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SHORT COMMUNICATION

Yan Zhang
Changchun Wang
Wuli Yang
Bin Shi
Shoukuan Fu

Tri-component diblock copolymers of poly(ethylene glycol)–poly(ε-caprolactone-*co*-lactide): synthesis, characterization and loading camptothecin

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Y. Zhang · C. Wang · W. Yang · S. Fu (✉)
Department of macromolecular Science of Fudan University and the Key Laboratory of Molecular Engineering of Polymers, Ministry of Education, Shanghai 200433, China
E-mail: skfu@fudan.edu.cn
Tel.: +86-21-65642385
Fax: +86-21-65640293

Y. Zhang
Modern Experiment Technology Center, Anhui University, Hefei 230039, China

B. Shi
School of Pharmacy of Fudan University, Shanghai 200032, China

Abstract Biodegradable tri-component diblock copolymer was synthesized by bulk copolymerization of ε-caprolactone (CL) and D, L-lactide (LA) in the presence of methoxy poly(ethylene glycol) (MePEG), using stannous octoate as catalyst. Their chemical structure and physical properties were investigated by GPC, NMR, DSC, TGAand XRD. The increase of CL/LA ratio in the diblock copolymer leads to lower T_g, higher decomposition temperature and crystallinity. Nanoparticles formulated from MePEG–poly(CL-*co*-LA) (PCAE) possess spherical structure, which was characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). The DLS results indicate that the particle size increased with the increase of CL/LA ratio and the hydrophobic fragment length in the copolymer. The drug encapsulation efficiency and the drug release behavior in vitro conditions of camptothecin were measured by high performance liquid chromatography (HPLC). The encapsulation efficiency can be achieved as high as 84.4% and the release behavior can be made well-controlled. MePEG–poly(CL-*co*-LA) nanoparticles might have a great potential as carriers for hydrophobic drugs.

Keywords Methoxy poly(ethylene glycol)–poly(ε-caprolactone-*co*-lactide) · Diblock copolymer · Nanoparticles · Camptothecin · Drug carrier

Introduction

Most conventional polymeric drug carriers (polymeric nanoparticles) are subjected to rapid elimination from the bloodstream through phagocytosis by cells of the reticuloendothelial system after intravenous administration and recognition by the macrophages of the mononuclear phagocyte system (MPS) [1, 2]. Long-circulating drug-loaded nanoparticles may be used to maintain a required level of a pharmaceutical agent in the blood for extended time intervals for better drug availability [3, 4]. Long-circulating polymer nanoparticles can be obtained from amphiphilic block copolymers modified with hydrophilic, flexible and non-ionic polymers, such as poly(ethylene glycol) (PEG). The non-toxic and non-immunogenic nature of PEG provides a great advantage in utilizing it as a component for medical purposes, and its strong hydration power contributes to regulation of the hydrophilicity of the materials [5–7].

Amphiphilic block copolymers are classified into several types according to sequential arrangement of component segment, such as AB-type diblock copolymer, ABA-type triblock copolymer, $(AB)_n$ type multiblock copolymer and star block copolymer. Compared with other type of amphiphilic block copolymers, AB

type amphiphilic diblock copolymers are the most appropriate candidates for forming polymer nanoparticles in size design, aggregation number, and nanoparticle stability due to simple architecture of their molecules [8]. Methoxy poly(ethylene glycol)–poly(lactic acid) (MePEG–PLA) copolymer can self-assemble into polymeric nanoparticles characterized by a core-shell architecture in aqueous systems, in which a segregated core of associated hydrophobic segments (PLA) is surrounded by a hydrophilic and sterically stabilized shell (PEG), which was used as drug carriers extensively [9–13].

As well known, polycarolactone is a biodegradable and biocompatible polymer with high permability to drugs [14]. Methoxy poly(ethylene glycol)–poly(ε-caprolactone), was much attractive for drug-delivery applications [15–17]. However, it has a slow rate of biodegradation in human tissues due to the high hydrophobicity and crystallinity of poly(ε-caprolactone) fragment [14]. For the controlled delivery of bioactive agents, it is necessary to adjust carefully both drug release rate and polymer degradation properties to achieve desired formulation properties. Copolymerization is a simple and most widely used approach for modifying polymer properties to meet specific requirements.

In previous studies, much attention was paid to the drug-loaded nanoparticles with different hydrophobic/hydrophilic ratio, different interactions between the drug and carriers, different drug-release behavior and triblock copolymers [18–22], few works were done to modify the hydrophobic segment of diblock copolymers. In this paper, AB type diblock copolymers composed of poly(CL-*co*-LA) (A) and MePEG (B) segments were synthesized, and a wide range of copolymers with various properties were obtained by adjusting the ratio of CL/LA in the hydrophobic block. A series of physicochemical characterization was carried out in order to study the properties of the MePEG–poly(CL-*co*-LA) copolymers and confirm the formation of compolymer nanoparticles. Camptothecin (CPT), one of the best anticancer drugs [23–25] was encapsulated into MePEG–poly(CL-*co*-LA) copolymer nanoparticles as model drug.The release of CPT from these nanoparticles was investigated to identify that the nanoparticles could serve as useful carriers of hydrophobic drugs, and the drug-release behavior is releated to the composition and structure of the copolymers.

Experimental

Materials

Methoxy poly(ethylene glycol) (MePEG, $M_n = 5{,}000$) was supplied by Aldrich and dried under vacuum in a desiccator with P_2O_5 overnight before use. D, L-lactide (purity, 99.5%) was purchased from PURAC and purified by twice recrystallization from dried ethyl acetate. ε-caprolactone (ε-CL, Aldrich) was purified by drying over CaH_2 and distilled under reduced pressure. Stannous octoate (stannous content, 26.5–27.5%) was supplied by Shanghai Chemical Reagent Company and distilled before use. CPT was purchased from Fudan Zishan New Technology Co. (Shagnhai, China). All other chemicals used were reagent grade and used without further purification. Cellulose dialysis bag (molecular weight cut off, 14,000) was supplied by Luniao Technology Co.

Synthesis of MePEG–poly(CL-*co*-LA) copolymer

The copolymers made of MePEG, D, L-lactide and ε-CL block were synthesized by a bulk polymerization procedure. In brief, a predetermined amount of MePEG (0.5 g), and D, L-lactide (4.50 g) were placed in a dried round-bottomed bottle connected with a vacuum joint, and the appropriate amount of stannous octoate (0.5%) was added as a solution (40%) in dried toluene. The reactants were dried under reduced pressure at 70 °C for 1 h. A predetermined ε-CL (0.5 g) was added by a syringe, and then the reaction was allowed to proceed under vacuum at 160 °C for 4 h. Detailed recipes are given in Table 1. The cooled product was dissolved in dichloromethane, recovered by precipitation into an excessive

Table 1 Molecular weight and chemical composition of MePEG–poly(CL-*co*-LA) copolymers

Sample	Feed MePEG (wt%)	[CL]/[LA] (mol%)		M_n^a (×10^4)	M_n^b (×10^4)	M_w/M_n^b
		Feed	Product			
PCAE1	9.0	6/94	5.9/94.1	4.75	1.95	1.7
PCAE2	9.0	22/78	20.7/79.3	4.84	2.11	1.8
PCAE3	9.0	38/62	38.2/61.8	4.93	2.13	1.9
PCAE4	9.0	60/40	59.7/40.3	5.24	2.24	1.9
PCAE5	9.0	85/15	86.9/13.1	4.35	2.74	1.6
PCAE6	14.3	22/78	19.0/81.0	2.96	1.50	1.5
PCAE7	20.0	22/78	20.6/79.4	2.07	1.34	1.4

[a]Number average molecular weight calculated by NMR[b]Obtained by GPC with respect to polystyrene standards

amount of methanol. The precipitates were filtered out and dried at 40 °C under vacuum for 2 days, and then stored in a desiccator under vacuum. The copolymer product was abbreviated as PCAE.

Preparation of polymeric nanoparticles

The PCAE copolymer nanoparticles were prepared by dialysis method. Copolymer (8 mg) was dissolved in 1 mL acetone, then 4 mL water was added to induce micellization under agitation. The aqueous solution was placed in a dialysis bag and dialyzed against doubly distilled water for 24 h to remove acetone.

To prepare the CPT-loaded PCAE nanoparticles, 100 mg PCAE copolymer and 5 mg CPT were dissolved in 5 mL tetrahydrofuran and dimethylsulfoxide (v/v, 4/1) co-solvent and then the organic phase was dropped into 30 mL water under moderate stirring. The organic solvent was removed by dialysis against doubly distilled water for 24 h.

In vitro release of CPT-loaded nanoparticles

The release test of the CPT in vitro was performed by incubating approximately 1 mL CPT-loaded nanoparticles in a dialysis bag and immersed in phosphate buffer solution (0.1 M, pH 7.4). The entire system was kept at 37 ± 1 °C with horizontal shaking at about 60 rpm. At selected time intervals, 5 mL of the release medium was withdrawn from the media and the same volume of fresh dissolution medium was added. The aliquotos were acidified with 0.1 M HCl, and 20 μL was used to analyze by HPLC.

Measurement

Polymer characterization

The molecular weight and molecular weight distribution of PCAE copolymers were characterized using GPC (HP1100, Hewlett Packard) with tetrahydrofuran as eluent at a flow rate of 1 mL/min. ^{1}HNMR spectra of the copolymers in deuterated chloroform solution were recorded on a 500 MHz NMR (DMX-500, Bruker) with tetramethylsilane as internal standard to determine the chemical structure and molecular weight of the copolymers. DSC measurements were carried out under nitrogen atmosphere at a heating rate of 10 °C/min on a Perkin Elmer Instrument DSC7 thermal analyzer. Thermogravimetric analysis was run on Perkin Elmer Instrument (TGA, Pyrolysis-1, PE) from 40 °C to 500 °C at a heating rate of 10 °C/min, and the decomposition temperature (T_d) was obtained. X-ray diffraction patterns of the copolymers were recorded on a D/max-rB X-ray diffractionmeter (Rigaku) equipped with a graphite monochromator-filtered Cu–K_α radiation.

Particle size and morphology of the nanoparticles

The hydrodynamic radii of nanoparticles were determined by dynamic light scattering (DLS) measurement at 25 °C using a light scattering spectrophotometer (Autosizer 4700, Malvern) with a vertically polarized incident beam at 532 nm supplied by an argon ion laser, and scattering angle 60°, 80°, 90°, 100° and 120° were used in this study. Before measurement, all samples were filtered through a 0.45 μm filter (Millipore). Nanoparticles morphology was performed on a transmission electron microscopy (TEM) (Hitachi H-600).

HPLC analysis of camptothecin

To determine the drug content, a predetermined aliquot of nanoparticles suspension was withdrawn and frozen, then lyophilized by freeze dryer system to obtain dried nanoparticle product. The CPT content of these nanoparticles was determined by HPLC analysis [26]. HPLC was based on a Hypersil ODS (USA 5 μm), 150×4.6 mm column with isocratic elution (65:35 mixture of aqueous triethylamine-acetate buffer (pH 5.5) and acetonitrile at a flow rate of 0.55 ml/min) and detection of camptocin by a Shimadzu UV detector. CPT was monitored at 360 nm. Drug loading, content and encapsulation efficiency were obtained by Eqs. 1 and 2.

$$\text{Drug loading content (mg/g)} = \frac{\text{Weight of the drug in nanoparticles (mg)}}{\text{Weight of the nanoparticles (g)}} \quad (1)$$

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Weight of the drug in nanoparticles (mg)}}{\text{Weight of the feeding drugs}} \times 100 \quad (2)$$

Results and discussion

The composition and molecular weight of MePEG–PLA–PCL block copolymers

A series of amphiphilic diblock copolymers, MePEG–poly(CL-*co*-LA) (PCAE), were synthesized by a ring

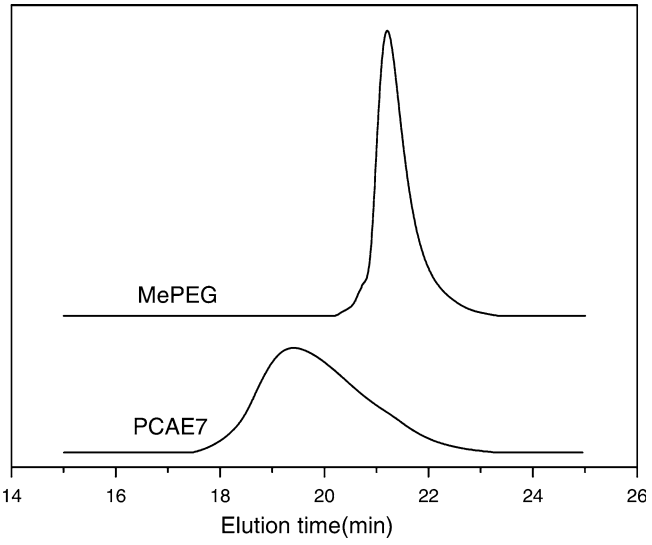

Fig. 1 Typical GPC chromatogram of the synthesized copolymer (sample PCAE7) and MePEG

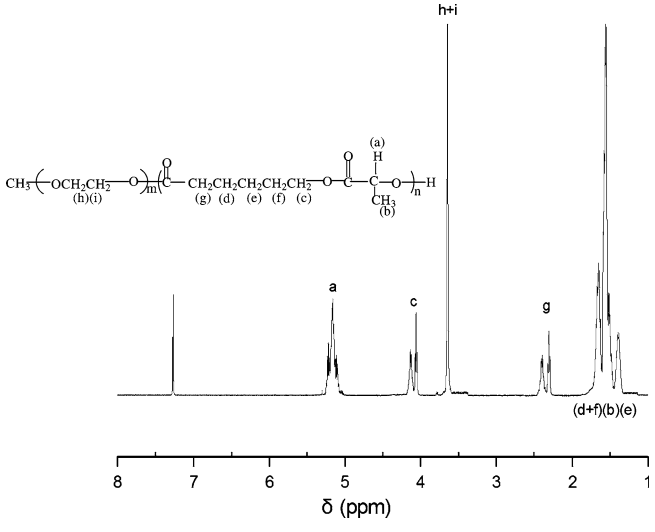

Fig. 2 The ^{1}HNMR spectrum of MePEG–poly(CL-*co*-LA) block copolymer in deuterated chloroform, Sample PCAE2

opening copolymerization of D, L-lactide and ε-caprolactone initiated with the hydroxyl group of methoxy poly(ethylene glycol) using stannous octoate as catalyst. Figure 1 shows the GPC results of a representive copolymer (PCAE7) and the precursor MePEG5000. Compared with MePEG5000, there is a clear shift to high molecular weights for PCAE7 copolymers, which had a symmetric peak and a relatively narrow polydispersitiy. All of these data indicated that the synthesis of the diblock copolymers were succesful. In order to gain insight into their chemical structure, the various PCAE copolymers were subjected to ^{1}HNMR measurements. A typical spectrum of PCAE2 is shown in Fig. 2. Typical signals of PLA, PCL, MePEG components were observed: signals at 1.5(−CH_3) and 5.1 ppm(−CH) were assigned to PLA segments, 1.3, 1.6, 4.0 ppm to the different methylene protons (–CH_2) of PCL segments, and 3.6 ppm(–CH_2) to PEG blocks, respectively [27, 28]. The [CL]/[LA]/[EO] molar ratio of the copolymers was determined from the integrations of the signals due to PEG blocks at 3.6 ppm, PCL segments at 4.0 ppm and PLA segments at 5.1 ppm.

Table 2 Thermal properties of MePEG–poly(CL-*co*-LA) block copolymers

Sample	T_g (°C)	T_{d1}^{a} (°C)
PCAE1	16.4	216
PCAE2	4.7	222
PCAE3	−20.3	228
PCAE4	−33.9	253
PCAE5	−44.2	283

[a]Data obtained with respect to 5% weight loss

Table 1 summarizes the molecular characteristics of these polymers. The M_n values calculated from GPC were lower than those from NMR spectra, which could be assigned to changes of hydrodynamic volume of MePEG–poly(CL-*co*-LA) copolymers bearing both hydrophilic PEG and hydrophobic PCL and PLA segments, as compared with the parent homopolymers [29].

Thermal properties of the copolymers

For T_g measurements, all samples were scanned from −70 °C to 100 °C for twice, the heating rate were 10 °C/min, and T_g values were obtained from the second scan. As shown in Table 2, through introducing the CL segment into the copolymer chains by random copolymerization of CL and LA, T_g was decreased a considerable amount, which is attributed to the CL segment enhanced the copolymer chain mobility greatly.

The TGA traces were obtained by heating the samples from 50 °C to 500 °C, at a heating rate of 10 °C/min in nitrogen. The first decomposition temperature (T_{d1}) was read with respect to 5% weight loss of the samples, as shown in Table 2. The thermal stability of PCAE copolymer depends on the intrinsic properties of the materials, which is one important property for practical applications, always increases with the CL content in the copolymers. The TGA curve for PCAE2 is shown in Fig. 3. The T_d and ΔW values suggest that the first degradation step was due to poly(CL-*co*-LA) blocks and the second to the PEG portion [30, 31]. Conse-

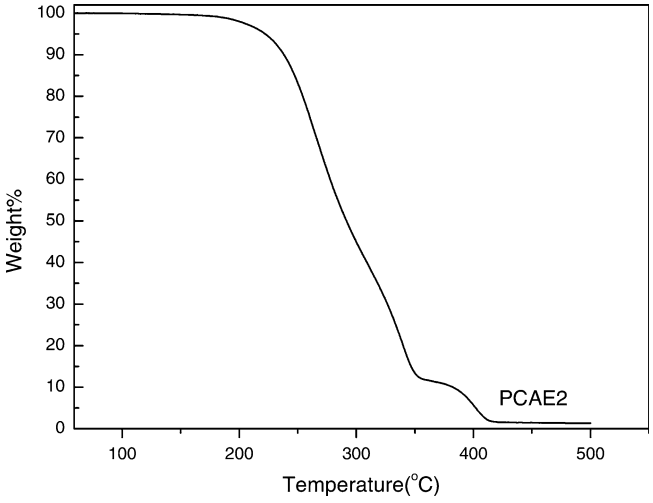

Fig. 3 Typical TGA trace of MePEG–poly(CL-*co*-LA) copolymer, sample PCAE2

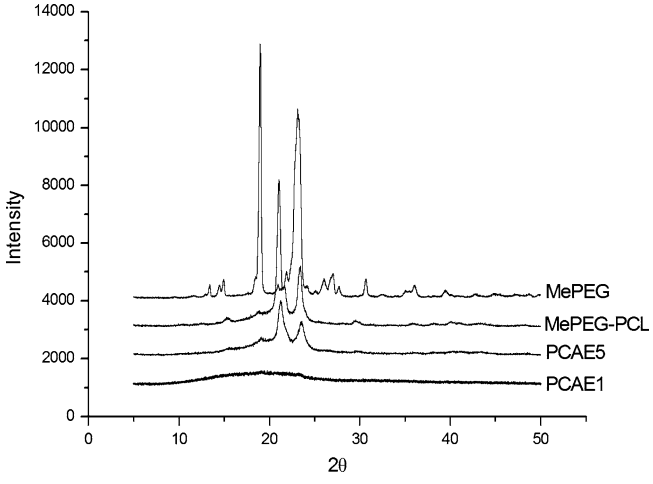

Fig. 4 X-ray diffraction patterns of MePEG, MePEG–PCL, PCAE5, PCAE1

quently, for tri-component di-block copolymer (MePEG–poly(CL-*co*-LA)), we could draw a conclusion that CL and LA are copolymerized randomly not blocky in the copolymer because only two degradation processes are detected.

X-Ray diffraction (XRD) analysis of the copolymers

Figure 4 depicts the X-ray diffraction patterns of the block copolymers in comparison with those of MePEG, MePEG–PCL. The PEG diffraction patterns exhibited two main peaks at $2\theta = 18.8°$ and 23.0°, MePEG–PCL shows an intense peak at $\theta = 21.2°$ and 23.6° [28], no characteristic diffraction peaks of PEG were well resolved in the spectra of the PCAE copolymers because the copolymers consisted of a relatively higher fraction of PCL units. When amorphous LA was introduced into the copolymer, the crystalline microstructure of the copolymers was not well developed, the intensity of the diffraction peaks with respect to the diffusion background greatly decreased (PCAE5). With more and more PLA being introduced, the crystallization structure of PCL was disrupted completely (PCAE1), no characteristic diffraction peaks were observed in the spectra.

Table 3 Hydrodynamic diameter of MePEG–poly(CL-*co*-LA) copolymer nanoparticles, scattering angle is 90°

Nanoparticles	Copolymer	Particle size (nm)	Polydispersity
NPCAE1	PCAE1	143	0.10
NPCAE2	PCAE2	167	0.20
NPCAE5	PCAE5	179	0.23
NPCAE6	PCAE6	135	0.22
NPCAE7	PCAE7	114	0.31

Particle size and morphology

The particle size and size distribution were measured by DLS. The mean diameters of the copolymer nanoparticles, which the scattering angle was 90°, are listed in Table 3. From Table 3, when the contents of MePEG were maintained constant, it could be seen that the mean diameter decreases with the ratio of CL/LA decreasing in the copolymers (NPCAE5 → NPCAE2 → NPCAE1, also see Table 1). When there was no change with the ratio of CL/LA in the copolymers, the more the MePEG in the copolymer, the smaller the nanoparticles were (NPCAE2 → NPCAE6 → NPCAE7). The preparation conditions and the MePEG content are same, so the diameters of the nanoparticles are determined by the chemical composition of the core, the more hydrophobic of the core, the bigger the nanoparticles were [32, 33]. PCL is more hydrophobic than PLA, so when the PCL content in the copolymer increased, the mean diameter also increased. Accordingly, the MePEG was more in the copolymer, the poly(CL-*co*-LA) fragment will have lower molecular weight, and the hydrophibic content was less in the copolymer. Consequently, the core was smaller and the diameter of the nanoparticles became smaller. When changing the scattering angle, the diameters of the nanoparticles are almost the same (Fig. 5), it could be concluded that the nanoparticles had a regular spherical shape and hold narrow size distribution on the nano scale (the system error is ± 5 nm).

The TEM measurement was carried out to confirm it (Fig. 6a). It could be seen that the nanoparticles had a

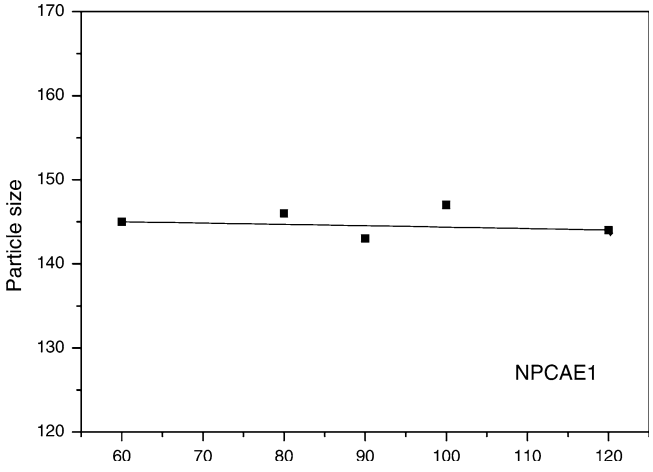

Fig. 5 Angular DLS measurement of MePEG–PCL–PLA nanoparticles, sample NPCAE1

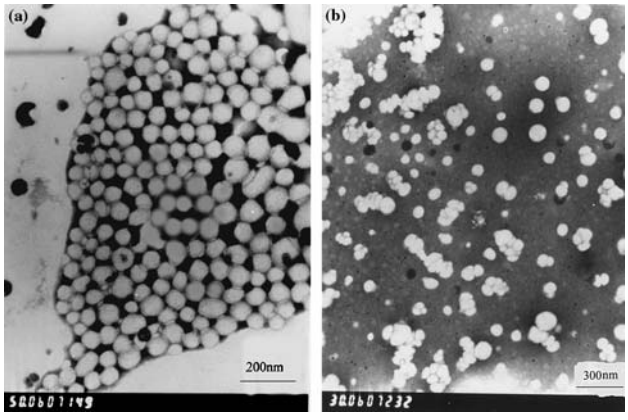

Fig. 6 TEM image of MePEG–PCL–PLA nanoparticles: **a** NPCAE1; **b** DPCAE6

regular spherical shape, which also proved that these block copolymers could form micelle-like nanoparticles in water.

In vitro release behavior of CPT-loaded nanoparticles

Camptothecin has a very poor solubility in water and most organic solvents, which leads to the limit of the application. In this study, CPT was loaded into the nanoparticles by dialysis method. When CPT was loaded into the nanoparticles, from TEM and DLS results, they still preserved spherical shape (Fig. 6b) and were nano scale (Table 4). However, from TEM image, some aggregation were observed, which may attribute to the lower stability of the drug-loaded nanoparticle. The drug-loading content could get high

Table 4 Characteristics of the CPT-loaded nanoparticles

CPT-loaded nanoparticles	Copolymer	Particle size	DLE (mg/g)[a]	EE (%)[b]
DPCAE2	PCAE2	167	42.2	84.4
DPCAE5	PCAE5	186	34.9	69.8
DPCAE6	PCAE6	121	18.4	36.8

[a]Drug loading efficiency
[b]Encapsulation efficiency

as 42.2 mg/g and the encapsulation efficiency reached 84.4%. Thus, CPT solubility in the aqueous nanoparticle suspension would be improved greatly. Figure 7 shows that the copolymer nanoparticles could retard CPT release, and the release behavior was significantly affected by the chemical composition of the copolymers, which may be assigned to that T_g and crystallinity affected the release of the drug molecules to some extent. Izumikawa et al. [34] have reported that the crystallinity of the polymer matrixes greatly affected the drug-release rate. From Fig. 7, the higher the crystallinity (PCAE5 > PCAE2) of the polymer matrix was, the faster the CPT released from the nanoparticles, because the higher crystallinity could lead to forming a microchannel structure and higher surface area in the polymer matrix, and make the drug easily released from nanoparticles. Nanoparticles formed from the copolymers with low crystallization behavior would be soft and deformable at body temperature in aqueous medium, where the hydrophilic PEG shell is in its solvation state. Deformable nanoparticles are more capable of surviving the splenic filtration than rigid ones. T_g also affected the release of the drug molecules to some extent. T_g of PCAE5 copolymer is lower than that of PCAE2 copolymer, however, the release rate of PCAE5 is higher due to that the movement of the small drug molecules in a relatively mobile polymeric matrix is much easier than in a glassy core [35]. Crystallinity and T_g can be tailored via adjusting the CL/LA ratio in the copolymes. We also observed that the release rate decreased as the hydrophobic/hydrophiphilic ratio (i.e. PCAE2 > PCAE6) increased, which is attributed to that the nanoparticles have a bigger hydrophobic cores and small surface area, the drug would spend more time to diffuse across the matrix to the surface [16].

Conclusion

Biodegradable MePEG–poly(CL-*co*-LA) copolymers were synthesized by ring open polymerization in bulk, and stealthy nanoparticles were prepared by the dialysis method for controlled release of an anticancer drug CPT. The properties of the copolymers were deter-

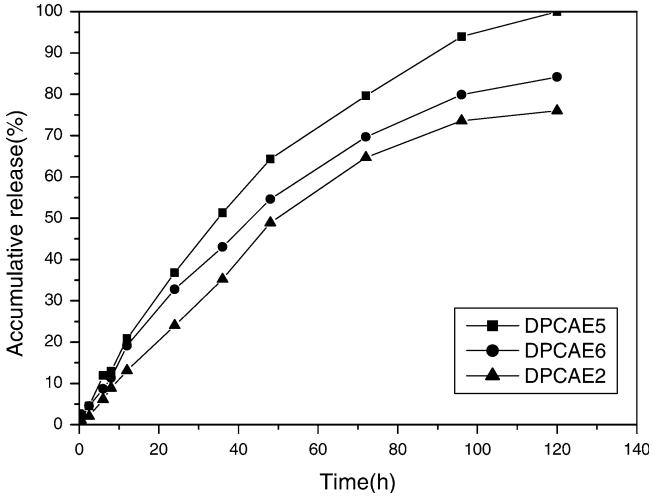

Fig. 7 Camptothecin release profiles from MePEG–poly(CL-*co*-LA) nanoparticles

mined by the chemical composition. The size and size distribution of the prepared nanoparticles and the drug-release behavior are affected significantly by the composition of the polymeric matrix. These results show that the copolymers of CL, D, L-lactide are promising materials for preparing nanoparticles as drug carriers and the release rate from nanoparticles can be adjusted by changing the composition of polymer matrix. This research may have potential to provide an alternative dosage form for CPT, one of the best anticancer drugs found from the nature in the past decades and commercially, the most successful anticancer agents.

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