Preparation of surfactant-free nanoparticles of methoxy poly(ethylene glycol)-b- poly(ε-caprolactone-co-D,L-lactide) diblock copolymers

Presented in The 2nd Progress in Advanced Materials: Micro/Nano Meterials and Applications

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Abstract

Surfactant-free nanoparticles of methoxy poly(ethylene glycol)-*b*-poly(ε-caprolactone-*co*-D, L-lactide) diblock copolymers [MPEG-*b*-P(CL-*co*-DLL)] were prepared by modified-spontaneous emulsification-solvent diffusion method. The diblock copolymers were synthesized by ring-opening polymerization of CL and DLL using MPEG with molecular weight of 5,000 g/mol and stannous octoate as the initiating system. Influences of CL:DLL ratios of the diblock copolymers on characteristics of the nanoparticles were investigated and discussed. The sizes of colloidal nanoparticles from light - scattering analysis were in the range of 84 - 639 nm. The nanoparticle sizes decreased with increasing the DLL ratio. Transmission electron microscopy demonstrated that the almost nanoparticles have spherical shape with smooth surface.

Keywords: Biodegradable polymers, MPEG-*b*-poly(ε-caprolactone), MPEG-*b*-poly(ε-caprolactone-*co*-D,L-lactide), spontaneous emulsification solvent diffusion method, nanoparticles

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1. Introduction

Poly(ε-caprolactone) (PCL) and poly (D,L-lactide) (PDLL) are biodegradable and biocompatible polymers that are widely used in medicine and pharmaceutical applications. Diblock copolymers composed of methoxy poly(ethylene glycol) (MPEG) and PCL/PDLL have been synthesized to attain versatile biodegradable polymers having more water-absorbing capacity because of the inclusion of hydrophilic MPEG segment within the relative hydrophobic PCL (He et al., 2004; Shuai et al., 2004; Aliabadi et al., 2005; Hyun et al., 2006) / PDLL (Kim et al., 1998; Kim et al., 2005; De Faria et al., 2005) / P(CL-co-DLL) (Zhang et al., 2005) segments. These diblock copolymers have been used for the preparation of drug-loaded nanoparticles (Shuai et al., 2004; Aliabadi et al., 2005; Kim et al., 1998; Kim et al., 2005; De Faria et al., 2005; Zhang et al., 2005). The nanoparticles have shown potential as drug delivery systems because of the small size of nanoparticles, which improves circulation times in the body and creates more available routes of administration than do microparticles, which are rapidly cleared by the reticulo-endothelial tissue (Kumar, 2000).

The modified spontaneous emulsification-solvent diffusion method (modified-SESD method) for nanoparticle preparation was proposed first by Murakami et al. (1999). Poly(D,L-lactide-co-glycolide) was dissolved in volatile water-miscible organic solvents, acetone and ethanol instead of acetone and dichloromethane, which were used in the original SESD method (Niwa et al., 1993). Higher energy apparatus, such as a homogenizer or a sonicator (usually applied in larger scale preparation of polymer nanoparticles), was not used for this technique. However, preparation of nanoparticles of MPEG-*b*-P(CL-*co*-DLL) diblock copolymers by the modified-SESD method has not been studied.

Therefore, the aims of this study were to attain MPEG-*b*-P(CL-*co*-DLL) nanoparticles and to investigate the influences of CL:DLL ratio on the nanoparticle characteristics. The size and size distribution of the prepared nanoparticles can be characterized by using the light-scattering apparatus. Meanwhile, the surface and the morphological analyses will be studied by using transmission electron microscopy (TEM).

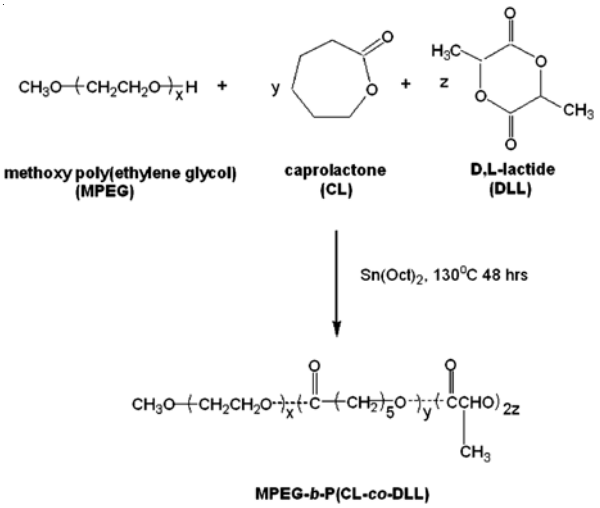
2. Experimental

2.1. Materials

Methoxy poly(ethylene glycol) (MPEG) with a molecular weight of 5,000 g/mol (Fluka, Germany) was used after it was dried in vacuo at 120°C for 4 hrs. The ε-caprolactone) (CL) monomer (99%, Acro, USA) was purified by drying with CaH_2 followed by distillation under reduced pressure before storage over molecular sieves in a refrigerator. D,L-lactide (DLL) was synthesized by well-established procedures from D,L-lactic acid (90%, Fluka, Switzerland). It was purified by repeated recrystallization from ethyl acetate and dried in vacuo at 50°C for 48 hrs before used. The stannous octoacte ($Sn(Oct)_2$, 95%, Sigma, USA), acetone (AR, Merck, Germany) and ethanol (AR, Merck, Germany) were used without further purification.

2.2. Synthesis of MPEG-b-P(CL-co-DLL)

The diblock copolymers with different CL:DLL monomer feed mole ratios (100:0, 90:10, 80:20 and 50:50 mol%) were each synthesized in bulk at 130°C for 48 hrs under a dry nitrogen atmosphere, as represented in Scheme 1.



Scheme 1. Polymerization reaction of the MPEG-*b*-P(CL-*co*-DLL).

The molecular weights of the P(CL-*co*-DLL) blocks were approximately 60,000 g/mol. MPEG and $Sn(Oct)_2$ were used as the initiating system with the $Sn(Oct)_2$ concentration kept constant at 0.04 mol%. The as-polymerized diblock copolymers were purified by dissolving in chloroform before being precipitated in cool n-hexane. Finally, they were dried to constant weight in a vacuum oven at room temperature before characterization and nanoparticle preparation.

2.3. Characterization of MPEG-b-P(CL-co-DLL)

Copolymer composition and number-average molecular weight ($\overline{M}_n$) of the MPEG-*b*-P(CL-*co*-DLL) were determined by ^1H magnetic resonance imaging (MRI) spectrometry using a Bruker Advance DPX 300 ^1H-MRI spectrometer. $CDCl_3$ was used as a solvent at room temperature, and tetramethylsilane was used as the internal standard. The thermal properties of polymers were characterized by nonisothermal differential scanning calorimetry (DSC) using a Perkin-Elmer Pyris Diamond DSC. For, DSC, the approximate 10 mg of sample was heated at the rate of 10°C/min under helium flow to observe thermal transition temperatures.

2.4. Preparation of MPEG-b-P(CL-co-DLL) nanoparticles

The nanoparticles of MPEG-*b*-P(CL-*co*-DLL) without the addition of the surfactant were prepared according to the modified-SESD method (Murakami *et al.*, 1999). This procedure was explained as follows.

Approximate 0.4 g of diblock copolymer was dissolved in 20 mL of the 1/1 (v/v) acetone/ethanol organic mixture. The solution was added dropwise into 160 mL distilled water in a 250-mL beaker with stirring at 600 rpm. Organic solvents were evaporated at room temperature for 6 hrs in a fume hood. Then, the nanoparticle colloid was obtained.

2.5. Characterization of MPEG-b-P(CL-co-DLL) nanoparticles

Particle sizes and size distributions of the nanoparticle colloids were determined by light-scattering analysis using a Coulter LS230 light scattering particle size analyzer at 25°C. Morphology of the nanoparticles was investigated by transmission electron microscopy (TEM) (JEOL JEM 1230).

3. Results and discussion

3.1. Characterization of MPEG-b-P(CL-co-DLL)

All purified diblock copolymers had percentage yields higher than 90%. The compositions of diblock copolymers were determined from their ^{1}H-MRI spectra by ratioing the peak areas corresponding to the ethylene oxide (EO) methylene protons at δ = 3.4-3.6 ppm, the CL ε-methylene protons at δ = 3.9-4.2 ppm and the DLL methine protons at δ = 5.0-5.3 ppm. The ^{1}H-MRI spectrum of the MPEG-*b*-P(CL-*co*-DLL) diblock copolymer with CL:DLL ratio of 50:50 mol% is shown as example in Figure 1 and the calculated compositions of CL:DLL and EO:CL:DLL mol% are given in Table 1. As would be expected, the copolymer compositions are very similar to the comonomer feed ratios, indicating that the synthesis reactions proceeded to near-quantitative conversion.

Molecular weight characteristics of the diblock copolymers were carried out by mean of EO:CL:DLL ratios from ^{1}H-MRI spectra. The molecular weight characteristics are also summarized in Table 2. It was found that the $_n$ of all of the diblock copolymers obtained from ^{1}H-MRI are similar to that obtained from the feed ratios (65,000 g/mol).

The chain microstructures of P(CL-*co*-DLL) blocks are reflected in the fine structures of the ^{1}H-MRI spectra. The appearance of multiple resonances for the same proton can be attributed to the presence of difference monomer sequences and therefore slightly different chemical environments in the copolymer chain. The α-CH_2 and ε-CH_2 protons in the CL units are seen to be particularly sensitive to this. The bands at 2.4 and 4.1 ppm, corresponding to the α-CH_2 and ε-CH_2 protons in the CL units, respectively are split into to quit distinct triplets adjacent to one another suggesting randomization of the CL units in the copolyester blocks (Baimark and Molloy, 2004).

Thermal analysis of the diblock copolymers was carried out by means of differential scanning calorimetry (DSC). The DSC thermograms of the diblock copolymers are shown in Figure 2 and their thermal properties obtained from DSC curves are summarized in Table 2. The T_m and ΔH_m of the diblock copolymer decreased when the DLL units were copolymerized. However, the T_m of diblock copolymer with CL:DLL ratio of 50:50 mol% can not be observed suggested that it is an amorphous polymer. The results indicating that the crystallizability of the PCL block was suppressed when the DLL ratio increased up to 50 mol%.

Table 1 Copolymer compositions and $\overline{M}_n$ of the diblock copolymers.

Diblock copolymer (CL:DLL)	CL:DLL (mol%)		EO:CL:DLL (mol%)		$\overline{M}_n$[b] (g/mol)
	Feed ratio[a]	^{1}H-MRI[b]	Feed ratio[a]	^{1}H-MRI[b]	
100:0	100:0	100:0	20:80:0	18:82:0	62,300
90:10	90:10	91:9	20:72:8	18:75:7	65,600
80:20	80:20	81:19	20:64:16	19:66:15	63,200
50:50	50:50	49:51	20:40:40	20:39:41	64,000

[a] Calculated from comonomer feed ratios

[b] Calculated from ^{1}H-MRI spectra

Table 2 Thermal properties and particle sizes of the diblock copolymers.

Diblock copolymer (CL:DLL)	T_m[a] (°C)	ΔH_m[a] (J/g)	Particle size[b] (nm)
100:0	52	71.0	639 ± 213
90:10	46	52.2	595 ± 159
80:20	39	39.2	457 ± 72
50:50	-	-	84 ± 33

[a] Measured from DSC thermograms

[b] Measured from light-scattering analysis

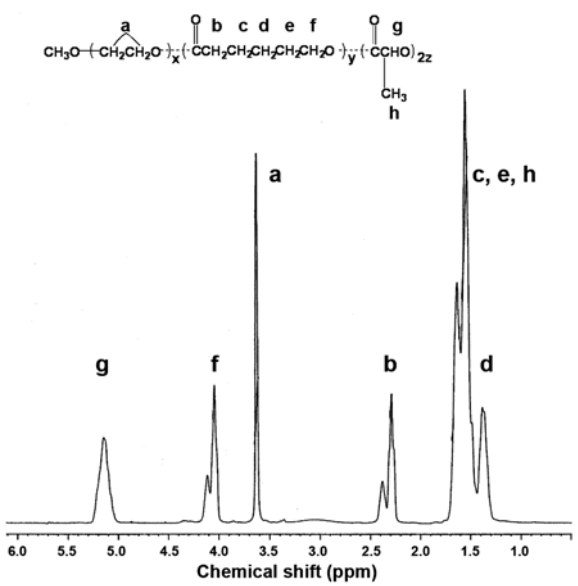


Figure 1. ^{1}H-MRI spectrum of MPEG-*b*-P(CL-*co*-DLL) with CL:DLL ratio of 50:50 (mol%).

3.2. Characterization of MPEG-b-P(CL-co-DLL) nanoparticles

All surfactant-free nanoparticle colloids of the diblock copolymers show clear aqueous suspensions. The particle size results of diblock copolymers obtained from light-scattering analysis are reported in Table 2. The particle sizes were in the range of 84-639 nm. The results could be proposed that the MPEG block of diblock copolymer can be formed as shell of nanodroplets during the solvent diffusion process, and its protective effect is adequate, then nanocolloids could be formed as core-shell structure before solidification. This suggested that the mechanism of modified-SESD method can be used to prepare surfactant-free nanoparticles of the hydrophilic-hydrophobic diblock copolymers without any surfactant used. The sizes and size distributions of the nanoparticles were increased with decreasing

the DLL ratio. This may be explained that the diblock copolymers containing higher crystallizable PCL blocks give faster solidification of nanodroplets during solvent diffusion process. For proposed mechanism of the modified-SESD method, the droplet sizes of copolymer solution were reduced in the solvent diffusion stage. Therefore, the diblock copolymers with higher CL ratio show larger particle sizes.

From TEM images, the dried nanoparticles morphology show nearly spherical shape and smooth surface as shown in Figure 3. The morphology result is more clearly illustrated as example of which is show in Figure 4 for the diblock copolymer with CL:DLL ratio of 50:50 mol%. It should be note that this method is easy for larger scale preparation of polymer colloidal nanoparticles than the other methods and it is important that the surfactant-free nanoparticles can be prepared for used in pharmaceutical and food coating applications.

4. Conclusions

The MPEG-*b*-P(CL-*co*-DLL) diblock copolymers with different CL:DLL ratios were successfully synthesized by ring-opening polymerization of CL and DLL monomers by using MPEG with molecular weight of 5,000 g/mol and stannous octoate as the initiating system at 130°C for 48 hrs. The $\overline{M}_n$ of diblock copolymers were in the range of 62,000 - 65,000 g/mol. The thermal properties of diblock copolymers were strongly depended upon the CL:DLL ratios. The surfactant-free nanoparticles of diblock copolymers with particle sizes in the range of 84-639 nm can be prepared by the modified-SESD method. The nanoparticle size decreased with increasing the DLL ratio. Almost nanoparticles were spherical shape with smooth surface. These promising results encourage the further development of surfactant-free biodegradable nanoparticles forming as novel drug delivery systems.

Acknowledgement

The authors would like to acknowledge the Research Development and Support Unit, Mahasarakham University, fiscal year 2008 for financial support.

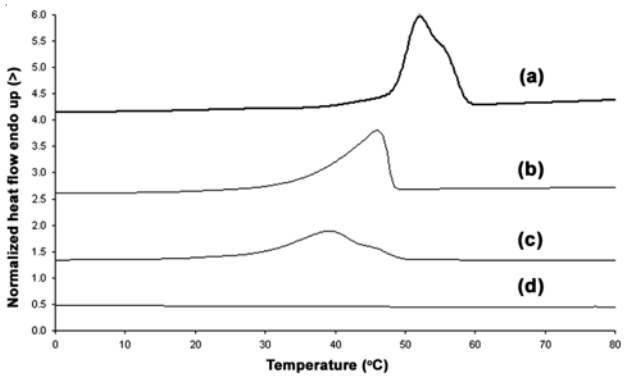


Figure 2. DSC thermograms of the MPEG-*b*-P(CL-*co*-DLL) with CL:DLL ratios of (a) 100:0, (b) 90:10, (c) 80:20 and (d) 50:50 (mol%).

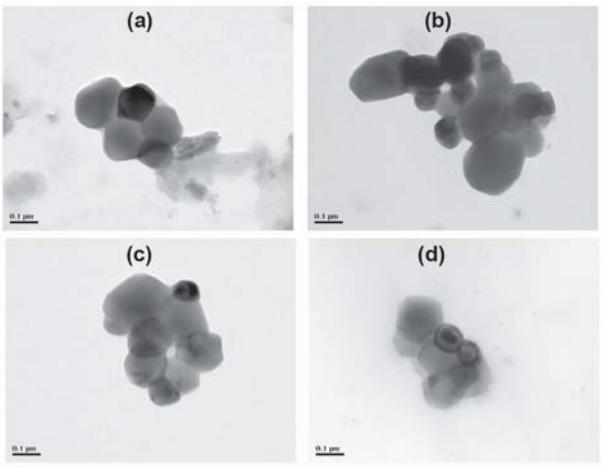


Figure 3. TEM micrographs of the MPEG-*b*-P(CL-*co*-DLL) with CL:DLL ratios of (a) 100:0, (b) 90:10, (c) 80:20 and (d) 50:50 (mol%).

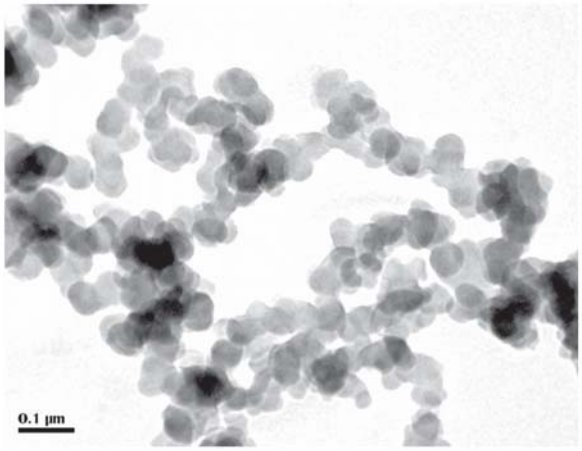


Figure 4. TEM micrograph of the MPEG-*b*-P(CL-*co*-DLL) with CL:DLL ratio of 50:50 (mol%).

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