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Preparation of Organic Solvent/Surfactant-Free Microspheres of Methoxy Poly(Ethylene Glycol)-*b*-Poly(ϵ-Caprolactone) by a Melt Dispersion Method

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Abstract: Aim of this research is to prepare organic solvent and surfactant-free microspheres of biodegradable methoxy poly(ethylene glycol)-*b*-poly(ϵ-caprolactone) diblock copolymer. The microspheres were produced in 90-100°C glycerol by melt dispersion method. Morphology of the microspheres was spherical in shape with rough surfaces. Almost microspheres were in the size range of 300-500 μm. Microsphere cross-sections showed condensed phases throughout the microsphere matrices. Melting temperatures and heats of melting of the MPEG-*b*-PCL were decreased in the microsphere form. In conclusion, the use of melt dispersion method results in organic solvent and surfactant-free biodegradable microspheres of diblock copolymer that showing a potentially useful drug delivery systems with free from surfactants and organic solvents.

Key words: Biodegradable polymers, diblock copolymer, microspheres

INTRODUCTION

In recent years, interest in biodegradable microspheres of polyesters has increased steadily because of an important application in controlled release drug delivery (Stolnik *et al*., 1995; Kumar, 2000; Sinha *et al*., 2004). Usually, the biodegradable microspheres have been prepared by an oil-in-water emulsion solvent evaporation method. For this method, organic solvents and surfactants must be used. However, it is of utmost interest to develop preparation procedures that avoid residue organic solvents and surfactants in the resulted microspheres. The value of biodegradable microspheres, which today are used in medical and pharmaceutical applications, will increase if we can design biodegradable microspheres that free from organic solvents and surfactants. However, preparation of biodegradable microspheres by a melt dispersion method without organic solvent and surfactant used has not been reported.

Methoxy poly (ethylene glycol)-*b*-poly(ϵ-caprolactone) diblock copolymers (MPEG-*b*-PCL) were biodegradable and biocompatible polymers that widely synthesized and investigated for using in biomaterial applications (Aliabadi *et al*., 2005; Kim and Lee, 2001; Zhang and Zhuo, 2005). The MPEG block enhanced hydrophilicity of the PCL blocks.

In this study, we propose a simple melt dispersion method for preparing the biodegradable microspheres of MPEG-*b*-PCL in 90-100°C. The MPEG block would be decrease melt viscosity of PCL block during melt dispersion process. The molten MPEG-*b*-PCL will be disrupted into droplets due to stirring force before solidification.

MATERIALS AND METHODS

Materials

ϵ-Caprolactone (CL) monomer (99%, Acros, 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) was used as received. Chloroform and n-hexane in analytical grade were used.

Methods

Synthesis and Characterization of MPEG-*b*-PCL

The MPEG-*b*-PCL diblock copolymers were synthesized in bulk at 130°C for 48 h under a dry nitrogen atmosphere as earlier described (Baimark *et al*., 2008). The MPEG with molecular weight of 5,000 g mol^{-1} and stannous octoate were used as the initiating system. The stannous octoate 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 microsphere preparation.

Chemical composition of the MPEG-*b*-PCL was determined by ^{1}H-NMR spectroscopy using a Bruker Advanced 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 number-average molecular weight (M_n) and Molecular Weight Distribution (MWD) were measured by Gel Permeation Chromatography (GPC) using a Waters 717 plus Autosampler GPC equipped with a 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^{-1}. The thermal properties of the polymer were characterized by non-isothermal Differential Scanning Calorimetry (DSC) using a Perkin-Elmer Pyris Diamond DSC. For DSC, approximately 10 mg of the sample were placed in a sealed aluminium pan and heat at the rate of 10°C min^{-1} under helium flow to measure the melting temperature (T_m) and heat of melting (ΔH_m).

Preparation and Characterization of Microspheres

The microspheres of MPEG-*b*-PCL were prepared by melt dispersion method without any organic solvents and surfactants. This method was explained as follows. Approximately 0.5 g of MPEG-*b*-PCL was melted in 150 mL of 90-100°C glycerol with magnetic stirring at 800 rpm for 30 min before cooling to room temperature. The microspheres were filtered through sieve with 300 and 500 μm in sizes. Then, the microspheres were separated into three size ranges of <300 μm, 300-500 μm and >500 μm. The mirospheres were shaking in distilled water for rinsing glycerol before centrifugation and freeze-drying. This rinsing procedure was repeated for 5 times.

Particle size distribution of the microspheres was determined from weighing the microspheres with different particle size ranges obtained from sieving method as above. Morphology of microsphere surfaces and matrices was investigated by Scanning Electron Microscopy (SEM) using a JEOL JSM-6460LV SEM. Before SEM measurement, the samples were sputter coated with gold for enhancing the surface conductivity. Thermal properties of the microspheres were determined by DSC as described earlier.

RESULTS AND DISCUSSION

MPEG-*b*-PCL Characterization

The chemical composition of MPEG-*b*-PCL was determined from the ^{1}H-NMR spectrum by calculating the ratio of the integral peak areas corresponding to the ethylene oxide (EO, repeating units of MPEG block) methylene protons at chemical shift = 3.6-3.7 ppm and the CL ϵ-methylene protons at chemical shift = 4.0 - 4.2 ppm, as shown in Fig. 1. From the peak area integrations of the peaks a and b in Fig. 1, the copolymer composition can be determined as EO:CL = 18:82 (mol%) corresponding

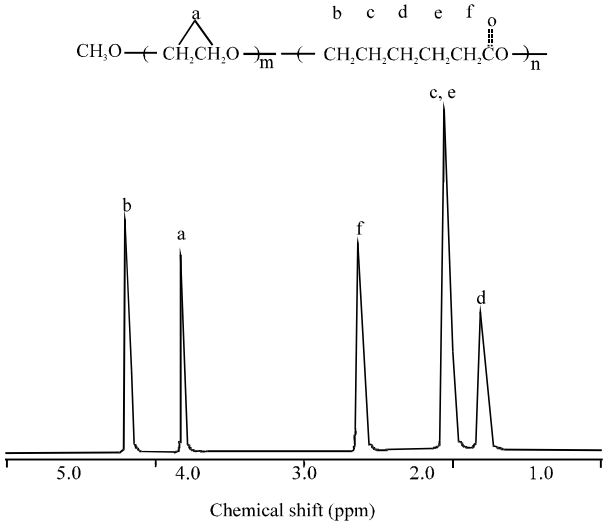

Fig. 1: ^{1}H-NMR spectrum of MPEG-*b*-PCL

to the MPEG:CL mole ratio of 1:502. As could be expected, this copolymer composition is similar to the MPEG:CL feed ratio (1:526). Therefore, the synthesized reaction was taken to near-quantitative conversion.

The M_n and MWD of MPEG-*b*-PCL obtained from GPC were 62,300 g mol^{-1} and 1.8, respectively. The T_m and ΔH_m of MPEG-*b*-PCL obtained from DSC were 58°C and 84.8 J g^{-1}, respectively.

Microsphere Characterization

From early study, homoPCL in similar molecular weight shows difficult to form the microspheres by the melt dispersion method (data did not show) whereas the MPEG-*b*-PCL microspheres were easily fabricated by the same method. This can be explained that the MPEG block may act as a plasticizer to decreased melt viscosity of PCL blocks for sphere droplet formation during melt dispersion process.

The obtained microspheres were separated into three size ranges of <300 μm, 300-500 μm and >500 μm by the sieving method. It was found that the almost microspheres (76% by weigh fraction) were 300-500 μm in size suggested that the microspheres could be prepared in narrow particle size distribution by the melt dispersion method (Fig. 2).

Microsphere morphology was investigated from their SEM micrographs. The results indicate that melt viscosity of the molten MPEG-*b*-PCL had lower enough to form as sphere droplets by magnetic stirring force in 90-100°C glycerol (Fig. 3). The surface roughness of microspheres increased when the microsphere sizes were increased as presented. It seems that the large microspheres consisted of many compressed small microspheres resulted in highly surface roughness of the large microspheres. This can be clearly observed in expanded microsphere surfaces in Fig. 4. In addition, the surface of microspheres contained holes which the depth of holes also increased with the microsphere sizes.

Internal morphology of microspheres was observed from its cross-sections as examples of which are shown in Fig. 5 and 6 for the microspheres with <300 μm and >500 μm in size ranges, respectively. It can be observed that the cross-sections of microspheres were condensed throughout the microsphere matrices. This shows that the small microspheres were completely melted and fused together. Then,

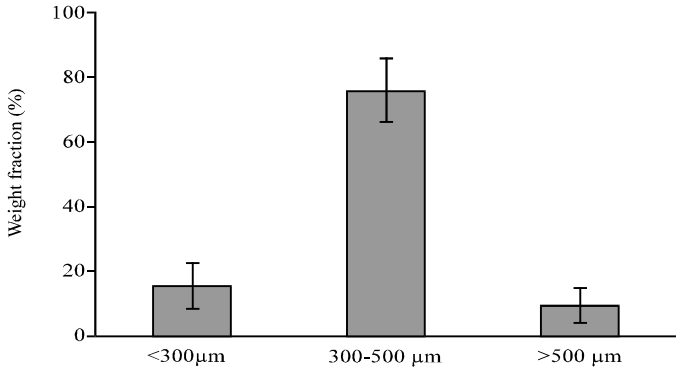

Fig. 2: Particle size distribution of microspheres measured by sieving method

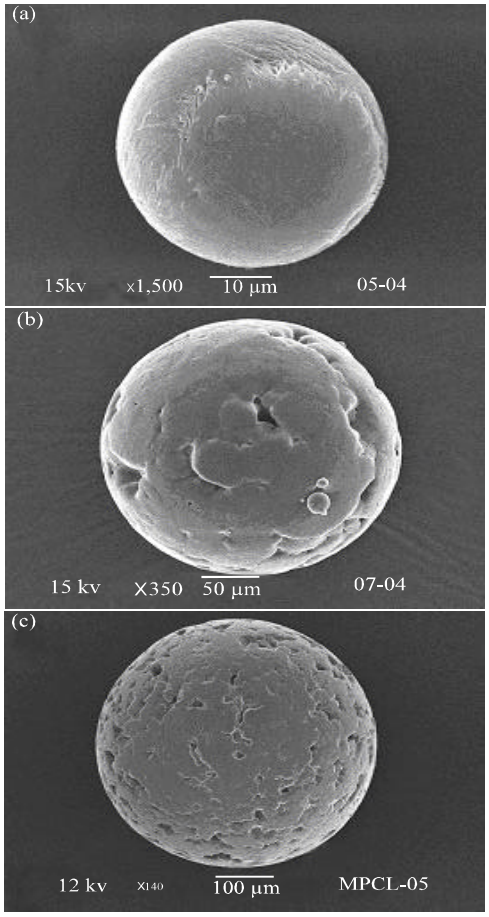

Fig. 3: SEM micrographs of microspheres in the size ranges of (a) < 300 μm, (b) 300-500 μm and (c) > 500 μm

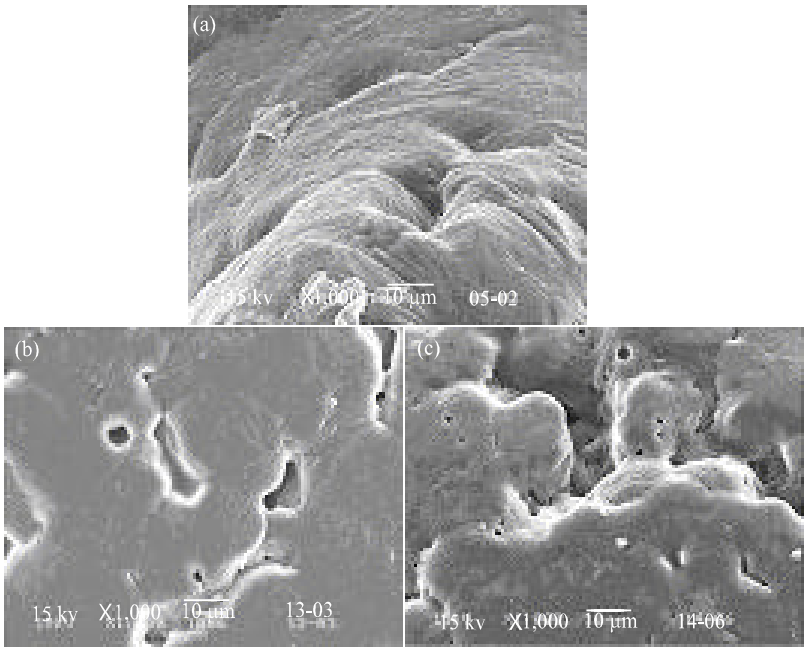

Fig. 4: Expanded SEM micrographs of surfaces of microspheres in the size ranges of (a) <300 µm, (b) 300-500 µm and (c) >500 µm

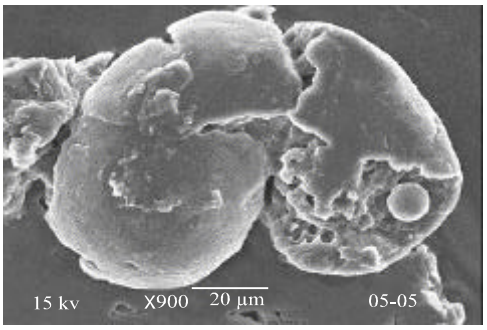

Fig. 5: SEM micrograph of cross-section of microspheres with < 300 µm in size

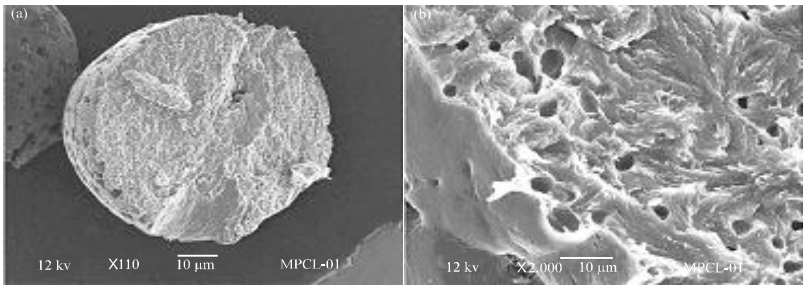

Fig. 6: SEM micrographs of cross-sections of microspheres with >500 µm in size; magnification of (a) 110 and (b) 2,000 times

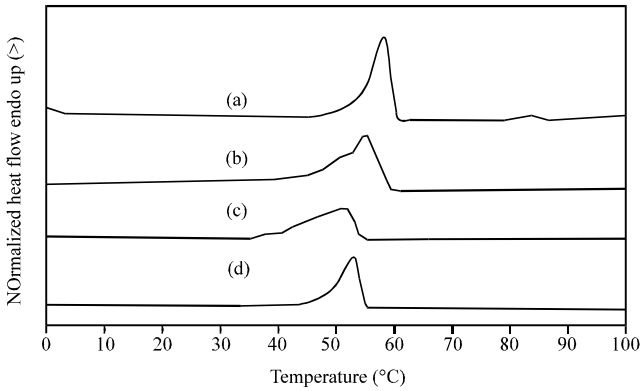

Fig. 7: DSC thermograms of (a) MPEG-*b*-PCL powders and MPEG-*b*-PCL microspheres in the size ranges of (a) <300 μm, (b) 300-500 μm and (c) >500 μm

Table 1: Thermal properties of MPEG-*b*-PCL powders and microspheres

MPEG-*b*-PCL forms	T_m (°C)	ΔH_m J g^{-1}
Powders	58	84.8
Microspheres size (μm)		
<300	55	80.4
300-500	51	64.9
>500	53	65.5

the separated microspheres can not be detected for the cross-section of large microspheres as shown in Fig. 6 while their microsphere surfaces show clearly microsphere separation as show in Fig. 4c.

The thermal properties of microspheres were investigated from DSC thermograms as illustrated in Fig. 7 and shown in Table 1. It was found that their T_m and ΔH_m were lower than the MPEG-*b*-PCL in powder form. This may be explained that the microspheres were rapidly quenched from 90-100°C to room temperature. Thus the smaller crystallisable PCL fractions were formed. The results of thermal properties indicated that the microspheres contained higher amorphous phases than the original MPEG-*b*-PCL. This amorphous characteristic may enhance the drug distribution into the microsphere matrices for used as drug carrier application.

It is important to note that this method is easy, safe and fast for larger scale preparation of biodegradable microspheres than the oil-in-water emulsion solvent evaporation method because the organic solvents, surfactants and longer time for solvent evaporation can be neglected. Thus the organic solvent and surfactant-free biodegradable microspheres can be obtained by the melt dispersion method. It is possible to prepare drug-loaded microspheres of MPEG-*b*-PCL by this melt dispersion method, especially poorly water-soluble or hydrophobic drugs. Firstly, the hydrophobic drug and MPEG-*b*-PCL were dissolved in low toxic organic solvent such as acetone before solvent evaporation and microsphere fabrication.

CONCLUSION

The microspheres of MPEG-*b*-PCL were successfully prepared by the melt dispersion method in 90-100°C glycerol. The organic solvents and surfactants can be avoided for this method. The largest weight fraction of microspheres with spherical shape and rough surface was 300-500 μm in size range. The surface roughness increased with the microsphere size. The microsphere matrices showed condensed phase with some closed voids. These biodegradable microspheres of diblock copolymer

with free from organic solvents and surfactants might be of interest for use as biomaterials in drug delivery and scaffold template applications. Further preparation and characterization of hydrophobic drug-loaded MPEG-*b*-PCL microspheres is currently being investigated.

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