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Preparation and Characterization of Biodegradable Nanospheres of Amphiphilic Methoxy poly(ethylene glycol)-*b*-poly(D,L-lactide-*co*-glycolide-*co*-ε-caprolactone) for Controlled Drug Delivery

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Abstract

Methoxy poly(ethylene glycol)-*b*-poly(D,L-lactide-*co*-glycolide-*co*-ε-caprolactone) (MPEG-*b*-PDLLGCL) amphiphilic diblock copolymers were synthesized and used as nanosphere matrices. The drug-loaded nanospheres were prepared by the modified-spontaneous emulsification diffusion method without any emulsifiers for controlled drug delivery of poorly water-soluble model drug, indomethacin. Effect of DLL/G/CL ratio on nanoparticle and drug release characteristics was evaluated. The resultant drug-loaded nanospheres with average size in range of 126 – 134 nm had spherical in shape and smooth in surface. The drug entrapped inside the nanospheres had amorphous state. Drug loading efficiency was not found to be influenced by the DLL/G/CL ratio. The drug release profiles exhibited biphasic with a fast release followed by a slow release. The amounts of drug release increased when the G and CL units were incorporated in the polyester blocks. This indicates that MPEG-*b*-PDLLGCL nanospheres might be useful candidate as sustained drug carriers for poorly water-soluble drugs.

Keywords: Lactide, glycolide, ε-caprolactone, diblock copolymers, nanospheres, drug delivery

Introduction

Biodegradable polyesters including poly(D,L-lactide) (PDLL), polyglycolide (PG), poly(ε-caprolactone) (PCL) and their copolymers have widely investigated for use in biomedical and pharmaceutical applications due to their biocompatibility and biodegradability [1-4]. Amphiphilic diblock copolymers composed of methoxy poly(ethylene glycol) (MPEG) and polyesters such as poly(D,L-lactide) (PDLL) [5-7], poly(ε-caprolactone) (PCL) [8], polyglycolide (PG) [9] and their copolymers [10, 11] have been synthesized to attain versatile biodegradable polymers having more water-absorbing capacity because of the inclusion of hydrophilic MPEG segments within the relative hydrophobic polyester segments. These diblock copolymers have been used for the preparation of drug-loaded nanoparticles [8, 9]. Their nanoparticles have shown potential as drug delivery systems because of their small sizes that improving circulation times in the body and creates more available routes of administration than do microparticles, which are rapidly cleared by the reticulo-endothelial tissues. Most of the previous works have concentrated on diblock copolymer nanoparticles consisting of MPEG and homo-/copolyester blocks. However, the diblock copolymer nanoparticles comprising MPEG and PDLLGCL blocks for controlled drug release applications have not been reported.

The modified-spontaneous emulsification-solvent diffusion method (modified-SESD method) for the preparing emulsifier-free nanoparticles of hydrophilic-hydrophobic diblock copolymer was first proposed as previously described [12]. MPEG-*b*-poly(D,L-lactide) was dissolved in volatile water-miscible organic solvents with lower toxicity, acetone and ethanol. 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.

In our previous work [13], nanoparticles of MPEG-*b*-PDLLGCL copolymers have been prepared and characterized. The nanoparticles of diblock copolymers with varying DLL/G/CL ratios have been investigated and their chemical structure-particle character relationships are discussed. The aims of present study were to prepare emulsifier-free drug-loaded MPEG-*b*-PDLLGCL nanospheres by the modified-SESD method and to investigate the

influence of DLL/G/CL ratio on the drug-loaded nanosphere characteristics and drug release behaviors. Indomethacin was used as a poorly-water soluble model drug.

Materials and Methods

Materials

Methoxy poly(ethylene glycol) (MPEG) with a molecular weight of 5,000 g/mol (Fluka, Germany) was dried at 120 °C under reduced pressure for 4 h before use. D,L-lactide (DLL) and glycolide (G) monomers were synthesized by well established procedures from D,L-lactic acid (90%, Fluka, Switzerland) and glycolic acid (99%, Acros, USA), respectively. Each was purified by repeated re-crystallization from distilled ethyl acetate. 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. Stannous octoate ($Sn(Oct)_2$, 95%, Sigma, USA) and indomethacin (98%, Sigma) were used without further purification. Acetone (Merck, Germany) and ethanol (Merck, Germany) in analytical grade were used.

Synthesis and Characterization of Diblock Copolymers

MPEG-*b*-PDLLGCL with DLL/G/CL feed ratios of 100/0/0, 80/20/0 and 80/15/5 mol% were synthesized by the ring-opening polymerization in bulk at 130 °C for 24 h under nitrogen atmosphere [14]. The PDLLGCL block length with approximately 60,000 g/mol was synthesized to attach the MPEG block. MPEG and $Sn(Oct)_2$ were used as the initiating system. The $Sn(Oct)_2$ concentration was kept constant at 0.02 mol%. The as-polymerized copolymers were purified by dissolving in chloroform before precipitating in cool *n*-hexane. They were then dried to constant weight in a vacuum oven at room temperature. The chemical composition and number-average molecular weight ($\overline{M}_n$) were determined using ^{1}H-NMR spectrometer (Bruker Advanced DPX 300). $CDCl_3$ was used as a solvent at room temperature, and tetramethysilane was used as the internal standard. The thermal properties of copolymers were characterized using a differential scanning calorimeter (DSC, Perkin-Elmer Pyris Diamond) at heating rate of 10 °C/min under helium flow.

Preparation of Drug-loaded Nanospheres

Indomethacin-loaded nanospheres were prepared by the modified SESD method without any emulsifiers [13]. Briefly, indomethacin (4 mg) and copolymer (36 mg) were dissolved in 2 mL of 1/1 (v/v) acetone/ethanol mixture. The solution was added drop-wise into 16 mL of deionized water under magnetic stirring. The nanospheres were immediately formed after solvent diffusion. The organic solvents were then evaporated at room temperature for 6 h in a fume hood. The resulting nanosphere suspension was centrifuged for 1 h at 12,000 rpm at 4 °C. The supernatant was carefully discarded and the pellet was re-suspended in water. The final nanosphere suspension volume was adjusted to 16 mL with deionized water. The dried nanospheres were obtained by centrifugation at 12,000 rpm for 1 h at 4 °C before freeze-drying for 48 h.

Characterization of Drug-loaded Nanospheres

Morphology of the nanospheres was investigated by a transmission electron microscope (TEM, JEOL JEM 1230). For TEM analysis, a drop of nanosphere suspension was placed on a formvar film coated on the copper grid. The specimen on the copper grid did not stain. Average particle size and size distribution of the drug-loaded nanospheres were determined from the nanosphere susprensions by a light-scattering particle size analyzer (Coulter LS230) at 25 °C. Thermal properties of the dried nanospheres were measured by DSC as described above. Theoretical drug loading content ($DLC_{theoretical}$), actual drug loading content (DLC_{actual}) and drug loading efficiency (DLE) were calculated from equations (1) - (3), respectively. The DLC_{actual} is an average value from three measurements. For measurement of DLC_{actual}, freeze-dried samples of drug-loaded nanospheres were dissolved in dichloromethane. The weight of actual drug entrapped in the dried nanospheres was determined by UV-Vis spectrophotometry (Perkin-Elmer Lambda 25) at 319 nm compared to standard curve of indomethacin.

$$DLC_{theoretical}\ (\%) = \frac{\text{Weight of feed drug}}{\text{Weights of feed drug and copolymer}} \times 100 \quad (1)$$

$$DLC_{actual}\ (\%) = \frac{\text{Weight of actual drug entrapped in nanospheres}}{\text{Weight of drug-loaded nanospheres}} \times 100 \quad (2)$$

$$DLE\ (\%) = \frac{DLC_{actual}}{DLC_{theoretical}} \times 100 \quad (3)$$

In vitro Drug Release Test

In vitro drug release test of the drug-loaded nanospheres was performed in a phosphate buffer solution (PBS, 0.1 M, pH 7.4). Two mL of nanosphere suspension was put in a dialysis bag (molecular weight cut-off = 8,000 – 12,000 Da), and immersed into a flask containing 150 mL of release medium, PBS. The flask was placed in an incubator shaker at 37 °C. At predetermined

time interval, 10 mL of release medium was withdrawn, and 10 mL of fresh PBS was re-placed into the flask for continuing the release test. The released indomethacin was measured by an UV-Vis spectrophotometer at 319 nm. According to a predetermined indomethacin concentration-UV absorbance standard curve, indomethacin concentration of the release medium was obtained. Percentage of indomethacin release was calculated based on ratio of drug release in each releasing time and initial drug content in nanospheres. The average %release was calculated from the three measurements.

Results and Discussion

Characterization of Diblock Copolymers

MPEG-*b*-PDLLGCL copolymers were synthesized from D,L-lactide (DLL), glycolide (G) and ε-caprolactone (CL) monomers in the presence of MPEG and stannous octoate. The hydroxyl end group of MPEG initiated the ring-opening of these monomers. Chemical compositions of the copolymers were determined from the ^{1}H-NMR spectra by ratioing the peak areas corresponding to the ethylene oxide (EO) methylene protons at δ = 3.4-3.6 ppm (repeating units of MPEG block), the DLL methine protons at δ = 5.0-5.3 ppm, the G methylene protons at δ = 4.5-4.9 ppm and the CL ε-methylene protons at δ = 3.9-4.2 ppm. The ^{1}H-NMR spectra of the copolymers are shown in Figure 1 and the copolymer compositions of EO/DLL/G/CL and DLL/G/CL (mol %) calculated from ^{1}H-NMR spectra are summarized in Table 1. As would be expected, the copolymer compositions are similar to the comonomer feed ratios, indicating that the synthesis reactions proceeded to near-quantitative conversion. The $\overline{M}_n$ s calculated from ^{1}H-NMR spectra are also reported in Table 1. It can be seen that the $\overline{M}_n$ s of copolymers calculated from ^{1}H-NMR spectra are similar calculated from the feed ratio.

Thermal transition properties of the copolymers were carried out by means of differential scanning calorimetry (DSC). The MPEG shows a melting temperature (T_m) at 56 ^{o}C. The DSC curves of the copolymers exhibited only glass transition temperatures (T_g) in range of 22 - 37 ^{o}C but did not T_m, as summarized in Table 2. It is significant to note that the crystallizability of the MPEG blocks in all copolymers was suppressed as connected to the amorphous polyester blocks. The T_g of the copolymers significantly decreased when glycolide and ε-caprolactone units were incorporated into the polyester chain.

Characterization of Nanospheres

The drug-loaded nanospheres were prepared by the modified-SESD method without any emulsifiers. The nanospheres were solidified after ethanol and acetone diffused out from emulsion droplets to continuous water phase. The MPEG blocks attached to PDLLGCL blocks can act as the emulsifier to prevent nanoparticle aggregation [12].

The morphology of nanospheres was investigated from TEM micrographs, as shown in Figure 2. The nanospheres had a nearly spherical shape and smooth in surfaces. The nanoparticles are in nanometer size range. The average sizes of nanospheres determined by the light-scattering method are summarized in Table 3. Their average sizes were found in range of 126 - 134 nm with narrow size distribution. Figure 3 shows an example of LS particle size graph. The results suggested that the DLL/G/CL ratios did not affect morphology and average size of the drug-loaded nanospheres.

The indomethacin drug is a crystalline drug that have a melting temperature about 155 °C. However, the melting peak of indomethacin entrapped in the nanospheres did not be detected from DSC thermograms of drug-loaded nanospheres. The results showed that the indomethacin crystallizability in the drug-loaded nanospheres was suppressed. This suggested that the drug was homogeneous dispersed in an amorphous state inside the nanosphere matrix. The indomethacin entrapped in the nanospheres can decrease slightly the T_g of copolymer nanosphere matrix, as reported in Table 2. This may be explained that the drug molecules entrapped in the copolymer matrix increase the copolymer molecule's free volume.

The calculated DLC_{actual} and DLE values are also summarized in Table 3. The DLC_{actual} and DLE of drug-loaded nanospheres are approximately 3% and 30%, respectively. This indicated that the partial drug has diffused out during the nanosphere formation. The different DLL/G/CL ratios did not affect the drug loading of nanospheres.

In vitro Drug Release

The *in vitro* drug release profiles of the nanospheres in PBS, pH 7.4 at 37 °C for 7 days are illustrated in Figure 4. The drug release profiles show biphasic containing rapid initial burst release and sustaining release. The rapid initial burst release of drug from the nanospheres was probably due to the releasing of drug that entrapped or adsorbed near to the nanosphere surfaces. After that the slow release may be due to drug diffusion mechanism.

The initial burst releases from the nanospheres were found within the first 12 h of release time. Then, release profiles were followed by a constant slow release until to 62, 79 and 88% indomethacin release within 7 days for DLL/G/CL ratios of 100/0/0, 80/20/0 and 80/15/5 mol%, respectively. The drug release results indicated that the DLL/G/CL ratio was an important factor of the drug release behavior. The results may be explained that the lower T_g nanosphere matrix exhibited higher drug releasing. The drug can easier diffuse out for the lower T_g nanosphere matrix.

Conclusions

In present work, the nanospheres were prepared from synthesized MPEG-*b*-PDLLGCL by the modified-SESD method for controlled-release of a poorly water-soluble model drug, indomethacin. The drug-loaded nanospheres were of size about 130 nm with narrow size distribution. The drug-loaded

nanospheres had nearly spherical in shape and smooth in surface. The DLL/G/CL ratios did not influence on the average size, shape and DLE of the drug-loaded nanospheres. The drug release content from copolymer nanospheres are in order DLL/G/CL of 80/15/5 > 80/20/0 > 100/0/0 mol%. The drug release rate can be optimally controlled by adjusting the DLL/G/CL ratios of MPEG-*b*-PDLLGCL nanosphere matrix. These nanospheres may have potential to provide other poorly water-soluble drugs for controlled-release drug delivery applications.

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Table. 1. Copolymer composition and M_n of the MPEG-*b*-PDLLGCL.

DLL/G/CL	EO/DLL/G/CL ratio (mol %)		DLL/G/CL ratio (mol %)		$\overline{M}_n$ (g/mol)	
	Feed ratio[a]	^{1}H NMR[b]	Feed ratio[a]	^{1}H NMR[b]	Feed ratio[a]	^{1}H-NMR[c]
100/0/0	22/78/0/0	21/79/0/0	100/0/0	100/0/0	65,000	67,700
80/20/0	21/63/16/0	21/67/12/0	80/20/0	85/15/0	65,000	65,000
80/15/5	21/63/12/4	20/66/10/4	80/15/5	83/12/5	65,000	67,300

[a] Calculated from comonomer feed ratios
[b] Calculated from ^{1}H-NMR spectra
[c] Calculated from EO/DLL/G/CL ratio (^{1}H-NMR spectra)

Table. 2. Glass transition temperatures (T_g) of the copolymers and drug-loaded copolymer nanospheres.

DLL/G/CL ratio (mol%)	T_g[a] (°C)
Copolymers	
100/0/0	**37**
80/20/0	**28**
80/15/5	**22**
Nanospheres	
100/0/0	**35**
80/20/0	**25**
80/15/5	**21**

[a] Mid-point of DSC glass transition from DSC curves

Table. 3. Average particle size and drug loading of the drug-loaded copolymer nanospheres.

DLL/G/CL ratio (mol%)	Average particle size[a] (nm)	DLC_{actual}[b] (%)	DLE[c] (%)
100/0/0	**126 ± 15**	**2.8**	**28**
80/20/0	**134 ± 18**	**3.1**	**31**
80/15/5	**129 ± 15**	**2.6**	**26**

[a] obtained from light-scattering analysis
[b] calculated from equation (2)
[c] calculated from equation (3); $DLC_{theoretical}$ = 10%

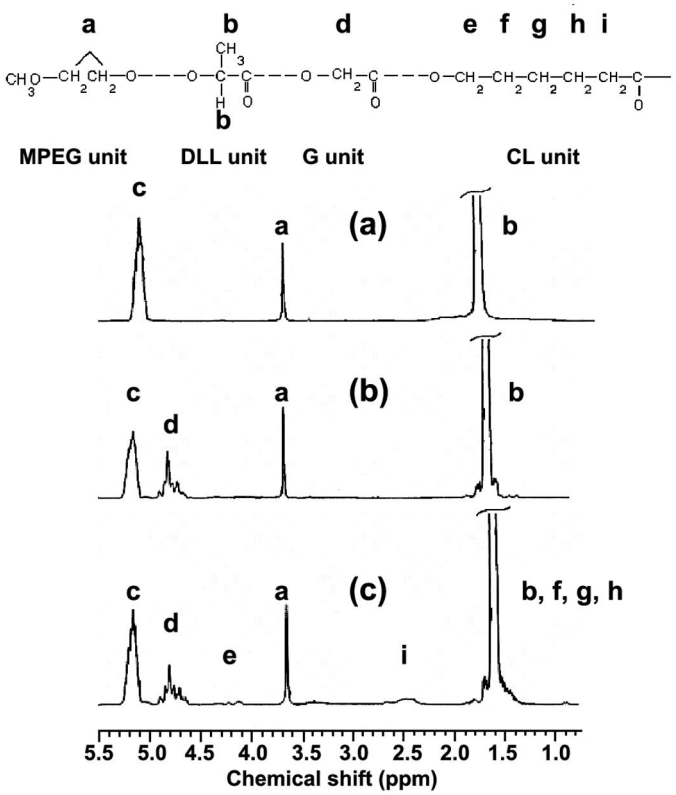

Figure 1 ^{1}H-NMR spectra of MPEG-*b*-PDLLGCL with DLL/G/CL ratios of (a) 100/0/0, (b) 80/20/0 and (c) 80/15/5 mol% (Peak assignments as shown).

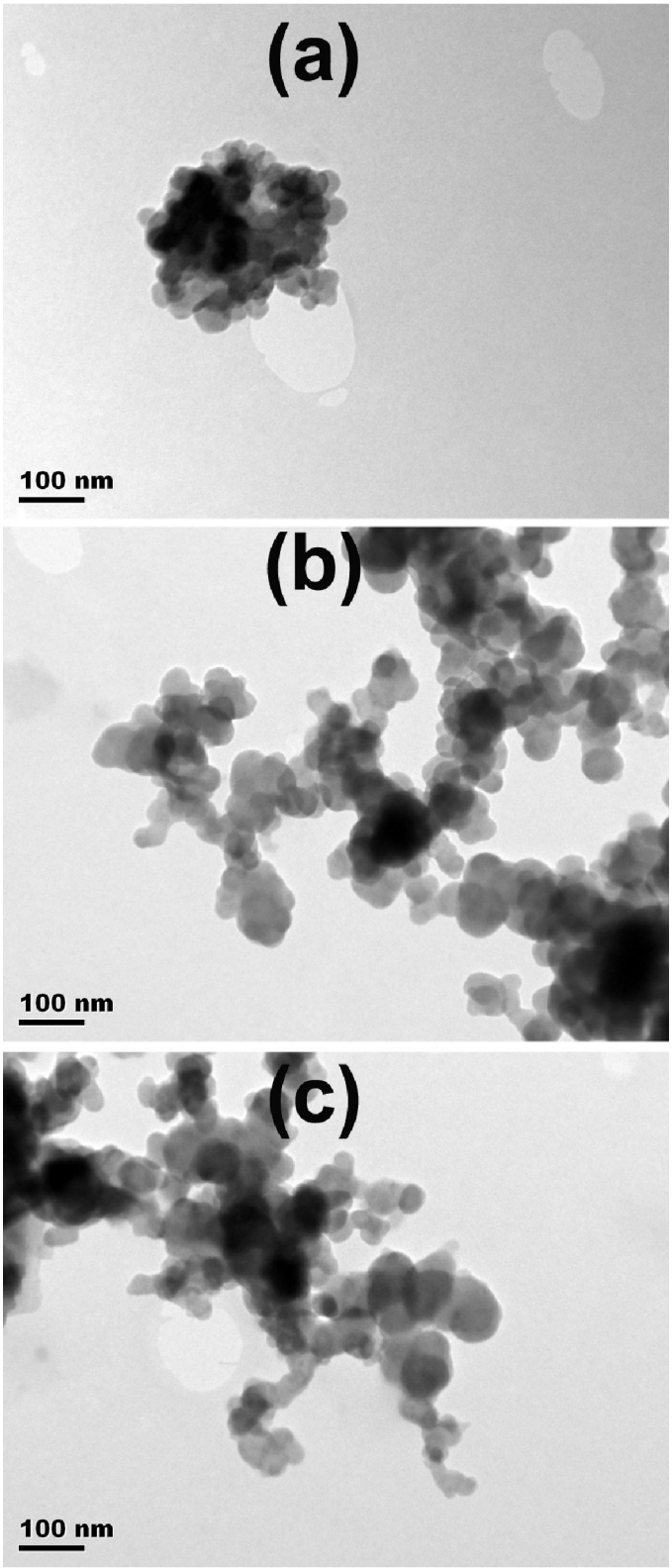

Figure 2 TEM micrographs of drug-loaded nanospheres with DLL/G/CL ratios of (a) 100/0/0, (b) 80/20/0 and (c) 80/15/5 mol% (All bars = 100 nm).

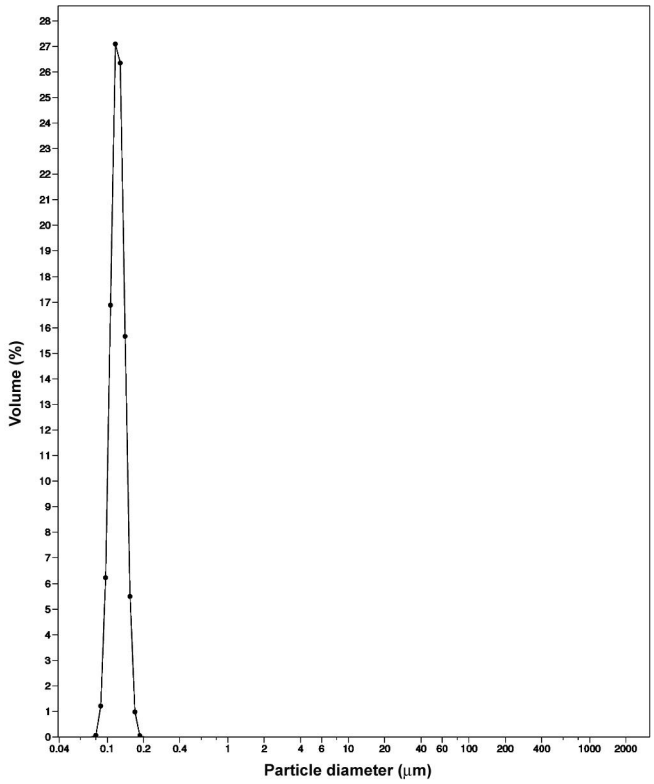

Figure 3 Light-scattering (LS) particle size graph of MPEG-*b*-PDLLGCL nanospheres with DLL/G/CL ratio of 80/15/5 mol%.

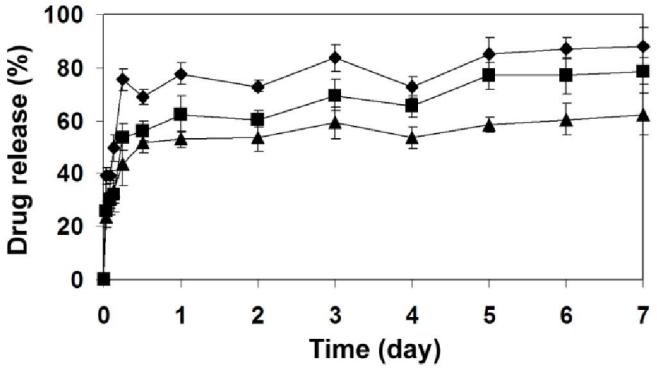

Figure 4 *In vitro* drug release profile from diblock copolymer nanospheres with DLL/G/CL ratios of (▲) 100/0/0, (■) 80/20/0 and (t) 80/15/5 mol%.