles suspension containing 0.1 wt.% phosphotungstic acid was placed on a carbon film coated on a copper grid for TEM. The specimen on the copper grid was not stained. Observation was done at 80 kV in a JEOL JEM-2000 FX II.

Photon Correlation Spectroscopy (PCS) Measurements. PCS was measured with a Zetasizer 3000 (Malvern instruments, England) 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.

Differential Scanning Calorimetry (DSC) Measurement. The melting temperature (T_m) was measured by a Mettler DSC-30 differential scanning calorimeter. The measurement was carried out in the range of room temperature to 200 °C under nitrogen at a scanning rate of 10 °C/min.

***In vitro* Degradation Test of CEC Triblock Copolymer Nanoparticles.** In order to study the degradation behavior of CEC-2 core-shell type nanoparticles, the dialyzed nanoparticles were incubated in PBS (0.1 M, pH 7.4). 100 mg of CEC triblock copolymer was dissolved in 20 mL of 1,4-Dioxane and the solution was stirred at room temperature to solubilize it entirely. The solution was dialyzed using molecular cut-off 2,000 or 12,000 g/gmol dialysis tube and then dialyzed against phosphate buffered saline (PBS, 0.1 M, pH 7.4) for 2 days with an exchange of fresh PBS at intervals of 3-6 hrs. The resultant dialyzed aqueous solution was adjusted to 50 mL with PBS solution and each 10 mL (*i.e.*, 10 mL of aqueous solution contain 20 mg of CEC triblock copolymer nanoparticles) was subsequently introduced into the dialysis tube (molecular weight cut-off 2,000 g/gmol). The dialysis tubes were then introduced into a 100 mL bottle with 50 mL PBS and incubated at 100 rpm in 37 °C. The whole media was exchanged with fresh PBS media at intervals of 2 days. At specific time intervals, dialysis tube samples were taken and dialyzed against distilled water for 6 hrs to remove trace elements. The resultant solution was freeze-dried for analysis of molecular weight changes of the PCL block by ^{1}H NMR as described above.

***In vitro* Release Studies.** The release experiment in vitro was carried out as previously reported.[10,17] 7 mg of CNZ loaded CEC core-shell type nanoparticles were suspended in 2 mL PBS by sonication for 10 s at 15 watts using a bar type sonicator (Ultrasonic homogenizer, UH-50, SMT Co. Ltd., Japan) and subsequently put into a dialysis tubes (molecular cut-off 12,000 g/gmole). 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. A whole-media change method was used for prevention of drug saturation in the drug release study. At specific time intervals, the whole medium (50 mL) was taken and replaced with the same volume of fresh PBS (50 mL). The concentration of released CNZ in the PBS was determined by UV spectrophotometer (Shimadzu UV-1201) at 310 nm.

Results and Discussion

The composition and molecular weights of the polymers were determined by ^{1}H NMR spectroscopy and the unit ratio of MePEG and ε-caprolactone was calculated from the peak intensities of the methylene proton of the PEG and the methylene protone of ε-caprolactone units. Value of 3.65 ppm and 4.13 ppm were assigned to proton peaks of PEG and PCL, respectively, as shown in Figure 1. The composition of the CEC block copolymer was estimated from each of the number-average molecular weights of the PCL and PEG blocks as a monomeric unit. The CEC triblock copolymers with different molecular weights were prepared by changing the molar ratio of PEG homopolymer/ε-caprolactone monomer. The calculated results of the molecular weight and composition of CEC are summarized in Table 1. PCL is a known semicrystalline polymer, melting in the range of 59-64 °C, depending on the crystallite size.[27] DSC results are shown in Figure 2. In the DSC results of CEC-2 and 3 block copolymers, the thermal curves showed two endotherms. The higher temperature endotherm at 61.5 °C of CEC-2 and 61.0 °C of CEC-3, respectively, can be attributed to the melting of the PCL crystal phase. The low temperature peaks at 48.9 °C of CEC-2 and 49.3 °C of CEC-3, respectively, correspond to the melting of the PEG crystal phase. Similar to our results, the bimodal melting peaks of the CEC triblock copolymer system was also reported by Bogdanov *et al.*[28] The bimodal

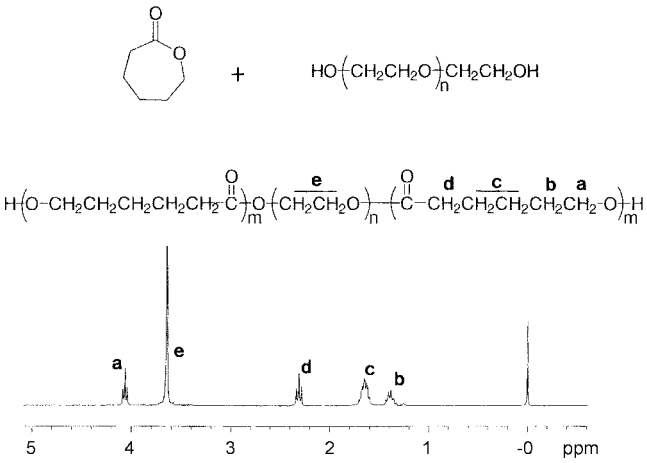

Figure 1. Synthesis scheme of poly(ε-caprolactone)/poly(ethylene glycol)/poly(ε-caprolactone) (CEC) triblock copolymers and typical ^{1}H NMR spectrum of CEC dissolved in $CDCl_3$.

Table 1. Characterization of CEC triblock copolymer

Sample	Mol-% as a monomeric units		$\overline{Mn}$ of PCL[a]	Total $\overline{Mn}$[b]	CAC (mol)[c]	Melting temperature, T_m (°C)[d]	
	PCL	PEG				PCL	PEG
CEC-1	18.8	81.2	4,810	12,810	2.58×10^{-7}	57.4	–[e]
CEC-2	26.7	73.3	7,550	15,550	1.93×10^{-7}	61.5	48.9
CEC-3	43.3	56.7	15,860	23,860	8.17×10^{-8}	61.0	49.3

[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. PEG M.W. was 8,000 from Sigma Co. Ltd. USA. [c]Evaluated from the pyrene excitation spectra measured by fluorescence spectroscopy as described in experimental section. [d]Measured by DSC. [e]Not detected.

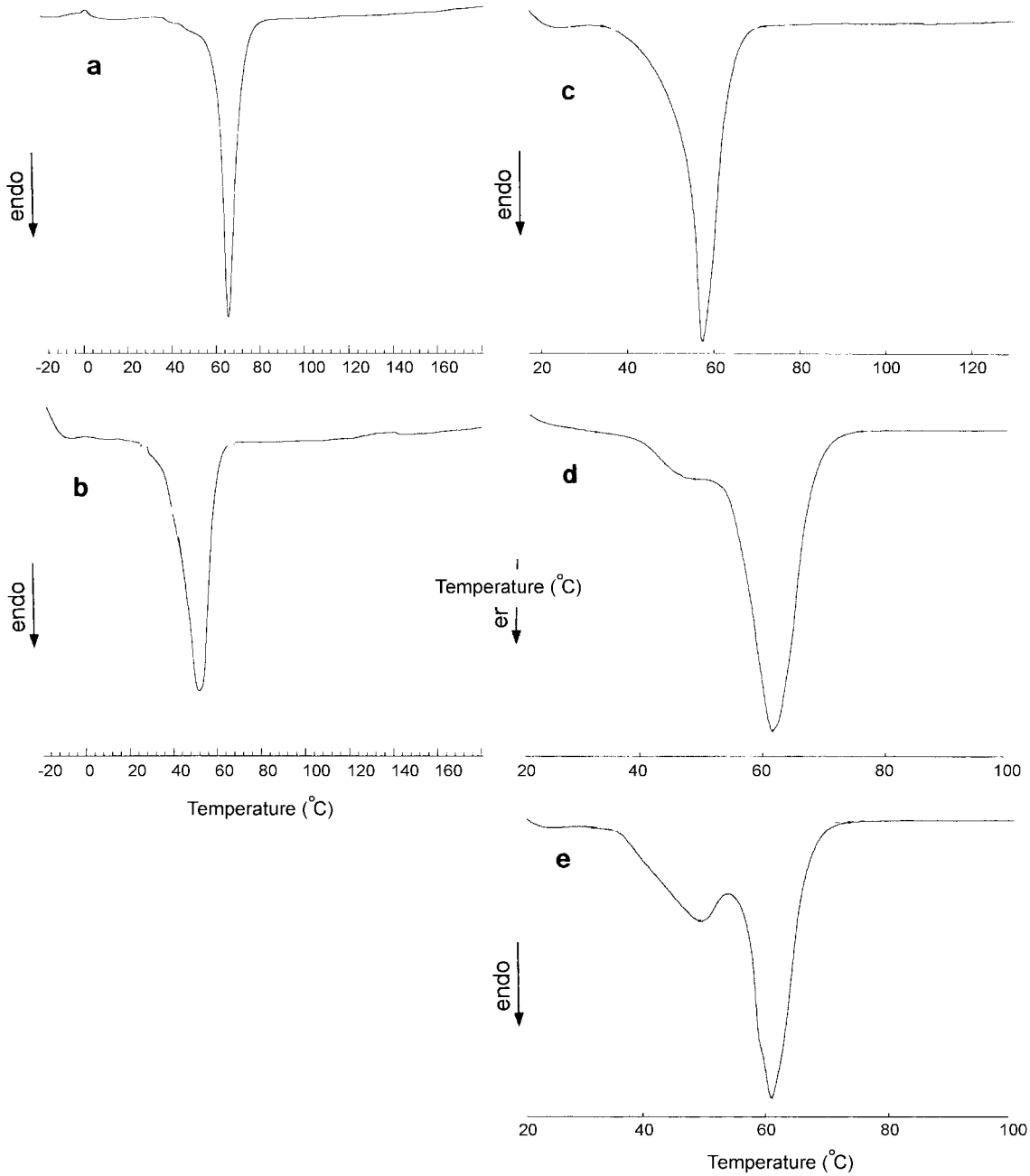

Figure 2. DSC thergrams of PCL (10K) (a), PEG (8K) (b), CEC-1 (c), CEC-2 (d), and CEC-3 (e) block copolymers.

melting peak observed for PEG is again most likely due to the existence of folded-chain lamellae with different fold numbers.[20] In our DSC results for the CEC triblock copolymer. the melting endotherm of CEC-1 showed monomodal

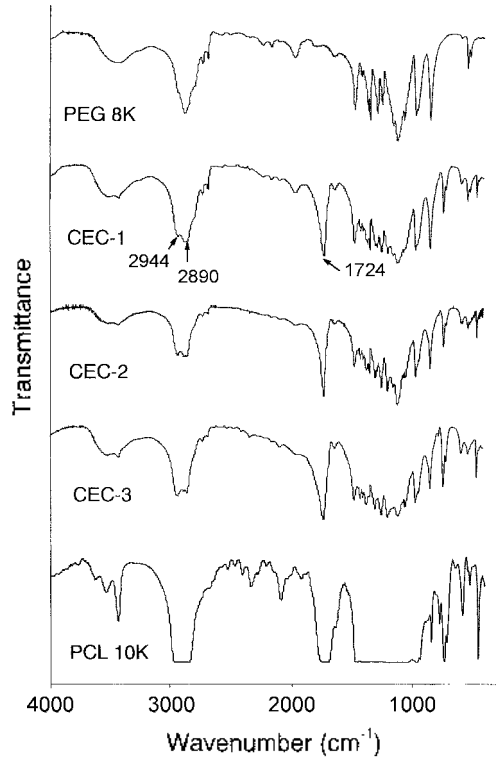

Figure 3. FT-IR spectra of CEC triblock copolymers.

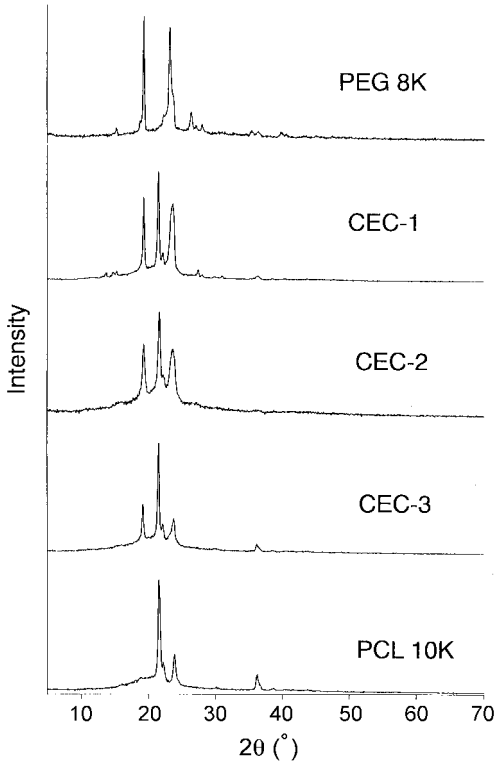

Figure 4. WAXD patterns of CEC triblock copolymers.

melting peaks. These results may be because the melting endotherm of the PEG block is shifted and less pronounced in the low Mw ranges. The melting temperature of PCL in the CEC-1, which has a relatively higher PEG content, was also inner-shifted to a lower temperature.[28,30]

Figure 3 shows the FT-IR spectra of PEG, PCL homopolymer, and CEC block copolymers with various compositions. A new strong carbonyl band appears at 1724 cm^{-1} and is attributed to the formation of block copolymer, and the aliphatic CH stretching band of ε-caprolactone at 2944 cm^{-1} increased with the rising molar ratio of the block copolymer, whereas the absorption band of CH stretching vibration of PEG at 2890 cm^{-1} decreased. The absorption band at 3436 cm^{-1} is determined to be the terminal hydroxyl groups in block copolymers and the absorption at 1187-1085 cm^{-1} is due to C-O stretching. Figure 4 shows the WAXD patterns of CEC block copolymers, PEG, and PCL homopolymers. It was observed that PEG has three characteristic crystalline peaks and that PCL homopolymer also has characteristic crystalline peaks. As shown in Figure 4, the sharp crystalline peak of PEG was reduced by copolymerization with ε-caprolactone, while the characteristic peak of PCL was increased. These results indicated that the relative crystallinities of copolymers were increased with the increased ratio of ε-caprolactone unit in the block copolymers. Since the PCL homopolymer is a semicrystalline polymer and has hydrophobic characteristics contributing to long *in vivo* degradation times, the introduction of ε-caprolactone into PEG, resulting in the PCL-PEG block copolymer, could control the degradation shortening the time and achieving better physicochemical properties and processibility. In particular, it is expected that block copolymers composed of PCL and PEG can be easily prepared as nanoparticles or polymeric micellar carriers with core-shell type structures.

It was expected that CEC block copolymers could self-associate at a critical concentration (*i.e.*, critical association concentration (CAC)) due to the core-shell type micellar structures of CEC block copolymer. Wilhelm *et al.*[25] reported a micelle formation of poly(styrene) (PS) and PEO di- or triblock copolymers in water using a fluorescence technique with pyrene as a hydrophobic probe and determined the critical micelle concentration (CMC) from the fluorescence and excitation spectra, as pyrene partitions between aqueous and micellar environments. The formation of core-shell micellar structures was confirmed by a fluorescence probe technique using pyrene as a hydrophobic probe (6.0×10^{-7} M). The fluorescence spectra of CEC block copolymers at various concentrations in the presence of pyrene were obtained (data not shown) in a manner similar to the previously repoted method.[10,17] Pyrene will be preferentially partitioned into hydrophobic cores with a change to the photophysical properties of the molecules. The fluorescence intensity was increased and a change in the vibrational structure of the monomer fluorescence was observed with increasing concentrations of CEC block copolymer. A red shift in the excitation spectrum was also observed with increasing concentration of CEC block copolymer. 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

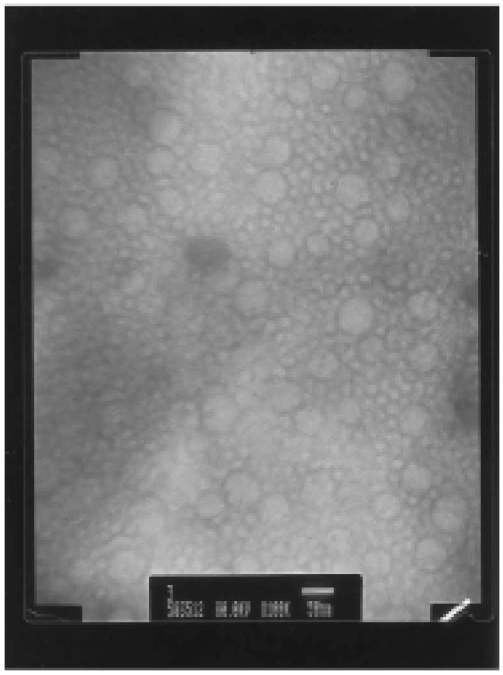

Figure 5. TEM photograph of CEC-2 core-shell type nanoparticles. Nanoparticles were negatively staining with 0.1 wt.-% phosphotungstic acid.

in the hydrophobic domain. In the plot of $I_{337.2}/I_{334.2}$ versus log c (not shown), the flat region in the low concentration extreme and sigmoidal region in the crossover region was noted and the signal change in the region of 0.0030 g/l (1.93×10^{7} M) can be evaluated as to the CAC values of CEC-2 block copolymer. The results of the CAC values of the CEC block copolymers are shown in Table 1. The CAC values of the 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 demonstrated a trend similar to previous reports[10,17] as expected. CEC-2 core-shell type nanoparticles were observed by transmission electron microscopy to obtain the morphology. Figure 5 shows TEM photographs of CEC-2 core-shell type nanoparticles. It was shown that CEC-2 core-shell type nanoparticles have relatively spherical shapes and their size was about 20-70 nm. Additionally, the particle size of the core-shell type nanoparticles of CEC-2 block copolymer was measured using photon correlation spectroscopy as shown in Figure 6. The particle size of CEC-2 was 32.3 ± 17.3 nm as a monomodal and narrow distribution. These CEC block copolymer nanoparticles have a similar particle size with polymeric micelles that generally have a particle size of 10-100 nm.[8,25] In the results of particle size measurement and TEM observations, CEC block copolymer can have sufficiently spherical nanoparticles with particle sizes the same as polymeric micelles.

In the block copolymeric micelles, the block lengths and compositions of each of the segments affect the micellar properties such as the critical micelle concentration, the micelle size, the aggregation number, the micelle stability, and the shape.[26,31,32] As the solvophobicity of the solvophobic part

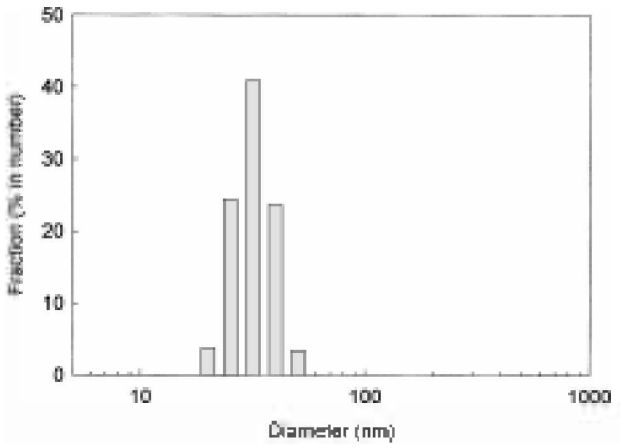

Figure 6. Particle size distribution of CEC-2 core-shell type nanoparticles.

of the block copolymers increases, the CMC of the block copolymers decreases, and the average aggregation number and size of the micelle is increased since the solvent repulsion force of the solvophobic block and the interfacial tension between the solvent and the surface of the micellar core increases.[26,31] Additionally, the selected solvent used to dissolve the block copolymer, can be affected by the micellar properties described above due to the different polymer solubilities in the solvent, differences in the diffusion rate of the solvent into the aqueous environment, differences of each block of the copolymer in the solvent/water mixtures, the solubility of the drug, etc.[5,32] These parameters can also affected by the particle size of the CEC block copolymer micelles.

Various water-miscible solvents such as 1,4-dioxane, acetone, DMF, DMSO, DMAc, and THF were used for the preparation of core-shell type nanoparticles of CEC block copolymers. Among these, 1,4-dioxane resulted in a small particle size with a narrow size distribution and maintained a stable nanoparticle solution after the dialysis procedure, *i.e.* the nanoparticle solution prepared with 1,4-dioxane resulted in a transparent aqueous solution without precipitants and the particle size was not significantly changed after one week (stored at 4 °C, data not shown). The use of THF, DMF, DMSO, and DMAc resulted in an increased particle size. The particle size of CEC core-shell type nanoparticles against the block copolymer composition and initial drug amount are summarized in Table 2. As shown in the results of the particle size of CEC block copolymer, particle size increased according to the increased block length of PCL. These results indicated that the particle sizes were dependent on the chain length and molecular weight of the PCL block on the CEC block copolymer. Additionally, the drug loading contents were relatively increased with the increased PCL block length in the CEC block copolymer. These results may be due to the fact that the longer hydrophobic PCL block chain length in the higher molecular weight of CEC block copolymer can be induced by a strong hydrophobic interaction with a hydrophobic drug. Moreover, the loading efficiency was increased in accordance with the increased PCL block length

Table 2. Particle size of CEC core-shell type nanoparticles against polymer composition and drug loading contents

Sample	Polymer (mg)	Drug (mg)	Particle size (nm)	Drug loading contents (wt.-%)	Loading efficiency (wt.-%)
CEC-1	40	20	25.5±1.2	7.7	16.7
CEC-2-1	40	20	30.8±16.5	8.3	18.1
CEC-2-2	40	40	32.9±11.3	12.1	13.8
CEC-3	40	20	61.9±9.1	11.7	26.5

of the CEC block copolymer. When initial drug amounts were supplied differently, the higher initial drug amount induced higher drug loading contents and lowered the loading efficiency in the CEC-2 block copolymer core-shell type nanoparticles. The particle size of the CEC-2 block copolymer was not significantly changed when the initial drug amounts were increased. From these results, it was indicated that the PCL block length of the CEC block copolymer, and the initial amount of drug can affected the drug loading contents, particle size, and physicochemical properties of CEC nanoparticles.

The CNZ-entrapped core-shell type nanoparticles of CEC block copolymer against block copolymer composition and drug loading contents were simply redistributed in PBS (pH 7.4, 0.1 M) and a drug release study was performed *in vitro*. Figure 7 shows the release kinetics of CNZ from the nanoparticles of CEC block copolymer. The release pattern of CNZ demonstrated an initial burst for the first 12 hrs and then almost continuously released in vitro for approximately 3 days as a pseudo zero-order kinetics. The drug release kinetics demonstrated that the higher PCL block length induced slower drug release kinetics. Matsumoto *et al.*[33] also reported that the amount of drug released increased as the PEG content increased and total M.W. of poly(L-lactide)-poly(ethylene glycol)-poly(L-lactide) (LEL) block copolymer decreased. Additionally, Ha *et al.*[30] reported that the increased PCL weight fraction in the PCL/PEG semi-interpenetrating polymer networks induced decreased a drug release rate. Furthermore, it was observed that the higher drug loading contents resulted in slower drug release kinetics. These phenomena have been reported by several authors.[4,5,10] Hydrophobic drug can be crystallized inside the nanoparticles at higher drug loading contents and then a phase separation occurs, leading to the crystallization of a part of the drug in the nanoparticles.[4] Hydrophobic drugs entrapped into nanoparticles release more slowly at higher drug loading contents. Additionally, the CNZ release rate from the nanoparticles was shown to be slow at higher drug loading contents. On the other hand, at low drug loading, CNZ is relatively present as a molecular dispersion inside the nanoparticles.[5] The crystallized drug should dissolve and diffuse more slowly into the outer aqueous phase than in that of molecular dispersion. Moreover, because of differences in the diffusivity of drug molecules to the outer aqueous phase, the drug-release kinetics are affected by the drug loading contents, the nature of the polymer used, and the size of the nanoparticles.[34-36]

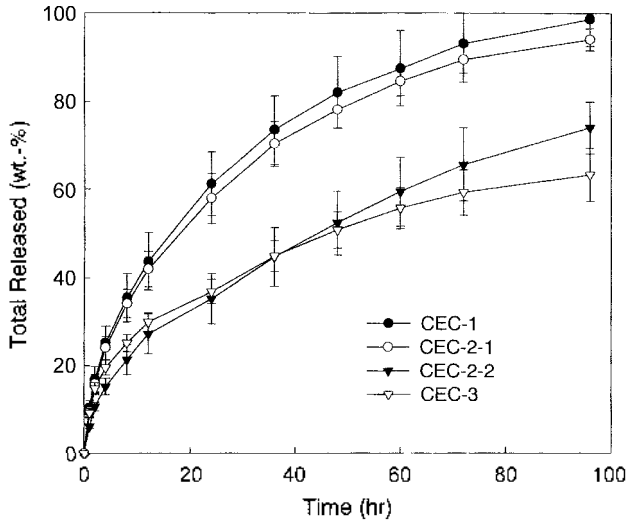

Figure 7. Clonazepam release from CEC core-shell type nanoparticles as a function of polymer composition and drug loading contents. Drug loading contents were described in Table 2.

To elucidate the release mechanism of CNZ from nanoparticles, a degradation test of the CEC nanoparticles was performed *in vitro*. PCL is known to degrade very slowly due to its hydrophobic properties which does not allow fast water penetration. PCL degradation by random hydrolytic chain scission of the ester linkages has been documented by Pitt *et al.*[37] The PCL homopolymer itself is degraded very slowly when compared with polyglycolic acid and polyglycolic acid-co-lactic acid, and is most suitable for long-term delivery systems such as Capronor, a 1-year contraceptive.[38] There were reports that the biodegradability of PCL homopolymer can be enhanced by copolymerization with less hydrophobic materials such as dilactide, diglycolide, δ-valerolactone, and ε-decalactone.[37] It is expected that the biodegradability of

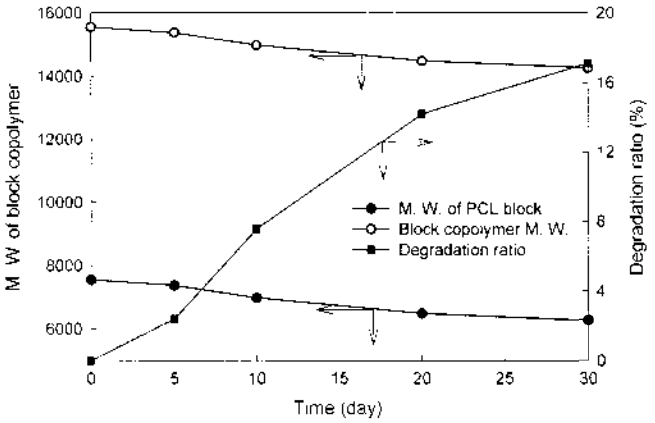

Figure 8. Degradation profiles of CEC-2 core-shell type nanoparticles.

Table 3. Degradation ratio of core-shell type nanoparticles of CEC triblock after 10 days

Sample	Initial $\overline{Mn}$ of PCL[a]	Initial Total $\overline{Mn}$[b]	Residual M.W. of PCL	Residual total M.W.	Degradation ratio (%)[c]
CEC-1	4,810	12,810	4,370	12,370	9.1
CEC-2	7,550	15,550	6,980	14,980	7.6
CEC-3	15,860	23,860	15,270	23,270	3.7

[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. PEG M. W. was 8,000 from Sigma Co. Ltd. USA. [c]Degradation ratio = [(Initial M. W. of PCL-PCL M. W. at time. T)/Initial M. W. of PCL] 100

PCL can be greatly enhanced by block copolymerization with the hydrophilic polymer, PEG. Since the PEG block is not biodegradable no matter the PCL block biodegradability, the molecular weight of PEG is consistent during the degradation test, although the PCL block can be expected to continuously degrade. The residual PCL block was calculated by ^{1}H NMR spectroscopy as described above and the results are summarized in Figure 8. In spite of the very large surface area of the nanoparticles, the CEC-2 triblock copolymer was degraded much more slowly than we expected, *i.e.*, only 17.1% of the PCL block was degraded after 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 a diffusion mechanism rather than polymer degradation. Moreover, Matsumoto *et al.*,[33] reported that the degradation of LEL block copolymer does not contribute to drug release since only a slight Mw loss was observed during the initial main release period. Degradation of their LEL copolymer was also very slow, *i.e.* 10-20% over 4days. In particular, the CEC block copolymer was degraded much more slowly than the LEL block copolymers, although a different PEG M.W. and total M.W. was used and therefore a direct comparison cannot be performed. The degradation ratio of the CEC block copolymer against polymer composition after 10 days is summarized in Table 3. The degradation ratio of CEC block copolymer after 10 days was very low and the degradation ratio against PCL block length demonstrated only small differences.

Conclusion

The CEC triblock copolymer was synthesized by a ring-opening mechanism of ε-caprolactone with poly(ethylene glycol) and characterized using various analysis equipment such as ^{1}H NMR, FT-IR, and WAXD. Core-shell type nanoparticles of CEC triblock copolymers were prepared by a dialysis technique in order to estimate their potential as a colloidal drug carrier using a hydrophobic drug. From the results of particle size analysis and TEM observation, the particle size of CEC core-shell type nanoparticles prepared by the dialysis method was about 20-60 nm with a nice spherical shape. Since CEC block copolymer can self-associate at a certain concentration, their CAC was determined by fluorescence probe techniques and the CAC values of the CEC block copolymer were decreased with the increase of the PCL block chain length. CNZ-loaded CEC core-shell type nanoparticles were prepared by the dialysis method and the drug loading contents were dependent on the PCL block length. The larger the PCL block length, the higher the drug loading contents. The drug release from the CEC core-shell type nanoparticles demonstrated an initial burst release for the first 12 hrs followed by pseudo-zero order release kinetics for 2 or 3 days. The CEC-2 block copolymer core-shell type nanoparticles were very slowly degraded, suggesting that drug release kinetics are governed by a diffusion mechanism rather than a degradation mechanism irrelevant to the CEC block copolymer composition.

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