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Amphiphilic biodegradable poly(ε-caprolactone)-poly(ethylene glycol)-poly(ε-caprolactone) triblock copolymers: synthesis, characterization and their use as drug carriers for folic acid

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Abstract Amphiphilic biodegradable poly(ε-caprolactone)-poly(ethylene glycol)-poly (ε-caprolactone) (PCEC) triblock copolymers have been successfully synthesized by the ring-opening polymerization of ε-caprolactone (ε-CL) employing SnOct as catalyst and double-hydroxyl capped PEG (DHPEG) as macro-initiator. The triblock structure and copolymer composition were conformed by FT-IR, ^{1}H-NMR, and GPC. Using a membrane dialysis method, PCEC micelles were prepared with a core–shell type. The critical micelle concentration (CMC) of PCEC triblock copolymers was determined by fluorescence technique using pyrene as probe, and CMC values decreased with the increase of PCL chain length. From the observation of transmission electron microscopy (TEM), the morphology of polymer micelles was spherical in shape. Micelles size measured by dynamic light scattering (DLS) exhibited a narrow size distribution. Folic acid (FA) was then used as a model drug to incorporate into PCEC micelles. The diameter, drug loading, and drug release rate of PCEC micelles were influenced by the feed weight ratio of FA and copolymer, and polymer composition. In addition, in vitro release experiments of the drug-loaded PCEC micelles exhibited sustained release behavior without any burst effects and the release behavior was also affected by the pH of release media.

Keywords Amphiphilic biodegradable triblock copolymer · Polymer micelles · Preparation · Controlled drug release

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Introduction

Recently, more and more attention has been paid for applying biodegradable polymers, especially aliphatic polyesters such as poly(ε-caprolactone) (PCL), polylactide (PLA), and polyglycolide (PGA), as biomaterials due to their biocompatibility, degradability, and excellent shaping and molding properties [1–4]. These polymers have been widely used in surgical repair, carriers in drug delivery, and temporary matrixes or scaffolds in tissue engineering [5, 6]. In the family of polyesters, PCL occupies a unique position: it is at the same time biodegradable and miscible with a variety of polymers and it crystallizes very readily [7]. A lack of toxicity and great permeability has already found wide use for PCL in medical applications [7].

However, the potential applications of PCL are considerably restrained by the high hydrophobicity, rather high crystallinity and the inadequate interaction between PCL and cells, leading to in vivo foreign body reactions, such as inflammation, infections, local tissue necrosis, and implant encapsulation as well as thrombosis [8, 9]. These drawbacks may obstruct its application in drug-controlled release systems. So as to improve these problems, hydrophilic polyether blocks, poly(ethylene glycol) (PEG), or poly(ethylene oxide) (PEO), have been incorporated into degradable polyester backbones for its hydrophilicity, non-toxicity, biocompatibility, nonimmunogenicity and filterability through kidney when the molar mass is below 40,000 [10]. In addition, PEG is able to form a palisade avoiding protein adsorption and subsequent non-specific uptake by the reticuloendothelial system (RES) after intravenous injection. These properties are very useful for a polymer used as a drug delivery system.

Over the last years, PCL/PEG block copolymers have been widely investigated as drug carriers in the form of microparticles, nanoparticles, hydrogels, and micelles [11–14]. Among them, the application of PCL/PEG polymer micelles as drug carriers in the medicinal field has been more universally studied, which is based on the micelle-forming propensity of PCL/PEG block copolymers in an aqueous milieu through multi-molecular association. Polymer micelles prepared from various types of amphiphilic block copolymers including PCL/PEG are formed by a core–shell architecture, in which the hydrophobic segments aggregate to form an inner core able to accommodate hydrophobic drugs with improved solubility and the hydrophilic shell consists of a protective corona that stabilizes the micelles in aqueous solution [15–17]. Therefore, various drugs with a hydrophobic nature can be loaded with high efficacy into the core of the micelles, allowing them to be solubilized in an aqueous milieu. Meantime, the hydrophilic and stabilized shell reduces interaction with biological components so as to make the micelles have a long half-life in the blood compartment [8]. Besides, polymer micelles possess a nano-scale size range with a narrow distribution, and they can achieve higher accumulation at the target site through an enhanced permeation retention effect (EPR effect) [18]. As expected, micelles prepared from PCL/PEG block copolymers have also been applied in this field because of their safe and biodegradable properties [19–22]. Furthermore, the best advantage of using PCL/PEG micelles as a drug carrier is their bioresorbability and improved biocompability, which make

them be eliminated after use by biodegradation. Therefore, many research groups have reported the synthesis of PCL/PEG block copolymers and their micelles using drug carriers [21–24].

Up to now, however, there are still scarce research groups who have systematically studied the copolymer composition controlled by ε-CL/PEG ratios in feeding and its effects on micelle physicochemical properties, including the particle size, drug loading content, and drug release properties. In addition, as a model drug, folic acid is a relative hydrophilic drug, that is to say water-soluble in alkaline aqueous environment, but non-water-soluble in neutral or acidulous environment. Though hydrophobic drugs [19, 21, 25] and hydrophilic drug [11] as a model drug loaded within nanoparticles have been extensively investigated, such kind of drug (FA) release study was scarcely reported. In this article, we successfully synthesized a series of PCEC triblock copolymers with different PCL block lengths. PCEC micelles with a core–shell type were prepared using a membrane dialysis method. The critical micelle concentration (CMC) of PCEC triblock copolymers determined by fluorescence technique decreased with the increase of PCL chain length. Folic acid (FA) was then used as a model drug for this study. Micelles size and size distribution, surface morphology, drug loading content and encapsulation efficiency of FA-loaded micelles were investigated. We also studied the drug release properties against various conditions in vitro.

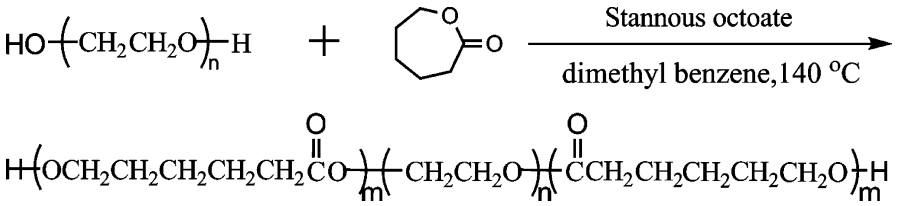
Materials and methods

Materials

DHPEG with a molecular weight of 6,000, ε-caprolactone, stannous 2-ethyl hexanoate (stannous octoate, SnOct), pyrene, and folic acid (FA) were purchased from Aldrich Chemical Co., USA. ε-Caprolactone was dried using CaH_2 and distilled under reduced pressure. Toluene and xylene were obtained from Chemical Regent Co., Ltd., Shanghai China, which were refluxed over calcium chloride and redistilled under reduced pressure. All other chemicals were analytical grade and used without further purification.

Synthesis of PCEC triblock copolymer

PCEC triblock copolymers with different PCL block lengths were synthesized by ring-opening polymerization of ε-caprolactone in the presence of DHPEG (HO-PEG-OH, Mn = 6,000) as an initiator with a trace amount of SnOct as a catalyst. The entire synthesis process of PCEC is presented in Scheme 1. Briefly, 0.1 mmol DHPEG, 10 mL toluene, and 8 mL xylene were introduced into a 50 mL round-bottomed, three necked flask, and residual moisture in the solution was removed by azeotropic drying with evaporation of a part of the toluene at 120 °C. After drying and cooling to about 60 °C, one drop of SnOct ($\sim$100 μL) and different amounts of ε-caprolactone monomer (16.4, 23.2, 28.7, and 37.0 mmol) were added into the



Scheme 1 Scheduler illustration of the synthesis of PCEC triblock polymer

above solution. Under a nitrogen atmosphere, the polymerization reaction was performed at 140 °C for 24 h with vigorous stirring. After the reaction was complete, the reaction mixture was precipitated in diethyl ether. The obtained product was purified by washing with cold methanol three times and dried at 40 °C in vacuum for 48 h. The copolymers were then stored in a desiccator.

Characterization of PCEC triblock polymers

The FT-IR spectra of polymers were studied using an AVATAR-360FTIR spectrometer by incorporating samples in KBr disks. Samples were pressed into potassium bromide pellets. The ^{1}H-NMR spectra were recorded on an AVANCE400M nuclear magnetic resonance (NMR) instrument at 400 MHz, using $CDCl_3$ as solvent.

The molecular weight of the polymers and its distribution were determined by a gel permeation chromatographic (GPC) equipped with laser light scattering detector (LSD) and deflection refractive index detector (DRID). THF was used as the eluent at flow rate of 1.0 mL/min, and the calibration was carried out with a narrow molecular weight distribution poly(styrene) standard.

Critical micelle concentration (CMC) study

The CMCs of copolymers in aqueous solution were estimated by fluorescence spectroscopy using pyrene as a hydrophobic fluorescence probe. Sixteen samples of polymer solution with concentrations ranging from 1.07×10^{-4} to 4.40×10^{-1} g/L were prepared and then left to equilibrate with constant pyrene concentration of 6×10^{-7} mol/L for 24 h at 25 °C. Fluorescence spectra of pyrene were recorded by a fluorescence spectrophotometer (F-7000 FL, Hiachi corp., JP) at room temperature. The excitation wavelength used was 340 nm and the emission spectra were recorded from 250 to 470 nm. Both excitation and emission bandwidths were set at 2 nm. The peak height intensity ratio (I_3/I_1) of the first peak (I_3 at 393 nm) to the third peak (I_1 at 373 nm) was plotted against the logarithm of polymer concentration. Two tangents were then drawn, one to the curve at high concentrations and another through the points at low concentrations. The CMC value was taken from the intersection between the two tangents.

Preparation of PCEC micelles before and after loading FA

Membrane dialysis method was used to prepare polymer micelles. Briefly, 10 mg PCEC was dissolved in 5 mL DMSO and then the solution was stirred for 5 h. After that, 10 mL distilled water was added at a rate of 10 μL every 30 s to induce micellization. Fifteen milliliters above solution was placed into a dialysis bag (MW 8,000–14,000) and dialyzed against distilled water 200 mL at room temperature for 2 days to remove any organic solvents. The external-distilled water phase was completely replaced by 200 mL fresh-distilled water every 4 h. Dialyzed products were centrifuged at 4,000 rpm for 10 min to remove aggregated particles during dialysis process. Then the supernatant was collected and lyophilized to obtain dried PCL–PEG–PCL micelles.

To prepare the drug-loaded nanomicelles, 10 mg of triblock PCEC and different amounts of FA were dissolved in 5 mL DMSO. After stirring the solution for 5 h, 10 mL distilled water was added at a rate of 10 μL every 30 s to induce micellization. Then, 15 mL above solution was placed into a dialysis bag (MW 8,000–14,000) and dialyzed against distilled water 200 mL at room temperature for 2 days to remove any organic solvents and unloaded FA molecule. The external-distilled water phase was completely replaced by 200 mL fresh distilled water every 4 h until no trace of FA in the external aqueous phase could be detected. After dialysis, the solution in the dialysis bag was also centrifuged as described above. Finally, the supernatant was collected and lyophilized to obtain dried FA-loaded PCL-PEG-PCL micelles (PCEC/FA).

Drug encapsulation efficiency and loading content

The FA content was measured by UV spectrophotometer and the detection wavelength was set at 286 nm. To determine the drug loading content and encapsulation efficiency of drug-loaded micelles, the lyophilized FA-loaded micelles were dissolved in DMSO. Then the FA amount (W_1) was measured using an established calibration curve in FA/DMSO solutions and was considered as the drug amount encapsulated in the micelles. In the dialysis process, the drug amount (W_2) contained by external aqueous phase was also measured. The drug loading content (DLC%) and encapsulation efficiency (EE%) of FA-loaded micelles could be calculated by the following equations: $\mathrm{DLC\%} = W_1/(W_1 + W_{\mathrm{PCEC}}) \times 100\%$; $\mathrm{EE\%} = W_1/(W_1 + W_2) \times 100\%$.

Micelles particle size and morphology analysis

After dialyzed and filtered, all the micelles were characterized by dynamic light scattering instrument (DLS) and transmission electron microscopy (TEM). The size and size distribution of polymer micelles were determined by dynamic light scattering using a Nano-ZS90 laser nano-granulometric analysis instrument (Malvern Instrument, Watts, UK). Each measurement was repeated three times, and an average value was obtained. TEM image of the micelles was conducted on a JEM-100cX II instrument (JP) operating at an acceleration voltage of 80 kV. TEM

sample was prepared by dipping a copper grid with formvar film into the prepared micelles aqueous solution. After the deposition, the aqueous solution was blotted away with a strip of filter paper and dried in air.

In vitro drug release

In vitro release of FA from drug-loaded micelles was studied at PBS with different pH. The dialysis bags containing 4 mL FA-loaded micelles solution were submerged fully into beakers containing 100 mL buffer solutions. The beakers were kept in a 37.0 ± 0.1 °C water bath and stirred at 60 rpm. At regular time intervals, 5 mL external buffer solution was withdrawn and the same volume fresh buffer solution was added in order to hold the volume of solution constant. The amount of FA released from drug-loaded micelles was measured using UV absorbance at 286 nm. The release profile was plotted using cumulative drug release (mg) as a function of time (h).

Results and discussion

Characterization of polymers

A series of PCEC triblock copolymers with different molecular weights were synthesized by adjusting the feeding weight ratio of ε-CL to PEG as shown in Table 1. IR was employed to characterize the structure of PCEC-2 copolymer and the IR spectra were shown in Fig. 1 (a: DHPEG; b: PCEC-2). Comparing to the IR spectra of DHPEG (curve a), the IR spectra of PCEC-2 (curve b) has new peaks appearing at 1,726 and 1,240 cm^{-1}, corresponding to the –C = O stretching vibrations of the ester carbonyl group and –COO– bonds stretching vibrations, respectively, which approved the successful ring-opening polymerization of ε-CL. In curve a and b, the signals at 1,110 cm^{-1} were attributed to the characteristic absorption of the $–CH_2–O–CH_2–$. All the C–H stretching bonds are centered at 2,945–2,891 cm^{-1}. All these signals indicate that the PCEC block copolymer may be formed [26].

Table 1 Characterization of PCEC triblock copolymers

Sample	Feed molar ratio of ε-CL (mmol):DHPEG (mmol)	ε-CL/EO molar ratio R^a	Mn^b	Mn^c	PDI^c	CMC (mg/L)
PCEC-1	16.4:0.1	0.94	11,640	11,032	1.23	11.3
PCEC-2	23.2:0.1	1.50	15,000	13,963	1.41	7.71
PCEC-3	28.7:0.1	1.81	16,860	15,354	1.43	6.23
PCEC-4	37.0:0.1	2.30	19,800	18,387	1.59	1.44

[a] Calculated according to the integrated area ratio of the resonance peaks due to the PCL block at 1.28 ppm and the PEG block at 3.58 ppm

[b] Calculated from M_{PEG} and the ε-CL/EO molar ratio

[c] Measured by the GPC analysis

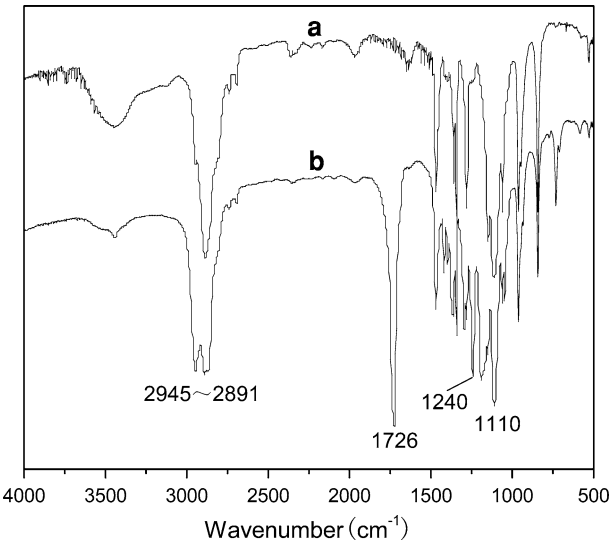


Fig. 1 IR spectra of DHPEG (**a**) and PCEC-2 copolymer (**b**)

To further determine the structure of the triblock copolymers, the copolymers were investigated by ^{1}H-NMR spectroscopy. Typical ^{1}H-NMR spectra of the copolymer are illustrated in Fig. 2. As shown in Fig. 2, the sharp single peak at 3.58 ppm is attributed to the methylene protons of homosequences of the PEG oxyethylene units. The very weak peak at 4.13 ppm is attributed to the methylene proton of PEG end unit. In addition, Peaks at 1.28, 1.64, 2.20, and 3.96 ppm (e, d, c, and f) are assigned to methylene protons of $OCOCH_2CH_2–C\mathit{H}_2–CH_2CH_2$,

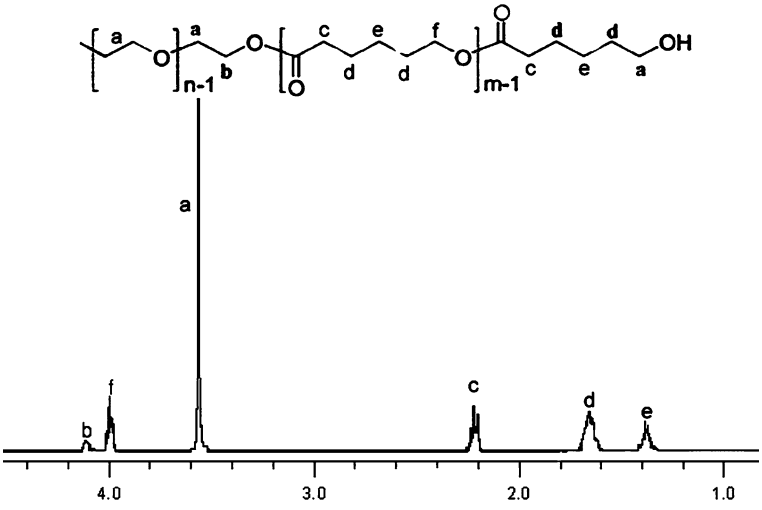


Fig. 2 ^{1}H-NMR spectra of PCEC-2 in $CDCl_3$ at 25 °C

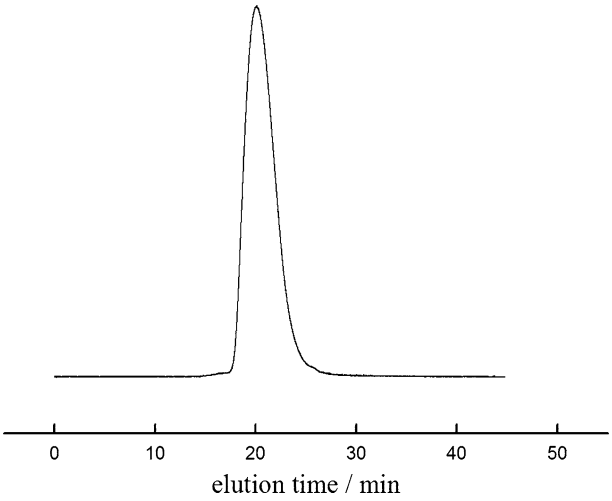


Fig. 3 CPC curve of PCEC-2 copolymer

$OCOCH_2$–CH_2–CH_2–CH_2–CH_2, OCO–CH_2–$(CH_2)_4$, and $OCOCH_2CH_2CH_2CH_2$–CH_2 in PCL units, respectively. These results are consistent with those obtained from triblock copolymers prepared by the ring opening polymerization of ε-CL with alkali metal alkoxide derivatives of poly(ethylene glycol) [27]. Since the proton resonance from the ethylene group of the PEG block was observed at 3.58 ppm and the resonance from the ε-methylene protons of $OCCH_2CH_2$–CH_2–CH_2CH_2 in the PCL block was observed at 1.28 ppm, ε-CL/EO molar ratio R in the block copolymer could be determined from the intensity area ratio of the ^{1}H-NMR peaks at 1.28 ppm to that at 3.58 ppm. Then the number average molecular weight of PCEC and PCL block length were calculated by $M_{\mathrm{nPCL}} = R \times M_{\mathrm{nPEG}}$ and $M_{\mathrm{n}} = (1 + R) \times M_{\mathrm{nPEG}}$, where M_{nPEG} was the molecular weight of used DHPEG ($M_{\mathrm{nPEG}} = 6{,}000$ g/mol). The results are summarized in Table 1.

The average molecular weight and polydispersity index (PDI) were also measured by GPC using THF as an elution solvent and monodisperse poly(styrene) as standard. The GPC results (Table 1) clearly show the average molecular weight of triblock copolymers increased with increasing ε-CL/PEG in feeding. The copolymers had narrow molecular weight distributions with PDI varying from 1.23 to 1.59 seen in Table 1. In Fig. 3, it can be seen that only a single peak exists, which suggests the monodispersion of M_{w} and the absence of any homopolymer of ε-CL and PEG monomers, which indicate that no transesterification and/or backbiting reactions occurred during the copolymerization process [26].

Determination of critical micelle concentration

The CMC is an effective parameter of micellar stability and a low critical value is desired. In the study, micelles formation was monitored by using pyrene as a hydrophobic probe. Several fluorescence patterns were obtained from pyrene equilibrated with aqueous solutions of copolymers at various concentrations. In the

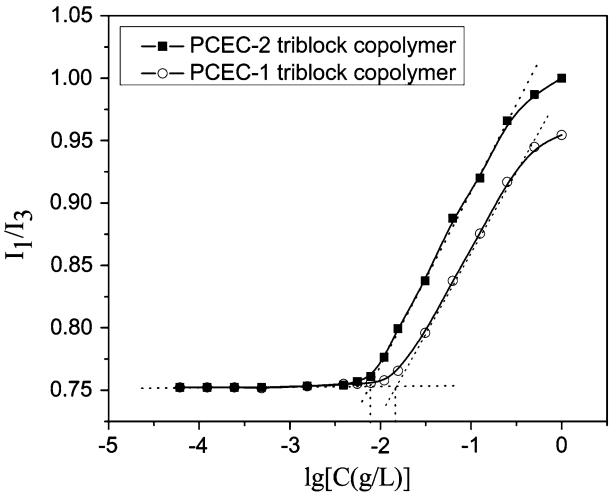


Fig. 4 Plot of the I_3/I_1 ratio as a function of logarithm of PCEC-1 and PCEC-2 copolymers concentration

emission spectra, fluorescence intensity increases with increasing polymer concentration, due to the fact that pyrene in water has a very small absorption, which increases when it is transferred into a hydrophobic environment. Of special interest are the third (I_3 at 393 nm) and the first (I_1 at 373 nm) peaks, since their peak height-intensity ratio (I_3/I_1) can be used as a sensitive parameter to represent the polarity of the microenvironment [28]. The respective I_3/I_1 values have been plotted as a function of copolymer concentration (Fig. 4). From the figure, the ratio of I_3/I_1 is essentially constant at low concentration. Above a certain concentration, the ratio increases dramatically, indicating formation of micelles and the transfer of pyrene into the hydrophobic core of the micelles. The CMC values for these triblock copolymers are summarized in Table 1, which show that these triblock copolymers all have lower CMC values, providing evidence for an apparent stability of micelles and allowing their use in very dilute aqueous milieu such as body fluid. The CMC decreased with the increase of PCL block length, indicating that the micelles formation became easier for the triblock copolymers with larger hydrophobic blocks.

Characterization of PCEC micelles

PCEC triblock copolymer micelles were prepared by a membrane dialysis method. The size and size distribution of these micelles were determined by DLS. Table 2 showed the effect of the block copolymer composition and feed molar ratio of drug/copolymers on the size of PCEC triblock copolymer micelles. The size of PCEC micelles gradually increased with the increase of the hydrophobic PCL block length, i.e., the longer the hydrophobic PCL block, the larger the micelle size. It was recognized that the aggregation of hydrophobic PCL block built up the core of micelles. The core of micelles became larger with the increase of the length of PCL

Table 2 Physicochemical properties of PCEC copolymer micelles before and after loading FA

Sample	Feed weight ratio of FA (mg):Polymer (mg)	DLC (%)	EE (%)	Micelle Size ± SD (nm)	Polydispersity Index ± SD
PCEC-1a	0:10	–	–	47 ± 3.1	0.171 ± 0.033
PCEC-1b	5:10	9.5	74.1	66 ± 9.4	0.199 ± 0.029
PCEC-2a	0:10	–	–	55 ± 4.2	0.179 ± 0.064
PCEC-2b	2:10	8.7	82.8	68 ± 8.5	0.183 ± 0.053
PCEC-2c	5:10	10.6	76.3	72 ± 6.3	0.206 ± 0.031
PCEC-2d	8:10	13.1	73.2	75 ± 9.7	0.201 ± 0.042
PCEC-3a	0:10	–	–	73 ± 6.8	0.187 ± 0.017
PCEC-3b	5:10	18.4	74.9	94 ± 7.9	0.197 ± 0.020
PCEC-4a	0:10	–	–	81 ± 6.7	0.186 ± 0.020
PCEC-4b	5:10	19.2	75.8	106 ± 8.8	0.202 ± 0.020

blocks, so that the micelle size was larger. Also, the drug loading content (DLC) was increased with increased molecular weight of PCEC block copolymer. These results might be due to the strong hydrophobic interaction between the longer hydrophobic PCL block chain and hydrophobic drug. When initial drug feed amounts were differently supplied, the higher the feed amount of the drug, the higher the drug loading content. Meantime, the encapsulation efficiency (EE) of PCEC-2 micelles also decreased accordingly. After the drug loading, the micelles size increased. The result is expected, because incorporation of FA into the hydrophobic cores increases the volume of the micelles. In addition, the size of PCEC-2 micelle was slightly increased when the feed drug amount was increased. Their size distribution, however, was not markedly influenced by the drug loading. The results were similar to the previous reports [24, 25, 29].

Furthermore, the morphology and size of drug-unloaded and drug-loaded PCEC-2 micelles (DLC = 8.7) were also investigated by TEM. Figure 5 showed that both micelles were spherical in shape. After loading drug, the morphology of polymer micelles maintained about the same. The diameters of micelles observed by TEM were generally smaller than those obtained by DLS. There is a reason that the diameter of micelle obtained by DLS reflected the hydrodynamic diameter of micelle swelling in aqueous solution, while that observed by TEM was the diameter of dried micelle.

In vitro drug release study

To study the effect of the copolymer composition and drug loading content on the drug release behavior, the FA-loaded PCEC core–shell type micelles were immersed in PBS (pH 7.4, 37 °C) and drug release studies were performed in vitro using a dialysis bag (MW 8,000–14,000). Figures 6 and 7 show the release profiles of FA from the drug-loaded PCEC micelles against the drug loading content and various copolymer composition, respectively. As shown in Fig. 6, it is observed that as the drug loading content increases, the drug release rate decreases.

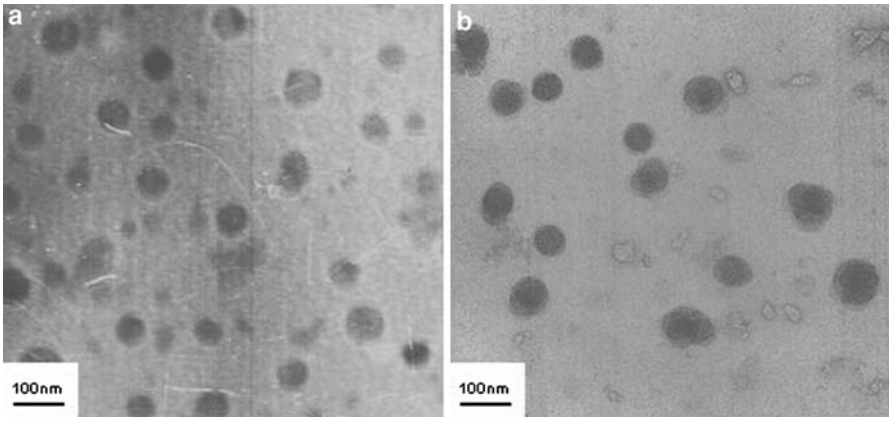


Fig. 5 The TEM micrograph of drug-unloaded (**a**) and drug-loaded PCEC-2 triblock copolymer micelles (DLC = 8.7) (**b**)

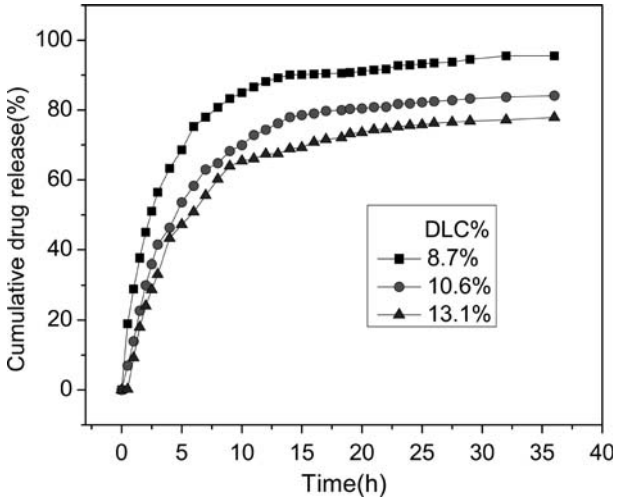


Fig. 6 In vitro release profiles of FA from PCEC-2 copolymer micelles with various DLCs in pH 7.4 PBS buffer solution at 37 ± 0.1 °C

The comparatively accepted mechanism is that crystallization of hydrophobic drug occurs inside the micelles and especially, at higher drug loading content, a phase separation occurs, leading to the crystallization of drug in the inner core of the polymer micelles [24, 30]. Then, hydrophobic drugs loaded into micelles are released more slowly at higher drug loading content. On the other hand, at lower drug loading content, the drug exists as a molecular dispersion inside the micelles. The crystallized drug should be dissolved and diffused more slowly into the outer aqueous phase than that of molecular dispersion. In addition, an increase of the amount of model drug with hydrophobic character enhances an interaction between drug and hydrophobic PCL block, leading to a decreased drug release.

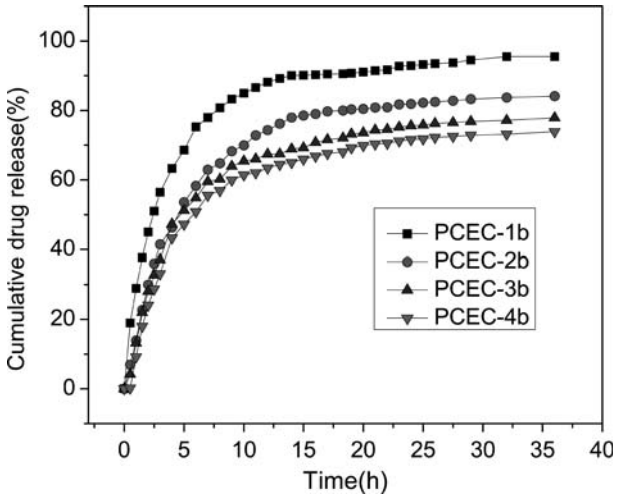


Fig. 7 In vitro release profiles of FA from drug-loaded PCEC micelles with various PCL block lengths in pH 7.4 PBS buffer solution at 37 ± 0.1 °C

Figure 7 showed the effect of the molecular weight on the drug release profiles. It seems that the release rate of FA from drug-loaded polymer micelles decreases with an increase in molecular weight of PCEC, where the PEG block has a fixed molecular weight of 6,000, and the molecular weight of the PCL blocks is changed by the feed weight ratio. When we compared PCEC-1b and PCEC-2b which have a similar DLC (%) and different molecular weights, the drug release rate from PCEC-1b micelle, formed by the PCEC-1 copolymer with a molecular weight of 11,640, was faster than that from PCEC-2b micelle, formed by the PCEC-2 copolymer with a molecular weight of 15,000. If comparing the drug release rate from PCEC-3b and PCEC-4b micelles, a consistent conclusion was drawn. From the results, it is considered that the drug binding affinity with PCL block affects the drug release behaviours [30]. FA is physically entrapped in hydrophobic inner core of micelles. Therefore, the release behaviours of a drug molecular from loaded-drug micelle system are affected by the hydrophobic property of its inner core. In this study, PCEC triblock copolymers were synthesized with PEG segments having a fixed molecular of 6,000. Thus, when the molecular weight of PCEC copolymers increases, the hydrophobic PCL block length increases accordingly, leading to the enhancement of the binding affinity between drug and PCL blocks, which lowers the drug release rate.

To determine the effect of pH values of release medium on the release behaviours of FA, the studies on the release of FA were carried out by immersing the FA-incorporated micelle solutions in pH 7.4 and 9.18 buffers. As shown in Fig. 8, no initial burst drug release at earlier stage was observed in the release patterns of the drug-loaded PCEC-2 micelles (DLC = 8.7). Furthermore, it showed a significant sustained release characteristic. However, the FA release rate in the release media of pH 9.18 is larger than that in the media of pH 7.4. This can be explained by the effect of the pH values on the solubility of FA in external buffers. Folic acid (FA) is an acidity drug with carboxyl on it and so, the larger the pH value of external release

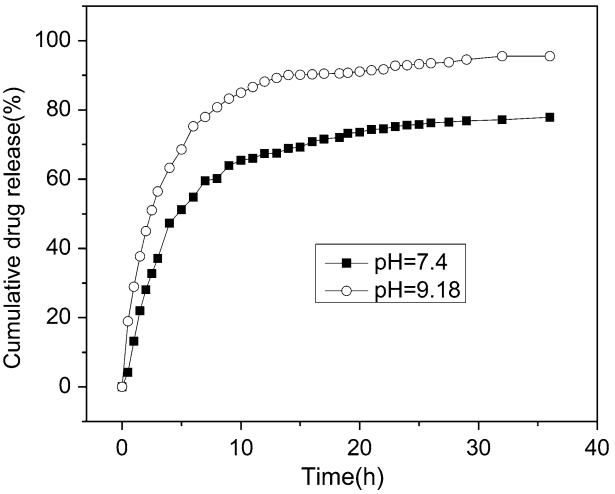


Fig. 8 In vitro release profiles of FA from drug-loaded PCEC-2 micelles (DLC = 8.7) in pH 7.4 and pH 9.18 PBS buffer solutions at 37 ± 0.1 °C

media, the easier the solubility of FA in it. Accordingly, the enhanced solubility results in larger concentration difference of FA between the inner core and external buffers, which accelerate the diffusion of FA into the external buffers.

Conclusion

Amphiphilic triblock copolymers PCEC with different molar compositions were successfully synthesized by the ring-opening polymerization of ε-caprolactone (ε-CL). Using a membrane dialysis method, PCEC micelles entrapped with model drug (FA) were prepared with a spherical core–shell type. The critical micelle concentration (CMC) of PCEC triblock copolymers determined by fluorescence technique depended on the length of PCL block. The micelles size and drug loading content (DLC) increased with the increase of the feed weight ratio of FA and copolymers and PCL chain length. In addition, in vitro release experiments of the FA-loaded PCEC micelles exhibited sustained release behavior without any burst effects and the release behavior could be tailored by adjusting the copolymer molar composition, drug loading content, and pH of release media.

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