# Articles

## Preparation and Characterizations of Poly(ethylene glycol)-Poly(ε-caprolactone) Block Copolymer Nanoparticles

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Diblock copolymers with different poly(ε-caprolactone) (PCL) block lengths were synthesized by ring-opening polymerization of ε-caprolactone in the presence of monomethoxy poly(ethylene glycol) (mPEG-OH, MW 2000) as initiator. The self-aggregation behaviors of the diblock copolymer nanoparticle, prepared by the diafiltration method, were investigated by using $^1$H NMR, dynamic light scattering (DLS), and fluorescence spectroscopy. The PEG-PCL block copolymers formed the nano-sized self-aggregate in an aqueous environment by intrsa- and/or intermolecular association between hydrophobic PCL chains. The critical aggregation concentrations (cac) of the block copolymer self-aggregate became lower with increasing hydrophobic PCL block length. On the other hand, reverse trends of mean hydrodynamic diameters were measured by DLS owing to the increasing bulkiness of the hydrophobic chains and hydrophobic interaction between the PCL microdomains. The hydrodynamic diameters of the block copolymer nanoparticles, measured by DLS, were in the range of 65-270 nm. Furthermore, the size of the nanoparticles was scarcely affected by the concentration of the block copolymers in the range of 0.125-5 mg/mL owing to the negligible interparticular aggregation between the self-aggregated nanoparticles. Considered with the fairly low cac and nanoparticle stability, the PEG-PCL nanoparticles can be considered a potential candidate for biomedical applications such as drug carrier or imaging agent.

**Key Words :** Poly(ethylene glycol), Poly(ε-caprolactone), Block copolymer, Self-aggregate, Nanoparticle

### Introduction

Amphiphilic block copolymers have been extensively studied in biotechnology and pharmaceutical fields for their unique properties of micelle or micelle-like self-aggregate formation in aqueous milieu.[1-4] Because of the limited water solubility of hydrophobic block and the hydrophobic interaction between the water-insoluble segments in aqueous environment, the polymeric amphiphiles spontaneously form micelle or micelle like self-aggregates *via* intra- and/or intermolecular segregation, mainly to achieve thermodynamic stability by minimizing interfacial free energy.[3,5]

In particular, the polymeric micelles have been considered one of the promising candidates for drug delivery system owing more to the increment of drug concentration in an aqueous milieu than to the solubility limit of the hydrophobic free drug by partitioning the drugs into the hydrophobic core.[1,3-7] The hydrophilic corona, mostly composed by poly(ethylene glycol) (PEG), endowed the polymeric micelles with escape from non-specific uptake by reticuloendothelial systems (RES). In the systemic circulation, the RES plays a crucial role for clearance of colloidal particle from the blood stream. Therefore, long systemic circulation can be achieved by introducing the PEG component into the polymeric micelle systems.[8,9] Coupled with the long circulation effect, remarkably low critical micelle concentration (cmc) is guaranteed to the polymeric micelle on excellent stability during the systemic circulation. Furthermore, the enhanced permeation and retention (EPR) effect in tumor site gave the possibility of passive targeting of the polymeric micelles or macromolecular pro-drugs into tumor site.[10,11] Therefore, a number of studies have focused on the applications of the polymeric micelles and/or nanoparticles into cancer drug delivery systems.[8,10-14] As nanoparticle forming materials, amphiphilic block copolymers,[4,6-9,11-13] hydrophobically modified water-soluble polymers,[15-17] and hydrophobized polysaccharides[3,14,18,19] have been extensively investigated.

In this study, we focused on the PEG-PCL diblock copolymers. The hydrophobic block of PCL is well-known biodegradable polyester with excellent biocompatibility and degradability. Furthermore, it is recently reported that the PEG-PCL block copolymer showed an excellent potential for internalization into cellular cytoplasm by endocytosis.[20-22] Although a number of studies of PCL based amphiphilic block copolymers have well documented for the synthesis and characterization,[23-28] biodegradation,[29-31] and application

for drug delivery systems,[32-34] the microscopic physicochemical properties of the diblock copolymers with different hydrophilic/hydrophobic balances still remain unclear. In this paper, we synthesized PEG-PCL diblock copolymers that have different PCL lengths with the fixed PEG chain length (MW 2000 Da), investigating their nanoparticle formations and physicochemical properties of the formed nanoparticles.

## Experimental Section

**Materials.** Methoxy-PEG (mPEG, MW 2000) with a molecular weight of 2000, $\varepsilon$-caprolactone, stannous 2-ethyl hexanoate (stannous octoate, SnOct), and pyrene, were purchased from Aldrich Chemical Co. The mPEG was purified by re-crystallization on the dichloromethane/diethyl ether system, and $\varepsilon$-caprolactone was dried using $CaH_2$ and distilled under reduced pressure. All other chemicals and solvents were analytical and/or reagent grades and used without further purification.

**Synthesis of PEG-PCL block copolymers.** PEG-PCL diblock copolymers with different PCL block lengths were synthesized by ring opening polymerization of $\varepsilon$-caprolactone in the presence of mPEG as an initiator with a trace amount of SnOct as catalyst. The target molecular weights of PCL blocks were 1000, 2000, and 3000.

**Characterization of PEG-PCL block copolymers.** The block copolymer formations were confirmed by FT-IR, $^1$H NMR, and gel permeation chromatography (GPC). To measure the FT-IR spectra by FT-IR spectrometer (Shimadzu, FT-IR 8700), the block copolymers-KBr discs were prepared from the polymer-KBr mixtures. $^1$H NMR spectra of the diblock copolymers were recorded by using a Bruker spectrometer operating at 400 MHz using $CDCl_3$ as solvent. Chemical shifts (d) were given in ppm using tetramethylsilane (TMS) as internal reference. Average molecular weights and their distribution of the copolymers were measured by gel permeation chromatography (Waters, USA) using THF as an elution solvent and monodisperse polystyrene standards.

**Preparation of self-assembled nanopatricles.** PEG-PCL nanoparticles were prepared by dissolving the copolymers in DMF (10 mg/mL), followed by dialysis against distilled water using dialysis membranes of 15000 molecular weight cut-off (MWCO) at 20 °C for 24 h. After the nanoparticles were formed, the solutions were filtered through a 0.8 $\mu$m pore sized filter to remove large aggregates, and the copolymer nanoparticles were obtained by lyophilization. Nanoparticle formations of the PEG-PCL diblock copolymers in aqueous milieu were confirmed by $^1$H NMR spectroscopy with adopting different locking solvents.

**Measurement of fluorescence spectroscopy (pyrene).** The self-aggregation behavior and critical aggregation concentrations of the PEG-PCL block copolymers in aqueous environment were investigated by photophysical methods using pyrene as fluorescence probe. The pyrene solution in acetone ($6 \times 10^{-5}$ M, prepared prior to use) was added to the deionized water to make a pyrene concentration of $1.2 \times 10^{-6}$ M, and the acetone was removed at reduced pressure at 40 °C for 2h. This solution was mixed with the block copolymer nanoparticle solutions to make a copolymer concentration from 2 mg/mL to $1 \times 10^{-5}$ mg/mL, resulting in a pyrene concentration of $6 \times 10^{-7}$ M. Pyrene emission fluorescence spectra were obtained by using spectrofluorophotometer (RF-5301PC, Shimadzu). The emission wavelength was 390 nm.

**Measurement of dynamic light scattering.** The particle size and size distribution of PEG-PCL diblock copolymer nanoparticles were investigated on a dynamic light scattering (DLS) instrument. The DLS measurements were carried out using an ELS-8000 electro phoretic LS spectrophotometer (Otsuka Electronics Co., Japan) equipped with a He-Ne laser operating at 632.8 nm at 25 °C and a fixed scattering angle of 90°. Before measurement, the nanoparticles were re-dispersed in deionized water (1 mg/mL), sonicated for 30 sec, and they were filtered through a 0.8 $\mu$m pore size filter. Measurements were carried out at a higher concentration of the critical aggregation concentration (cac) measured by fluorescence spectroscopy. The hydrodynamic diameters of the block copolymer nanoparticles were calculated by the Stokes-Einstein equation, and the polydispersity factors represented as $\mu_2/\Gamma^2$ were evaluated form the cumulant method ($\mu_2$; second cumulant of the decay function, $\Gamma^2$; average characteristic line width).[35,36]

To investigate the effect of the nanoparticle concentration on hydrodynamic properties of the self-aggregate, the concentration dependent stabilities of the nanoparticles were also investigated by DLS and UV-VIS spectrometer. Briefly, the formed nanoparticles were dispersed into deionized water with different concentration (5, 2, 1, 0.5, 0.25, and 0.125 mg/mL). Then the particle sizes and optical transmittance were measured by DLS and UV-VIS spectrometer, respectively.

## Results and Discussion

**Synthesis and characterization of PEG-PCL diblock copolymers.** The block copolymers of PEG-PCLs with different hydrophobic PCL blocks were synthesized by ring opening polymerization as illustrated in Figure 1. The ring opening polymerization of $\varepsilon$-caprolactone was confirmed by FT-IR and $^1$H NMR spectroscopy. As shown in Figure 2, the FT-IR spectra of the PEG-PCL block copolymers showed typical absorption bands originated from PEG and PCL blocks. Furthermore, the optical density of characteristic PCL peaks, such as carbonyl stretching (1725 $cm^{-1}$) and CH stretching (2945 $cm^{-1}$) peaks, were increased with increasing PCL block length.

Typical $^1$H NMR spectrum of the PEG-PCL block copolymer (PEG-PCL21) was illustrated in Figure 3B. The number average molecular weights of PEG-PCLs and PCL block lengths were calculated from the integration data of $^1$H NMR spectra ($DP_{PCL}$ = g/c +1, $Mn_{PCL} = DP_{PCL} \times 114.14$, and $Mn = Mn_{PCL} + 2000$). The results are summarized in Table 1.

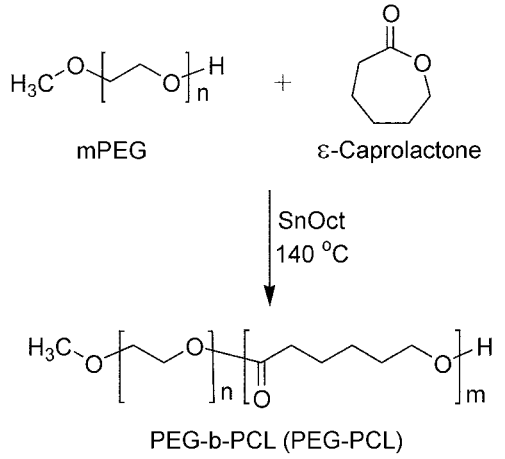


**Figure 1**. Synthetic scheme for PEG-PCL diblock copolymers.

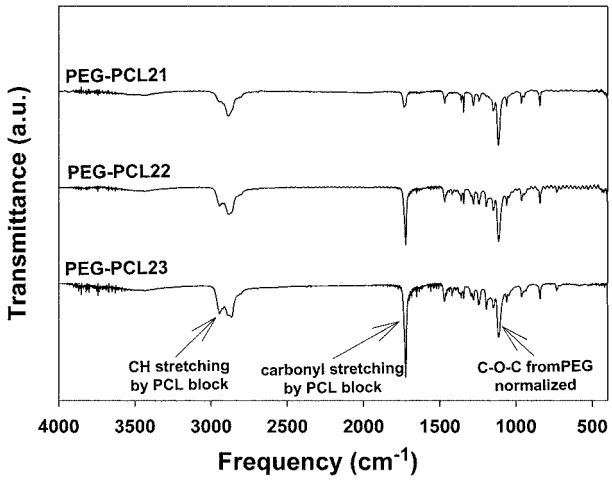


**Figure 2**. FT-IR characterization of PEG-PCL diblock copolymers.

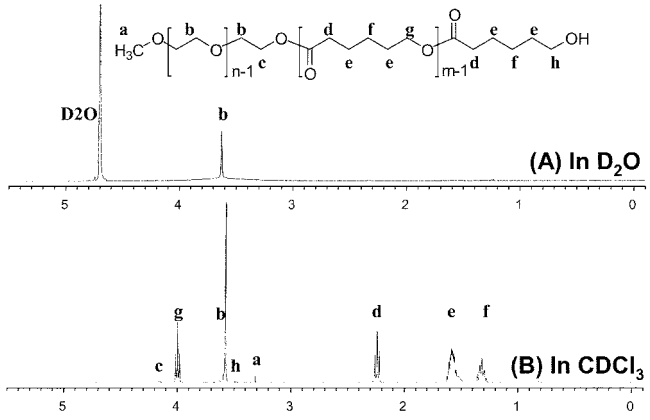


**Figure 3**. $^1$H NMR spectra of PEG-PCL22 in (A) $D_2O$ and (B) $CDCl_3$, and peak assignment of the synthesized copolymer.

The average molecular weights and polydispersity indexes (PDIs) of the PEG-PCLs were also measured by GPC using THF as an elution solvent and monodisperse poly(styrene) as standards. The GPC results and chromatograms clearly showed the formation of diblock copolymer by increasing MW and shifting chromatograms to a high MW region with increasing PCL block length (Figure 4). Furthermore, the PEG-PCLs showed narrow molecular distributions with PDI values around 1.23-1.46. The GPC results are listed in Table 1.

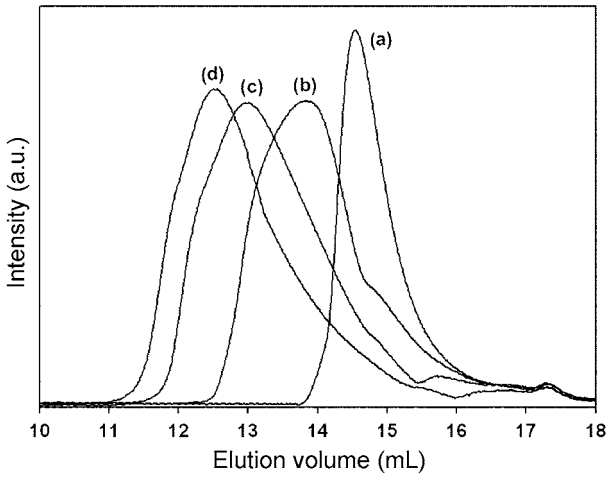


**Figure 4**. GPC chromatograms of PEG-PCL diblock copolymers. (a) mPEG 2000, (b) PEG-PCL21, (c) PEG-PCL22, and (d) PEG-PCL23.

**Table 1**. Characterizations of the synthesized PEG-PCL diblock copolymers

| Samples | PEG MW | PCL MW[b] | PCL MW[c] | Mn[c] | Mn[d] | PDI[d] |
|---|---|---|---|---|---|---|
| PEG-PCL21[a] | 2 K | 1 K | 890 | 2890 | 3160 | 1.23 |
| PEG-PCL22 | 2 K | 2 K | 1910 | 3910 | 4840 | 1.37 |
| PEG-PCL23 | 2 K | 3 K | 2780 | 4780 | 6100 | 1.42 |

[a]PEG-PCL21, PEG2K-block-PCL1K (theoretical). [b]theoretical PCL MW based on feed ratio. [c]calculated from $^1$H NMR data. [d]number average MW and polydispersity index by GPC

**Self aggregation of PEG-PCL diblock copolymers.** The amphiphilic PEG-PCLs with hydrophilic PEG blocks and hydrophobic PCL blocks can self-associate to form micelle like self-aggregates in an aqueous environment. The formation of the hydrophobic PCL domain of the PEG-PCLs in an aqueous environment can be easily verified by $^1$H NMR spectra with different NMR locking solvents of $D_2O$ and $CDCl_3$. The results, as demonstrated in Figure 3B, showed that in $CDCl_3$, a nonselective solvent for the PEG and PCL blocks, the completed structural resolution of PEG and PCL blocks were observed. However, in $D_2O$ (Figure 3A), the characteristic PEG signal was only detected, which mainly originated from the selective salvation of exterior hydrophilic PEG corona through hydrogen bond formation with $D_2O$. The results clearly revealed that the PCL blocks in PEG-PCLs were self-aggregated into a hydrophobic domain and remained in solid and/or semi-solid state, which resulted in the disappearance of PCL peaks with $D_2O$. Similar trends of $^1$H NMR spectra are consistent with other amphiphilic block copolymer systems and hydrophobized poly- saccharides.[3,6,37]

To investigate the self-aggregation behavior of PEG-PCL block copolymers in an aqueous milieu, pyrene was

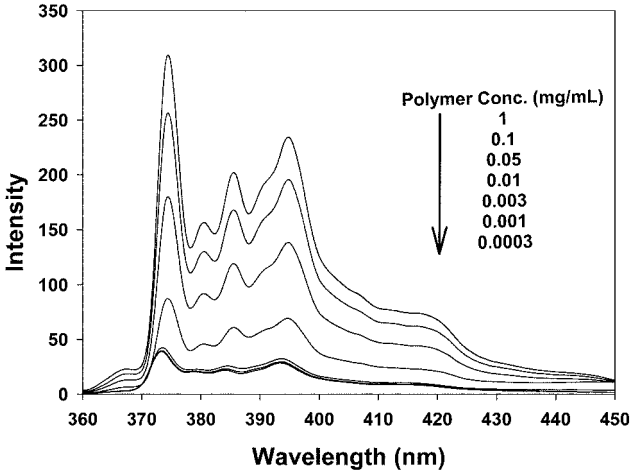


**Figure 5**. Pyrene emission spectra ([Py] = $6.0 \times 10^{-7}$ M) of PEG-PCL22 nanoparticle aqueous solutions.

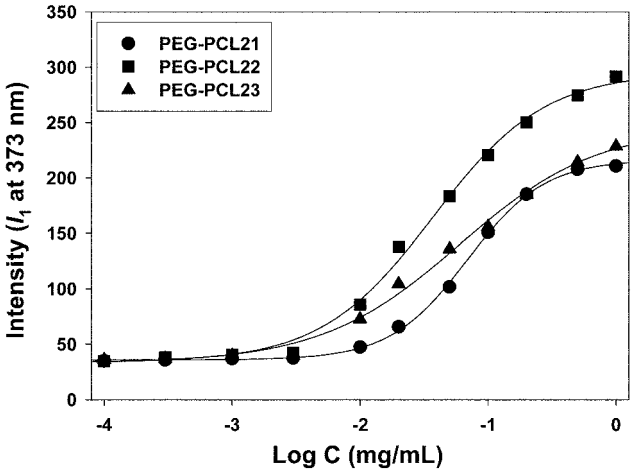


**Figure 6**. Total fluorescence emission intensity ($I_1$ at 373 nm) versus polymer concentrations plots of PEG-PCL diblock copolymers.

used as a fluorescence probe. When exposed to a polymeric micelle aqueous solution, pyrene molecules preferably participated into the hydrophobic microdomains of micelles rather than the aqueous phase. Combined with strong fluorescence illumination of pyrene in a non-polar environment, the localization, showed different photophysical characteristics depending on the concentration of micelle forming materials.[3,38,39] Therefore, to investigate

**Table 2**. Characterizations of the PEG-PCL nanoparticles by DLS and fluorescence probe method

| Samples | CAC (mg/L) | Particle Size (mean ± SD, nm) | Polydispersity ($\mu_2/\Gamma^2$) |
|---|---|---|---|
| PEG-PCL21 | 5.95 | 64.6 ± 7.7 | 0.2134 |
| PEG-PCL22 | 2.06 | 192.3 ± 23.9 | 0.1884 |
| PEG-PCL23 | 0.83 | 266.2 ± 35.4 | 0.1523 |

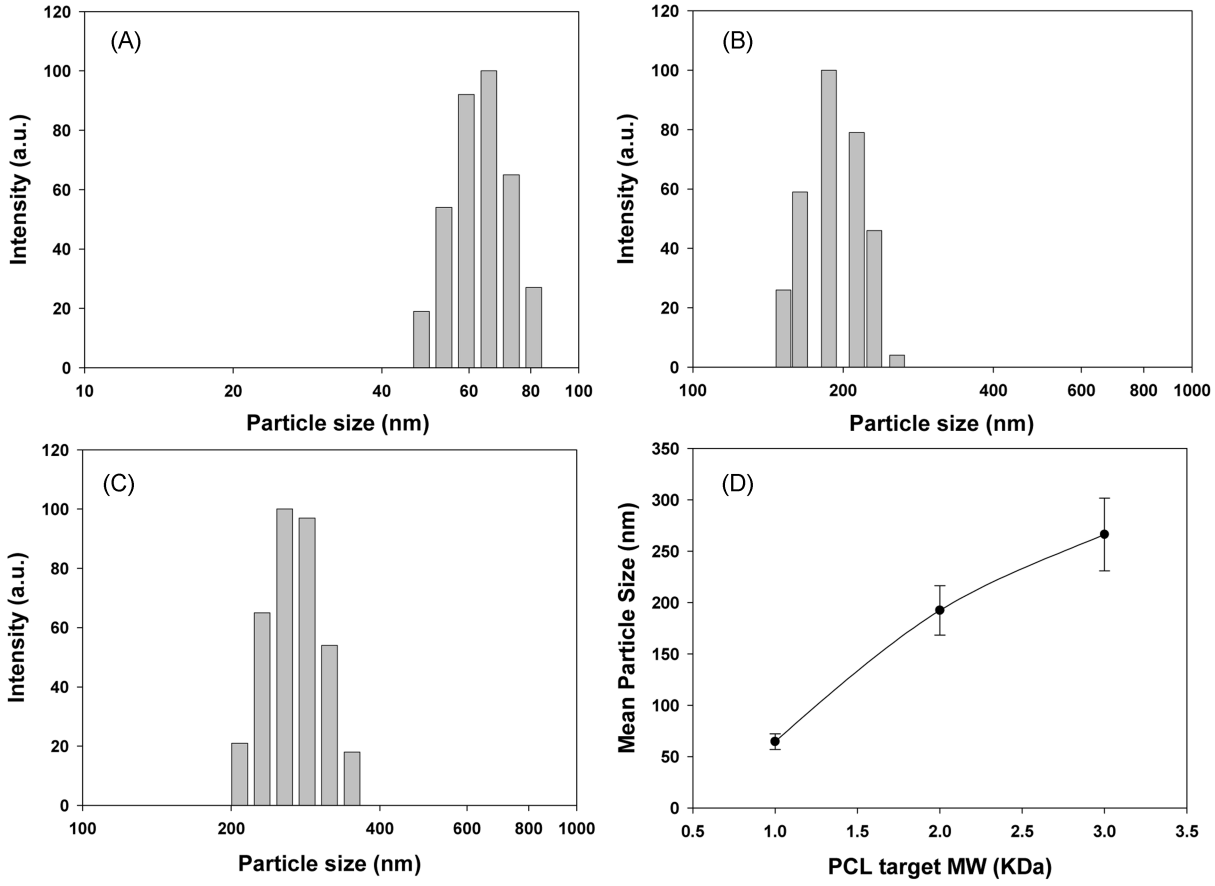


**Figure 7**. Particle size distributions of PEG-PCL nanoparticles (A-C), and their relationships on hydrophobic block lengths (D). (A) PEG-PCL21, (B) PEG-PCL22, and (C) PEG-PCL23.

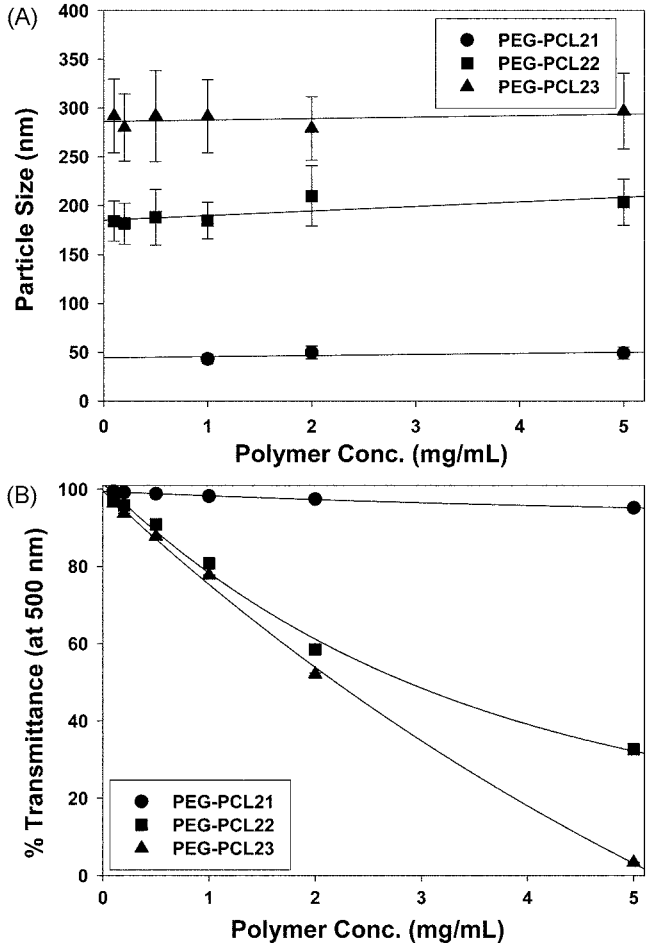


**Figure 8**. Concentration dependent particle sizes (A), and transmittances (B) of PEG-PCL nanoparticles at room temperature.

the critical aggregation concentration (cac), the self-aggregation behaviors of the PEG-PCL copolymers in an aqueous phase were investigated by using fluorescence emission spectra of the copolymer solutions with various concentrations, in the presence of $6.0 \times 10^{-7}$ M pyrene, and the results are illustrated in Figure 5. At a low concentration ($c$ < cac), there were negligible changes in total fluorescence emission intensity ($I_1$ at 373 nm). As the concentration increased, a remarkable increase of the total fluorescence intensity ($I_1$) was observed. Figure 6 shows the change in intensity ($I_1$) of the first peak in the emission spectra plotted against polymer concentration. Based on the intensity versus concentration data, the cac values of PEG-PCLs were calculated by the crossover point at low concentration ranges. The cac values of PEG-PCL block copolymers were in the range of 0.83-5.95 mg/L (Table 2). The cac values of PEG-PCLs were similar to the reported values of amphiphilic block copolymers such as PEG-PLA, poly(2-ethyl-2-oxazoline)-PCL, and PVP-PCL block copolymers.[23,27,38]

The mean diameters of PEG-PCL nanoparticles, prepared by the dialysis method and measured by DLS technique, were in the range of 65-270 nm (Table 2, Figure 7). The particle size increased as the PCL block length increased. The increment of particle size with increasing PCL block length originated mainly from the increase of hydrophobic property by the longer hydrophobic PCL chain and hydrophobic interactions between hydrophobic PCL microdomains in aqueous milieu. Furthermore, the fairly low polydispersity factors ($\mu_2/\Gamma^2$, 0.15-0.21) estimated by the cumulant method, suggest that the nanoparticles showed a narrow size distribution.

Another interesting finding for the hydrodynamic properties of the PEG-PCL block copolymer nanoparticles are the stability of the self-assembled nanoparticles. As shown in Figure 8A, the hydrodynamic diameters of the nanoparticles were scarcely affected by the concentration of PEG-PCL block copolymers in the range of 0.125-5 mg/mL. Considered with the transmittance data (Figure 8B), this may imply that the negligible inter particle interaction between the self-assembled nanoparticles. Therefore, increasing block copolymer concentration resulted in the increase of the number of nanoparticles, which decreased the optical transmittance of the solutions. Furthermore, the negligible change of the hydrodynamic diameter and optical transmittance with time (up to 1 week at 37 °C) revealed that the PEG-PCL block copolymers formed stable nanoparticles without particle-particle segregation (data not shown).

## Conclusions

The present study demonstrated the synthesis and self-aggregation behavior of PEG-PCL diblock copolymer in an aqueous milieu. Applying various technical procedures such as $^1$H NMR, dynamic light scattering, and fluorescence spectroscopy, we found that the PEG-PCL block copolymer formed nanoparticles and the characteristics of the nanoparticles were closely related to the hydrophobic/hydrophilic balance resulting from different PCL block lengths. These findings opened the possibility of introducing the PEG-PCL self-aggregates into various biomedical fields such as drug delivery and imaging.

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