Preparation of Poly(L-lactide) Microparticles by a Supercritical Antisolvent Process with a Mixed Solvent

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ABSTRACT: Poly(L-lactide) (PLLA) microparticles were prepared by a supercritical antisolvent (SAS) process with a mixed solvent. Five factors, namely, the molar percentage of acetone, pressure, temperature, flow rate, and concentration of the solution, were optimized by a four-level orthogonal array design. By analysis of variance, the concentration of the solution showed a significant effect on the PLLA microparticle size. The effects of the mixed solvent (dichloromethane/acetone) at different mixing ratios, pressures, and temperatures on the morphology of the PLLA microparticles were also investigated. The thermal properties of PLLA before and after the SAS process were studied by differential scanning calorimetry. The results indicate that the molar percentage of acetone had a significant effect on the morphology of the PLLA microparticles. The microparticles prepared with the mixed solvent were much smaller than those prepared with dichloromethane alone under the same conditions. Furthermore, the particle size distribution was more uniform in the case of the mixed solvent. The particle size decreased with increasing pressure, whereas it showed no significant change with increasing temperature. The results also show that the thermal properties of PLLA could be improved through the SAS process. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 3744–3750, 2012

Key words: biological applications of polymers; nanoparticle; processing

INTRODUCTION

The incorporation of a pharmaceutical ingredient into polymer-based microparticles is of great interest for controlled drug-delivery systems.[1] The targeted drug-delivery function can be realized by the control of the particle size. Microparticles less than 100 μm in size are suitable for intravenous delivery for absorption. When the particle size is less than 5 μm, the microparticles can be administered via inhalation drug delivery.[1] The drug with a mean particle size of 2–5 μm can be directly delivered to the lung;[2] however, when the particle size is less than 1 μm, the microparticles are absorbed by the reticuloendothelial system, which is abundantly present in the liver and spleen tissues, and fail to get into the systemic circulation.

Recently, poly(L-lactide) (PLLA) has been the most widely used biodegradable polymer because of its favorable biocompatibility and biodegradability. PLLA-based particles can be obtained by conventional techniques, such as a mechanical technique, recrystallization of the solute particles from solution, freeze drying, and spray drying.[3] The mechanical technique is simple but not suitable for making small particles, whereas organic solvent residue in the final products is a major problem for the other techniques. The supercritical fluid (SCF) technique has been recently introduced into particle preparation to solve these problems. SCF has the advantages of both a high dissolving power and a high transport rate. Supercritical carbon dioxide ($ScCO_2$) is one of the most commonly used SCFs because of its mild supercritical conditions (critical pressure [P_c] = 7.38 MPa and critical temperature [T_c] = 31.2°C).[4] The low critical temperature of CO_2 makes it attractive for preparing heat-sensitive drugs.[5] Besides, CO_2 is nontoxic, nonflammable, relatively cheap, and easy to obtain.

Many studies have been conducted on the development SCF techniques for preparing single-component particles with uniform particle size distributions. Extensively reported have been methods of preparing microparticles with the rapid expansion of a supercritical solution[6,7] and a variety of antisolvent processes, such as supercritical antisolvent (SAS)[8–10] and gas-saturated solution processes.[11,12]

For preparing PLLA microparticles by an SAS process, a single solvent (dichloromethane) has been studied in recent years, and the process always results in a large particle size and a broad size distribution.[13] The objective of this study was to

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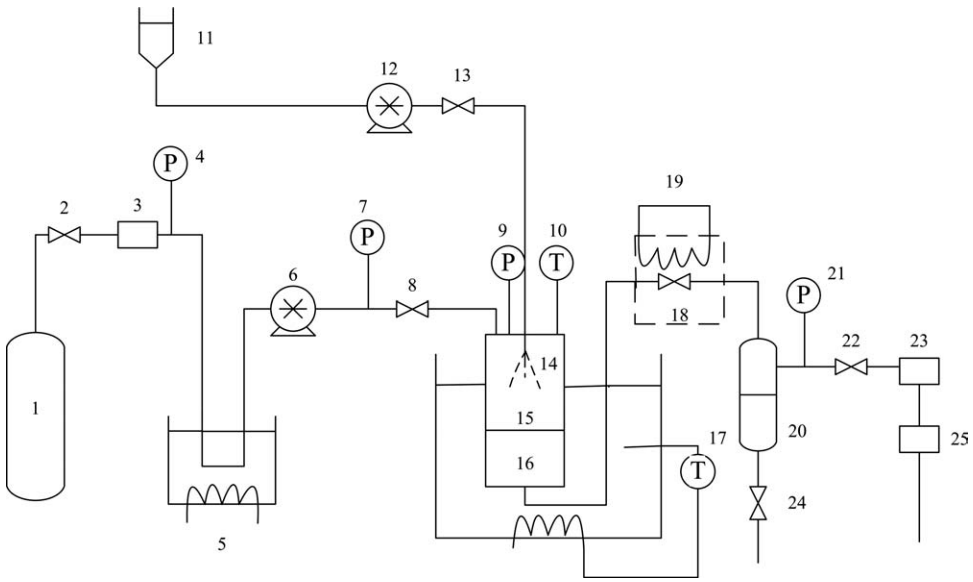

Figure 1 Schematic experimental apparatus of the SAS process: 1, CO_2 cylinder; 2, cylinder outlet valve; 3, CO_2 filter; 4, 7, 9, 21, pressure gages; 5, cooler; 6, plunger metering pump; 8, 13, 22, 24, stop valves; 10, 17, temperature controller; 11, organic solution; 12, plunger pump; 14, nozzle; 15, precipitator; 16, high pressure vessel; 18, micrometering valve; 19, heater band; 20, liquid–vapor separator; 23, mass flowmeter; 25, flow integration meters.

investigate the effects of a mixed solvent (dichloromethane and acetone) on the morphology and properties of particles prepared by the SAS process. Moreover, the effects of pressure and temperature on the PLLA microparticles were investigated with a constant mixing ratio.

EXPERIMENTAL

Materials

PLLA (weight-average molecular weight = 100,000) was purchased from Shandong Institute of Medical Instruments (Shandong, China) and dichloromethane, absolute alcohol, and acetone were from Tianjin Damao Chemical reagent Factory (Tianjin, China). Carbon dioxide (purity = 99.9%) was purchased from the Credit Co. (Dalian, China). All of the reagents were used without any further purification.

Particle characterization

The microparticles were characterized by scanning electron microscopy (SEM; KYKY-2800B, KYKY, Beijing, China). The samples were gold-sputtered under high vacuum, and photographs were taken at magnifications of 5000×.

The particle size and size distribution of the microparticles were analyzed by a Zetasizer (Zeta-PLUS, Brookhaven Instruments Corp., New York, USA). The samples (2 mg) were dispersed in 10 mL of absolute alcohol. The suspensions were stirred for 5 min and sonicated for 2 min and were finally ready for analysis.

The thermal properties of the PLLA samples were measured by differential scanning calorimetry (CDR-34P, Shanghai Precision & Scientific Instruments Corp., Shanghai, China) with aluminum oxide as the standard. The particles (2–10 mg) were loaded onto standard aluminum pans. The melting temperature (T_m), glass-transition temperature (T_g), and enthalpy of melting (ΔH_m) of each sample were measured from 20 to 200°C at a heating rate of 10°C/min.

Gas chromatography (G-3900, Hitachi Co., Tokyo, Japan) was used to determine the organic solvent residue in the particles. A nonpolar column was used to analyze the organic solvent residue. The oven temperature program consisted of temperatures of 40°C (5-min hold) to 180°C (1-min hold) at a rate of 30°C/min. Nitrogen was used as the carrier gas with a flow rate of 1.0 mL/min. Chloroform (1 mL) was used to dissolve 20 mg of particles, and 0.1 μL of solution was injected in the split mode with the temperature of the injector at 250°C.

Experimental method

The experimental apparatus is schematically shown in Figure 1. After CO_2 was chilled to about 0°C by a cooling system, it was pumped into the high-pressure chamber with a volume of 500 mL of by a plunger metering pump. We established a steady flow of CO_2 by adjusting the micrometering valve and plunger metering pump when the predetermined operating conditions were reached. Through a plunger pump, the PLLA solution was sent through a capillary nozzle (i.d. = 100 μm) into the

precipitator that was installed in the high-pressure chamber. A sintered metal plate was located at the bottom of the precipitator to separate the gas–solid mixtures. The $ScCO_2$ dissolved into the liquid solution rapidly, and the microparticles were precipitated on the bottom of the precipitator. After spraying, the fresh CO_2 was supplied continuously to make sure that organic solvents could be removed thoroughly. Finally, the system was depressurized, and the dry microparticles were collected on the precipitator bottom.

RESULTS AND DISCUSSION

The phase behavior of the ternary mixture in the high-pressure vessel plays an important role in the atomization and particle formation. For preparing smaller particles with narrow size distribution, the PLLA concentration and CO_2 molar fraction in the liquid solution should be as high as possible to enhance the yield of PLLA particles and to improve the atomization effect.[14] However, an increase in PLLA concentration may result in a viscous solution that can influence atomization. For this reason, the PLLA solution should be maintained at a relatively low concentration. Increasing the CO_2 molar fraction in the solution is a good way to improve the particles size. Because the ternary phase behavior is very complicated, the exact equilibrium data of $ScCO_2$–dichloromethane/acetone–PLLA system are barely presented in the literature. The operation parameters were selected according the binary data of $ScCO_2$–dichloromethane[15] and $ScCO_2$–acetone[16] systems. The vapor–liquid equilibrium pressure of acetone was less than one of dichloromethane at the same temperature, so dichloromethane was selected as a standard in determining the operation parameters. The vapor–liquid equilibrium pressure of dichloromethane was less than 8 MPa below 50°C, so the temperature was chosen below 50°C.

Optimization study

Because various parameters should be considered in the SAS process, the optimization of the experimental conditions is a critical step in obtaining a minimum mean particle size of PLLA microparticles. In fact, the pressure, temperature, concentration, and flow rate of the PLLA solution and the molar percentage of acetone are generally considered to be the most important factors. All of the selected factors were examined with an orthogonal OA_{16} (4^5) test design (Table I).

The arrangement of the experiment and the collected data for the mean particle size of PLLA microparticles are shown in Table II. Table III lists the data of the analysis of variance (ANOVA) table of the experiment. The results show that the maximum mean particle size of the PLLA microparticles was 3.65 ± 0.45 μm, and the minimum was 0.86 ± 0.20 μm. We concluded from Table II that the effect of the parameters on the mean particle size of the PLLA microparticles was in the following order: Concentration of the PLLA solution (C) > Molar percentage of acetone (M) > Flow rate of the PLLA solution (F) > Temperature (T) > Pressure (P), according to the range (R) values. The minimum mean particle size (0.78 ± 0.15 μm) of the PLLA microparticles was obtained when the considered parameters were $P_2T_2C_2F_2M_3$ [12 MPa, 38°C, 1.0% (w/v), 1.0 mL/min, and 47%, respectively]. Repeated experiments confirmed this result.

TABLE I
Factors and Levels of Orthogonal Array Design

	Factor				
Level	P (MPa)	T (°C)	C (% w/v)	F (mL/min)	M (%)
1	8	33	0.5	0.5	0
2	12	38	1.0	1.0	30
3	16	43	1.5	1.5	47
4	20	48	2.0	2.0	64

Effects of the mixed solvent on the morphology of the products

The PLLA microparticles were prepared by an SAS process with a mixed solvent of acetone and dichloromethane at different molar percentages of acetone, whereas the other parameters were kept constant: P = 10 MPa, T = 38°C, C = 1.0% (w/v), F = 1.0 mL/min, and depressurization rate = 5 L/min. Figure 2 shows the SEM pictures of the microparticles prepared at the following molar percentages of acetone: 0, 30, 47, and 64 mol %. The PLLA microparticles prepared with acetone and dichloromethane as a mixed solvent [Fig. 2(b–d)] were much smaller in size than those prepared with dichloromethane alone at the same conditions [Fig. 2(a)]. Figure 3 shows the size distribution of the microparticles. It can be intuitively seen that the PLLA microparticles prepared with acetone and dichloromethane as a mixed solvent had a more uniform particle size distribution than those prepared with dichloromethane alone. Table IV reports the geometrical parameters of the PLLA microparticles prepared at different mixed-solvents ratios.

Figure 2(a) shows that the microparticles prepared with dichloromethane as solvent had a mean particle size of 1.89 ± 0.32 μm. The system of $ScCO_2$–dichloromethane was under the vapor–liquid equilibrium state at 10 MPa and 38°C.[15] Dichloromethane dissolved in $ScCO_2$ when the solution was pumped into the high-pressure chamber. The same

TABLE II
Orthogonal Array Design OA_{16} $(4)^5$ and Experimental Results

Code	P (MPa)	T (°C)	C (% w/v)	F (mL/min)	M (%)	Mean particle size (μm) ± SD (n = 3)
1	1	1	1	1	1	3.65 ± 0.45
2	1	2	2	2	2	0.86 ± 0.20
3	1	3	3	3	3	2.06 ± 0.31
4	1	4	4	4	4	3.35 ± 0.40
5	2	1	2	3	4	1.22 ± 0.16
6	2	2	1	4	3	1.25 ± 0.16
7	2	3	4	1	2	3.06 ± 0.40
8	2	4	3	2	1	1.45 ± 0.22
9	3	1	3	4	2	2.06 ± 0.25
10	3	2	4	3	1	3.42 ± 0.41
11	3	3	1	2	4	1.86 ± 0.31
12	3	4	2	1	3	1.09 ± 0.18
13	4	1	4	2	3	2.18 ± 0.20
14	4	2	3	1	4	1.33 ± 0.16
15	4	3	2	4	1	2.94 ± 0.29
16	4	4	1	3	2	1.84 ± 0.22
K_1[a]	2.48 ± 0.34	2.28 ± 0.27	2.15 ± 0.35	2.28 ± 0.30	2.87 ± 0.34	
K_2	1.74 ± 0.24	1.72 ± 0.23	1.53 ± 0.21	1.59 ± 0.23	2.00 ± 0.27	
K_3	2.11 ± 0.29	2.48 ± 0.33	1.73 ± 0.24	2.14 ± 0.28	1.65 ± 0.21	
K_4	2.10 ± 0.22	1.93 ± 0.26	3.00 ± 0.35	2.40 ± 0.28	1.94 ± 0.26	
R[b]	0.74	0.77	1.48	0.81	1.22	
Optimal level	P2	T2	C2	F2	M3	

SD, standard deviation.
[a] K_i^P = σ(Mean particle size at P_i)/4, the mean values of mean particle size for a certain factor at each level with SD.
[b] R_i^P = Maximum K_i^P − Minimum K_i^P.

results have been reported in other articles.[4,13,17] Although the particles were well-shaped [Fig. 2(a)], the particle size distribution was broad, with a range of 1.30–2.30 μm (Fig. 3). The reason may have been that the supersaturation degree of the low concentration of solution was low. The high PLLA concentration may have resulted in a viscous solution; this can influence atomization, and this problem can be solved by the addition of acetone into the solution. PLLA with a high molecular weight hardly dissolves in acetone, which can mix well with $ScCO_2$, so the addition of acetone can decrease the percentage of dichloromethane in solution and relatively increase the supersaturation degree of the solution.

Figure 2(b) shows the microparticles prepared with dichloromethane and acetone as mixed solvent at the mixing ratios of 30 mol %. The particles size [Fig. 2(b)] was smaller than the one shown in Figure 2(a), and the particle size distribution was narrower (Fig. 3). Because there were no equilibrium data reported for a ternary mixture ($ScCO_2$+acetone+ dichloromethane), it is impossible to guarantee that a higher acetone composition would lead to an increase in the amount of solvent in the fluid phase that could favor the SAS process. From the binary data of $ScCO_2$–dichloromethane[15] and $ScCO_2$–acetone[16] systems, we estimated that acetone and dichloromethane could greatly dissolve in $ScCO_2$. When acetone was added to the solvent, the concentration of PLLA solution was constant, whereas the supersaturation degree of the solution increased because the PLLA could not dissolve in acetone. The particle size decreased with the increase of the supersaturation degree of the solution in the SAS process.[18]

With the increase of the acetone concentration, the supersaturation degree of the solution increased, thus, the microparticle size decreased, and the particle size distribution became narrow gradually. Figure 2(c) shows that the morphology of most particles was spherical or approximately spherical and that the diameter of most particles was less than 1 μm. The increase of the acetone content had no significant effect on the particle size distribution, whereas the particles tended to cohere [Fig. 2(d)]. The explanation for this may be that the PLLA incompletely dissolved in the solvent when the percentage of acetone was greater than 64 mol %.

TABLE III
ANOVAs of the Five Parameters of the SAS Process

Source	Sum of squares	Degrees of freedom	F-test (F) ratio	$F_{0.1}$	Type of effect
P (MPa)	3.339	3	0.505	2.27	
T (°C)	4.310	3	0.651	2.27	
C (%, w/v)	15.509	3	2.344	2.27	Significant
F (mL/min)	4.730	3	0.715	2.27	
M (%)	10.150	3	1.534	2.27	

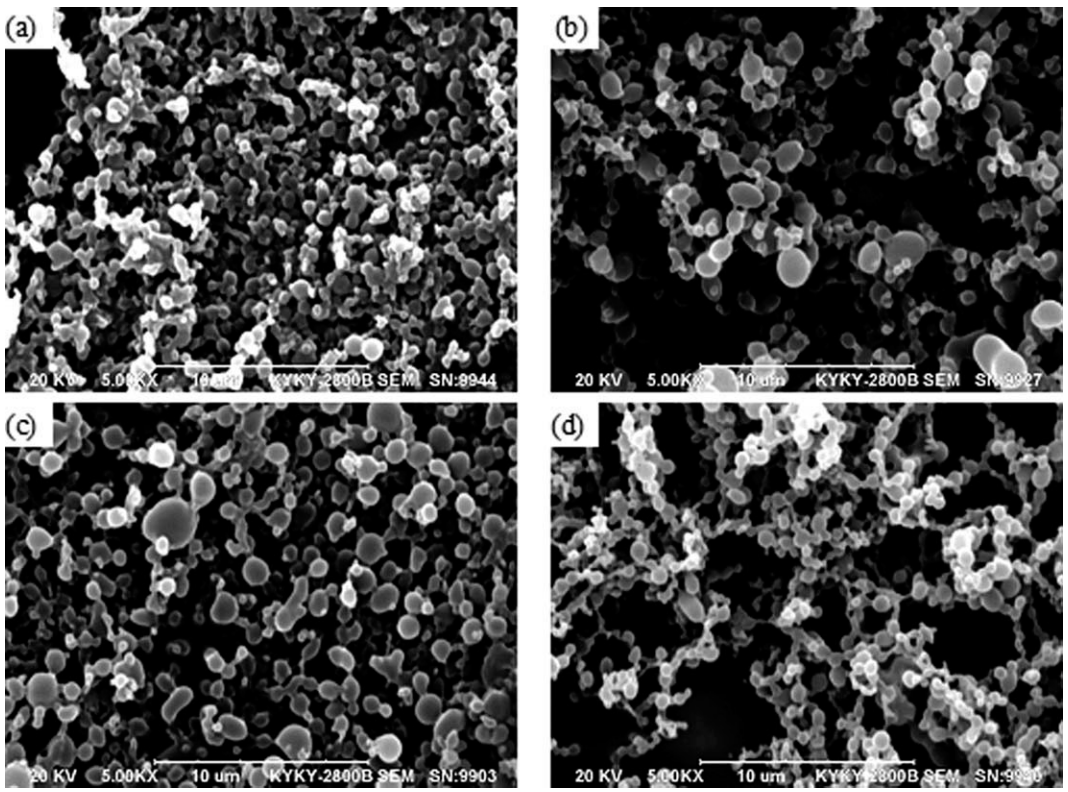

Figure 2 SEM photographs of the PLLA microparticles prepared at different molar percentages of acetone: (a) 0, (b) 30, (c) 47, and (d) 64%.

Gas chromatography was used to determine the organic solvent residue. The results show that the PLLA microparticles had no trace of acetone or dichloromethane. The limit of detection of the organic solvent was 0.002% with a signal-to-noise ratio of 3. Moreover, the time of organic solvent removal was also shortened. With the increasing percentage of acetone, the time decreased from 30 to 10 min. The reason may have been that the acetone and the dichloromethane could dissolve in $ScCO_2$ at the same time; this would have increased the content of solvent in $ScCO_2$ and decreased the solvent residue in the microparticles.

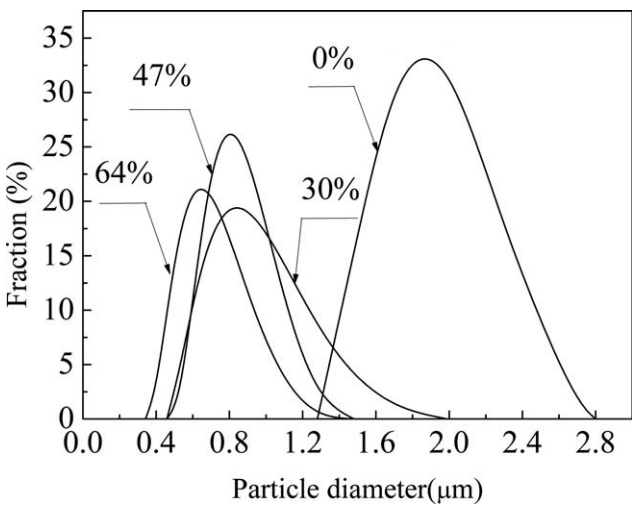

Figure 3 Size distributions of the PLLA microparticles prepared at different molar percentages of acetone.

Effects of the pressure and temperature on the morphology of the products

Figure 4 shows the SEM photographs of the PLLA microparticles prepared at 38°C and different pressures. It can be seen that the PLLA microparticles prepared at 16 MPa [Fig. 4(b)] were much smaller in size than those prepared at 8 MPa [Fig. 4(a)]. The results shown in Figures 4(a), 2(c), and 4(b) indicate that the microparticle size decreased with the increase of pressure. At the near critical pressure, the diffusion coefficient of $ScCO_2$ was small; this could have led to the slow diffusion into the solvent,

TABLE IV
Geometrical Parameters of the PLLA Microparticles

	M			
Parameter	0%	30%	47%	64%
Mean particle size	1.89	0.83	0.68	0.90
SD	0.28	0.16	0.17	0.25
Kurtosis	2.68	2.78	2.98	3.22

SD, standard deviation.

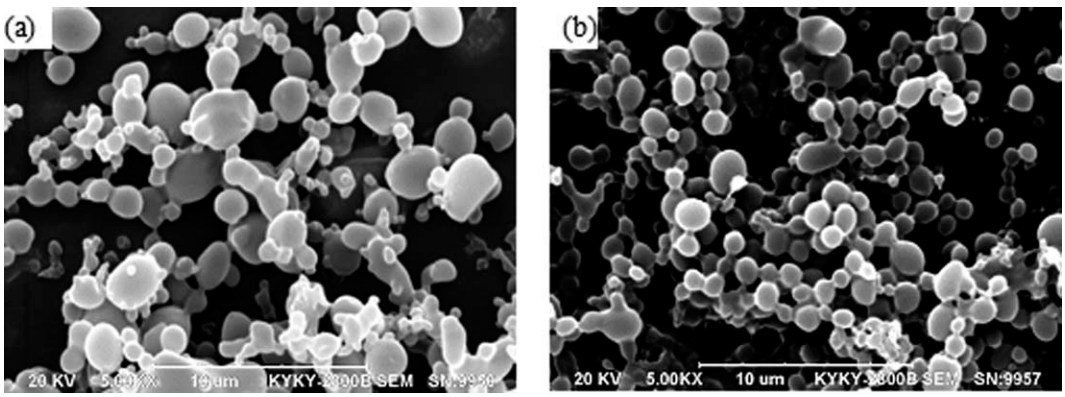

Figure 4 SEM photographs of the PLLA microparticles prepared at different pressures: (a) 8 and (b) 16 MPa.

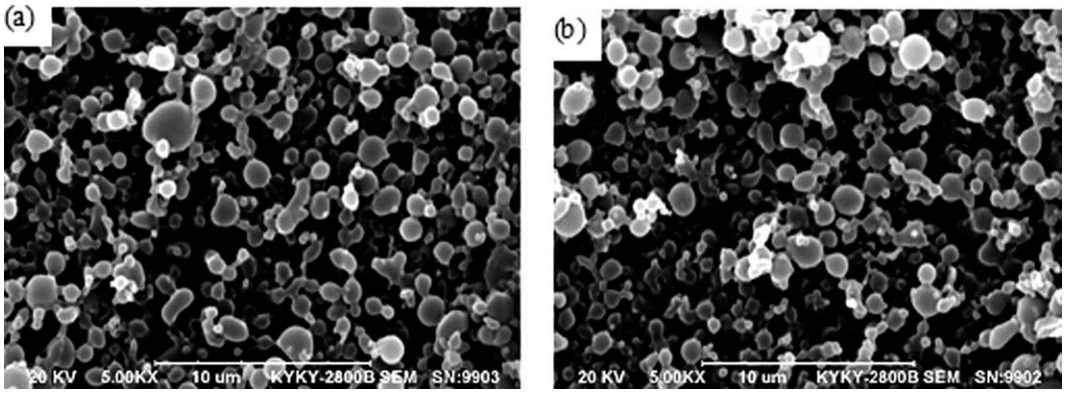

Figure 5 SEM photographs of the PLLA microparticles prepared at the different temperatures: (a) 38 and (b) 43°C

the slow precipitation rate, and the large particle size. At the higher pressure, a higher diffusion coefficient of $ScCO_2$ resulted in complete mixing with the solution before precipitation.

Figure 5 shows the SEM photographs of the PLLA microparticles prepared at 10 MPa and different temperatures. It can be seen that the morphology of the PLLA microparticles prepared at 38°C [Fig. 5(a)] and 43°C [Fig. 5(b)] changed a little. Because the density of $ScCO_2$ decreased and the diffusion coefficient of $ScCO_2$ increased with the increase of temperature, these two opposite effects compensated each other, and the change of particles size was not obvious with the increase of temperature.

Modification of the thermal properties

The crystallinity of the polymer and copolymer is known to play an important role in the determination of degradability, erosion, and permeability of water, and drugs become more inaccessible to water diffusion due to the bulk crystalline phase.[19] T_m was taken at the end of the melting peak, and T_g was considered as the minimum of the exothermic peak. The crystallinity (χ) of PLLA was calculated on the basis of the enthalpy value of a 100% crystalline PLLA sample (ΔH_{m^*}) of 135 J/g[20] from the following equation:

$$\chi = \Delta H_m / \Delta H_{m^*} \times 100\%$$

From Figure 6, it can be known that the crystallinity of PLLA was 29.38% after processing, whereas it was 34.37% before processing. This result was in

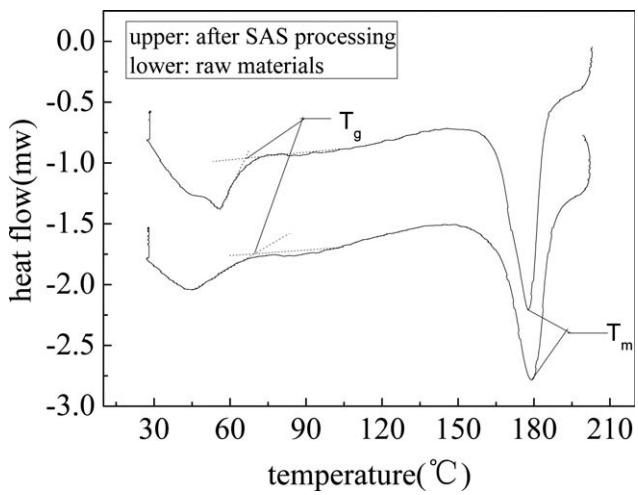

Figure 6 Differential scanning calorimetry curves of PLLA after and before SAS processing.

accordance with the conclusion that $ScCO_2$ could reduce the crystallinity of the processed polymeric materials[21] and lower crystallinity could improve degradation.[22]

CONCLUSIONS

PLLA microparticles were prepared by an SAS process with acetone and dichloromethane as a mixed solvent. The effects of the operating parameters on the mean particle size of the PLLA microparticles were in this order: $C > M > F > T > P$. The minimum mean particle size (0.78 ± 0.15 μm) of the PLLA microparticles was obtained under the following conditions: $P = 12$ MPa, $T = 38°C$, $C = 1.0\%$ (w/v), $F = 1.0$ mL/min, and $M = 47\%$. The microparticles prepared with the mixed solvent were much smaller in size and more uniform in size distribution than those prepared with dichloromethane alone under the same conditions. The microparticle size decreased with increasing pressure, and it had no significant change with the increase of temperature. The prepared PLLA microparticles had no trace of acetone or dichloromethane by SAS processing. Besides, the crystallinity of PLLA was lowered, and the thermal properties of PLLA could be improved.

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