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Preparation of Surfactant-Free and Core-Shell Type Nanoparticles of Methoxy Poly(ethylene Glycol)-*b*-Poly(ε-caprolactone-*co*-d,l-lactide) Diblock Copolymers

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Abstract: Methoxy poly(ethylene glycol)-*b*-poly(ε-caprolactone-*co*-D,L-lactide) diblock copolymers [MPEG-*b*-P(CL-*co*-DLL)] were synthesized by ring-opening polymerization of CL and DLL using MPEG with molecular weight of 5,000 g mol^{-1} and stannous octoate as the initiating system. Surfactant-free and core-shell nanoparticles of MPEG-*b*-P(CL-*co*-DLL) were prepared by modified-spontaneous emulsification solvent diffusion method without any surfactants. Influences of CL:DLL ratio of the diblock copolymers on nanoparticle characteristics were investigated and discussed. The sizes of colloidal nanoparticles obtained from light-scattering analysis were in the range of 84-639 nm. The nanoparticle size decreased with increasing the DLL ratio. Scanning and transmission electron micrographs indicated that the nanoparticles were spherical in shape and smooth surface. Core-shell structure of the nanoparticles consisting of the hydrophilic outer shell of MPEG and the hydrophobic inner core of P(CL-*co*-DLL) was confirmed by ^{1}H-NMR spectroscopy.

Key words: Biodegradable polymers, MPEG-*b*-poly(ε-caprolactone-*co*-D,L-lactide), modified-spontaneous emulsification solvent diffusion method, nanoparticles, core-shell structure

INTRODUCTION

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 (Kumar, 2000). Moreover, the micelles and nanoparticles consisted of hydrophobic core and hydrophilic shell of hydrophobic-hydrophilic diblock copolymers have more interested due to the hydrophilic shell is generally accepted to minimize the adsorption of circulating blood components (Stolnik *et al*., 1995). Diblock copolymers consisted of hydrophilic methoxy poly(ethylene glycol) (MPEG) and hydrophobic aliphatic polyester block have been widely investigated for this propose.

The diblock copolymers composed of hydrophilic MPEG and hydrophobic poly(ε-caprolactone) (PCL)/poly(D,L-lactide) (PDLL) blocks 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, 2005; De Faria *et al*., 2005) and 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, 2005; De Faria *et al*., 2005; Zhang *et al*., 2005).

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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) and working with toxic solvents (dichloromethane, dimethylsulfoxide, etc.) were not used for this technique. While, the micelles formation by dialysis method was greatly limited by difficult in larger-scale nanoparticle preparation. The surfactant-free nanoparticles of MPEG-*b*-PDLL were successfully prepared in our previous work (Baimark *et al*., 2007). However, characteristics of the obtained nanoparticles of MPEG-*b*-P(CL-*co*-DLL) with different CL:DLL ratios prepared by the modified-SESD method have not been investigated.

Therefore, the aim of this study was to attain surfactant-free MPEG-*b*-P(CL-*co*-DLL) nanoparticles prepared by the modified-SESD method and to investigate the influence of CL:DLL ratio on the nanoparticle characteristics. The size and size distribution of the resulted nanoparticles were characterized by using the light-scattering apparatus. Meanwhile, the morphological and the surface analyses will be studied by using scanning and transmission electron microscopy. ^{1}H-NMR spectroscopy and transmission electron microscopy was used to investigate the core-shell structure of the nanoparticles.

MATERIALS AND METHODS

Materials

Methoxy poly(ethylene glycol) (MPEG) with a molecular weight of 5,000 g mol^{-1} (Fluka, Germany) was used after it was dried in a vacuum oven at 120°C for 4 h. 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 distilled ethyl acetate and dried in a vacuum oven at 50°C for 48 h 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.

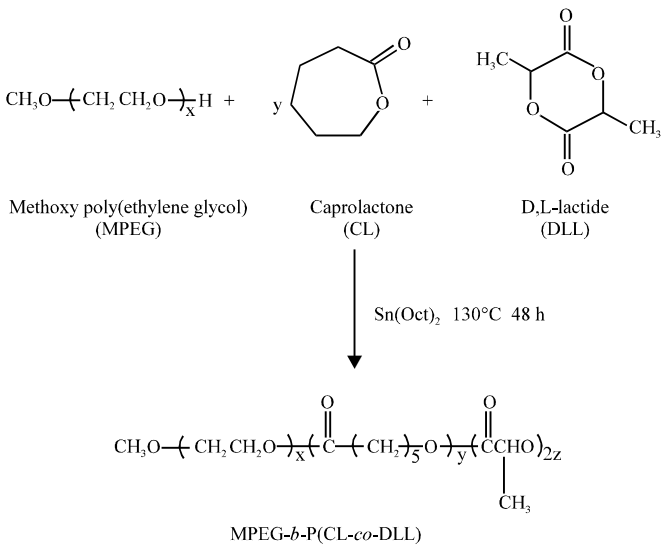
Methods

Synthesis of MPEG-*b*-P(CL-*co*-DLL)

The diblock copolymers with CL:DLL monomer feed mole ratios of 100:0, 90:10, 80:20 and 50:50 mol% were synthesized in bulk at 130°C for 48 h under a dry nitrogen atmosphere. Polymerization reaction is presented in Scheme 1. The calculated molecular weights of the P(CL-*co*-DLL) blocks from feed ratios were approximately 60,000 g mol^{-1}. The MPEG and the $Sn(Oct)_2$ were used as the initiating system. The $Sn(Oct)_2$ concentration was 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.

Characterization of MPEG-*b*-P(CL-*co*-DLL)

Copolymer composition and number-average molecular weight ($\bar{M}_n$) of the MPEG-*b*-P(CL-*co*-DLL) were determined by ^{1}H-NMR spectrometry using Bruker Advance DPX 300 ^{1}H-NMR 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 non-isothermal Differential Scanning Calorimetry (DSC) using Perkin-Elmer Pyris Diamond DSC. For DSC, the sample (~10 mg) was heated at the rate of 10°C min^{-1} under helium flow to observe thermal transition temperatures.



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

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

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

The diblock copolymer (~0.4 g) was dissolved in 20 mL acetone/ethanol [1/1 (v/v)] 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.

Characterization of MPEG-*b*-P(CL-*co*-DLL) Nanoparticles

Particle sizes and size distributions of the colloidal nanoparticles were determined by light-scattering analysis using Coulter LS230 light-scattering particle size analyzer at 25°C. Shape and surface of the nanoparticles were investigated by Scanning Electron Microscopy (SEM) and transmission electron microscopy (TEM) using JEOL JSM-6460LV scanning electron microscope and JEOL JEM 1230 transmission electron microscope, respectively. For SEM, the nanoparticle colloid was dropped on the stab before freeze-dried for over night. Prior to examination, the samples were sputter-coated with gold for enhanced surface conductivity. For TEM, a drop of nanoparticle colloid was placed on a formvar film coated on the copper grid. The specimen on the copper grid was not stained.

Core-shell structure of the nanoparticles was studied by ^{1}H-NMR spectrometry using Bruker Advance DPX 300 ^{1}H-NMR spectrometer. For ^{1}H-NMR analysis, the nanoparticles were prepared in D_2O instead of distilled water.

RESULTS AND DISCUSSION

Characterization of MPEG-*b*-P(CL-*co*-DLL)

All purified diblock copolymers had percentage yields higher than 90%. The copolymer compositions of diblock copolymers were determined from the ^{1}H-NMR spectra by ratioing the peak

Table 1: Copolymer compositions and $\bar{M}_n$ of the diblock copolymers

Diblock copolymer [CL:DLL (mol%)]	CL:DLL (mol%)		EO:CL:DLL (mol%)		$\bar{M}_n$ [b] (g mol^{-1})
	Feed ratio[a]	^{1}H-NMR[b]	Feed ratio[a]	^{1}H-NMR[b]	
100:0	100:00	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-NMR spectra

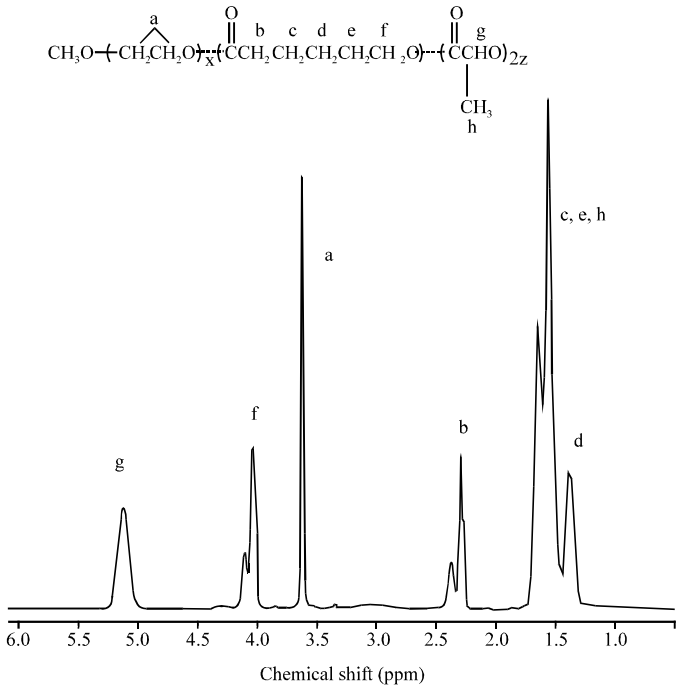


Fig. 1: ^{1}H-NMR spectrum of MPEG-*b*-P(CL-*co*-DLL) with CL:DLL ratio of 50:50 mol% in $CDCl_3$ (peak assignments as shown)

areas corresponding to the ethylene oxide (EO, repeating units of MPEG) 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-NMR spectrum of MPEG-*b*-P(CL-*co*-DLL) diblock copolymer with CL:DLL ratio of 50:50 mol% is shown as example in Fig. 1. The calculated compositions of CL:DLL and EO:CL:DLL ratios (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.

The $\bar{M}_n$ of diblock copolymers was carried out by mean of EO:CL:DLL ratio from ^{1}H-NMR spectra and MPEG molecular weight (5,000 g mol^{-1}). The calculated $\bar{M}_n$ values are also summarized in Table 2. It was found that the $\bar{M}_n$ of all diblock copolymers obtained from ^{1}H-NMR are similar to that obtained from the feed ratios (65,000 g mol^{-1}) suggested that the degradation side-reactions were small occurred.

The chain microstructures of P(CL-*co*-DLL) blocks are reflected in the fine structures of the ^{1}H-NMR 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

Table 2: Thermal properties and particle size of the diblock copolymers

Diblock copolymer [CL:DLL (mol%)]	T_m[a] (°C)	Δh_m[a] (J g^{-1})	Particle size[b] (nm)
100:0	52	71.0	639.0±213
90:10	46	52.2	595.0±159
80:20	39	39.2	457.0±72
50:50	-	-	84.0±33

[a]Determined from DSC thermograms, [b]Determined from light-scattering analysis

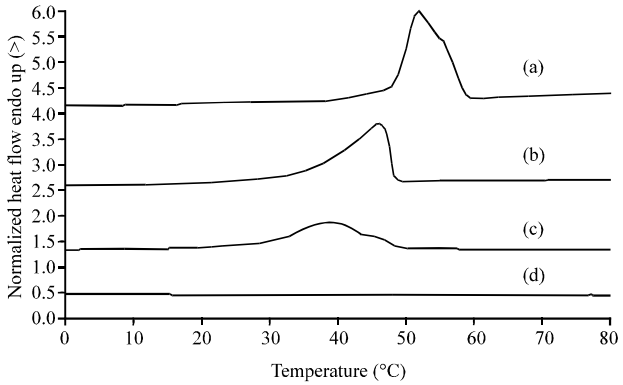


Fig. 2: DSC thermograms of the MPEG-*b*-P(CL-*co*-DLL) with CL:DLL ratios of (a) 100:0, (b) 90:10, (c) 80:20 and (d) 50:50 mol%

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). DSC thermograms of the diblock copolymers are shown in Fig. 2 and the thermal properties are summarized in Table 2. The T_m and ΔH_m of diblock copolymer decreased when the DLL units were copolymerized. In addition, the T_m of diblock copolymer with CL:DLL ratio of 50:50 mol% can not be observed indicated that it is an complete amorphous polymer. The results suggested that the crystallizability of PCL block was suppressed when the DLL ratio was increased up to 50 mol%.

Characterization of MPEG-*b*-P(CL-*co*-DLL) Nanoparticles

All surfactant-free nanoparticle colloids of the diblock copolymers are clear aqueous suspensions. The nanoparticle sizes obtained from light-scattering analysis are shown in Table 2. The nanoparticle sizes were in the range of 84-639 nm. The results can be proposed that the MPEG block of diblock copolymer can be formed as the shell of nanodroplets during the solvent diffusion stage and its protective effect is adequate, then colloidal nanoparticles were formed after solidification. This suggested that the mechanism of modified-SESD method can be successfully used to prepare surfactant-free nanoparticles of the hydrophilic-hydrophobic diblock copolymers without any surfactant use. The size of the nanoparticles was increased as decreasing the DLL ratio. This may be explained that the diblock copolymers with higher crystalline PCL content give faster solidification of nanodroplets during solvent diffusion stage. From proposed mechanism of the modified-SESD method (Murakami *et al*., 1999), the droplet sizes of copolymer solution were reduced during the solvent diffusion stage. Therefore, if the diblock copolymer nanodroplets are faster solidified during the solvent diffusion stage, the nanoparticles with larger size are obtained. It can be concluded that the diblock

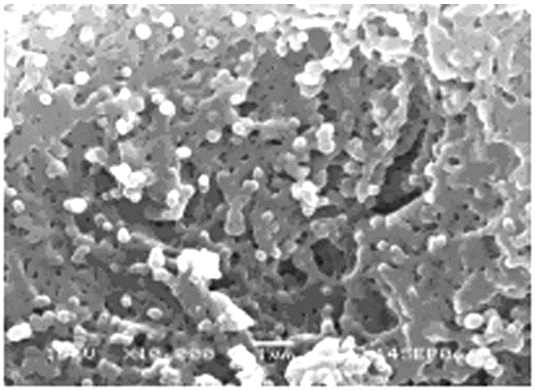


Fig. 3: SEM micrograph of the MPEG-*b*-P(CL-*co*-DLL) with CL:DLL ratio of 50:50 mol% (bar = 1 μm)

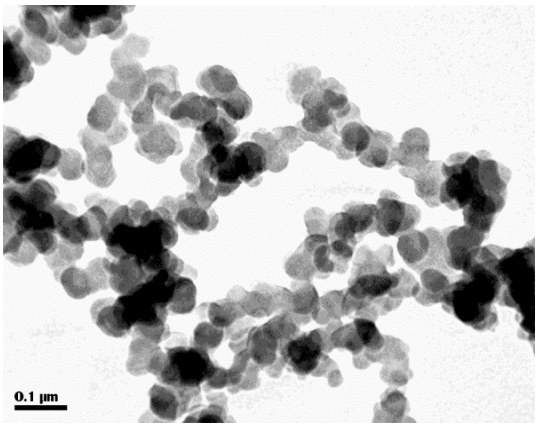


Fig. 4: TEM micrograph of the MPEG-*b*-P(CL-*co*-DLL) with CL:DLL ratio of 50:50 mol% (bar = 100 nm)

copolymer with higher crystalline CL content gave larger nanoparticle size. In addition, the particle sizes of nanoparticles seem to large distribution. However, these nanoparticles may be separated into similar size group by syringe filter (Zhang and Zhuo, 2005) and fractional centrifugation techniques (Kim *et al*., 1999). From SEM micrographs, it was found that the resulted nanoparticles were spherical shapes with smooth surfaces, as example of which is shown in Fig. 3. Figure 4 shows TEM micrograph of MPEG-*b*-P(CL-*co*-DLL) with CL:DLL ratio of 50:50 mol% confirmed that the resulted nanoparticles were spherical in shape with smooth surface according to SEM result.

Core-shell structure of the nanoparticles was demonstrated by comparing the ^{1}H-NMR spectra of the MPEG-*b*-P(CL-*co*-DLL) in $CDCl_3$ (Fig. 1) and the MPEG-*b*-P(CL-*co*-DLL) nanoparticles suspended in D_2O (Fig. 5). In $CDCl_3$ (Fig. 1), peaks of MPEG (peak a) and P(CL-*co*-DLL) (peaks b-h) blocks are observed. In contrast, only the MPEG peak (peak a) is found in D_2O (Fig. 5) while the P(CL-*co*-DLL) peaks are not detected. The results clearly indicate the core-shell structure of MPEG-*b*-P(CL-*co*-DLL) nanoparticles, according with literature reports base on similar ^{1}H-NMR data (Hrkach *et al*., 1997; Kim *et al*., 1999, 2000; Kim and Lee, 2001; Heald *et al*., 2002; Shuai *et al*., 2004; Zhang and Zhuo, 2005). It was concluded that core-shell type MPEG-*b*-P(CL-*co*-DLL) nanoparticles

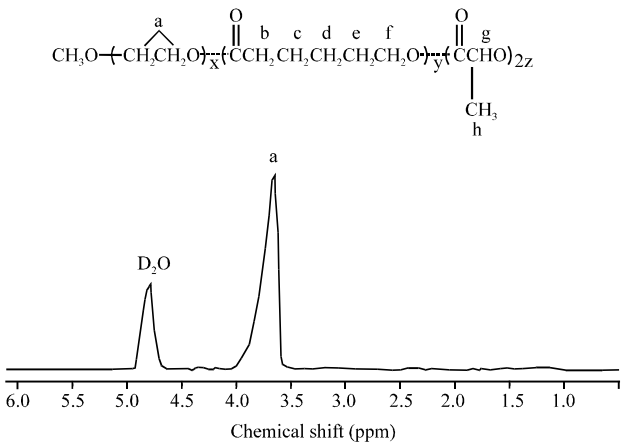


Fig. 5: ^{1}H-NMR spectrum of nanoparticles of MPEG-*b*-P(CL-*co*-DLL) with CL:DLL ratio of 50:50 mol% suspended in D_2O (peak assignments as shown)

composed of hydrophilic outer shell of MPEG and hydrophobic core of P(CL-*co*-DLL) were successfully prepared by the modified-SESD method. It should be note that this method is easy for larger scale preparation of surfactant-free and core-shell type nanoparticles of biodegradable polymers than the other methods, especially dialysis method.

CONCLUSION

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^{-1} and stannous octoate as the initiating system at 130°C for 48 h. The $\bar{M}_n$ values of diblock copolymers from ^{1}H-NMR spectra were in the range of 62,300-65,600 g mol^{-1}. 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 were prepared by the modified-SESD method. The nanoparticle size decreased with increasing the DLL ratio. From SEM and TEM images, the obtained nanoparticles were spherical in shape and smooth surface. The structure of the nanoparticles studied from ^{1}H-NMR is of the core-shell type, with the P(CL-*co*-DLL) block forming the inner core and the anchored MPEG blocks on the nanoparticle surface. These surfactant-free and core-shell type biodegradable nanoparticles might be of interest for used as drug delivery systems.

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