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SHORT COMMUNICATION

Preparation of nanoparticle colloids of methoxy poly(ethylene glycol)-*b*-poly(D,L-lactide): effects of surfactant and organic solvent

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Abstract Nanoparticle colloids of methoxy poly(ethylene glycol)-*b*-poly(D,L-lactide) (MPEG-*b*-PDLL) diblock copolymer were prepared by a modified spontaneous emulsification solvent diffusion method using acetone/ethanol as the mixture organic solvents. The MPEG-*b*-PDLL was synthesized by ring-opening polymerization of D,L-lactide using stannous octoate and MPEG with molecular weight of 5,000 g/mol as the initiating system. The MPEG-*b*-PDLL obtained was an amorphous polymer with molecular weight of 73,600 g/mol. Influences of acetone/ethanol (v/v) ratios and Tween 80 surfactant concentrations on characteristics of the colloidal nanoparticles were investigated and discussed. Light-scattering analysis showed that average diameters of the surfactant-free colloidal nanoparticles were in the range of 86–124 nm. The nanoparticle sizes decreased as the ethanol ratio increased. The Tween 80 did not show the significant effect on the nanoparticle sizes. Scanning electron micrographs of dried nanoparticles that demonstrated the aggregation of most particles suggested they were the soft nanoparticles. However, the dried nanoparticle morphology can be observed from scanning electron microscopy as having a spherical shape and smooth surfaces.

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Introduction

Amphiphilic block copolymers have recently attracted many attentions for their theoretical studies in self-assembled micelles, nanoparticles, and films. Poly(D,L-lactide) (PDLL) is a biodegradable and biocompatible material that is widely used in medicine and bioengineering. Diblock copolymers composed of PDLL and methoxy poly(ethylene glycol) (MPEG) have been synthesized to attain versatile biodegradable polymers having more water-absorbing capacity because of the inclusion of hydrophilic MPEG segments within the relatively hydrophobic PDLL segment [1–3]. MPEG-PDLL diblock copolymers have been used for the preparation of drug-loaded nanoparticles [4–7] with composed of the MPEG chains on the nanoparticle surface [8]. Polylactide–poly(ethylene glycol) 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 [9, 10].

Many methods have been reported for the preparation of polymer nanoparticles such as emulsion solvent evaporation method [11], nano-precipitation method [12, 13], salting-out method [14], and micelles formation method [15]. However, the application of these techniques was greatly limited by some problems, such as working with toxic solvents (dichloromethane, 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 of polymer nanoparticles (micelles formation method).

A modified spontaneous emulsification solvent diffusion method (modified-SESD method) for pharmaceutical use was proposed first by Murakami et al. [16] where a poly (D,L-lactide-*co*-glycolide) was dissolved in a volatile water-miscible organic solvent, acetone/ethanol, instead of acetone/dichloromethane, which was used in the original SESD method [17]. The use of a higher energy apparatus such as the homogenizer and sonicator is avoided for this technique. Hence, the large-scale preparation of the polymer nanoparticles would be possible. However, preparation of nanoparticles of amphiphilic block copolymers by the modified-SESD method has not been studied, and it is of utmost interest to develop preparation procedures that avoid surfactants. The value of polymer colloids, which today are used in medical applications, will increase if we can design colloids with specified properties, especially surfactant-free nanoparticles colloids.

Therefore, the aims of this study were to attain MPEG-*b*-PDLL nanoparticles colloids and to investigate the influences of acetone/ethanol mixture organic solvents and surfactant concentrations on the colloidal nanoparticle characteristics. Tween 80 was selected as the surfactant studied in this work. The size and the size distribution of the prepared colloidal nanoparticles can be characterized by using the light-scattering apparatus. Meanwhile, the surface and the morphological analyses will be studied by using scanning electron microscopy (SEM).

Experimental

Materials

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 h. D,L-lactide (DLL) was synthesized by well-established procedures from D,L-lactic acid solution (90%, Fluka, Switzerland). It was purified by repeated recrystallization from ethyl acetate and dried in vacuo at 50 °C for 48 h before used. The stannous octoate ($Sn(Oct)_2$, Sigma, USA), Tween 80 (Lab Chem, Australia), acetone (AR, Merck, Germany), and ethanol (AR, Merck, Germany) were used without further purification. The Tween 80, systematic name: polyoxyethylene (20) sorbitan monooleate, was a nonionic surfactant with critical micelle concentration of 1.2×10^{-5} M.

Synthesis of MPEG-*b*-PDLL

MPEG-*b*-PDLL was polymerized in bulk at 130 °C for 24 h under nitrogen atmosphere. MPEG:DLL feed mole ratio of 1:416 was used. $Sn(Oct)_2$ and MPEG were used as the initiating system. $Sn(Oct)_2$ concentration of 0.02 mol% was used. As-polymerized diblock copolymer was purified by dissolving it in chloroform before precipitating in cool *n*-hexane and then dried to constant weight in vacuo at room temperature. According to this procedure, the purified diblock copolymer was obtained with more than 95% yield.

Characterization of MPEG-*b*-PDLL

The structure of the obtained MPEG-*b*-PDLL was studied by Fourier transform infrared (FT-IR) spectroscopy using a Perkin-Elmer Spectrum GX FT-IR spectrophotometer with air as the reference. The resolution of 4 cm^{-1} and 32 scans were chosen in this work. FT-IR spectra were collected using a KBr disk method. The sample was dissolved in dichloromethane before cast on the KBr disk and dried in vacuo at room temperature for 1 week. Chemical composition of the MPEG-*b*-PDLL was 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 tetra methysilane was used as the internal standard. The number-average molecular weight (M_n) and molecular weight distribution (MWD) were determined by gel permeation chromatography (GPC) using a Waters 717 plus Autosampler GPC equipped with an Ultrastyragel® column operating at 30 °C and employing a refractive index detector. Tetrahydrofuran was used as the solvent at a flow rate of 1 mL/min. 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.

Preparation of MPEG-*b*-PDLL nanoparticle colloids

The nanoparticle colloids of MPEG-*b*-PDLL with and without the addition of the surfactant (Tween 80) were prepared according to the modified-SESD method [16]. This procedure was explained as follows.

Approximate 0.4 g of MPEG-*b*-PDLL was dissolved in 20 mL of the organic mixtures. These organic mixtures were prepared from different acetone/ethanol ratios (3/1, 3/2, and 3/3 [v/v]). Each formula is reported in Table 1. These solutions were 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 h in a fume hood. Then, the surfactant-free nanoparticle colloids were centrifuged at 10,000 rpm for 1 h to eliminate particle aggregates before characterization. For study on the effect of surfactant concentrations, 160 mL of different Tween 80

Table 1 Preparing condition and the size of the MPEG-*b*-PDLL nanoparticle colloids

Nanoparticle colloids	Organic solvent (acetone/ethanol [v/v])	Aqueous phase	Particle size[a] (nm)
A	3/1	Water	124±16
B	3/2	Water	118±15
C	3/3	Water	86±29
D	3/2	1% (w/v) Tween 80	125±16
E	3/2	2% (w/v) Tween 80	118±16

[a] From light-scattering particle size analysis

concentrations (1 and 2% [w/v]) were used instead of distilled water. Each formula is also reported in Table 1.

Characterization of MPEG-*b*-PDLL nanoparticle colloids

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 SEM (JEOL JSM-6460LV). The nanoparticle colloid was dropped onto a stab and then dried in vacuo at room temperature for 1 week. Before SEM measurement, the nanoparticles were sputter coated with gold for enhancing the surface conductivity.

Results and discussion

Characterization of MPEG-*b*-PDLL diblock copolymer

Identity of the diblock copolymer was investigated by FT-IR spectroscopy. Typical FT-IR spectra of the MPEG and MPEG-*b*-PDLL are shown in Fig. 1. The major peaks assigned to the structure of MPEG-*b*-PDLL are 2,900–3,000 (C–H stretching), 1,760 (ester C=O stretching), and 1,100 cm^{-1} (O–CH_2 stretching). The comparison of the FT-IR spectrum of MPEG with MPEG-*b*-PDLL confirmed that the reaction between MPEG and PDLL have been taken place. In addition, the broad absorption band at 3,500 cm^{-1} (O–H stretching) in the spectrum of MPEG (in Fig. 1a) was practically eliminated from the spectrum of MPEG-*b*-PDLL (in Fig. 1b). This result indicated that the free hydroxyl groups of MPEG were reacted with the carbonyl groups of lactide in ring-opening polymerization.

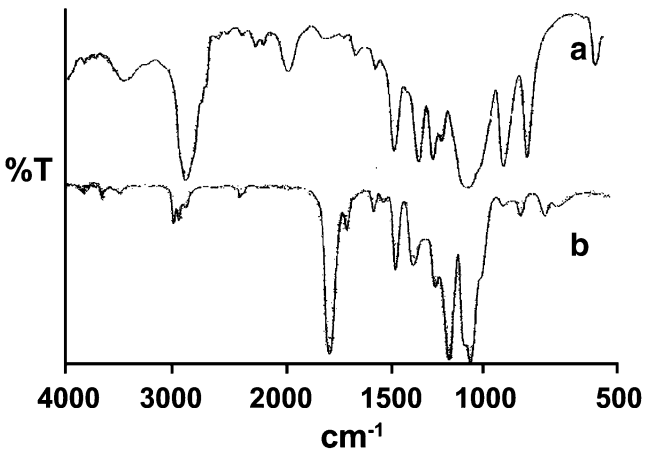

Fig. 1 FT-IR spectra of *a* MPEG and *b* MPEG-*b*-PDLL

The chemical composition of MPEG-*b*-PDLL was determined from the ^{1}H-MRI spectrum by ratioing the integral peak areas corresponding to 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 ^{1}H-MRI spectrum of MPEG-*b*-PDLL is shown in Fig. 2. From the peak area integrations of the peaks a and b in Fig. 2, the copolymer composition can be determined as EO:DLL=21:79 (mol%) corresponding to the MPEG:DLL mole ratio of 1:429. As would be expected, this copolymer composition is very similar to the MPEG:DLL feed mole ratio (1:416). Therefore, the synthesis reaction was taken to quantitative conversion.

The M_n and MWD of MPEG-*b*-PDLL obtained from GPC were 73,600 g/mol and 1.88, respectively. The M_n

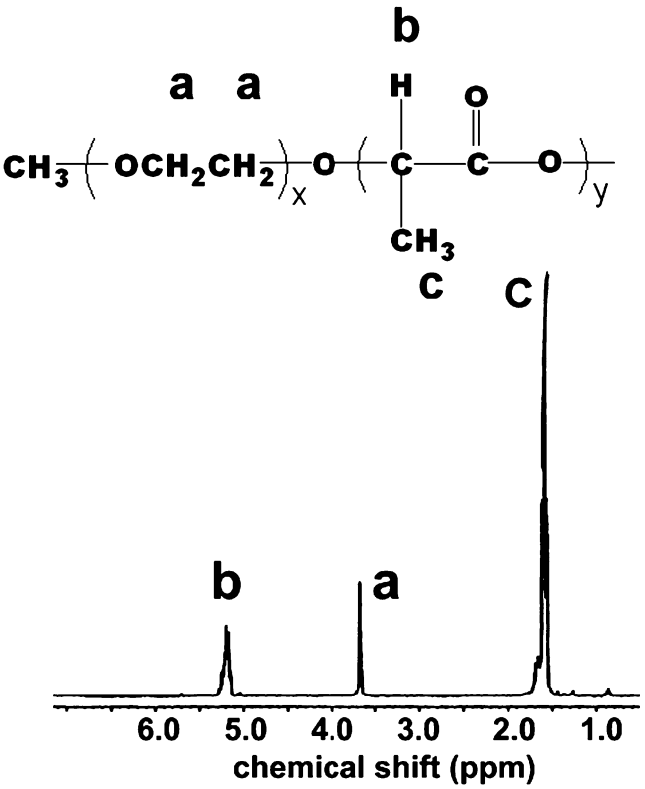

Fig. 2 ^{1}H-MRI spectrum of MPEG-*b*-PDLL (peaks assignment as shown)

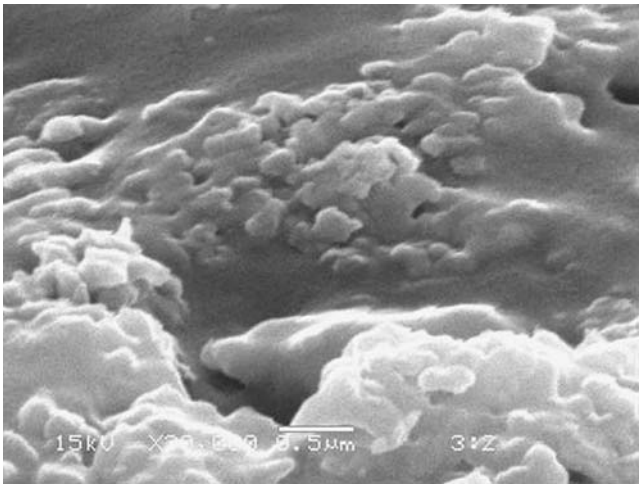

Fig. 3 SEM micrograph of the aggregated nanoparticles of colloid B (Preparing system of colloid B as shown in Table 1)

obtained from GPC was closely similar to that obtained from the feed ratio (65,000 g/mole). The DSC curve of MPEG showed the melting temperature and the heat of melting, and they were found to be 56 °C and 174.7 J/g, respectively. The DSC curve of MPEG-*b*-PDLL revealed an amorphous morphology with a single-glass transition temperature (T_g) at 36 °C (DSC curve not shown). However, the crystallinity of the MPEG block was suppressed when it connected to the amorphous PDLL block. In addition, the T_g of MPEG-*b*-PDLL from the DSC curve was lower than the T_g of homo-PDLL (~65 °C). These results suggested that the MPEG block of MPEG-*b*-PDLL acted as a plasticizer to decrease the T_g of the diblock copolymer.

Characterization of MPEG-*b*-PDLL nanoparticle colloids

In our previous study, the MPEG-*b*-PDLL cannot completely dissolved when the ethanol ratio of the acetone/ethanol mixture solvents was higher than 3/3 (v/v). Therefore, in this study, the 3/1, 3/2, and 3/3 (v/v) acetone/ethanol mixtures were used as the solvent. The nanoparticle colloids were clear aqueous suspensions. The sizes of the colloidal nanoparticles were studied by the light-scattering analysis. Their sizes were found in the range of 86 to 125 nm. These data are summarized in Table 1. The particle size of surfactant-free nanoparticle colloids significantly decreased as the ethanol ratios increased. These results indicated that the mixture ratio of the organic solvent used for dissolving the MPEG-*b*-PDLL showed a strong effect on the size of nanoparticles prepared by the modified-SESD method. From the possible mechanism of the modified-SESD method as described by Murakami et al. [16], the size reduction of polymer solution droplets occurred in the stage of alcohol diffusion. Then, the results in this work supported that the different acetone/ethanol ratios of the mixture solvent resulted in the different ethanol diffusion rates. Thus, increasing the ethanol ratio led to the decrease of the polymer droplets before particle solidification.

It has been reported that the nature and amount of surfactant are the important factors for the size and size distribution of resulted nanoparticles [18]. However, the different result was obtained by this work; that is, Tween 80 concentrations did not influence on the size and size distribution of nanoparticles prepared by the modified-SESD method. These results are shown in Table 1. The results indicated that the MPEG block of the diblock copolymer was acted as the shell of nanodroplets, and its protective effect is adequate. Then, the nanoparticles can be formed as a core–shell structure [19]. This result could realize that the surfactant-free nanoparticle colloids seemed to be the core–shell structure. Consequently, the surfactant-free of hydrophilic–hydrophobic diblock copolymers can be prepared by using the modified-SESD method. This method is simple, and it is easy for larger-scale preparation of the polymer colloidal nanoparticles. Moreover, this proposed method is superior to the formation of micelles by dialysis method.

The SEM micrograph of the dried colloidal nanoparticles is shown in Fig. 3 as a typical example. It was found that most nanoparticles were aggregated after drying. This suggested that the colloidal nanoparticles are soft particles because of its low T_g. However, nanoparticle morphology can be observed on the aggregate surface, where an example of which is shown in Fig. 4. It was found that the colloidal nanoparticles have a spherical shape in the nanometer size range and smooth nanoparticle surfaces. The sizes of the nanoparticle obtained from SEM were larger than those obtained from the light-scattering analysis.

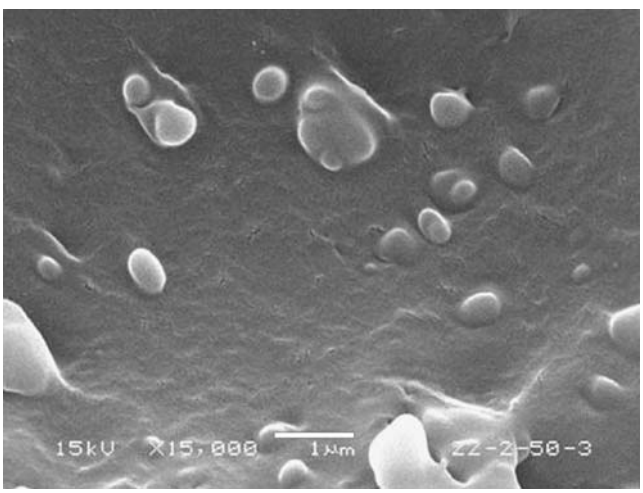

Fig. 4 SEM micrograph of the nanoparticles of colloid A (Preparing system of colloid A as shown in Table 1)

It was due to the colloidal nanoparticles, which were flatten during the drying step.

Conclusions

The MPEG-*b*-PDLL diblock copolymer was successfully synthesized by ring-opening polymerization of the DLL monomer using $Sn(Oct)_2$ and MPEG as the initiating system. It was an amorphous polymer. The nanoparticle colloids of the MPEG-*b*-PDLL diblock copolymer were successfully prepared by the modified-SESD method using an acetone/ethanol mixture as the organic solvent. The sizes of yielded nanoparticle were in nanometer size ranges, with narrow size distributions. The size of surfactant-free colloidal nanoparticles decreased as the ethanol ratio increased. The Tween 80 surfactant did not influence on the nanoparticle sizes. The colloidal nanoparticles were soft colloidal particles, with a spherical shape and smooth surface.

The obtained results suggested that these surfactant-free MPEG-*b*-PDLL nanoparticle colloids might be of interest in the use for biodegradable drug delivery systems and edible food-coating materials.

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