Synthesis and characterization of a novel amphiphilic poly (ethylene glycol)–poly (ε-caprolactone) graft copolymers

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ABSTRACT
A series of amphiphilic graft copolymers PEO-g-PCL with different poly (ε-caprolactone) (PCL) molecular weight were successfully synthesized by a combination of anionic ring-opening polymerization (AROP) and coordination-insertion ring-opening polymerization. The linear PEO was produced by AROP of ethylene oxide (EO) and ethoxyethyl glycidyl ether initiated by 2-(2-methoxyethoxy) ethoxide potassium, and the hydroxyl groups on the backbone were deprotected after hydrolysis. The ring-opening polymerization of CL was initiated using the linear poly (ethylene oxide) (PEO) with hydroxyl group on repeated monomer as macroinitiator and Sn(Oct)2 as catalyst, then amphiphilic graft copolymers PEO-g-PCL were obtained. By changing the ratio of monomer and macroinitiator, a series of PEO-g-PCL with well-defined structure, molecular weight control, and narrow molecular weight distribution were prepared. The expected intermediates and final products were confirmed by 1H NMR and GPC analyzes. In addition, these amphiphilic graft copolymers could form spherical aggregates in aqueous solution by self-assembly, which were characterized by transmission electron microscopy, and the critical micelle concentration values of graft copolymers PEO-g-PCL were also examined in this article.

1. Introduction
It is well known that amphiphilic block copolymers containing poly (ethylene oxide) (PEO) show the unique and outstanding properties, such as biocompatibility, lack of immunogenicity and easy clearance from the human body, it has caused widely concern in the field of medicine.[1–15] PEO is widely used not only for its hydrophilicity and biocompatibility, but also for some properties of POE present in the materials modified by POE.[6] Among the investigated amphiphilic block copolymers, the biodegradable copolymers are of special interest. The biodegradable polymers, such as poly (ε-caprolactone) (PCL) [12–18] have been used as important biomaterials for a wide variety of drug delivery carriers because of their biocompatibility and biodegradability. The biodegradable polymers PCL modified by PEO have attracted more attention in order to meet the increasing demands for better performances and some specific applications.

Amphiphilic graft copolymer is a kind of complex polymer with uniform structure, which shows unique microfacies pattern and possesses particular characteristics owing to the special structure,[19–24] such as spherical micelles, vesicles, cylindrical shapes, and petal shapes. Polymeric micelles self-assembled from amphiphilic block copolymers in aqueous media are considered to be promising drug carriers.[20,25] The CMC values of PEO-g-PCL change with the variation of molecular weight of PCL chains, and thus amphiphilic graft copolymers demonstrate different properties in drug delivery.

Typically, the graft polymers can be realized by ‘grafting from’,[26,27] ‘grafting onto’,[28–30] macromonomer method,[31] and self-assembly method.[32,33] However, each method has its own advantages and limitations. In the present work, the objective is to synthesize graft polymers with PEO as main chain and PCL as side chains, and the modulated grafting sites are specially spaced with a certain length of PEO segment. The graft copolymers PEO-g-PCL with PEO as main chain were successfully prepared by the ‘grafting from’ living polymerization techniques, and at the same time a novel method for the synthesis of graft polymers with complex structures via a combination of AROP and CROP was developed.

The amphiphilic graft copolymers PEO-g-PCL with designed structure are obtained. The relationship between CMC and the polymer with different ratios of hydrophilic and hydrophobic chain was studied, and morphology of...
the micelles was also observed with transmission electron microscopy (TEM).

2. Experimental section

2.1. Materials

ε-Caprolactone (CL, 99%, Aldrich) was dried over CaH₂ and distilled under reduced pressure. Ethylene oxide (EO, 98%, SCR) was dried with CaH₂ and then distilled under nitrogen atmosphere and stored at −22 °C before use. Tetrahydrofuran (THF) (99%) was refluxed over potassium wire and distilled from potassium naphthalene solution. 2-(methoxymethoxy) ethoxide potassium was synthesized by a method described previously.[28] All other reagents were purified by common purification procedures.

2.2. Measurements

1H NMR spectra was obtained on a DMX 500 MHz spectrometer with tetramethylsilane (TMS) as the internal standard, and all the samples were dissolved in CDCl₃.

Gel Permeation Chromatography (GPC) was performed using an Agilent 1100 with a G1310A pump, a G1362A refractive detector, and a G1314A variable wavelength detector, with THF as eluent at 35 °C at 1.0 mL/min. One 5 μm LP gel column (500 Å, molecular range 500 – 2 × 10⁴ g/mol) and two 5 μm LP gel mixed bed column (molecular range 200 – 3 × 10⁶ g/mol) were calibrated with polystyrene standard samples.

The critical micelle concentration (CMC) was determined by fluorescence measurement using pyrene as a fluorescent probe. Fluorescence excitation spectra were recorded on Hitachi F-4600 fluorescence spectrometer at 339 nm emission wavelength and 10.0 nm slit width.

TEM images were obtained using a FEI Tecnai G² 20 electron microscope operated at an accelerating voltage of 40 kV. A drop of micelle solution was placed on an electron microscopy copper grid coated with carbon film. Excess solution was blotted away using a strip of filter paper. The sample grid was observed after drying in air for 1 h.

2.3. Synthesis

2.3.1. Synthesis of EEGE

EEGE was prepared according to literature,[34] and the reaction route is illustrated as follows in Figure 1. The structure of the EEGE was confirmed by 1H-NMR analyses (CDCl₃, ppm): 4.76 (q, –O–C(H₃)–O–), 3.35–3.90 (m, –O–CH₂–CH₂O), 3.15 (m, methine C(H) of epoxy ring), 2.61–2.91 (d, methylene –C(H)₂– of epoxy ring), 1.33 (d, –OCH(CH₃)–O–), 1.19 (t, –O–CH₂–CH₃), and the 1H-NMR spectrum was shown in Figure 2.

2.3.2. Synthesis of poly (EO-co-EEGE), then hydrolyze to get macroinitiator poly (EO-co-Gly)

2-(methoxymethoxy) ethoxide potassium (6.497 mL, 0.055 mol) was added in a clean and dry ampoule charged with 50 mL THF. The solution was cooled to 0 °C in ice water bath before EEGE (8.66 g, 0.0593 mol) and EO (26.1 g, 0.593 mol) were added sequentially, the ampoule was then removed from the ice-water bath and the reaction was allowed to proceed at 60 °C for 48 h under stirring until a few drops of methanol were added to terminate it. Afterwards, all of the solvents in the ampoule were removed by reduced distillation. The resultant polymer was dissolved in dichloromethane and precipitated in diethyl ether to obtain poly(ethylene oxide-co-2, 3-epoxypropyl-1-ethoxyethyl ether) [poly(EO-co-EEGE)] was obtained in the yield of 90% after dichloromethane removed. Then poly (EO-co-EEGE) (0.63 g, 0.095 mmol) was dissolved into formic acid (30 mL) in a 50 mL flask equipped with a magnetic stirrer, and the excess formic acid was removed by atmospheric distillation after stirring for 2 h, followed by addition of a mixture of 1,4-dioxane and methanol (30 mL, 1:1, v/v). After the polymer was completely dissolved in the solvent, the solution was hydrolyzed by KOH (2.0 g) under refluxing for 24 h, and neutralized with HCl aqueous solution (1 mol/L). The product was obtained by dissolving in dichloromethane and precipitating in diethyl ether
after the solvents were removed under reduced pressure. This hydrolysis was complete to get \( \text{poly(ethylene oxide-co-glycidol)} \) [\( \text{poly(EO-co-Gly)} \)] with a hydroxyl group on repeated monomer unit. The reaction route is shown in Figures 3 and 4.

2.3.3. Synthetic of amphiphilic graft copolymers PEO-g-PCL by ROP

Amphiphilic graft copolymers PEO-g-PCL were synthesized by CROP of \( \varepsilon \)-CL with poly (EO-co-Gly) as macroinitiator and Sn(Oct)\(_2\) as catalyst. The flask charged with Sn(Oct)\(_2\) (0.4152 mmol), poly (EO-co-Gly) (0.37 g, 0.06270 mmol, 0.08306 mmol –OH), predetermined amounts of \( \varepsilon \)-CL (0.4153 g) was sealed with a glass stopper and exhausted under vacuum for degassing and purged 3 times with dry nitrogen before it was immersed in an oil bath at 100 °C for 24 h. The resultant polymer was dissolved in dichloromethane and precipitated in hexane. The structure of the polymer was confirmed by \( ^1 \text{H-NMR} \) analyses and the molecular weight was determined by GPC after dried in vacuum at 40 °C for 24 h. The reaction route is shown in Figure 5.

2.3.4. Determination of critical micelle concentration

The CMC was determined by the fluorescence measurement with pyrene as a fluorescent probe, which is a well-known method to determine the CMC value.[35] The concentration of pyrene (6.4 × 10\(^{-7}\) mol/L) was prepared by adding acetone solution of pyrene 1 mL (6.44 × 10\(^{-2}\) mg/mL, 3.2 × 10\(^{-4}\) mol/L) to 500 mL water. Next, different concentrations of graft polymer solution in THF (20, 10, 5, 2, 1, 0.5, 0.1, 0.05, 0.01 mg/mL) were added to pyrene-water solution to obtain the mixed solution in which the copolymer concentration ranges from 10\(^{-4}\) to 10\(^{-9}\) mg/mL. Each copolymer solution was placed for 1 h at room temperature to ensure equilibration of pyrene between the micelles and the aqueous solution. All fluorescence spectra were recorded at 25 °C.

2.3.5. Preparation of the aqueous micelle solution

Amphiphilic graft polymers PEO-g-PCL nanoparticles were acquired by a dialysis method. Briefly, PEO-g-PCL graft polymers with same hydrophilic segment and different hydrophobic segment (A, B, C, 20.0 mg) were dissolved in THF (1 mL), which is the common solvent for both the PEO and PCL segment. Then deionized water (20 mL) was slowly added to the copolymer solution at a rate of 1 drop for every 20 s with vigorous stirring after the copolymers were dissolved thoroughly. With the increase of the content of water, the mixed solvent became poorer for the PCL segment, and the solution was then dialyzed against deionized water to remove THF for 3 days after stirring for 24 h, during which the deionized water was replaced every 8 h. The final dialyzed micellar solution was diluted to 1.0 mg/mL.

### Table 1. Data of the copolymer poly (EO-co-EEGE).

| Sample | \( R^a \) | \( R^b \) | \( M^c \) | \( M_\text{w}/M_\text{n}^d \) | \( M^e_n \) | \( N^f \text{EEGE} \) | \( M^g \) |
|--------|----------|----------|--------|----------------|--------|----------|--------|
| A      | 10/1     | 9.46/1   | 4100   | 1.08           | 5900   | 10       | 6000   |

\( ^a \) The feed ratio of EO to EEE.
\( ^b \) The molar ratio of EEE to EO in poly(EO-co-EEGE) calculated by \( ^1 \text{H NMR} \).
\( ^c \) Number-average molecular weight determined by GPC.
\( ^d \) The polydispersity determined by GPC.
\( ^e \) Number-average molecular weight calculated by \( ^1 \text{H NMR} \).
\( ^f \) The number of EEE units in poly(EO-co-EEGE) calculated by \( ^1 \text{H NMR} \).
\( ^g \) Number-average molecular weight we designed.
3. Results and discussion

3.1. Characterization of poly (EO-co-EEGE) and poly (EO-co-Gly)

The main chain of PEO with a hydroxyl group on repeated monomer unit was prepared including synthesis of poly (EO-co-EEGE), and deprotection of EEGE units of poly (EO-co-EEGE). The molecular weight of poly (EO-co-EEGE) can be calculated according to Equation (1).

\[
RT = \frac{(A_{\text{sum}} - 7A_a)}{4} \quad A_{\text{EEGE}} = Mn + 44/R_T
\]

\[
M_n = \frac{(A_{\text{sum}} - 7A_a)}{A_j/3} \times 44 + \frac{A_a}{A_j/3} \times 146
\]

\[
M_n = \frac{M_n}{M_p + 1.5} \quad b
\]

*Figure 7.* (A) $^1$H NMR spectrum of poly (EO-co-EEGE) ($M_n = 5700$), (B) $^1$H NMR spectrum of poly (EO-co-Gly) ($M_n = 5400$, CDCl$_3$ as solvent).

*Figure 8.* $^1$H NMR spectra of PEO-g-PCL.

*Figure 9.* GPC traces of PEO-g-PCL with same hydrophilic segment and different hydrophobic segment (A: $M_{Peo}/M_{PCL} = 1.5$; B: $M_{Peo}/M_{PCL} = 1:1$ C: $M_{Peo}/M_{PCL} = 1:1.5$).

*Table 2.* Polymerization data of the PEO-g-PCL.

| Sample | Design weight | $M_n^a$ ($\times 10^3$) | $M_n^b$ ($\times 10^3$) | $M_n/M_w^a$ |
|--------|---------------|-------------------------|-------------------------|-------------|
| A      | 8.500         | 8.95 8.750 1.07         |                         |             |
| B      | 11.00         | 10.00 10.40 1.12        |                         |             |
| C      | 15.00         | 14.00 13.52 1.21        |                         |             |

*Figure 10.* Dependence of fluorescence intensity ratios of pyrene emission bands on the concentration of PEO-g-PCL (A: $M_{Peo}/M_{PCL} = 1.5$; B: $M_{Peo}/M_{PCL} = 1:1$ C: $M_{Peo}/M_{PCL} = 1:1.5$).
1.18–1.21 ppm (–O–CH₂CH₃) assigned to the ethoxyethyl group of poly (EO-co-EEGE) in Figure 7(A) disappeared completely after hydrolysis as shown in Figure 7(B), indicating the efficient hydrolysis of poly (EO-co-EEGE). The molecular weight of poly (EO-co-Gly) could be calculated according to Equation (2). The calculation result was 5180 g/mol.

\[ M_n \text{poly(EO-co-EEGE)} = M_n \text{poly(EO-co-Gly)} - N_{EEGE} \times 72 \]  

(2)

3.2. Characterization of PEO-g-PCL

As shown in Figure 7, the signals at 4.68–4.72 ppm [–O–CH(CH₃)–O–], 1.30, 1.29 ppm [–OCH(CH₃)–O–] and 1.18–1.21 ppm (–O–CH₂CH₃) assigned to the ethoxyethyl group of poly (EO-co-EEGE) in Figure 7(A) disappeared completely after hydrolysis as shown in Figure 7(B), indicating the efficient hydrolysis of poly (EO-co-EEGE). The molecular weight of poly (EO-co-Gly) could be calculated according to Equation (2). The calculation result was 5180 g/mol.

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\[ M_n \text{poly(EO-co-Gly)} = M_n \text{poly(EO-co-EEGE)} - N_{EEGE} \times 72 \]  

(2)

3.2. Characterization of PEO-g-PCL

The ¹H NMR spectrum in Figure 8 clearly showed that new peaks appeared at 1.49, 1.68, 2.41 and 4.16 ppm attributed
to the protons of PCL, suggesting that PEO-g-PCL had been obtained by CROP. The number average Molecular Weight of PEO-g-PCL could be calculated according to Equation (3). The calculation results were $8.750 \times 10^3$, $10.40 \times 10^3$, $13.52 \times 10^3$ g/mol.

$$N_{PCL} = \frac{A_1/2}{A_2/3} \quad M_{n(PCL)} = N_{PCL} \times 114 \quad (3)$$

Figure 9 showed the GPC traces of PEO-g-PCL copolymers with different length of PCL block. The analysis results were summarized in Table 2. According to the data, it showed that the polymerization could be controlled and the molecular weight distribution of the polymers was narrow.

### 3.3. Measurement of critical micelle concentration

The CMC of amphiphilic copolymer is a very important parameter which determines whether the polymer forms aggregates or exists as a unimer, and its values were examined by fluorescence technique using pyrene as probe in this paper. Pyrene fluorescence is a very sensitive technique for detecting the formation of copolymers micelles. Pyrene is highly hydrophobic and has very low solubility in water so it migrates preferentially into the hydrophobic micelle cores. A red shift can be observed in the pyrene fluorescence spectra, and changes in relative peak intensities can be also detected.[13,14] In this passage, the definition of CMC was the ratio of the first vibration peak ($I_1$) and the third vibration peak ($I_3$) based on the analysis of pyrene emission spectra.[36–39]

Figure 10 showed the variation of the intensity ratio ($I_1/I_3$) vs. solution concentration for amphiphilic graft copolymers PEO-g-PCL. The critical micelle concentration values were estimated from the reduced ($I_1/I_3$) ratio, which indicated a more hydrophobic environment for the pyrene probe. These results demonstrated that amphiphilic graft copolymers PEO-g-PCL with same PEO segment and longer PCL chains possessed the lower critical micelle concentration values. This difference may be mainly caused by the different percentage of PCL chains. Amphiphilic graft copolymers PEO-g-PCL with the longer PCL chains is more hydrophobic, leading to the formation of micelles with PCL cores in lower solution concentration values.

### 3.4. TEM studies on nanoparticle morphology of PEO-g-PCL copolymers micelle

Polymeric micelles of different morphologies from the amphiphilic graft copolymers PEO-g-PCL were fabricated by a Co-solvent method. THF is a common solvent for both the PEO and PCL segment. The effects of the graft copolymer compositions on the aggregation behavior were investigated. Figure 8 displayed TEM images of the various morphologies formed from PEO-g-PCL copolymers with different PCL chains, and the same concentration (1 mg/mL) in THF solution. Nano-spherical aggregates PEO-g-PCL were observed from PEO-g-PCL as shown in Figure 11 (a–c). It indicated that the morphologies were spherical, similar to the conventional core–shell-type micelles, which the core was composed of hydrophobic segment PCL and the shell was composed of hydrophilic segment PEO. The number average diameters of these micelles were ranging from 400 nm to 1 μm. However, it was observed that with further increasing the weight fraction of PCL segment, the sizes of these micelles were larger. Therefore, these results indicated that the PCL composition affected the dimension of the self-assembled aggregates. These graft copolymers could provide spherical nanoparticles with tunable size. Moreover, it should be noted that only the sizes of spherical micelles measured by TEM are in accordance with the CMC of PEO-g-PCL.

### 4. Conclusion

The copolymers of PEO and PCL with new structure were synthesized in this paper. A series of amphiphilic graft copolymers PEO-g-PCL with different PCL molecular weight were successfully prepared by AROP and CROP, and the percentage of hydrophobic segment and hydrophilic segment could be controlled. Moreover, the morphologies of the obtained micelles from the graft copolymers PEO-g-PCL were observed with transmission electron microscopy, the sizes of self-assembled spherical nanoparticles from PEO-g-PCL in aqueous solution could be controlled by the composition of the copolymer. Polymeric micelles self-assembled from amphiphilic graft copolymers in aqueous media are considered to be promising in drug carriers, gene therapy, and preparation of nanostructure materials. Further investigation on drug-loading capacity of PEO-g-PCL micelles is ongoing in our study.

### Disclosure statement

No potential conflict of interest was reported by the authors.

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