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Preparation of L-PLA Submicron Particles by a Continuous Supercritical Antisolvent Precipitation Process

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Abstract−Submicron particles of L-polylactic acid (L-PLA) without residual solvent were prepared by a continuous supercritical antisolvent (SAS) recrystallization process. Methylene chloride (CH_2Cl_2) was used as a carrier solvent of L-PLA. Experiments were performed with changing process parameters such as pressure and temperature at constant concentration. Also, L-PLA initial concentrations in methylene chloride were varied from 0.3 to 4 wt%. The flow rates of CO_2 and solution, which were introduced into the precipitator, and nozzle diameter were kept unchanged in all of the experiments. It was found that the SAS process gives fine tuning of particle size and particle size distribution (PSD) by simple manipulations of the process parameters. In all cases of SAS recrystallization experiments, the formed spherical fine particles with a smooth surface were non-agglomerated and free flowing. Mean particle size of the L-PLA microparticles formed was varied from 0.1 to 1 μm by means of adjusting the system pressure and/or temperature.

Key words: Biodegradable Polymer, Poly(L-Lactic Acid), Supercritical Antisolvent, Recrystallization, Atomization

INTRODUCTION

An increasing requirement for modulated delivery of both conventionally and biotechnologically generated drugs of a high molecular weight and short half-life has generated considerable interest in the development of biodegradable polymers and their formulation into drug delivery systems. Biodegradable polymers used in drug delivery research may be broadly classified as of natural or synthetic origin. The most widely investigated and advanced synthetic polymers in terms of the available toxicological and clinical data are the linear aliphatic polyesters based on the hydroxyacids lactic acid and glycolic acid. Poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(lactic-co-glycolide) (PLGA) display important advantages of biocompatibility, predictability of biodegradation kinetics, ease of fabrication, regulatory approval, and commercial availability. They are used clinically as absorbable sutures and have been investigated for bone plates, implant materials, bone graft substitutes, and nerve graft substitutes. Yolles et al. [1975] were among the first to use PLA for parenteral drug delivery. In recent years, these polymers have been applied widely in microsphere/microcapsule formulation and are already used clinically for drug delivery in the form of implants.

The biodegradable microparticles/microspheres can be prepared by different techniques recently reported in various articles, such as organic solution precipitation methods (polymer phase separation, solvent evaporation or solvent extraction), spray drying methods, or machinery comminution methods (crushing, grinding, and ball milling etc).

Organic solution precipitation methods and machinery comminution methods suffer from some shortcomings such as wide particle size distribution and environmental pollution problems associated with the use of organic solvent that not only can adversely affect the crystallinity and chemical stability of solutes, but also include the use of high shearing forces, long exposure time of the biodegradable polymers to organic solvents and the necessity of lyophilization to obtain a stable powder. The spray-drying process involves spraying a solution containing the solute into a hot gas to remove the solvent. This technique requires the removal of organic solvent and involves the use of high temperature. In the manufacture of microparticles intended for in vivo use, the removal of residual surfactants and solvents is a particularly important requirement. Therefore, there is an increasing demand in developing technology that allows microparticles with controlled particle size distribution and product quality (crystallinity, purity, morphology) to be produced under mild conditions.

During the last ten years, the most important methodologies using supercritical fluid (SCF) for the production of microparticles with Rapid Expansion of Supercritical Solutions (RESS) [Tom et al., 1993], Particles from Gas Saturated Solutions (PGSS) [Kerč et al., 1999], or Supercritical Anti Solvent (SAS) [Yeo et al., 1993; Reverchon et al., 1998] have been developed. The applicability of each technique depends mainly on the solute solubility in the SCF.

All gases can form SCFs above specific sets of critical conditions, although extremely high temperatures and/or pressures may be required. The most widely used SCF is carbon dioxide (CO_2) because of its low critical temperature (T_c=31.1 °C), attractiveness for heat-sensitive materials including products sourced from biologicals, as well as being non-flammable, non-toxic, and inexpensive.

In general, the SAS technique has been developed in several different modes. The first mode (semi-batch operation) is known as the Gas Anti Solvent (GAS) process in which the SCF is put into a

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stationary bulk liquid phase at the operating pressure [Yeo et al., 2000]. The second mode is continuous operation, in which a spray of liquid solution is fed through a capillary nozzle as fine droplets into a vessel containing the supercritical fluid [Randolph et al., 1993]. When in contact with the supercritical fluid, the liquid solution including solute dissolves into the supercritical fluid, so high supersaturation of solute in the mixed solution can be achieved and small particles of solute can be precipitated. The second mode is also known as Aerosol Spray Extraction System (ASES) [Bleich and Mueller, 1996]. If either liquid or high-pressure gas is employed as antisolvent, the alternative name of this second mode is Precipitation with Compressed Antisolvents (PCA) [Dixon et al., 1993]. In addition, a new process, Solution Enhanced Dispersion by Supercritical Fluids (SEDS) [Palakodaty et al., 1998], has been proposed in which continuous injection with a coaxial nozzles takes place. All these methods have been successfully used to produce ultrafine particles of high explosives and solid propellants, pharmaceutical compounds, proteins, and polymers.

A considerable amount of research has been done to determine the effect of different process parameters on the antisolvent precipitation process, but as yet there is not much knowledge on the quantitative aspects of the process. Contradictory results have been obtained by different authors regarding the influence of pressure and temperature on the particle size during the precipitation process. Some researchers found a decreasing trend in particle size with reducing pressures, others observed the process to independent of pressure and yet others obtained particle size increases with pressure. Analogous discrepancy in the results was also observed with respect to the temperature. However, it was found that the particle size is strongly affected by the concentration of solution in one set of experiments.

The aim of this work is to make a contribution by studying the use of carbon dioxide as antisolvent in an SAS process for the precipitation of poly(L-lactic acid) (L-PLA) from methylene chloride (CH_2Cl_2) solution. The specific goal is to determine critical process variables affecting particle size and particle size distribution, as well as the reproducibility of these parameters. Therefore, the effects of the operating temperature, pressure, the concentration of solution, the flow rates of solution and CO_2, nozzle diameter, nozzle geometry on the particle size, particle size distribution, and morphology of the final product have been experimentally investigated.

EXPERIMENTAL

1. Material

The Poly(L-lactic acid) (M.W.: 85,000-16,0000, i.v.: 1.645) used here was supplied by SIGMA. The untreated L-PLA particles ranged from 50-100 μm, as shown in Fig. 2. Technical grade (99.0%) carbon dioxide was supplied by a local gas company in Korea. Reagent grade methylene chloride (analytical reagent, Mallinckrodt) was used as the solvent. These materials were used without further purification.

2. Apparatus

The experimental apparatus, shown in Fig. 1, consisted of CO_2 supplying system, L-PLA solution feeding system, a precipitator with injector, filters, a pressure regulator, and gas-liquid separation section.

Methylene chloride solution containing L-PLA is sprayed by a high-pressure metering pump (Milton Roy) into the precipitator vessel (Jerguson Gage, 34 ml) through a nozzle (stainless steel, 0.03" I.D., 1/16" O.D.). The nozzle is mounted on the top of the precipitator to introduce the L-PLA solution as dispersed liquid droplets into the antisolvent. Carbon dioxide is compressed by another high-pressure metering pump (Milton Roy) and fed into on the side or top of the precipitator as a continuous phase. The carbon dioxide was controlled at a desired temperature by using a heat exchanger in front of the precipitator. The precipitator is placed in a thermo-

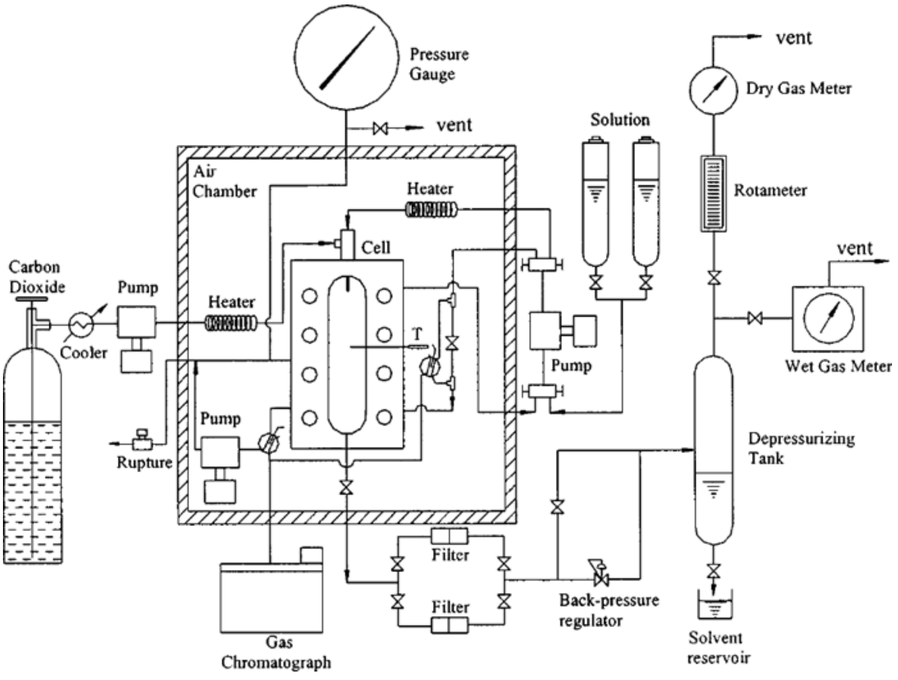


Fig. 1. Apparatus for recrystallization of microparticles.

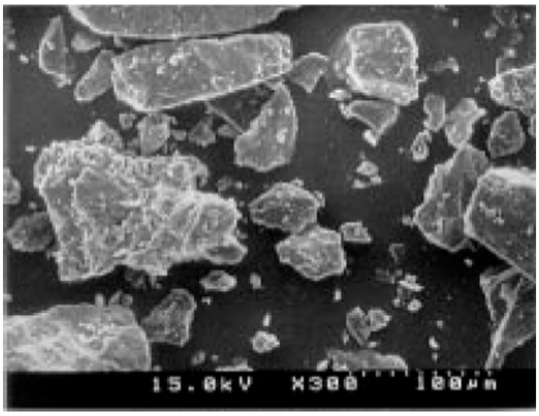


Fig. 2. SEM photographs of untreated L-PLA particles.

stat air chamber where the temperature is maintained with a PID temperature controller (Han Young Co.). The pressure of the precipitator is controlled by a back-pressure regulator (Tescom; 26-1721-24). Upon injection into the precipitator, L-PLA particles are formed and a fluid mixture of carbon dioxide and methylene chloride laden L-PLA particles leaves the precipitator. L-PLA particles are collected on filters (Tee-Type filter, 0.5 μm) which are located between the precipitator and the back pressure regulator. These filters were situated on both sizes by using a tee-type union in order to avoid a sudden pressure reduction in the precipitator as well as to investigate continuous experiments. The line from the back pressure regulator to the depressurizing tank is heated by electrical band heaters to prevent freezing due to CO_2 expansion. The effluent mixture is depressurized and expanded to atmospheric pressure as it passes through a depressurizing tank (SUS 316; 300 ml). The instantaneous and the accumulated flow rate of CO_2 are measured with a rotameter (Metheson 604 SUS ball) and a dry gas meter (Taekwang Energy S/T-3), respectively.

3. Procedure

Carbon dioxide was fed to the precipitator, in which the temperature was maintained constant with a value ranging from 25 °C to 50 °C, up to the desired pressure. After CO_2 flow rate, which was controlled at a designated value (8 L/min), was attained at a steady state condition, methylene chloride solution containing L-PLA was pumped into the precipitator through the nozzle (0.03") at a flow rate of 0.30 mL/min. At each experiment, the L-PLA solution of 0.30 mL was injected into the precipitator. Under normal conditions, a white fog of particles formed in the precipitator. Most of the particles formed were collected at the filter. Upon stopping injection, supercritical carbon dioxide continued to flow for several minutes to wash out any residual solvent from the particles, until the precipitator became completely clean. After the valve between precipitator and filter was closed, the filter was gradually depressurized by the back pressure regulator for the organic solvent to be condensed during the depressurization. The fluid mixture left the filter for the depressurization tank. Finally, the carbon dioxide was effluent up and the organic solvent was left down in the depressurization tank.

4. Particle Morphology

Morphological evaluation of the microparticles was conducted by scanning electron microscopy (SEM) (HITACHI S-4200). The microparticles were attached to the specimen holder with a double-coated adhesive tape.

5. Particle Size Measurement

The mean particle size of each sample was determined by using a laser diffraction particle size analyzer (LS 230, Coulter Electronics, Hialeah, FL). The method of sizing and counting particles is based on measurable changes in electrical resistance produced by nonconductive particles suspended in an electrolyte.

RESULTS AND DISCUSSION

In the continuous SAS process, there are two consecutive steps to form particles: droplet formation and precipitation in the droplets. The size of droplets is described by the dimensionless Weber number (N_{we}) [Lefebvre, 1989]:

$$N_{we}=\rho_{CO_2}V^2d/\sigma$$

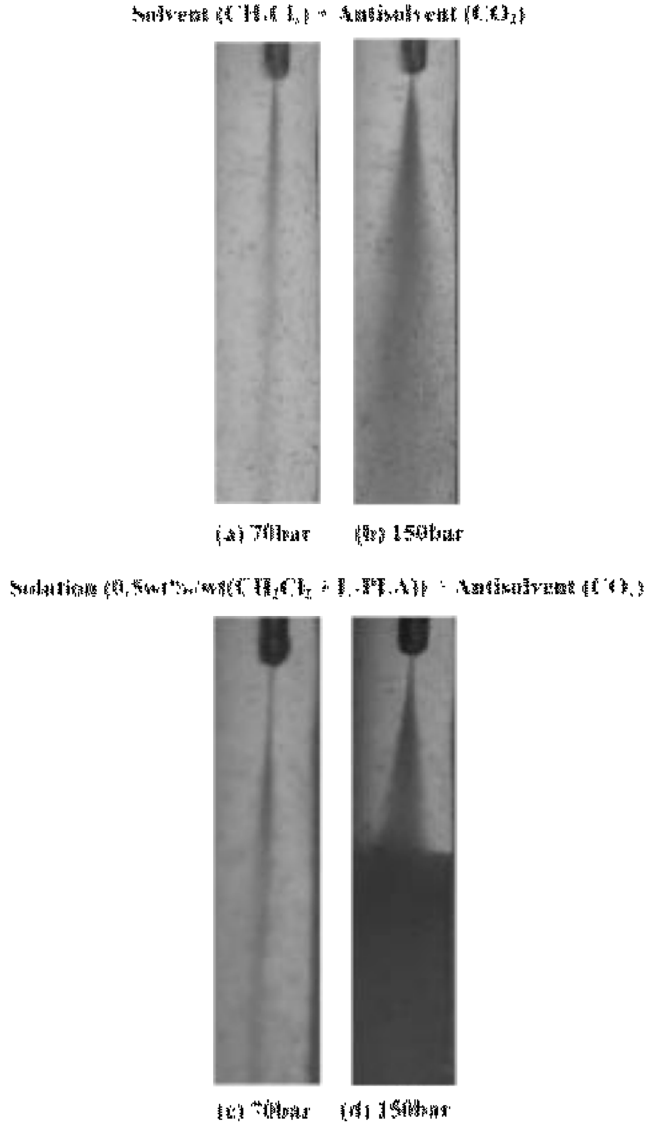


Fig. 3. Photographs of jet breakup at different pressures (70 bar and 150 bar), 40 °C and 0.01" nozzle diameter in the precipitator.

where ρ_{CO_2} is the antisolvent density, V is the relative velocity, d is the droplet diameter, and σ is the interfacial tension. The Weber number is defined as the ratio of the deforming forces to the reforming surface forces. As the density of carbon dioxide is increased, large deforming forces will break up the existing droplet further into smaller size.

We observed how the solvent (methylene chloride) jet or solution (methylene chloride+L-PLA) jet was atomized into very fine droplets through the capillary nozzle. For the pure methylene chloride, it was sprayed into CO_2 at temperature of 40 °C and nozzle diameter of 0.01'' I.D. with increasing pressure from 70 bar to 150 bar (Fig. 3). At low pressure of 70 bar, the methylene chloride jet was not atomized (Fig. 3a). However, the solvent jet broke up immediately into very fine droplets at a pressure of 80 bar or higher (Fig. 3b). These jet breakup phenomena were also observed at different temperatures (25, 31, 35, and 40 °C). When methylene chloride solution, including L-PLA, was introduced into CO_2 at the same condition (Fig. 3c and Fig. 3d), the polymer solution jet atomized to form fine droplets only at above 80 bar (Fig. 3d). In this case, we observed that the solution jet was not atomized immediately and its breakup was delayed a few millimeters from the nozzle tip (Fig. 3d). These results indicate that the large Weber number resulting from the higher antisolvent density (continuous phase density) leads to drop breakup into smaller droplet. In our experiments, the jet was not atomized at a CO_2 density of below ~0.3 g/ml, whereas the jet breakup occurred as CO_2 density increased above ~0.3 g/ml. At the CO_2 density is ~0.3 g/ml or below, no particle were formed.

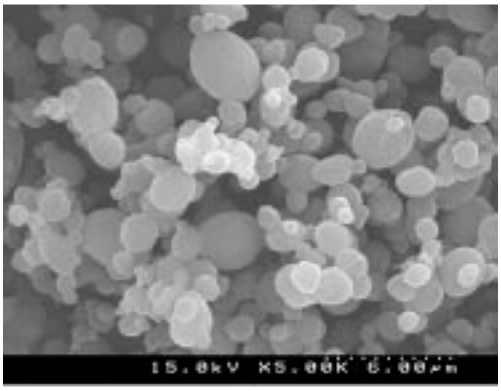
All experiments were conducted with increase of pressure from 80 bar to 150 bar at 31, 35, and 40 °C, respectively. Other process parameters such as concentration, nozzle diameter, flow rate of CO_2 and solution were fixed at 0.5 wt% L-PLA in methylene chloride, 0.03'', 8SLPM, and 0.3 ml/min, respectively.

In Table 1, the experimental conditions and results in this study are summarized. The particle size varied from 0.264 μm to 0.858 μm, when the pressure was increased from 80 to 150 bar at 31 °C. The same tendency is observed at 35 °C and 40 °C. Figs. 4(a)-(c) show SEMs of particles obtained in the experiments conducted at three different pressures at 35 °C. Most of the particles are found to be spherical and have a very narrow size distribution as listed in Table 1.

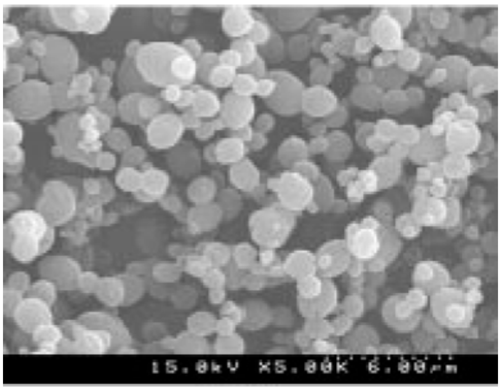
After the L-PLA solution is injected into the precipitator, it will form fine droplets which are contacted with supercritical carbon dioxide. Because the methylene chloride and supercritical CO_2 are fully miscible, fast diffusion of CO_2 into the liquid polymer droplet and evaporation of methylene chloride out of the droplet cause the droplet to swell rapidly. CO_2 diffusion decreases the L-PLA solubility within the droplet, whereas methylene chloride evaporation increases the L-PLA concentration. Therefore, high supersatura-

Table 1. Experimental conditions and results

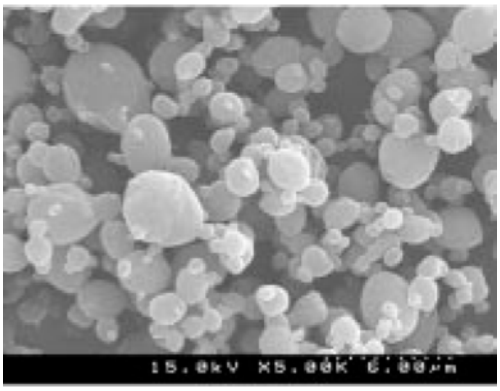
Temperature (°C)	Pressure (bar)	CO_2 density (g/ml)	Concentration (wt%)	Mean particle size (μm)
31	80	0.68049	0.5	0.264±0.1535
	100	0.76202	0.5	0.4030±0.2535
	150	0.84162	0.5	0.8580±0.3995
35	80	0.42599	0.5	0.1170±0.0720
	100	0.71399	0.5	0.3240±0.2100
	150	0.81595	0.5	0.7020±0.3595
40	90	0.48279	0.5	0.2570±0.1780
	100	0.62868	0.5	0.2970±0.1990
	150	0.78136	0.3	0.1350±0.0980
			0.5	0.3970±0.2450
			1.0	0.5110±0.3080
			2.0	0.5680±0.3050
			4.0	0.6220±0.2970



(a) 80 bar

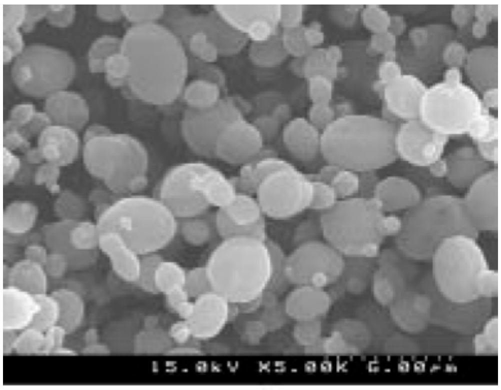


(b) 100 bar

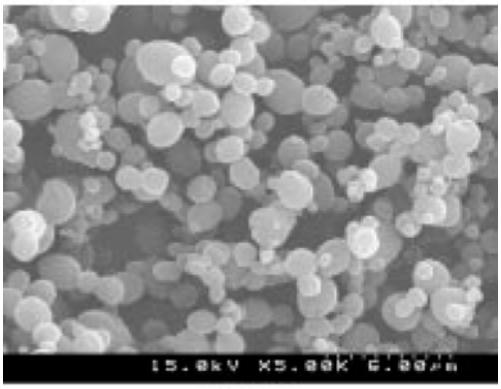


(c) 150 bar

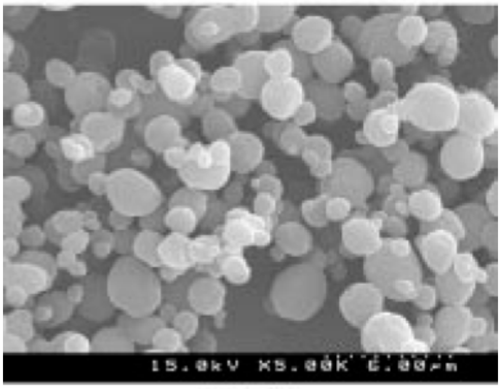
Fig. 4. SEM photographs of L-PLA microparticles prepared at 35 °C, 0.5 wt%/wt (L-PLA+CH_2Cl_2), 0.03" nozzle diameter, 0.3 mL/min solution flow rate, and 8 SLPM CO_2 flow rate.



(a) 31 °C



(b) 35 °C



(c) 40 °C

Fig. 5. SEM photographs of L-PLA microparticles prepared at 100 bar, 0.5 wt%/wt (L-PLA+CH_2Cl_2), 0.03" nozzle diameter, 0.3 mL/min solution flow rate, and 8 SLPM CO_2 flow rate.

tion and nucleation can be achieved and then fine particles are obtained.

In order to study the influence of temperature, the pressure was kept constant at the pressure of 100 bar while the temperature was varied from 31 °C to 40 °C. As shown in Table 1, the mean particle size changes from 0.4030 μm to 0.2970 μm as the temperature increases from 31 °C to 40 °C. Figs. 5(a)-(c) are SEMs of the experiments conducted at the three temperatures. In all the cases, spherical particles having a narrow size distribution are obtained. Also, the L-PLA microparticles formed over a temperature range of 31-40 °C are free-flowing and non-agglomerated with a smooth surface.

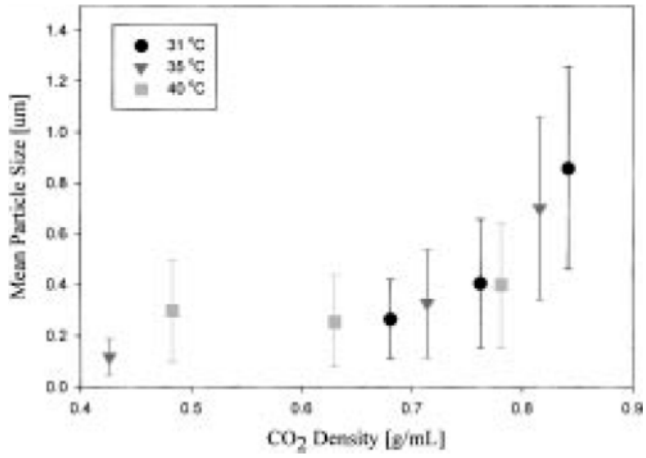


Fig. 6. The effect of CO_2 density on the L-PLA microparticles obtained at 0.5 wt%/wt (L-PLA+CH_2Cl_2).

As mentioned above, the density of CO_2 affects jet breakup by atomization and therefore the size of droplets formed by means of breakup of solution jet, which is sprayed through a capillary nozzle, changes as CO_2 density varies. The density of CO_2 is varied between 0.4 g/ml and 0.9 g/ml by changing the pressure from 80 bar to 150 bar over a temperature range of 31-40 °C. The mean particle size of L-PLA increases sharply as the density of CO_2 increases, as shown in Fig. 6.

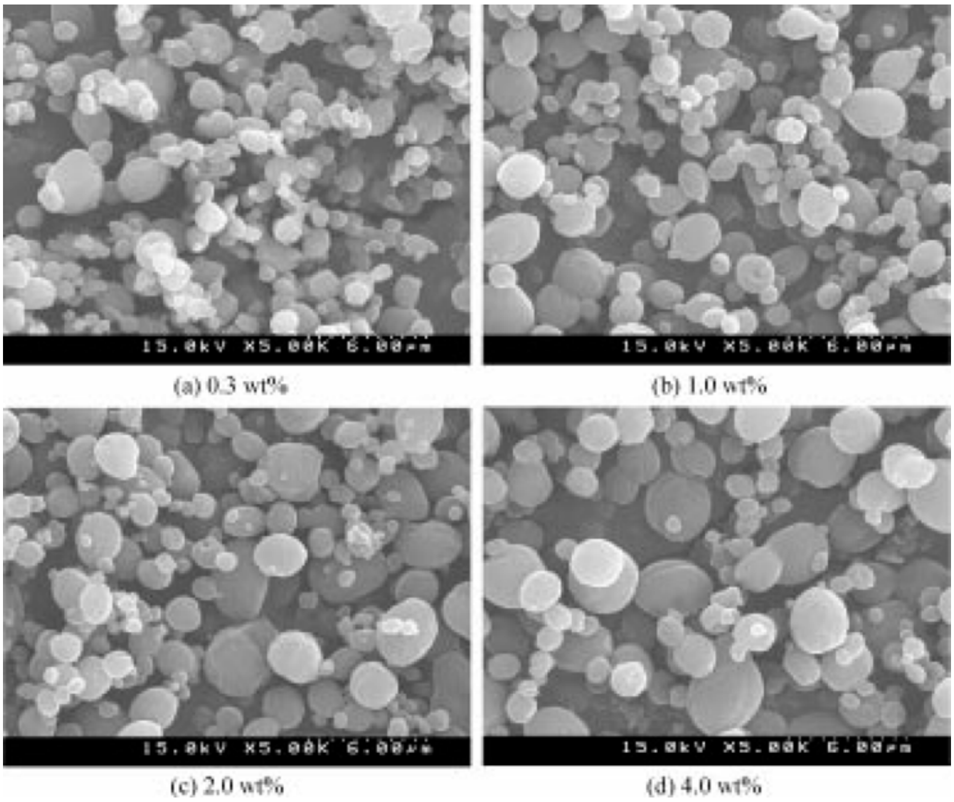
Since the variation of the diffusion coefficient is low when CO_2 density is lower than 0.6 g/ml, the particle size also changes a little in this region [Dixon et al., 1993]. The particle size sharply increases with increasing CO_2 density due to rapid decrease of the diffusion coefficients.

These results suggest that the smaller particles are produced as the diffusion coefficients of the solvent into CO_2 increase since the diffusion coefficient decreases with increasing CO_2 density.

The fast diffusion of CO_2 into and methylene chloride out of the liquid polymer droplet will cause high supersaturation followed by fast nucleation. As a result, smaller particles with narrow particle size distribution are produced.

Polymer concentration is another critical process variable. The effect of initial L-PLA concentration on particle size and particle size distribution was investigated at five different initial concentrations [0.3, 0.5, 1.0, 2.0, and 4.0 wt%/wt (L-PLA+CH_2Cl_2)] at 40 °C and 150 bar. Figs. 7(a)-(d) are the SEMs of L-PLA particles obtained at each concentration. Spherical microparticles were not agglomerated but formed as free-flowing powder. We do not observe any fibers in these concentration ranges. The effect of the different solution concentration on the mean particle size and PSD is shown in Fig. 8. The particle sizes increase from 0.135 μm to 0.622 μm when the initial concentration increases from 0.3 to 4.0 wt% at 40 °C and 150 bar. When the solution concentration is higher than 1.0 wt%, the size of L-PLA microparticles has shown a tendency to be invariable.

As the solution concentration increases, the interfacial tension between the sprayed polymer solution and CO_2 increases. Thus, the Weber number becomes small: the smaller the Weber number, the larger the droplets. Additionally, the high concentrated solution jet is not atomized immediately and then the breakup length increases. Consequently, supersaturation is delayed and nucleation rate be-



(a) 0.3 wt%

(b) 1.0 wt%

(c) 2.0 wt%

(d) 4.0 wt%

Fig. 7. SEM photographs of L-PLA microparticles prepared at 40 °C, 150 bar, 0.03" nozzle diameter, 0.3 mL/min solution flow rate, and 8 SLPM CO_2 flow rate.

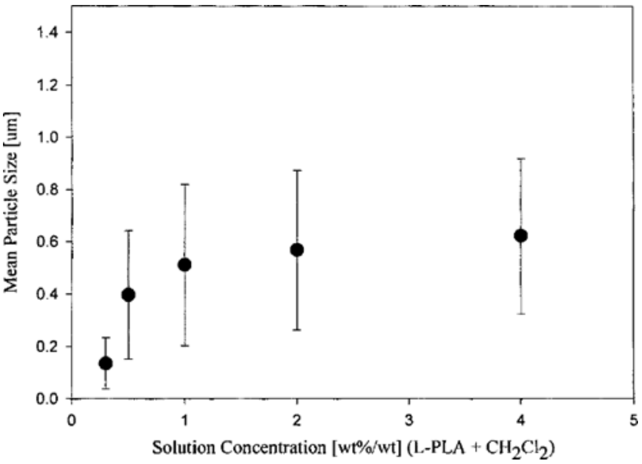


Fig. 8. The effect of initial solution concentration on the L-PLA microparticles at 40 °C, 150 bar.

comes slow. Therefore, particles are allowed to grow in the precipitator at high solution concentration since most of the L-PLA solutes are not consumed at nucleation stage. However, further particle growth is limited by the confined length of our precipitator at the 1 wt%/wt of L-PLA concentration or above.

CONCLUSIONS

L-PLA microparticles were prepared by a continuous SAS process in which methylene chloride solution was injected into carbon dioxide as an antisolvent. These precipitates were non-agglomerated and free-flowing, and solvent-free (or surfactant-free).

The size of the precipitated microparticles appears to be dependent on the pressure as well as the temperature. Particularly, the size of L-PLA microparticles may be adjusted by changing the density of CO_2 continuous phase, which in turn alters not only the mass transfer characteristics but also jet breakup by atomization.

It is found that the particle size (0.1-1 µm) and the particle size distribution can be manipulated easily by change of the pressure, temperature, and/or the initial solution concentration.

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