**Poly(ethylene glycol)-block-poly(ε-caprolactone)으로 제조된 nanoparticles의 특성**

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**Characterization of Nanoparticles Prepared by poly(ethylene glycol)-block-poly(ε-caprolactone)**

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**Abstract**

Amphiphilic diblock copolymer with different poly(ε-caprolactone) (PCL) block length were synthesized by ring-opening polymerization of ε-caprolactone in the presence of monomethoxy poly(ethylene glycol) (mPEG-OH, MW 2000) as initiator, using stannous octoate catalyst. The self-aggregation behavior and microscopic characteristics of the diblock copolymer self-aggregates, prepared by dialysis method, were investigated by $^1$H NMR, dynamic light scattering (DLS), fluorescence spectroscopy. The PEG-PCL block copolymer formed self-aggregates in an aqueous environment by intra- and/or intermolecular association between hydrophobic PCL chains. The microscopic characterization of the PEGCL self-aggregates revealed that the hydrophobic/hydrophilic balance, resulting from different PCL block length, played a crucial role for determination of the physicochemical properties of them.

1. **Introduction**

Polymeric amphiphiles, containing hydrophilic and hydrophobic components, have been extensively studied in biotechnology and pharmaceutical fields due to their unique properties of micelle or micelle-like self-aggregate formation in aqueous milieu [1-4]. In particular, the polymeric micelles have been considered one of the promising candidates for drug delivery system, mainly due to elevation of drug concentration in an aqueous milieu than above the solubility limit of hydrophobic free drug, by partitioning the drug into hydrophobic core[1, 3-5]. Because of the limited solubility of hydrophobic block in aqueous environment and hydrophobic interaction between the hydrophobic segments, the polymeric amphiphiles spontaneously formed 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 this study, we focused on the PEG-poly (ε-caprolactone) diblock copolymer. The hydrophobic block of PCL is well-known biodegradable polyester with excellent biocompatibility and degradability. Furthermore, it is recently revealed that the PEG–PCL block copolymer showed excellent potential for internalization into cellular cytoplasm by endocytosis [6–8]. Although a number of studies PCL based amphiphilic block copolymer concerning about synthesis and characterization, biodegradation [9–11], and application for drug delivery systems [12–14] are well documented, the microscopic physicochemical properties of the diblock copolymers with different hydrophilic/hydrophobic balances still remains unclear. Herein, we synthesized PEG–PCL diblock copolymers with different PCL length with fixing PEG molecular weight of 2000, and investigated their micelle formation and physicochemical properties of the formed micelle.

2. Experiment

2-1. Materials and methods

Methoxy PEG(mPEG–OH) with molecular weight of 2000, ε-caprolactone, stannous 2-ethyl hexanoate (SnOct), pyrene, and cetylpyridinium chloride (CPC) were purchased from Sigma–Aldrich co. The mPEG was purified by recrystallization on dichloromethane/diethyl ether system, and ε-caprolactone was dried using CaH2 and distilled at reduced pressure. All other chemicals and solvents were analytical and/or reagent grades used without further purification.

Amphiphilic diblock copolymer(PEGCLs) with different poly(ε-caprolactone) (PCL) block length were synthesized by ring-opening polymerization of ε-caprolactone in the presence of mPEG–OH as initiator, using SnOct.

2-2. Characterization of PEG–PCL block copolymers

1H NMR spectra of the diblock copolymer were recorded by using a Bruker spectrometer operating at 400 MHz using CDCl3 as solvent.

2-3. Preparation of self-assembled nanoparticles

Polymeric nanoparticles of PEGCLs were prepared by dissolving the copolymer in DMF (10 mg/mL), followed by dialysis against distilled water using dialysis membranes of 15000 molecular weight cut-off (MWCO) at 20 ºC for 24 h.

2-4. Measurement of fluorescence spectroscopy (pyrene)

Pyrene fluorescence spectra were obtain by using spectrofluorophotometer (RF-5301PC, Shimadzu). The excitation and emission wavelengths were 339 and 390nm, respectively. The aggregation number of PCL block per one hydrophobic domain was determined by the steady state fluorescence quenching method with CPC as a pyrene fluorescence quencher.

2-5. Measurement of dynamic light scattering

Particle size and size distribution of PEGCLs diblock copolymer self aggregates were investigated by dynamic light scattering (DLS) instrument. The DLS measurements were carried out using a ELS–8000 electro phoretic LS spectrophotometer (Otsuka Electronics Co., Japan) equipped with an He–Ne laser operating at 632.8 nm at 25 ºC.
and a fixed scattering angle of 90°.

3. Results and discussion

3-1. Synthesis and characterization of PEG–PCL diblock copolymers

The block copolymers of PEG–PCL (PEGCLs) with different hydrophobic PCL block were synthesized by ring opening polymerization. The ring opening polymerization of \( \varepsilon \)-caprolactone was confirmed by \(^1\)H NMR spectroscopy. Typical \(^1\)H NMR spectrum of the PEGCL block copolymer (PEGCL21) was illustrated in Figure 1B.

3-2. Self aggregation of PEG–PCL diblock copolymers

The formation of hydrophobic PCL domain of the PEGCLs in aqueous environment can easily be verified by \(^1\)H NMR spectra with different NMR solvents of D\(_2\)O and CDCl\(_3\) (Figure 1). The mean diameters of PEGCL copolymer nanoparticles, prepared by the dialysis method and measured by DLS technique, were in the range of 50 ~ 300 nm with increasing particle size by increasing PCL block length. The cac values of the PEGCL self-aggregates calculated based on pyrene excitation spectra were in the range of 0.83~5.95mg/L (Table 1), with lowering by increasing PCL block length (Figure 2).

3-3. Characteristics of PEG–PCL self aggregates

The partition equilibrium constants (K\(_v\)) of pyrene, measured by fluorescence spectroscopy, revealed that inner core hydrophobicity of the nanoparticles increased with increasing PCL chain length (Table 1). The aggregation number of PCL chain per one hydrophobic microdomain, investigated by the fluorescence quenching method using cetylpyridinium chloride as a quencher, revealed that 4 to 20 block copolymer chain were needed to from a microdomain, depending on PCL block length (Table 1).

4. Conclusion

The present study demonstrated the synthesis and self-aggregation behavior of PEG–PCL diblock copolymer in an aqueous environment. With introduction of various technical procedures such as \(^1\)H NMR, dynamic light scattering, and fluorescence spectroscopy, the physicochemical characteristics of PEG–PCL block copolymer self-aggregates were closely related to the hydrophobic/hydrophilic balance resulting from different PCL block length. Based on these results, the PEGCL self-aggregates can be considered as a potential candidates for biomedical application such as drug delivery and imaging.

References
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Figure 1. $^1$H NMR spectra of PEGCL21 in (A) D$_2$O and (B) CDCl$_3$
 at 25°C and peak assignment of the synthesized copolymer

Figure 2. Intensity ratio ($I_{339}/I_{335}$) from pyrene excitation spectra as a
function of PEGCL concentration in H$_2$O.

<table>
<thead>
<tr>
<th>sample</th>
<th>$X_{PEG}$ a</th>
<th>CAC (mg/L)</th>
<th>$K$ ($10^{-7}$)</th>
<th>$N_{agg}$ c</th>
<th>$d$ (nm)$^b$</th>
<th>$\eta$ (mPa)$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEGCL21</td>
<td>0.256</td>
<td>5.95</td>
<td>3.89</td>
<td>19.12</td>
<td>49.7</td>
<td>0.2134</td>
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<tr>
<td>PEGCL22</td>
<td>0.460</td>
<td>2.96</td>
<td>5.35</td>
<td>7.05</td>
<td>192.3</td>
<td>0.1884</td>
</tr>
<tr>
<td>PEGCL23</td>
<td>0.574</td>
<td>0.83</td>
<td>6.07</td>
<td>4.53</td>
<td>266.2</td>
<td>0.1523</td>
</tr>
</tbody>
</table>

a. PEGCL weight fraction calculated from $^1$H NMR data
b. Binding equilibrium constant of pyrene in water in the presence PEGCL nanoparticles
c. aggregation number of PCL blocks per one hydrophobic microdomain
d. mean diameter in water measured by DLS
e. polydispersity factor estimated by cumulant method

Table 1.