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Formation of MPEG-PLLA block copolymer microparticles using compressed carbon dioxide

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Abstract−Methoxy poly(ethylene glycol)-*b*-poly(L-lactide) (MPEG-PLLA) diblock copolymer was synthesized via ring-opening polymerization, and MPEG-PLLA microparticles were then prepared using an aerosol solvent extraction system (ASES) technique with compressed carbon dioxide as antisolvent. The MPEG-PLLA microparticles were prepared at temperatures ranging from 25 °C to 55 °C and at pressures from 85 bar to 150 bar. The concentration of MPEG-PLLA copolymer, solution flow rate, and CO_2 flow rate were adjusted to be 0.5-3.0% (w/v), 0.3-1.0 mL/min, and 19 g/min, respectively. Relatively small spherical microparticles were prepared in the subcritical region at 25 °C, while agglomerated particles were obtained at temperatures above the critical point. The mean particle sizes of the MPEG-PLLA microparticles prepared by the ASES varied from 9.53 μm to 46.9 μm depending upon the operating conditions.

Key words: ASES, MPEG-PLLA, Compressed Carbon Dioxide, Microparticles

INTRODUCTION

Drug-loaded biodegradable polymeric particles have been of great importance in the development of controlled-release devices for therapeutic applications. Biodegradable polymer nano- and microparticles have attracted considerable attention because of their flexibility of administration. Among many biodegradable polymers, poly (lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) have been widely used for a wide variety of pharmaceutical and biomedical applications such as scaffolds for tissue engineering and carriers for controlled release of drugs, because of their proven biocompatibility and favorable regulatory status. However, their high hydrophobicity often makes them unsuitable for controlled-release pharmaceutical formulations: for example, PLA or PLGA particles show too slow drug release and provide an unfavorable environment for hydrophilic drugs.

Polyethylene glycol (PEG) has been widely used to prepare stealthy or long-circulating particles. PEG is water-soluble, nonionic, nonantigenic, and non-immunogenic. The presence of PEG or PEG-containing copolymers on the surface of nano- and microparticles results in the formation of an effective protective layer, which inhibits the interaction of the particles with opsonin proteins via steric repulsion [1-4]. The blood clearance of BSA-loaded PEG-PLGA nanoparticles showed a prolonged circulatory half-life ($t_{1/2}$=270.9 min) compared to PLGA nanoparticles ($t_{1/2}$=13.6 min) [5].

Conventional methods of preparing drug-loaded particles include emulsion/solvent evaporation, spray drying, freeze-drying, phase separation, *etc.* [6]. However, most of these methods need large amounts of organic solvents and emulsifiers, which may cause serious environmental problems. Furthermore, these methods usually require a post-production drying step, lasting several days, to remove the residual solvent. To overcome these limitations, many innovative particle engineering techniques have been developed, among which supercritical fluid technology is one of the most promising methods [7-11].

Supercritical particle formation processes have many important advantages over the conventional methods, including minimal or no use of organic solvents, no residual solvents in the end products, environmental friendliness and reduced process complexity. Carbon dioxide is by far the most widely used fluid in supercritical fluid applications because of its mild critical conditions, non-toxicity, inflammability, good miscibility with organic solvents, and excellent transport properties. Supercritical carbon dioxide ($SCCO_2$) technologies have been applied in a variety of areas in the pharmaceutical industry, such as particle coating, encapsulation and particle size controlling.

Many fundamental studies focusing on the synthesis and physicochemical properties of MPEG-PLLA have been carried out [12-16]. MPEG-PLLA based micelles [17,18] and nanoparticles have been prepared and their properties have been investigated for drug delivery applications [19-21]. In the present study, MPEG-PLLA diblock copolymer was synthesized by ring opening polymerization, and MPEG-PLLA particles were then prepared by an aerosol solvent extraction system (ASES) technique using $SCCO_2$ to confirm the feasibility of supercritical fluid process for production of MPEG-PLLA based controlled release devices. $SCCO_2$ was used as antisolvent, and dichloromethane (DCM) was selected as solvent for MPEG-PLLA. The effect of process parameters (pressure, temperature, solution flow rate, and solution concentration) on the morphology and size of MPEG-PLLA particles was evaluated.

EXPERIMENTAL

1. Materials

L-lactide ((3S,6S)-3,6-dimethyl-1,4-dioxane-2,5-dione) was purchased from the Tokyo Chemical Industry and recrystallized from

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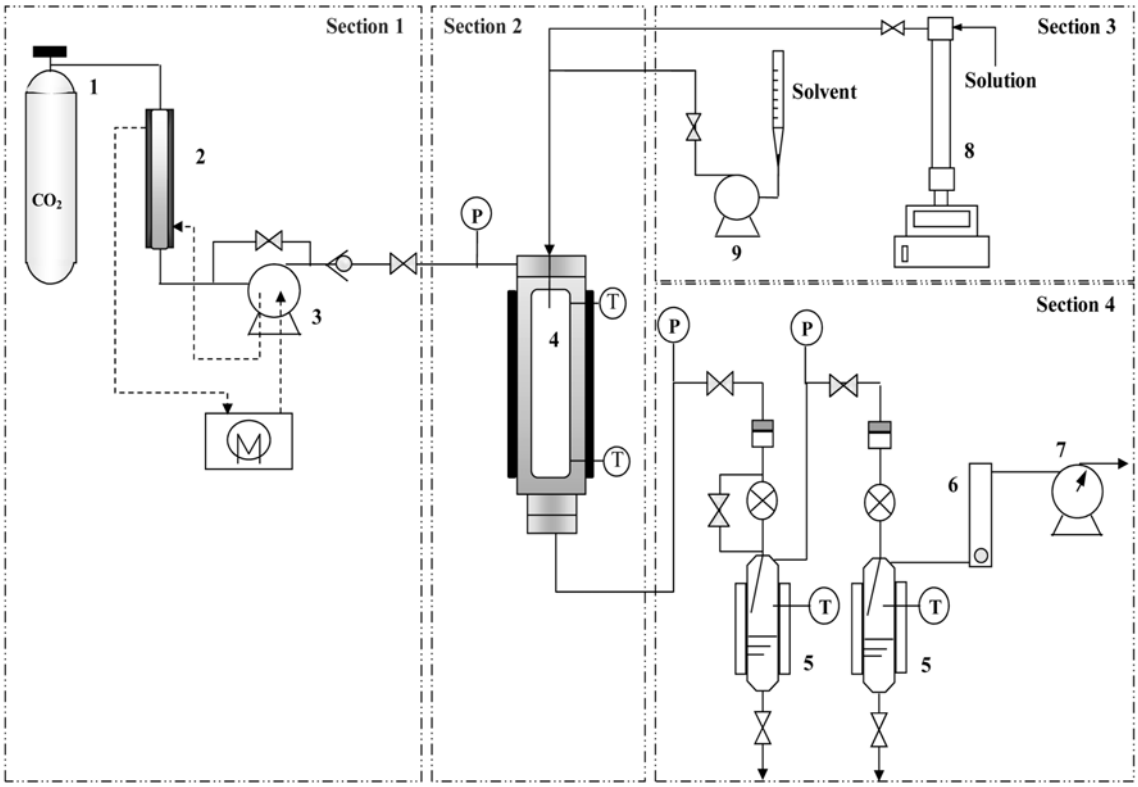

Fig. 1. Schematic diagram of the ASES apparatus.

1. CO_2 cylinder 3. High pressure pump 5. Sampling tank 7. Dry tester meter 9. LC pump
2. CO_2 cooler 4. Precipitator 6. Rotameter 8. Syringe pump

ethyl acetate before use. Methoxy (polyethylene glycol) (MPEG) with a molecular weight of 5,000 Da and stannous octoate ($Sn(Oct)_2$) were obtained from Sigma-Aldrich. Before polymerization, $Sn(Oct)_2$ was dissolved in distilled benzene to form a 0.1 M solution. All other reagents and solvents were of HPLC grade and used without further purification.

2. Synthesis of MPEG-PLLA Diblock Copolymer

MPEG-PLLA diblock copolymer was synthesized by solution ring-opening polymerization of L-lactide with hydroxyl-terminated MPEG as an initiator and $Sn(Oct)_2$ as a catalyst [21]. A certain amount of MPEG was introduced into a 250 mL three-necked flask along with 80 mL of toluene. Residual water in the solution was removed by azeotropic distillation at 130 °C for 5 h using an oil bath, resulting in 30 mL of MPEG/toluene solution. 2 g of L-lactide and 0.1 M $Sn(Oct)_2$ (0.5 wt% of MPEG and L-lactide) were then injected into the flask, and the reaction mixture was heated under reflux at 130 °C for 24 h under a nitrogen atmosphere. After the reaction was complete, the reaction mixture was precipitated in cold diethyl ether, producing MPEG-PLLA. The unreacted L-lactide monomer and PLLA homopolymer were removed by dissolving them in diethyl ether. The copolymer products were dried in a vacuum oven at 50 °C for 1 day. The molecular weight and the MPEG content of the synthesized MPEG-PLLA were determined by ^{1}H NMR (Unity Inova 500NB, Varian). Attenuated total reflection-Fourier transform infrared spectrophotometer (ATR-FTIR, Varian, 640-IR) equipped with PIKE MIRacle™ ATR accessory with a single reflection ZnSe ATR crystal was used to confirm the presence of the ester carbonyl group in the synthesized copolymer products.

3. Preparation of MPEG-PLLA Particles

MPEG-PLLA particles were prepared using a supercritical ASES process. A schematic diagram of the ASES apparatus used in this study is shown in Fig. 1. It consisted of four major sections: Section 1 is an antisolvent (CO_2) supply system, Section 2 is a high-pressure precipitator (a tubular vessel with an inside diameter of 25 mm and a length of 450 mm), and Sections 3 and 4 are a solution feeding unit and a separation part, respectively. Liquefied CO_2 was fed to the top of the precipitation vessel by a high-pressure liquid pump (ELM-1, Teikoku Electric, Japan). The CO_2 pressure was constantly controlled by a back-pressure regulator (BPR, 26-1723-24-043, Tescom, USA). The MPEG-PLLA particles were prepared at temperatures ranging from 25 °C to 55 °C and pressures ranging from 85 bar to 150 bar. The concentration and flow rate of polymer solution and the CO_2 flow rate were adjusted to 0.5-3.0% (w/v), 0.3-1.0 mL/min, and 19 g/min, respectively. Dichloromethane (DCM) was used as the solvent in all the experiments. Pure solvent was pumped through a nozzle with an HPLC pump (Prep 24, LapAlliance, USA) to the precipitator to obtain steady-state conditions in the fluid phase during solute precipitation and to avoid blockage of the injection nozzle from solute precipitation in the startup phase. The MPEG-PLLA solution was preheated before being introduced into the precipitator, and then sprayed into the high-pressure precipitation vessel via a nozzle with an inside diameter of 127 μm at a predetermined flow rate regulated by a high-pressure syringe pump (260D, Teledyne Isco, USA). When the sprayed solution comes into contact with the continuous

$SCCO_2$ phase cocurrently, two mass transfers occur between solution droplets and the $SCCO_2$ phase. One is fast diffusion of $SCCO_2$ into the liquid phase, and the other is evaporation of the organic solvent from the surface of liquid droplets into the continuous $SCCO_2$ phase. These two mass transfers rapidly lead to supersaturation, precipitating MPEG-PLLA in the form of fine particles. The precipitated MPEG-PLLA particles were collected on a metal filter of pore size 5 μm placed at the bottom of the vessel. The fluid mixture of $SCCO_2$ and solvent was passed through the filter and then separated in a depressurizing cylinder. After a sufficient quantity of particles was harvested, the liquid solution flow was stopped and the residual organic solvent on the particles was extracted using pure $SCCO_2$ for 30 min.

4. Size and Surface Morphology Analysis

The surface morphology of the polymer particles prepared by ASES was analyzed by a field emission scanning electron microscope (FE-SEM, JSM6700F, JEOL, Japan). The prepared particles were dispersed on a carbon tap stuck to an aluminum stub. Prior to analysis, samples were coated with gold-palladium by using a sputter module. The mean size distributions (MSD) of the particles were obtained as D (v, 0.5), the equivalent volume diameter at 50% cumulative volume determined by laser diffraction using a Mastersizer 2000 S (Malvern Instruments, UK) coupled with the Mastersizer S long bed software (Release 2.19, Malvern Instruments, UK) that controls the collection, manipulation, and presentation of the data. The particles were dispersed in deionized water and sonicated for 2 min before measurement.

RESULTS AND DISCUSSION

1. Synthesis of MPEG-PLLA Diblock Copolymer

MPEG-PLLA diblock copolymer was synthesized from L-lactide by ring-opening polymerization using MPEG as the initiator. The structure of the synthesized polymer was confirmed by a proton NMR spectrum in $CDCl_3$, which is shown in Fig. 2. The peaks at 1.55 and 5.15 ppm correspond to the methyl ($-CH_3$) and methine (-CH) hydrogens of the PLLA segment, respectively, while the methjylene ($-CH_2-$) hydrogens of the PEG segment appear at 3.65 ppm. No other peaks were detected. This demonstrates the successful synthesis of high-purity MPEG-PLLA diblock copolymer [15]. From the ratio of the peak areas at 5.15 and 3.65 ppm, the number-average molecular weight of the synthesized copolymer was determined to be 16,000, and the weight content of MPEG to be 31.2%.

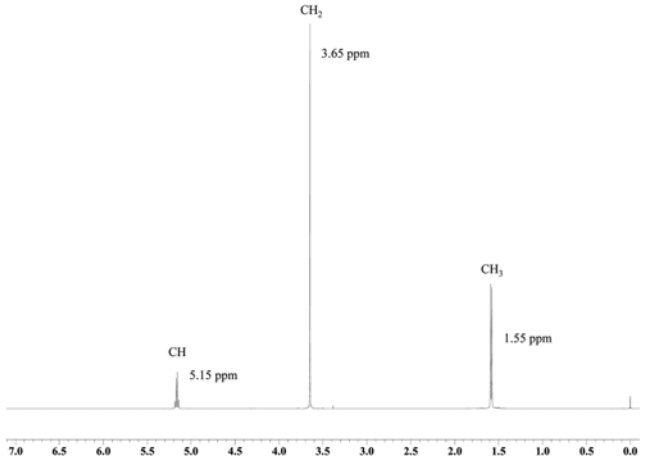

Fig. 2. ^{1}H NMR spectrum of MPEG-PLLA in $CDCl_3$.

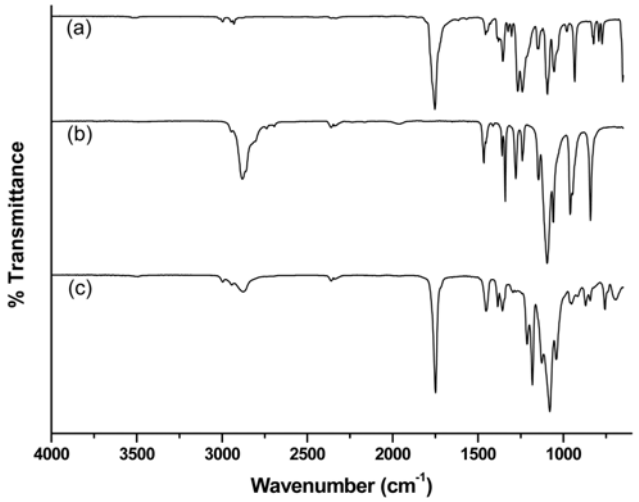

Fig. 3. ATR-FTIR spectra of (a) L-lactide, (b) MPEG, and (c) MPEG-PLLA.

The presence of ester groups originating from the reaction of the MPEG hydroxyl group with the acyl oxygen of L-lactide was investigated by ATR-FTIR spectroscopy (see Fig. 3). In the MPEG-PLLA IR spectrum, the absorption band at 2,876 cm^{-1} is attributed to the C-H stretch of methylene groups of the MPEG segment. A very strong band at 1,749 cm^{-1} is due to the C=O stretch of ester groups in the PLLA segment.

2. Effect of Temperature and Pressure

The main purpose of this work was to investigate the variation in the size and morphology of MPEG-PLLA particles with the ASES process parameters such as temperature, pressure, flow rate and solution concentration. The experimental conditions for the preparation of particles are summarized in Table 1. The miscibility of supercritical fluid with organic solvent is one of the key parameters controlling the efficiency of the supercritical ASES process. The CO_2 solubility in DCM depends mainly on operating temperature and pressure, and residence time in the precipitator [22]. The effect of temperature on the size and morphology of MPEG-PLLA particles is shown in Table 1 and Fig. 4, respectively. The process temperature was increased from 25 °C to 55 °C, in 10 °C increments, at a pressure of 130 bar, a solution flow rate of 0.5 mL/min, a concentration of 1.0% (w/v) and a CO_2 flow rate of 19 g/min.

An isobaric increase in temperature from 25 °C to 55 °C resulted in an increase in the mean particle size from 9.53 μm to 46.90 μm. As shown in Fig. 4, fine spherical particles were produced in the subcritical region (at 25 °C), while larger, agglomerated particles of irregular shape were produced at higher temperatures in the supercritical region. This indicates that the morphology and size of the particles have a strong correlation with the operating temperature.

An explanation for such a strong temperature dependence of the mean particle size and morphology is that the MPEG chain of the MPEG-PLLA block copolymer could be partially melted by $SCCO_2$. $SCCO_2$ is known to be able to plasticize polymers and to reduce

Table 1. Preparation conditions of MPEG-PLLA particles and their mean particle size

Experiments	T (°C)	P (bar)	Solution flow rate (mL/min)	Concentration (%, w/v)	Mean particle size (μm)
1	25	130	0.5	1	9.53
2	35				16.29
3	45				24.12
4	55				46.90
5	35	85	0.5	1	18.28
6		100			16.19
7		150			16.52
8	35	130	0.3	1	14.81
9			1		18.92
10	35	130	0.5	0.5	15.40
11				3	15.35

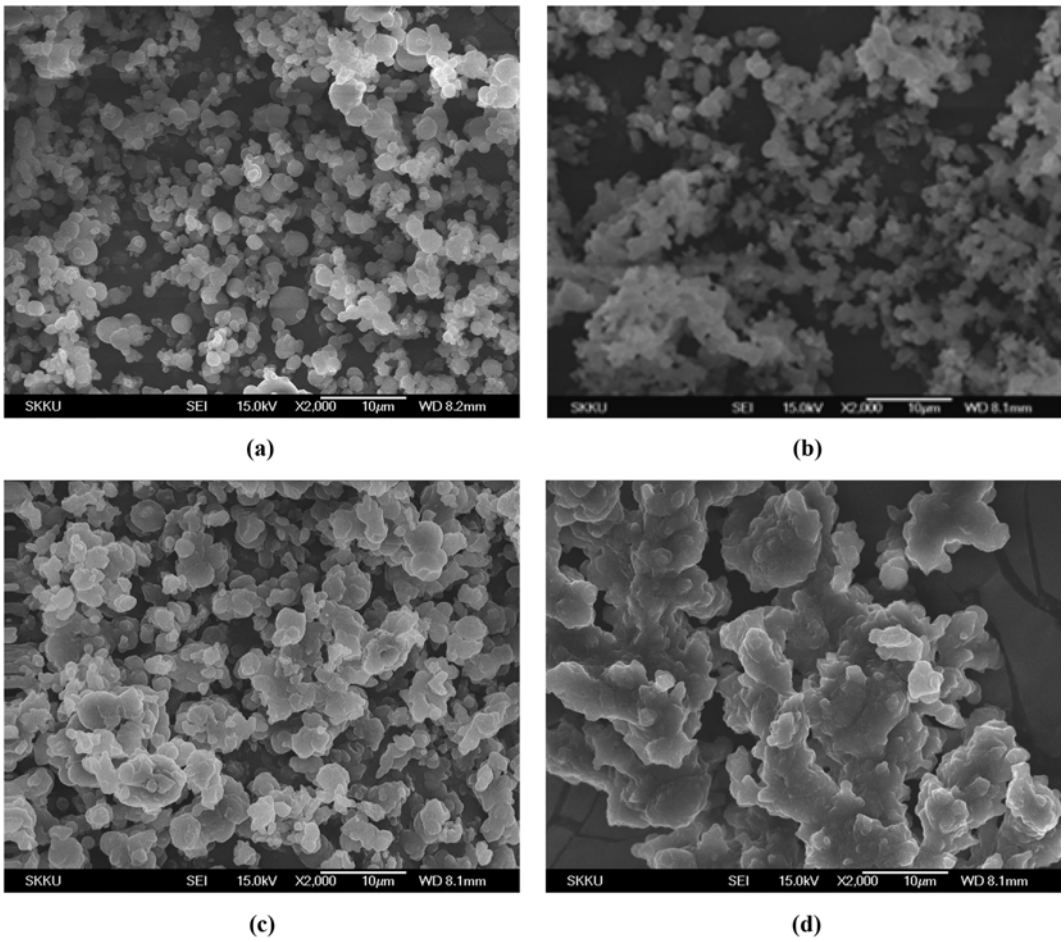

Fig. 4. SEM micrographs of various MPEG-PLLA particles prepared at 130 bar and temperatures of (a) 25 °C, (b) 35 °C, (c) 45 °C, and (d) 55 °C. The scale bar is 10 μm.

the glass transition temperature in the case of amorphous polymers, or the melting point in the case of semi-crystalline polymers. Thus, it is very difficult to produce PEG particles using $SCCO_2$ as the antisolvent because of its relatively high solubility in the PEG matrix [23]. Weidner et al. [24] have reported that the melting point of PEG 4000 decreases significantly with increasing pressure, especially up to 80 bar (from 56.2 °C at 1 bar to 45 °C at 80 bar) and is practically constant in the pressure range of 80-210 bar. In contrast, many researchers have confirmed that well-formed particles of the semi-crystalline PLLA are produced by the ASES processing using $SCCO_2$ as antisolvent [7,10,11,25]. Thus, it is very plausible that the particles collected on the metal filter at the bottom of the precipitator have aggregated in the supercritical region. However, the particles prepared in the subcritical region were spherical because the MPEG chain of the block copolymer has been little affected by CO_2 in the subcritical region.

The effect of pressure at a given temperature on mean particle size and morphology is shown in Table 1 and Fig. 5. The pressure was changed in the range of 85-150 bar at 35 °C, while keeping other operating parameters constant (solution flow rate: 0.5 mL/min, solution concentration: 1.0% (w/v), CO_2 flow rate: 19 g/min). As shown in Fig. 5, agglomerates of spherical particles smaller than 1 μm were obtained at all experimental pressures. The mean particle size, which was measured with a laser diffraction particle size analyzer, was in the range of 16.19-18.28 μm (see Table 1 for detail). It was found that the effect of pressure at a fixed temperature on the mean particle size is not significant. This observation is in qualitative agreement with the previous result of Reverchon et al. [25] that, for pressures higher than the asymptotic volume expansion, there is no significant pressure effect on the mean particle size or on the particle

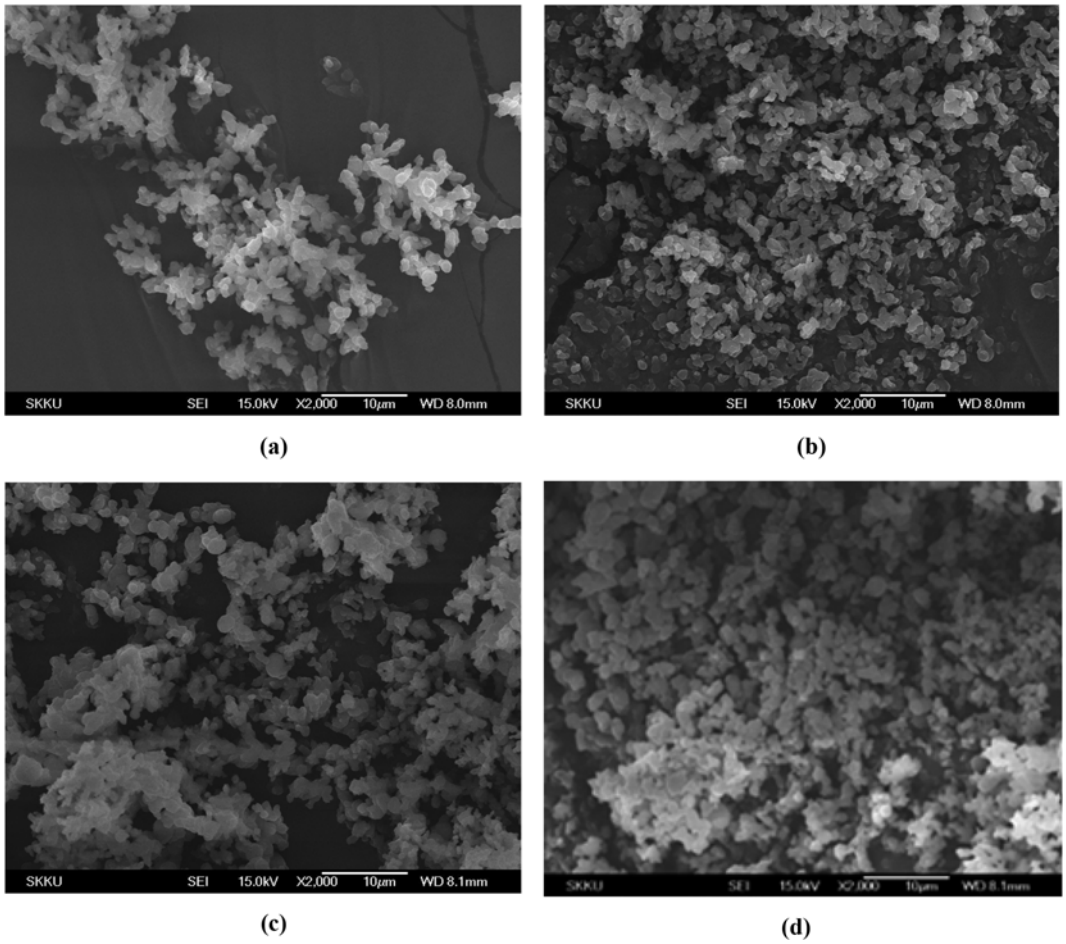

Fig. 5. SEM micrographs of various MPEG-PLLA particles prepared at 35 °C and pressures of (a) 85 bar, (b) 100 bar, (c) 130 bar, and (d) 150 bar. The scale bar is 10 μm.

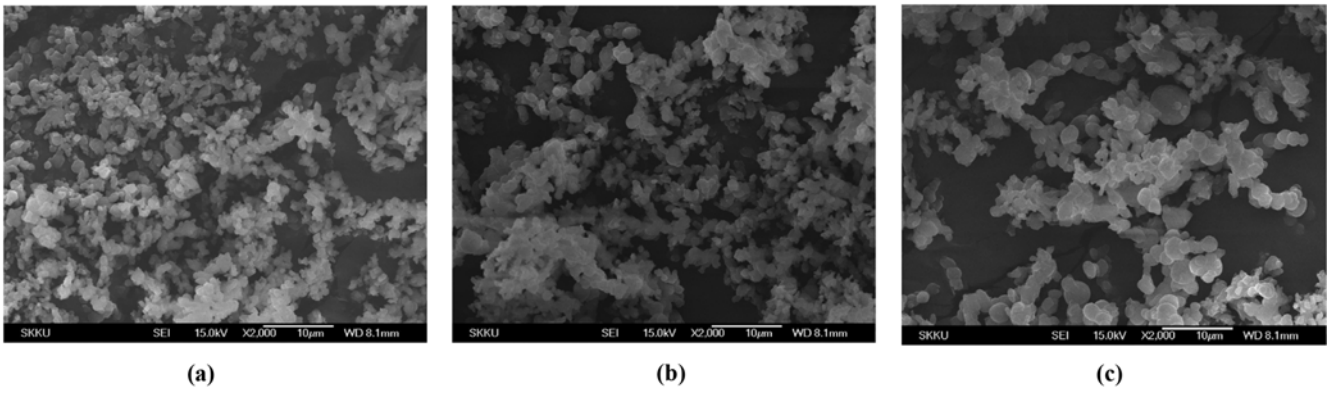

Fig. 6. SEM micrographs of various MPEG-PLLA particles prepared at 35 °C, 130 bar and solution flow rates of (a) 0.3 mL/min, (b) 0.5 mL/min, and (c) 1.0 mL/min. The scale bar is 10 μm.

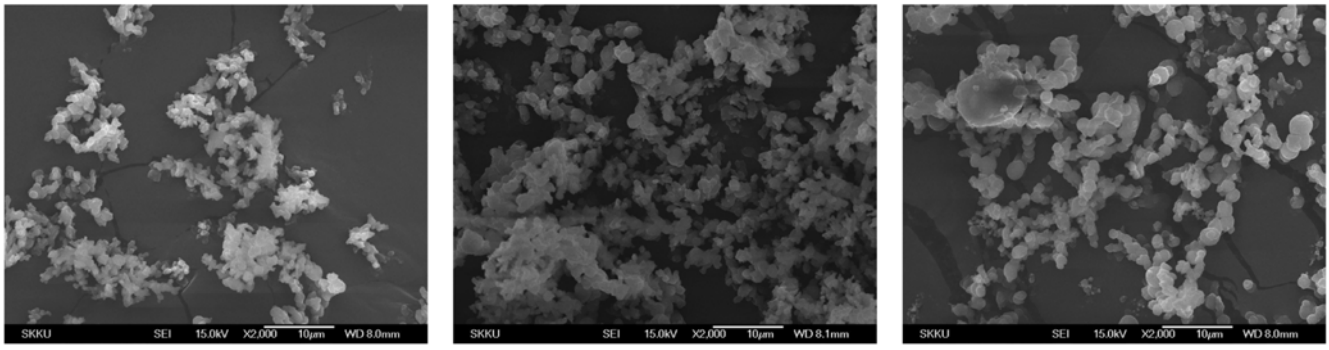

Fig. 7. SEM micrographs of various MPEG-PLLA particles prepared at 35 °C and 130 bar using different solution concentrations: (a) 0.5%, (b) 1.0%, and (c) 3.0% (w/v). The scale bar is 10 μm.

size distribution of precipitated powders.

3. Effect of Solution Flow Rate

The influence of solution flow rate on the formation of MPEG-PLLA particles is shown in Table 1 and Fig. 6. The solution flow rate was changed in the range of 0.3-1.0 mL/min. Fig. 6 shows that the degree of agglomeration increased as the solution flow rate increased. Therefore, the mean size of the MPEG-PLLA particles increased with the solution flow rate, as shown in Table 1. Higher flow rates decreased the mass-transfer rates of organic solvent out of the droplets, thus reducing the achievable supersaturation ratio. Lower supersaturation ratios engendered fewer nuclei, which in turn produced agglomerated and larger particles [26].

4. Effect of Concentration

The effect of concentration on the mean particle size and morphology is shown in Table 1 and Fig. 7. The MPEG-PLLA concentration in DCM was changed from 0.5% to 3.0% (w/v) at 35 °C and 130 bar, while the solution and CO_2 flow rates were kept to be constant as 0.5 mL/min, and 19 g/min, respectively. As shown in Fig. 7, the particle size increased with the MPEG-PLLA solution concentration and the morphology of the particles was changed from irregular to spherical. The size of unit particles composing the agglomerates increased with increasing solution concentration because of the higher solution viscosity. However, the MPEG-PLLA concentration did not exert a significant influence on the mean particle size, which was greatly affected by the degree of agglomeration, as shown in Table 1.

CONCLUSIONS

MPEG-PLLA diblock copolymer was synthesized, and MPEG-PLLA microparticles were then prepared using a supercritical ASES technique. It was found that the operating temperature significantly influences the size and morphology of MPEG-PLLA particles. Relatively small spherical particles were obtained in the subcritical region, while highly agglomerated particles were produced in the supercritical region. Laser-diffraction particles size analysis showed that the effect of pressure, solution flow rate and solution concentration on the mean particles size was not significant.

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