Preparation of Surfactant-Free Nanoparticle Colloids of Methoxy Poly(ethylene Glycol)-*b*-Poly(D,L-lactide) Diblock Copolymers

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ABSTRACT

Methoxy poly(ethylene glycol)-b-poly(D,L-lactide) diblock copolymers (MPEG-b-PDLL) was synthesized by ring-opening polymerization of D,L-lactide using MPEG with different molecular weights and stannous ctoate as the initiating system. The obtained diblock copolymers were amorphous polymers. Surfactant-free nanoparticle colloids of the MPEG-b-PDLL were prepared by modified spontaneous emulsification solvent diffusion method without any surfactant. Acetone and ethanol mixture was used as the organic solvent. Effect of different MPEG block lengths and acetone/ethanol mixture ratios on nanoparticle characteristics was investigated and discussed. Light scattering analysis showed that average particle sizes were in the range of 86 - 124 nm. The particle sizes decreased as decreasing the MPEG block length and increasing the ethanol ratio of mixture solvents. Scanning electron microscopy demonstrated the aggregation of the dried nanoparticles. However, the dried nanoparticle morphology can be observed with spherical in shape and smooth surface.

Key words: Biodegradable polymers, Poly(D,L-lactide), Diblock copolymers, Spontaneous emulsification solvent diffusion method, Nanoparticle colloids

INTRODUCTION

Methoxy poly(ethylene glycol)-b-poly(D,L-lactide) diblock copolymers (MPEG-*b*-PDLL) have been synthesized to attain versatile biodegradable polymers having more water-absorbing capacity because of the inclusion of hydrophilic MPEG segment within the relatively hydrophobic PDLL segment (Lucke et al., 2000). The MPEG-b-PDLL has been used for the preparation of drug-loaded nanoparticles (Faria et al., 2005).

Many researchers have reported the preparation methods of polymer nanoparticles such as emulsion solvent evaporation (Gorner et al., 1999), nanoprecipitation (Hu et al., 2003), salting-out (Allemann et al., 1993), and micelles formation methods (Dai et al., 2004). However, the application of these techniques was greatly limited by some problems, such as working with toxic solvents

(dichloromethane or dimethylsulfoxide, etc.), high-energy apparatus (homogenizer or sonicator, etc.), salts that are incompatible with bioactive compounds (salting-out method) and difficult for larger scale preparation (micelles formation method).

The modified-spontaneous emulsification solvent diffusion (SESD) method for pharmaceutical use was first proposed by Murakami et al., (1999) where a poly(D,L-lactide-*co*-glycolide) was dissolved in a volatile water-miscible organic solvents, acetone/ethanol. The use of higher energy apparatus is avoided for the SESD technique, which is able to apply for larger scale preparation of the polymer nanoparticles. However, the preparation of nanoparticles of amphiphilic block copolymers by the modified-SESD method has not been reported.

The aims of this study are to prepare the surfactant-free nanoparticle colloids of MPEG-*b*-PDLL, and to investigate the influence of MPEG block lengths and acetone/ethanol ratios of mixture solvent on the colloidal nanoparticle characteristics.

MATERIALS AND METHODS

Materials

MPEG with molecular weights of 2,000 and 5,000 g/mol (Fluka, Germany), hereafter called MPEG2,000 and MPEG5,000, respectively were used after it was dried in vacuo at 120°C for 4 hrs. D,L-lactide (DLL) monomer was synthesized by well established procedures from D,L-lactic acid aqueous solution (90% Fluka, Switzerland). It was purified by repeated recrystallization from distilled ethyl acetate and dried in vacuo at 50°C for 48 hrs before use. The stannous octoate (Sn(Oct)2, 95% Sigma, USA) was used without further purification. All reagents were analytical grade and used as received.

Synthesis and characterization of MPEG-b-PDLL

MPEG-b-DLL with DLL:MPEG feed ratio of 1:416 by mole were polymerized in bulk at 130°C for 24 hrs under nitrogen atmosphere. Sn(Oct)2 and MPEG were used as the initiating system. The MPEG2,000-PDLL and MPEG5,000-PDLL were obtained by using the MPEG2,000 and MPEG5,000, respectively. Polymerization reaction of the MPEG-*b*-PDLL is illustrated in Figure 2. $Sn(Oct)_2$ concentration of 0.02 mol% was used. The as-polymerized MPEG-b-PDLL was purified by dissolving it in chloroform before precipitating in cool n-hexane. Then it was dried to constant weight in vacuo at room temperature. Purified MPEG-*b*-PDLL were obtained with %yield more than 95%.

The structure of MPEG-*b*-PDLL was confirmed by Fourier transform infrared (FT-IR) spectroscopy using a Perkin-Elmer, Spectrum GX FT-IR spectrometer. The resolution of 4 cm^{-1} and 32 scans were chosen in this work. Copolymer composition of the MPEG-*b*-PDLL was determined by ^{1}H-NMR spectrometry using a Bruker Advance DPX 300 1H-NMR spectrometer. CDCl3 was used as solvent at room temperature. Tetramethylsilane was used as the internal standard. The number-average molecular weight ($\overline{M_n}$) was determined by gel permeation

chromatography (GPC) using a Waters 717 plus Autosampler GPC equipped with an Ultrastyragel® column operating at 40°C and employing a refractive index detector. Tetrahydrofuran was used as the solvent at a flow rate of 1 mL/min. Thermal properties were characterized at heating rate of 10°C/min by non-isothermal differential scanning calorimetry (DSC) using a Perkin-Elmer DSC Pyris Diamond under helium flow.

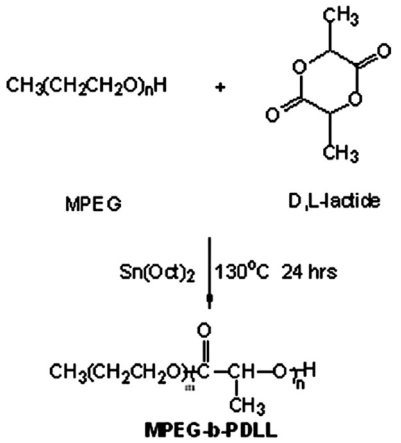

Figure 1. Polymerization reaction of MPEG-*b*-PDLL.

Preparation and characterization of nanoparticle colloids

Surfactant-free nanoparticle colloids of MPEG-*b*-PDLL were prepared according to the modified-SESD method (Murakami et al., 1999) as follows. 0.4 g MPEG-*b*-PDLL was dissolved in 20 mL acetone/ethanol mixture. The acetone/ethanol mixture ratios of 3/1, 3/2 and 3/3 (v/v) were investigated. The polymer solution was added drop-wise into 160 mL distilled water with stirring at 600 rpm. The organic solvents were evaporated at room temperature for 6 hrs in a fume hood. Then, the resulted nanoparticle colloids were filtered through 0.2 μm syringe filter to eliminate the particle aggregates. For study the effect of MPEG block length, the nanoparticle colloid of MPEG2,000-PDLL was prepared using 3/1 (v/v) acetone/ethanol mixture as the solvent.

Particle size and size distribution of the colloidal nanoparticles were determined by light scattering analysis using a Coulter, LS230 LS particle size analyzer at 25°C.

Morphology of the nanoparticles was investigated by scanning electron microscopy (SEM) using a JEOL, SEM JSM-6460LV. The nanoparticle colloid was dropped onto a stab before drying in vacuo at room temperature for 1 week. Prior to SEM examination, the dried nanoparticles were sputter-coated with gold for enhanced surface conductivity.

RESULTS AND DISCUSSION

Characterization of MPEG-*b*-PDLL

FT-IR spectra of the MPEG5,000 and the MPEG5,000-PDLL are shown as a typical example in Figure 2. The major peaks assigned in the structure of MPEG-*b*-PDLL are 2,900-3,000 cm^{-1} (C-H stretching), 1,760 cm^{-1} (ester C=O stretching), and 1,100 cm-1 (O-CH_2 stretching). Comparing the FT-IR spectrums of MPEG and MPEG-*b*-PDLL, it was found that the reaction between MPEG and PDLL was relatively taken place. It is characteristic that the broad absorption band at 3,500 cm-1 (O-H stretching) of the MPEG spectrum, in Figure 2 (a) was practically eliminated from the spectrum of MPEG-*b*-PDLL, in Figure 2 (b). These results indicating that the free hydroxyl groups of MPEG were reacted with the carbonyl groups of lactide monomer via the ring-opening polymerization. The FT-IR spectra of MPEG2,000 and MPEG2,000-PDLL showed the same evidence.

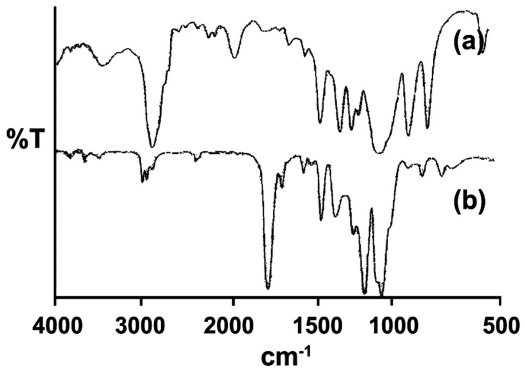

Figure 2. FTIR spectra of (a) MPEG5,000 and (b) MPEG5,000-PDLL.

The copolymer composition of MPEG-*b*-PDLL was determined from the ^{1}H-NMR spectrum. From the peak area integrations of peaks of the ethylene oxide (EO, repeating units of MPEG), methylene protons at δ = 3.4 - 3.6 ppm, and the DLL methine protons at δ = 5.0 - 5.3 ppm, the EO:DLL (mol%) of MPEG2,000-PDLL and MPEG5,000-PDLL were found to be 9:91 and 21:79, respectively. These values could correspond to the MPEG:DLL ratios of 1:455 and 1:429 by mole, respectively. As expected, these copolymer compositions are nearly values to the feed ratios (MPEG:DLL = 1:416 by mole) suggested that the synthesis reactions were taken place near with quantitative conversion.

The $\overline{M}_n$ of the MPEG2,000-PDLL and MPEG5,000-PDLL from their GPC curves were 58,200 and 73,600 g/mol, respectively. DSC curve of the MPEG2,000 and MPEG5,000 show the melting temperatures at 54 and 62°C, respectively. Therefore, the MPEG are semi-crystalline polymers. However, the melting peak of MPEG is not observed in DSC curves of the MPEG2,000-PDLL and MPEG5,000-PDLL suggested that the MPEG-*b*-PDLL are amorphous polymers. The glass transition temperatures (T_g) of the MPEG2,000-PDLL and MPEG5,000-PDLL were 48 and 37°C, respectively. In addition, the T_g values of MPEG-*b*-PDLL from DSC curves were lower than the homoPDLL (~ 65°C), suggested that the MPEG block

can act as plasticizer to increase mobility of PDLL molecules. Then, the Tg of PDLL block was decreased. The Tg of MPEG-*b*-PDLL decreased as increasing the MPEG block length. The results obtained in this work were accorded to the literatures (Lucke et al., 2000; Riley et al., 2003).

Characterization of nanoparticle colloids

The nanoparticle colloids of MPEG-*b*-PDLL prepared using the acetone/ethanol ratios of 3/1, 3/2 and 3/3 (v/v) as the mixture solvents obtained were clear aqueous suspensions.

The particle sizes of colloidal nanoparticles are in the nanometer scale, as summarized in Table 1. The results indicated that the MPEG block of MPEG-*b*-PDLL can act as hydrophilic droplet shell during solvent diffusion and evaporation stages and its protective effect was adequate, then the nanoparticles might be formed as the core-shell structure (Riley et al., 2003).

From Table 1, the particle size of the MPEG5,000-PDLL nanoparticles significantly decreased as the ethanol ratios increased indicated that the size of nanoparticles prepared by the modified-SESD method was significantly dependent on the initial mixture solvents. Increasing ethanol ratio caused increase diffusion rate of ethanol in the modified-SESD mechanisms, then resulting in the reduction of the droplet size (Murakami et al., 1999).

Table 1. Size of colloidal nanoparticles from light scattering analysis.

MPEG-*b*-PDLL	acetone/ethanol (v/v) mixture solvent	Particle size (nm)
MPEG2,000-PDLL	3/1	121±16
MPEG5,000-PDLL	3/1	124±16
MPEG5,000-PDLL	3/2	118±15
MPEG5,000-PDLL	3/3	86±29

In addition, it was found that the colloidal nanoparticle size of MPEG2,000-PDLL was nearly with the MPEG5,000-PDLL. This suggested that the MPEG block lengths used in this study did not show the effect on the nanoparticle sizes. Almost dried particles were aggregated, as shown in example in Figure 3, suggested that the colloidal nanoparticles were the soft particles.

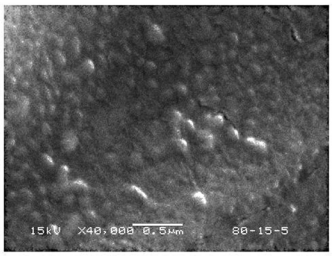

Figure 3. SEM image of dried nanoparticle aggregates of MPEG5,000-PDLL prepared using 3/1 (v/v) acetone/ethanol as the mixture solvent (bar = 500 nm).

However, morphology of the dried nanoparticles can be observed in somewhere of the aggregates as shown in Figure 3. It was found that the surfactant-free nanoparticles prepared by the modified-SESD method have the spherical shape, nanometer in size and smooth surfaces. Moreover, it was found that the nanoparticle size obtained from SEM method seemed to be larger than that obtained by the light scattering method. This was due to the colloidal nanoparticles were flatten during the drying process.

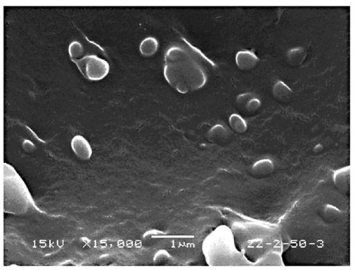

Figure 4. SEM image of dried nanoparticles of MPEG5,000-PDLL prepared by using 3/1 (v/v) acetone/ethanol mixture solvent (bar = 1,000 nm).

CONCLUSION

The MPEG-*b*-PDLL diblock copolymers were successfully synthesized using MPEG and Sn(Oct)2 as the initiating agents. The MPEG-*b*-PDLL with molecular weights of 58,200 and 73,600 g/mol were prepared from the MPEG with molecular weights of 2,000 and 5,000 g/mol, respectively. Their surfactant-free nanoparticle colloids can be prepared by the modified-SESD method using acetone/ethanol as the organic mixture solvent. The average sizes of nanoparticles were less than 124 nm with narrow size distribution and depended on the acetone/ethanol mixture ratios. The colloidal nanoparticles were the soft particles, spherical in shape and smooth surface.

The results suggested that the surfactant-free colloidal nanoparticles of MPEG-*b*-PDLL might be of interest for using in drug delivery systems and edible food coating applications.

ACKNOWLEDGEMENTS

This research was supported by grant from the Thailand Research Fund (TRF) (Grant no. DBG4880008).

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