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Synthesis and self-assembly of comb-like amphiphilic Doxifluridine–poly(ε-caprolactone)-*graft*-poly(γ-glutamic acid) copolymer

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ABSTRACT

Advances in amphiphilic copolymers can potentially be exploited in drug or gene delivery. This study develops novel comb-like amphiphilic copolymers that comprise poly(γ-glutamic acid) (γ-PGA) as a hydrophilic backbone and Doxifluridine–poly-(ε-caprolactone) (5′-deoxy-5-fluorouridine–poly(ε-caprolactone), 5′DFUR–PCL) as a hydrophobic side chain. A novel 5′DFUR–PCL polymer was synthesized with antitumor agent Doxifluridine (5′DFUR) as the initiator via the ring-opening polymerization of ε-caprolactone (ε-CL) using tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) as the catalyst. The 5′DFUR–PCL polymer was then grafted on γ-PGA to yield a 5′DFUR–PCL–γ-PGA comb-like copolymer with the help of 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC). The characteristics of these copolymers were examined by ^{1}H NMR, FT-IR, GPC, contact angle measurement and thermal properties. Grafting 5′DFUR–PCL would significantly increase the contact angle and decrease the melting temperature (T_m) of the copolymers. The micelles self-assembled from these amphiphilic copolymers were formed in an aqueous phase and were examined via fluorescence approaches, dynamic light scattering (DLS) and transmission electron microscopy (TEM). The average sizes of the micelles were in the range from 130 to 230 nm and their zeta potentials were negative and less than −16.7 mV. The critical micelle concentration (CMC) was from 1.49 mg/L to 4.63 mg/L at 25 °C. TEM images demonstrated that the micelles were spherical and clearly had a core–shell structure.

1. Introduction

Micelle-like aggregates formed from amphiphilic copolymers have attracted considerable interest in recent years owing to their unique phase behavior in aqueous media and their potential applications as carriers for genes and hydrophobic drugs [1–3]. Due to their amphiphilic characteristics, block or graft copolymers composed of hydrophilic and hydrophobic segments exhibit surfactant behavior and can form micelles with core–shell structure at critical micelle concentration (CMC) [4,5]. These micelles have a hydrophobic compact inner core and a hydrophilic swollen outer shell in aqueous media; both of which are thermodynamically stable in physiological solution because the hydrophobic blocks in an aqueous phase undergo macromolecular assembly to generate polymeric micelles and micelle-like aggregates [6]. Furthermore, polymer micelles could be easily designed on the nano-scale sizes with a narrow size distribution, which greatly facilitates the regulation of biodistribution [7].

The hydrophobic segments of the polymeric micelles normally comprise biocompatible and biodegradable materials such as polyester and its derivatives [8–10]. Poly(ε-caprolactone) (PCL) is an aliphatic polyester prepared by the ring-opening polymerization (ROP) of ε-caprolactone. PCL has various advantages such as non-toxicity, excellent biodegradability and biocompatibility, and high resistance to water, oil, solvent and chlorine [11]. These unique characteristics of PCL are responsible for its potential medical applications, such as drug carriers in the development of controlled drug delivery systems [12–15]. Polypeptide block and graft copolymers are also effective copolymers for fabricating drug carriers and can produce core–shell structure with various shapes [5,16–20]. Poly (γ-glutamic acid, γ-PGA) is a biosynthetic polypeptide consisting of D- and L-glutamic acid units that are connected by amide linkages between the α-amino and γ-carboxylic groups [21]. Interest in this biomaterial has recently been renewed because of its excellent properties including its water-solubility, biodegradability, biocompatibility, high capacity to absorb water and non-toxicity toward humans and the environment. Recently, several studies have investigated the hydrophilic segments of polymeric micelles such as PLLA-*block*-γ-PGA copolymer [22–24] and phenylalanine-*graft*-γ-PGA copolymer [25], because of their high hydrophilic property and excellent water-binding capacity.

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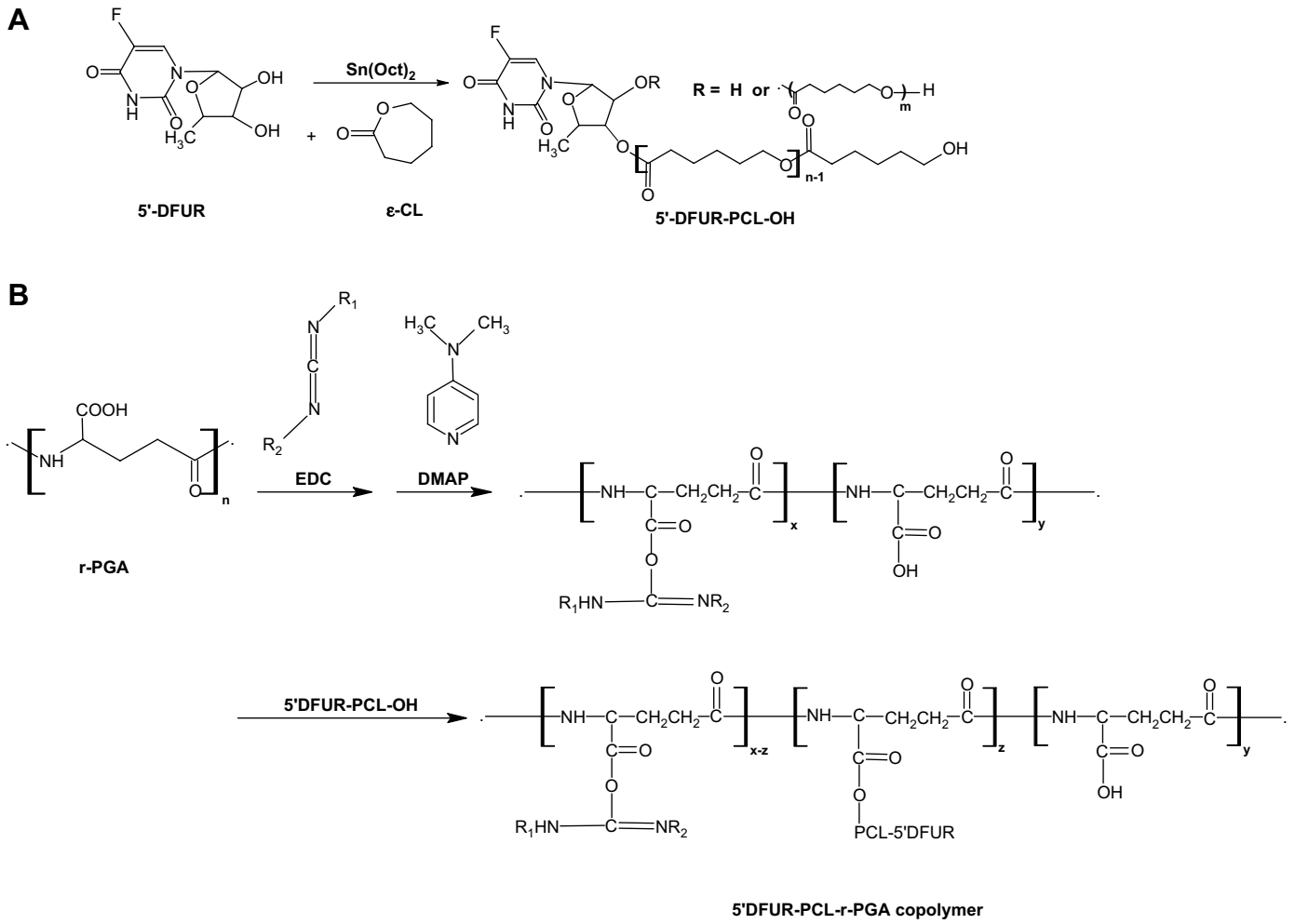

Scheme 1. Synthesis of (A) 5′DFUR–PCL polymer and (B) 5′DFUR–PCL–γ-PGA copolymer.

The aim of this study was to investigate the synthesis, characterization and properties of comb-like amphiphilic copolymers consisting of γ-PGA as a hydrophilic backbone and 5′-deoxy-5-fluorouridine–poly(ε-caprolactone) (5′DFUR–PCL) as a hydrophobic side chain. A novel 5′DFUR–PCL polymer was synthesized by the ROP of ε-caprolactone (ε-CL), initiated by antitumor agent Doxifluridine (5′DFUR). The 5′DFUR–PCL polymer was then grafted to γ-PGA forming a 5′DFUR–PCL–γ-PGA comb-like copolymer. These novel amphiphilic copolymers are capable of forming polymeric micelles in the aqueous solution and their physicochemical properties are discussed.

2. Experimental

2.1. Materials

Doxifluridine (5′-deoxy-5-fluorouridine, 5′DFUR) was purchased from Tokyo Chemical Industry Co., LTD (TCI) and dried at 110 °C for 8 h before use. ε-caprolactone (ε-CL) was purchased from Acros, dried over calcium hydride for 48 h and distilled at reduced pressure. The dry ε-CL was then stored in nitrogen gas covered molecular sieves (4 Å) before use. Tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) was purchased from Sigma and diluted in a hexane solution (0.025 g/mL). 4-Dimethylaminopyridine (DMAP) and 1-ethyl-3-(3-dimethyl-amino-propyl) carbodiimide (EDC) were purchased from Acros. [Poly(γ-glutamic acid), sodium salt] [γ-PGA(Na)] was kindly supplied by Vedan Enterprise Corp. (Taiwan) and was used without further purification. The molecular weight ($M_w = 12{,}000$, $M_w/M_n = 1.05$) of γ-PGA was measured by GPC and calibrated with poly(ethylene glycol) (PEG) as the standard. The mobile aqueous phase contained 0.01 M NaH_2PO_4 and 0.2 M $NaNO_3$ and was adjusted to a pH value of 7.0. Tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), dimethyl formamide (DMF), ethyl ether, hexane and other solvents were of analytic grade.

Table 1
Synthesis and some characteristic properties of 5′DFUR–PCL–γ-PGA_n copolymers.

Sample	Feed ratio[a]	Yield[b] (wt.%)	γ-PGA content[c] (wt.%)	Particle size[d] (nm)	μ_2/Γ^{2} [e]	Zeta potential[d] (mV)
5′DFUR–PCL–γ-PGA_{86}	1.0	60	86	129.7 ± 2.1	0.187	−28.2 ± 2.6
5′DFUR–PCL–γ-PGA_{71}	2.5	45	71	148.0 ± 5.5	0.243	−21.6 ± 4.0
5′DFUR–PCL–γ-PGA_{64}	7.5	24	64	187.0 ± 8.7	0.218	−20.1 ± 3.9
5′DFUR–PCL–γ-PGA_{22}	15.0	26	22	231.8 ± 4.6	0.142	−16.7 ± 1.9

[a] Feed ratio = 5′DFUR–PCL/γ-PGA (g/g).
[b] Yield = (5′DFUR–PCL–γ-PGA_n)/(5′DFUR–PCL + γ-PGA) × 100% (g/g).
[c] Determined by ^{1}H NMR analysis, content of γ-PGA unit in wt.% = (γ-PGA in g/grafted copolymer in g) × 100%.
[d] The particle size and zeta potential of 5′DFUR–PCL–γ-PGA nanoparticles were measured in deionized water at 0.1 mg/mL ($n = 4$).
[e] Polydispersity factors.

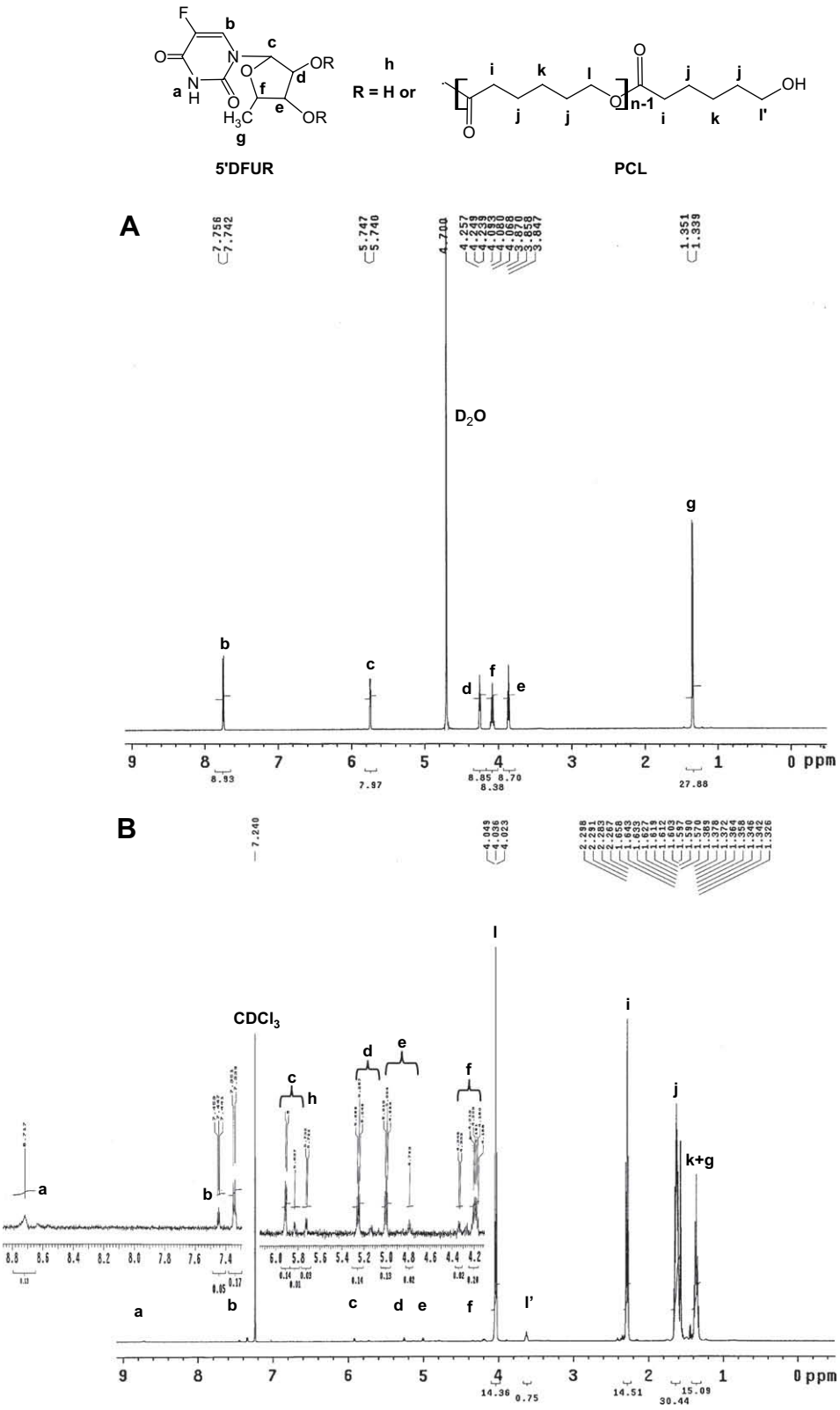

Fig. 1. ^{1}H NMR spectra of (A) 5′DFUR; (B) 5′DFUR–PCL polymer.

2.2. Synthesis of 5′DFUR–PCL

The synthesis of 5′DFUR–PCL was described in a previous report [26]. Briefly, $Sn(Oct)_2$ solution (0.5 wt.% of ε-CL) was accurately weighed and placed in a dried glass flask, then evaporated under vacuum at room temperature for 30 min to remove all of the hexane completely. After 5′DFUR (0.369 g) and ε-CL (5.479 g) had been added and mixed to homogeneity, the flask was exhausted under

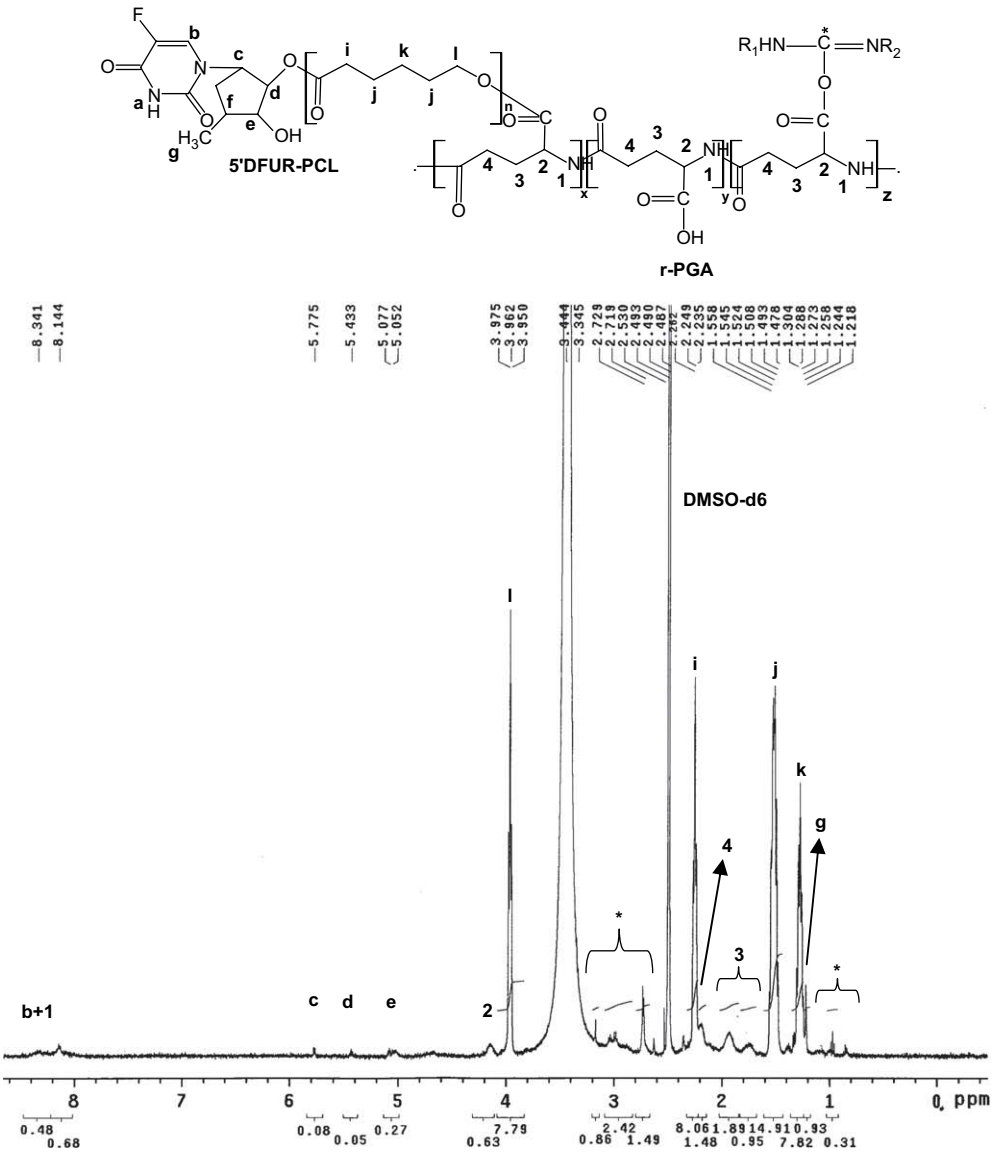

Fig. 2. ^{1}H NMR spectra of 5′DFUR–PCL–γ-PGA copolymer. *the signals of EDC residue [21].

vacuum for degassing and purged with dry nitrogen three times. Finally, 5′DFUR reacted with ε-CL in the presence of $Sn(Oct)_2$ as the catalyst at 140 °C for 24 h under nitrogen. After polymerization, the crude product was cooled to room temperature, dissolved in THF, and then precipitated into excess deionized water twice to remove any unreacted 5′DFUR. The obtained product was further purified with cold ethyl ether and dried under vacuum. Scheme 1A presents the method for preparing 5′DFUR–PCL polymer.

2.3. Synthesis of 5′DFUR–PCL–γ-PGA copolymer

γ-PGA(Na) was converted to γ-PGA(H) in a HCl solution and was adjusted to a pH value of 2.0 [27]. The carboxylic group of γ-PGA(H) was activated by EDC and then 5′DFUR–PCL was grafted to γ-PGA(H). Briefly, γ-PGA(H) (0.1 g) was dissolved in DMSO (9 mL) by ultrasonication and DMAP (0.095 g) was subsequently added to the solution. After predetermined amounts of 5′DFUR–PCL had been added and dissolved completely, the homogenous solution was mixed with 1 mL of EDC solution (0.148 g, in DMSO) at 40 °C in a nitrogen atmosphere and the reaction was allowed to continue for

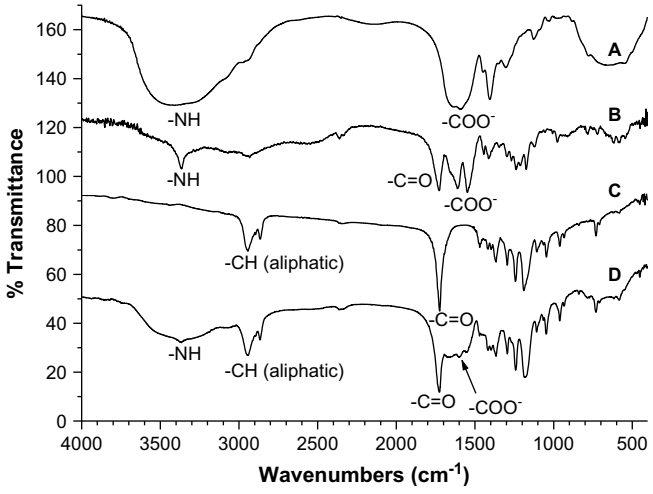

Fig. 3. FT-IR spectra of (A) γ-PGA(Na); (B) γ-PGA(H); (C) 5′DFUR–PCL polymers and (D) 5′DFUR–PCL–γ-PGA copolymers.

Table 2
Solubility of 5′DFUR–PCL–γ-PGA$_n$ in various solvent.

Sample	Concentration								
	2.5 mg/mL								5 mg/mL
	H_2O	THF	$CHCl_3$	CH_2Cl_2	DMF	Acetone	DMSO	co-solvent[a]	co-solvent[a]
γ-PGA(H)	–	–	–	–	–	–	+	+	+
5′DFUR–PCL–γ-PGA$_{86}$	–	–	–	–	–	–	–	+	–
5′DFUR–PCL–γ-PGA$_{71}$	–	–	–	–	–	–	–	+	–
5′DFUR–PCL–γ-PGA$_{64}$	–	–	–	–	–	–	–	+	+
5′DFUR–PCL–γ-PGA$_{22}$	–	–	–	–	–	–	–	+	+
5′DFUR–PCL	–	+	+	+	+	+	+	+	+

The solubility tests were performed by ultrasonication for 4 h at room temperature.
Key: +, soluble; –, insoluble.
[a] Co-solvent: 99% DMSO + 1% 2 N HCl (v/v).

24 h. It was then dialyzed with a membrane (spectrum, MWCO: 12,000–14,000) for 3 days to remove all the unreacted γ-PGA(H) and was lyophilized until dry. The obtained products were further purified twice in an excess of acetone to remove the un-grafted 5′DFUR–PCL. The final products were collected by centrifugation and dried in vacuum at room temperature for 24 h. Scheme 1B presents the procedures for preparing 5′DFUR–PCL–γ-PGA copolymer.

2.4. Preparation of polymeric micelles

Polymeric micelles were prepared by nanoprecipitation method [28]. 5′DFUR–PCL–γ-PGA copolymer (10 mg) was dispersed in acetone (2 mL) by ultrasonication. The obtained organic solution was dropwisely added into deionized water (10 mL) with stirring at room temperature and then dispersed by ultrasonication again. Subsequently, acetone was removed by reducing the pressure at room temperature for 1 h. Finally, the resulting aqueous solution was filtered through a 0.45 μm filter membrane to collect polymeric micelles.

2.5. Characterizations

^{1}H NMR spectra of the 5′DFUR–PCL polymers and 5′DFUR–PCL–γ-PGA copolymers were obtained from a ^{1}H NMR apparatus (VARIAN, UNIYTINOVA-500 NMR) at room temperature with $CDCl_3$ and co-solvent (99% DMSO-d_6 + 1% 2 N HCl) as the solvents. Fourier transformed infrared (FT-IR) spectra were recorded on an infrared spectrophotometer (Perkin Elmer, 842 IR spectroscopy). The dried samples were carefully embedded at ca. 1 wt.% in KBr pellets. GPC analysis (MILLIPORE, Waters-410) was performed to determine the distribution of molecular weight. Tetrahydrofuran (THF) was used as the mobile phase (1 mL/min) and a universal calibration was performed with polystyrene as the standard.

2.6. Contact angle measurements and thermal analyses

Water contact angles of the 5′DFUR–PCL–γ-PGA copolymers were obtained using the sessile drop method [29]. First, the 5′DFUR–PCL–γ-PGA copolymers (2.5 mg/mL) were dissolved in a co-solvent (99% DMSO + 1% 2 N HCl). The solution (50 μL) was then dropped on the surface of a clear glass slide (25 mm × 11 mm × 1 mm) and film was formed by a spin coater (Pentad, SSP-02A) and dried under vacuum at room temperature. The contact angle was measured at 45 s with a contact angle meter (Paul N, Gardner, PG3) at ambient temperature after a water droplet was dropped onto the film. Measurements were made at five positions and averaged. In thermal analyses, a differential scanning calorimeter (DSC, TA 2910) was employed in a heating mode from 20 to 200 °C at heating rate of 10 °C/min under nitrogen (5 mL/min).

2.7. Measurements of size and zeta potential

The size and size distribution of the micelles were measured by dynamic light scattering (DLS) via a Zetasizer 3000ES instrument (Malven Instruments, Malvern) equipped with an argon laser beam of wavelength 670-nm at a detector angle of 90°. The zeta potential of the micelles dispersed in deionized water was determined with a zeta potential analyzer (Malven Instruments, Malvern). Both the size and zeta potential of micelles were averaged across four measurements and each sample of the micelle solution was adjusted to a concentration of 0.1 mg/mL in deionized water.

2.8. Determination of the critical micelle concentration (CMC)

The formation of micelles was confirmed by a fluorescence probe technique with pyrene [30]. Stock solutions of pyrene (6.0×10^{-4} M) were prepared in acetone and stored at 4 °C until used. Pyrene solution was then diluted with deionized water to yield a pyrene concentration of 6×10^{-7} M. Acetone was subsequently removed under vacuum at 60 °C for 1 h. 5′DFUR–PCL–γ-PGA copolymers at concentrations from 0.02 to 167 mg/L were added into pyrene solution at room temperature for 24 h, respectively. The fluorescence spectra of pyrene at a fixed emission wavelength of 390 nm were obtained with a fluorescence spectrophotometer (Hitachi, F-2500).

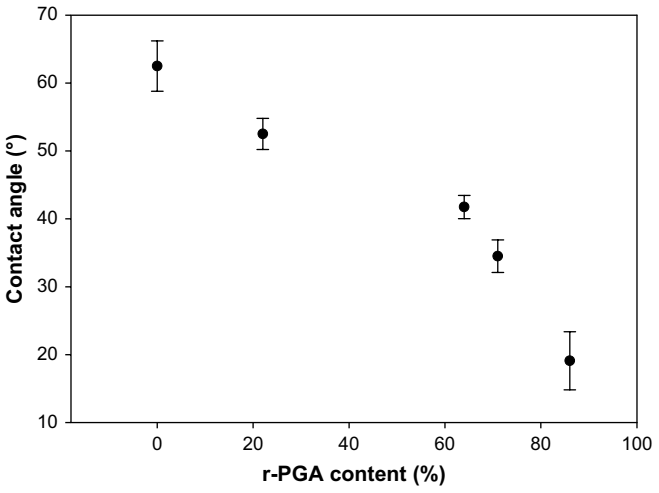

Fig. 4. Water contact angle of 5′DFUR–PCL–γ-PGA copolymers with various γ-PGA content.

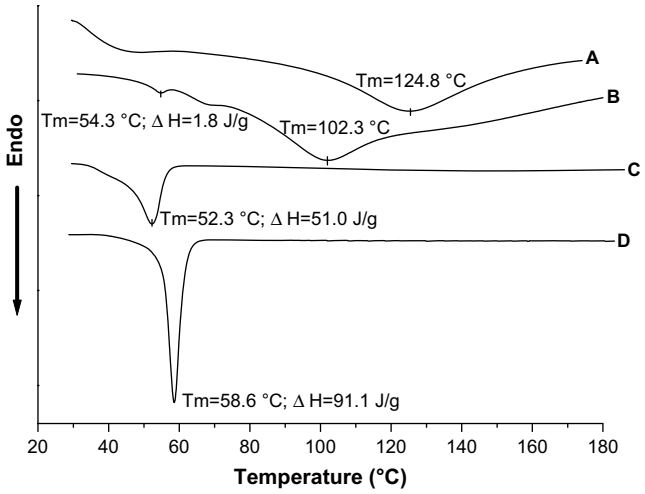

Fig. 5. DSC thermograms of various 5′DFUR–PCL–γ-PGA copolymers. (A) γ-PGA(H); (B) 5′DFUR–PCL–γ-PGA$_{86}$; (C) 5′DFUR–PCL–γ-PGA$_{22}$; (D) 5′DFUR–PCL.

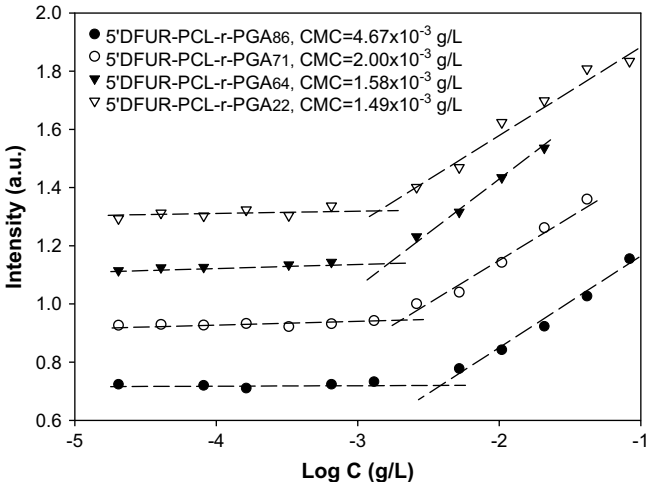

Fig. 7. Relationship of the intensity ratio (I_{338}/I_{335}) to the 5′DFUR–PCL–γ-PGA copolymer concentration.

2.9. Morphology of micelles

The morphology of the self-assembly structure for 5′DFUR–PCL–γ-PGA copolymers was observed by transmission electron microscopy (TEM, JEOL 2100(HT)). Samples were prepared by dropping the micelles suspension on a carbon-coated copper grid and then drying in vacuum. Finally, samples were coated with carbon before observation.

2.10. Statistical analysis

The data were statistically analyzed via the ANOVA test. Estimates of the standard uncertainty values were presented as mean ± standard deviation (SD). A value of $p < 0.05$ was regarded as statistically significant.

3. Results and discussion

3.1. Syntheses of 5′DFUR–PCL–γ-PGA copolymer

Scheme 1 describes the preparation of 5′DFUR–PCL–γ-PGA copolymer. First, 5′DFUR–PCL polymers were directly synthesized by the ring-opening polymerization (ROP) of ε-CL with 5′DFUR as the initiator and promoted with $Sn(Oct)_2$ [26]. The average molecular weight of 5′DFUR–PCL polymers was approximately 4×10^3 g/mol as estimated by ^{1}H NMR. The yields of the 5′DFUR–PCL products were about 81.4%, although the conversions of ε-CL monomers were near completion (>99%, data not shown) in the ring-opening step. This result followed from the fact that 5′DFUR–PCL polymer of small molecular weight would be dissolved in ethyl ether during the purification process. Furthermore, polydispersity index (M_w/M_n) of the 5′DFUR–PCL products was 1.32 as measured from the GPC data. Un-grafted 5′DFUR–PCL and 5′DFUR–PCL–γ-PGA copolymer were successfully separated by acetone. 5′DFUR–PCL–γ-PGA copolymer would precipitate in acetone and is collected by centrifugation. Table 1 presented the synthesis of 5′DFUR–PCL–γ-PGA copolymers at 40 °C for 24 h. It showed that the recovery yield and the content of γ-PGA in 5′DFUR–PCL–γ-PGA declined as the feed ratios (5′DFUR–PCL/γ-PGA) increased from 1 to 15. The amounts of γ-PGA in the 5′DFUR–PCL–γ-PGA copolymers were 86%, 71%, 62% and 22% with respect to different feed ratios, respectively. In this work, EDC played an important role to activate the carboxylic group of γ-PGA, which then reacted with the hydroxyl group of 5′DFUR–PCL to generate comb-like 5′DFUR–PCL–γ-PGA copolymer.

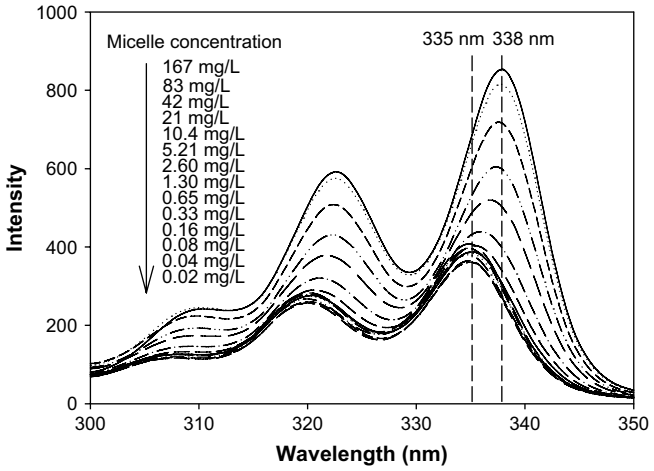

Fig. 6. Emission spectra ($\lambda_{em} = 390$ nm) of pyrene (6×10^{-7} M) recorded in the presence of increasing concentrations of 5′DFUR–PCL–γ-PGA$_{64}$ copolymer from 0.02 to 167 mg/L.

3.2. Characterizations of 5′DFUR–PCL–γ-PGA copolymer

The structure of the 5′DFUR–PCL polymer was characterized by ^{1}H NMR spectra, as presented in Fig. 1. The chemical shifts at 1.34 (k-CH_2), 1.59(j-CH_2), 2.28 (i-CH_2), 3.62(l′-CH_2) and 4.03(l-CH_2) ppm are assigned to the methylene protons in PCL while the peaks at $\delta = 1.42$(g-CH_3), 4.27–4.33(f-CH), 4.79–5.00(e-CH), 5.13–5.25(d-CH), 5.72(h-OH), 5.83–5.92(c-CH), 7.33–7.44(b-CH) and 8.78–8.93(a-NH) ppm are associated with 5′-DFUR. In the ^{1}H NMR spectra, the characteristic peaks (d and e) of 5′DFUR originally present at 3.86–4.25 ppm (Fig. 1A). However, these characteristic peaks (d and e) shift to 4.80–5.26 ppm as 5′DFUR conjugates with PCL (Fig. 1B) [26]. The NMR analyses demonstrated that the polymerization of ε-CL proceeded at the hydroxyl groups of 5′DFUR. Fig. 2 shows that the methine and methylene protons of γ-PGA adjacent to the amide group appear at 4.04 (2-CH), 1.82–1.98(3-CH_2) and 2.24(4-CH_2) ppm respectively. The peak observed at 8.14 ppm (1-NH) is the amide group of γ-PGA. The ^{1}H NMR spectrum shown in Fig. 2 verified that 5′DFUR–PCL–γ-PGA copolymer composed of γ-PGA and 5′DFUR–PCL segments.

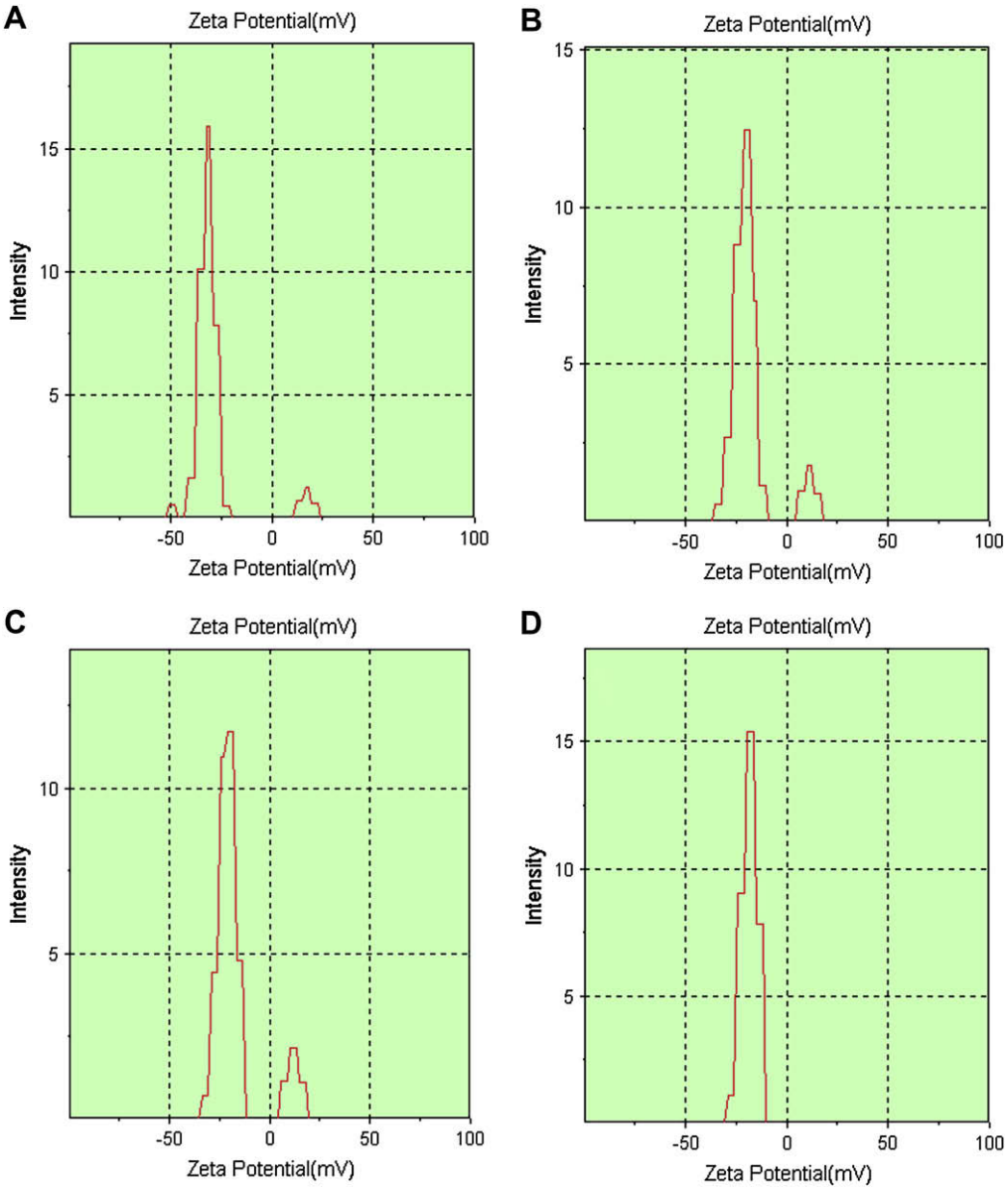

Fig. 8. Zeta potential measurements of various 5′DFUR–PCL–γ-PGA copolymers. (A) 5′DFUR–PCL–γ-PGA_{86}; (B) 5′DFUR–PCL–γ-PGA_{71}; (C) 5′DFUR–PCL–γ-PGA_{64}; (D) 5′DFUR–PCL–γ-PGA_{22}.

Fig. 3 presents the FT-IR spectra of the 5′DFUR–PCL polymer and 5′DFUR–PCL–γ-PGA copolymer respectively. Fig. 3A clearly reveals that the characteristic peaks at 1601 cm^{-1} and 3200–3600 cm^{-1} are the $–COO^-$ and –NH stretching of γ-PGA(Na). Fig. 3B shows that characteristic peaks at 1603 cm^{-1}, 1722 cm^{-1} and 3359 cm^{-1} correspond to the $–COO^-$, –C═O and –NH stretching of γ-PGA(H) respectively [23]. Therefore, γ-PGA(Na) was successfully transferred to γ-PGA(H) in HCl solution. Fig. 3C shows that the absorption peaks at 1722 cm^{-1} and 2943 cm^{-1} are associated with the functional groups –C═O and –CH (aliphatic) on the 5′DFUR–PCL polymer [31] and 5′DFUR–PCL–γ-PGA copolymer presents both peaks of γ-PGA(H) and 5′DFUR–PCL as shown in Fig. 3D.

3.3. Solubility and water contact angle of 5′DFUR–PCL–γ-PGA copolymer

γ-PGA(H), 5′DFUR–PCL and 5′DFUR–PCL–γ-PGA copolymers are soluble in some common organic solvents and co-solvents as listed in Table 2. It reveals that the grafted copolymers are soluble in the co-solvent, but insoluble in most aqueous or organic solvents such as acetone, THF, chloroform, dichloromethane, DMF and DMSO. It is probably due to the amphiphilic properties of the 5′DFUR–PCL–γ-PGA copolymer, which inhibits its dispersion and dissolution in common solvents. The solubility of the 5′DFUR–PCL–γ-PGA in DMSO will be improved if small amount of HCl is introduced so as to convert COO^- to COOH in the γ-PGA segments. The wetting behavior of coated glass surfaces containing various amounts of 5′DFUR–PCL–γ-PGA copolymers was investigated by measuring water contact angle (Fig. 4). The water contact angle of pristine 5′DFUR–PCL film was 62.5° while those of the 5′DFUR–PCL–γ-PGA copolymers could be reduced to 19° at a 86% γ-PGA content. Furthermore, the water contact angle increased with decreasing γ-PGA content. This strongly suggests that varying the amount of γ-PGA in 5′DFUR–PCL–γ-PGA copolymer can change hydrophilicity of the copolymer.

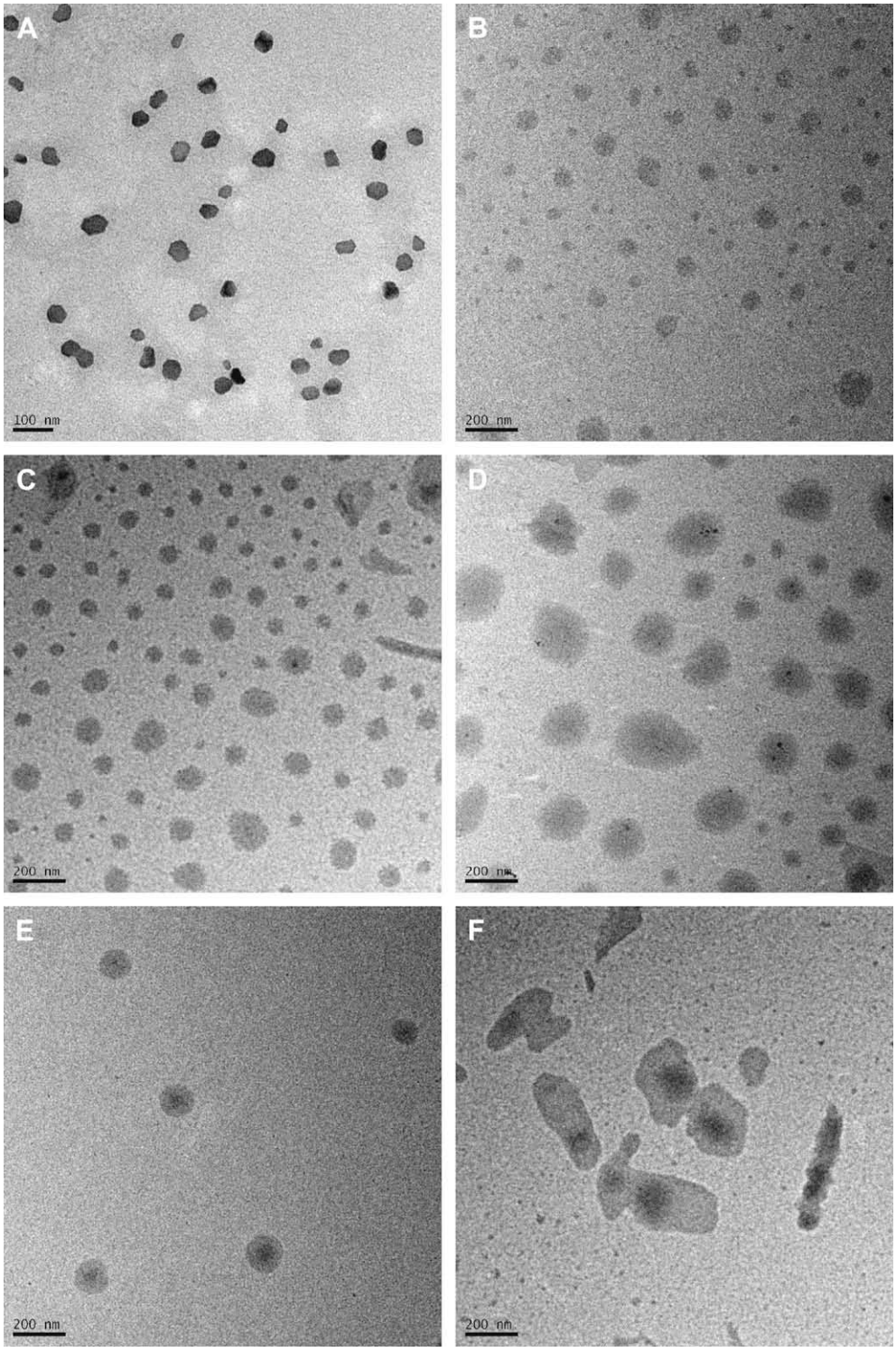

Fig. 9. TEM images of the 5′DFUR–PCL–γ-PGA copolymers. (A) 5′DFUR–PCL–γ-PGA_{86}; (B,E) 5′DFUR–PCL–γ-PGA_{71}; (C) 5′DFUR–PCL–γ-PGA_{64}; (D,F) 5′DFUR–PCL–γ-PGA_{22}.

3.4. Analyses of thermal properties

The melting behaviors of the 5′DFUR–PCL–γ-PGA copolymers were investigated by DSC as shown in Fig. 5. 5′DFUR–PCL is a semi-crystalline polyester and its melting transition temperature (T_m) is approximately 59 °C (Fig. 5D) while γ-PGA is a polyamino acid with a broad endothermal peak (Fig. 5A). Two endothermic peaks were observed during the heating process of 5′DFUR–PCL–γ-PGA_{86} (Fig. 5B) and the T_m values of γ-PGA and 5′DFUR–PCL segments in this copolymer were both lower than their corresponding homopolymers (Fig. 5A&D). The introduction of 5′DFUR–PCL into the copolymer causes the change of crystalline morphology of the γ-PGA and results in the decrease of its T_m value. Additionally, the endothermic peak of γ-PGA disappeared during the heating of 5′DFUR–PCL–γ-PGA_{22} (Fig. 5C). The DSC measurements suggested that the melting endotherm and enthalpy of fusion in γ-PGA and 5′DFUR–PCL segments of the 5′DFUR–PCL–γ-PGA copolymers affected each other strongly [32,33].

3.5. Critical micelle concentration (CMC)

The formation of micelles was monitored by examining the photophysics of pyrene, a hydrophobic probe that reveals changes in its microenvironment by the changes in its fine emission structure [34]. Pyrene strongly fluoresces in a non-polar environment however it has a weak fluorescence intensity in a polar environment such as water. This method is based on the difference between the fluorescence spectrum of pyrene in water and that of pyrene in the hydrophobic core of polymeric micelles [35,36]. Fig. 6 displays the excitation spectra of pyrene obtained in the presence of various concentrations of 5′DFUR–PCL–γ-PGA_{64} copolymer. The max absorption peak was shifted from 338 nm to 335 nm as the concentration of 5′DFUR–PCL–γ-PGA_{64} copolymer decreased. This shift indicates that pyrene preferentially lies close to or inside the hydrophobic core of the micellar assemblies, which is the evidence of the formation of micelles [34–36]. Fig. 7 plots the CMC of the 5′DFUR–PCL–γ-PGA copolymers. It shows that CMC values of the 5′DFUR–PCL–γ-PGA copolymers are 4.67 mg/L, 2.00 mg/L, 1.58 mg/L and 1.49 mg/L respectively as the γ-PGA content of the 5′DFUR–PCL–γ-PGA copolymer declines.

3.6. Size and zeta potential of micelles

5′DFUR–PCL–γ-PGA micelles were prepared by a precipitation method [28]. 5′DFUR–PCL–γ-PGA copolymer is able to form micelles because of its amphiphilic characteristics. The particle size, size distribution and surface charge of the 5′DFUR–PCL–γ-PGA micelles in deionized water were measured by DLS and zeta potential measurements, as shown in Table 1. In these experiments, the mean diameters of the 5′DFUR–PCL–γ-PGA micelles increased from 130 to 230 nm with increasing feed ratio (5′DFUR–PCL/γ-PGA) of the copolymer at constant γ-PGA content. These findings suggest that the hydrophobic segments in the core determine the size of the 5′DFUR–PCL–γ-PGA micelles [10]. Therefore, the mean diameters of 5′DFUR–PCL–γ-PGA micelles increase with the increasing 5′DFUR–PCL content. The 5′DFUR–PCL–γ-PGA micelles had a highly negative charge in deionized water due to the carboxyl groups in γ-PGA (Table 1). The zeta potentials of 5′DFUR–PCL–γ-PGA micelles were -28.2 ± 2.6 mV, -21.6 ± 4.0 mV, -20.1 ± 3.9 mV and -16.7 ± 1.9 mV respectively. However, some 5′DFUR–PCL–γ-PGA copolymers may show positive charges due to residuals of unactivated EDC (Fig. 8A–C). The γ-PGA content in 5′DFUR–PCL–γ-PGA_{86} is high so that its surface negative charges exceed those of other copolymers. On the other hand, the contents of EDC residues in the 5′DFUR–PCL–γ-PGA_{86} and 5′DFUR–PCL–γ-PGA_{22} copolymers were 15.6 wt.% and 3.5 wt.% respectively (data not shown, determined by ^{1}H NMR analysis). 5′DFUR–PCL–γ-PGA_{22} with more grafted 5′DFUR–PCL has small amount of residual unactivated EDC and so less surface negative charges and almost no positive charges (Fig. 8D).

3.7. Morphology of 5′DFUR–PCL–γ-PGA micelles

TEM was adopted to visualize directly the morphology, characteristics and the size of the micelles. TEM photographs demonstrate that the copolymer micelles are spherical or elliptical (Fig. 9). The well defined core–shell structure in Fig. 9E&F proved that these 5′DFUR–PCL–γ-PGA copolymers could form micelle-like nanoparticles in aqueous solution. However, the particle size estimated from the TEM images is slightly smaller than those determined by DLS analysis. This may be due to the DLS analysis reveals only the hydrodynamic diameter upon swelling in aqueous solution, whereas TEM reveals the diameter of dry micelles [8]. Additionally, elliptical morphologies (Fig. 9F) of the 5′DFUR–PCL–γ-PGA_{22} micelles were formed as the 5′DFUR–PCL content increased, perhaps because of the more the amount of the 5′DFUR–PCL in core of the micelles and the higher the hydrophobicity of the 5′DFUR–PCL [37].

4. Conclusion

In this study, novel comb-like amphiphilic copolymers consisting of hydrophilic segment (γ-PGA) and hydrophobic segment (5′DFUR–PCL) were successfully prepared. Structural analyses such as ^{1}H NMR and FT-IR were performed to confirm their conjugation. Thermal analysis reveals that 5′DFUR–PCL–γ-PGA copolymers have lower melting points than their corresponding homopolymers. Varying the amount of γ-PGA in 5′DFUR–PCL–γ-PGA copolymers would change their hydrophilic abilities. Furthermore, 5′DFUR–PCL–γ-PGA copolymers formed spherical self-aggregated micelles in the aqueous phase, as observed by TEM. The average sizes of the micelles were around 130–230 nm, and could be changed by varying the graft copolymer composition.

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References

[1] Determan MD, Cox JP, Seifert S, Thiyagarajan P, Mallapragada SK. Polymer 2005;46:6933–46.
[2] Deng C, Chen X, Yu H, Sun J, Lu T, Jing X. Polymer 2007;48:139–49.
[3] Kim SY, Shin IG, Lee YM. J Controlled Release 1998;56:197–208.
[4] Ye W, Leung MF, Xin J, Kwong TL, Lee DKL, Li P. Polymer 2005;46:10538–43.
[5] Jeong JH, Kang HS, Yang SR, Kim JD. Polymer 2003;44:583–91.
[6] Gao Z, Eisenberg A. Macromolecules 1993;26:7353–60.
[7] Aliabadi HM, Brocks DR, Lavasanifar A. Biomaterials 2005;26:7251–9.
[8] Lee J, Cho EC, Cho K. J Controlled Release 2004;94:323–35.
[9] Lee RS, Li HR, Yang JM, Tsai FY. Polymer 2005;46:10718–26.
[10] Lee CT, Huang CP, Lee YD. Biomacromolecules 2006;7:1179–86.
[11] Albertsson AC, Varma IK. Biomacromolecules 2003;4(6):1466–86.
[12] Choi C, Chae SY, Nah JW. Polymer 2006;47:4571–80.
[13] Geng Y, Discher DE. Polymer 2006;47:2519–25.
[14] Li YY, Zhang XZ, Cheng H, Zhu JL, Cheng SX, Zhuo RX. Macromol Rapid Commun 2006;27:1913–9.
[15] Guan X, Quan D, Shuai X, Liao K, Mai K. J Polym Sci Part A Polym Chem 2007;45:2556–68.
[16] Li T, Lin J, Chen T, Zhang S. Polymer 2006;47:4485–9.
[17] Nottelet B, Ghzaoui AE, Coudane J, Vert M. Biomacromolecules 2007;8:2594–601.
[18] Deng C, Rong G, Tian H, Tang Z, Chen X, Jing X. Polymer 2005;46:653–9.
[19] Xu N, Du FS, Li ZC. J Polym Sci Part A Polym Chem 2007;45:1889–98.
[20] Barboure E, Papon E, Rodríguez-Hernández J. Polymer 2007;48:3717–25.
[21] Murakami S, Aoki N. Biomacromolecules 2006;7:2122–7.
[22] Liang HF, Chen CT, Chen SC, Kulkarni AR, Chiu YL, Chen MC, et al. Biomaterials 2006;27:2051–9.
[23] Liang HF, Yang TF, Huang CT, Chen MC, Sung HW. J Controlled Release 2005;105:213–25.
[24] Liang HF, Chen SC, Chen MC, Lee PW, Chen CT, Sung HW. Bioconjugate Chem 2006;17:291–9.
[25] Matsusaki M, Hiwatari KI, Higashi M, Kaneko T, Akashi M. Chem Lett 2004;33(4):398–9.
[26] Chang KY, Lee YD. Acta Biomater, in press.
[27] Kunioka M, Furusawa K. J Appl Polym Sci 1997;65(10):1889–96.
[28] Hu Y, Jiang X, Ding Y, Zhang L, Yang C, Zhang J, et al. Biomaterials 2003;24:2395–404.
[29] Zhu Y, Shi M, Wu X, Yang SJ. Colloid Interface Sci 2007;315:580–7.
[30] Kalyanasundoram K, Thomas JK. J Am Chem Soc 1988;99:2039–44.
[31] Meng F, Zheng S, Liu T. Polymer 2006;47:7590–600.
[32] Rong G, Deng M, Deng C, Tang Z, Piao L, Chen X, et al. Biomacromolecules 2003;4:1800–4.
[33] Xie W, Zhu W, Shen Z. Polymer 2007;48:6791–8.
[34] Zhao C, Winnik MA, Riess G, Croucher MD. Langmuir 1990;6:514–6.
[35] Kwon G, Naito M, Yokoyama M, Okano T, Sakurai Y, Kataoka K. Langmuir 1993;9:945–9.
[36] Astafieva I, Zhong XF, Eisenberg A. Macromolecules 1993;26:7339–52.
[37] Zhou Z, Li Z, Ren Y, Hillmyer M, Lodge T. J Am Chem Soc 2003;125:10182–3.