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Study of poly(L-lactide) microparticles based on supercritical CO_2

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Abstract Poly(L-lactide) (PLLA) microparticles were prepared in supercritical anti-solvent process. The effects of several key factors on surface morphology, and particle size and particle size distribution were investigated. These factors included initial drops size, saturation ratio of PLLA solution, pressure, temperature, concentration of the organic solution, the flow rate of the solution and molecular weight of PLLA. The results indicated that the saturation ratio of PLLA solution, concentration of the organic solution and flow rate of the solution played important roles on the properties of products. Various microparticles with the mean particle size ranging from 0.64 to 6.64 μm, could be prepared by adjusting the operational parameters. Fine microparticles were obtained in a process namely solution-enhanced dispersion by supercritical fluids (SEDS) process with dichloromethane/acetone mixture as solution.

Introduction

Supercritical fluids have revealed great potential in particle engineering, CO_2 is non-toxic, non-flammable, relatively inexpensive and recyclable, its critical conditions ($Tc = 31.1$ °C, $Pc = 7.38$ MPa) are relatively mild. Hence the technologies based on supercritical CO_2 were wildly utilized for engineering of microparticles and microparticulate drug carrier systems, especially the supercritical anti-solvent (SAS) process has been wildly explored in particle design [1–3].

In a SAS process, the organic solution is sprayed into a precipitation chamber where a supercritical fluid (anti-solvent) already exists, causing rapid contact between the two media. This generates higher super-saturation ratio of the solution, resulting in fast nucleation and growth, and consequently creates smaller particles. An active substance and a carrier (often a polymer) dissolved in an organic solvent are sprayed together or separately in an anti-solvent. The anti-solvent expands the solvent that leads to the formation of active substance-loaded micro-/nano-particles. SEDS process is a modified version of SAS process, in which the liquid solution and supercritical fluid are sprayed together using a specially designed coaxial nozzle. It was developed by the Bradford University in order to achieve smaller droplet size and intense mixing of supercritical fluid and solution for increased transfer rates. The supercritical fluid is used as both anti-solvent for its chemical properties and 'spray enhancer' by mechanical effect. The spontaneous contact of high-speed streams of a liquid solution and a supercritical fluid generates the finely dispersed mixture and a prompt particle precipitation [4, 5].

Poly(L-lactide) (PLLA) was widely studied in the drug delivery system due to its biodegradability and biocompatibility. In this study, PLLA microparticles were investigated in SAS and SEDS process based on supercritical CO_2, and the experimental parameters such as pressure (P), temperature (T), flow rate of PLLA solution (F), PLLA concentration (C) and molecular weight (MW) of PLLA were also studied.

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Experimental part

Materials

PLLA, with molecular weight of 10, 50, 100 and 200 kDa, was purchased from Department of Medical Polymer Shandong Institute (Jinan, China). CO_2 with the purity of 99.9% was supplied by Chengdu Tuozhan gas Co. Ltd. (Chengdu, China). All other compounds were of analytical purity.

Methods

Preparation PLLA microparticles [6–8]

Figure 1 showed a schematic diagram of the SEDS apparatus, which consists of three major components: a CO_2 supply system, an organic solution delivery system, and a high-pressure vessel with a volume of 500 ml. In the running of the experiment, the CO_2 fed from a CO_2 cylinder was cooled down to around 0 °C by a cooler (Maneurop, France) in order to ensure the liquefaction of the gas and also to prevent cavitation. Then a high-pressure meter pump (2J-X8/32, Hangzhou Zhijiang Petrochemical Equipment Co., Ltd., China) was used to deliver liquefied CO_2 to the high-pressure vessel. After leaving the pump head, the liquefied CO_2 was pre-heated to desired operating temperature by using a heat exchanger. The high-pressure vessel was incubated in a gas bath (DHG-9070A Heating and Drying Oven, Shanghai Jing Hong Laboratory Instrument Co., Ltd., China) to keep the temperature constant during the experiment. When the desired pressure of the high-pressure vessel was reached, a steady flow of CO_2 was maintained, and the system pressure was controlled by adjusting a downstream valve and monitored by a pressure gauge (Shanghai Automation Instrumentation Co., Ltd., China) to keep the pressure constant. When the desired pressure and temperature were stabilized, the PLLA solution was delivered into the high-pressure vessel through a stainless steel coaxial nozzle (ID 800 μm, and the nozzle of PLLA solution with ID 330 μm was used in this study) by using an HPLC pump (P3000, Knauer, Germany). When the spraying was finished, fresh CO_2 was used continually to wash the products to remove the residual organic solvent for about 30 min. During the process of washing, the system operating conditions were maintained as described before. After washing, the high-pressure vessel was slowly depressurized and the products were collected for characterization.

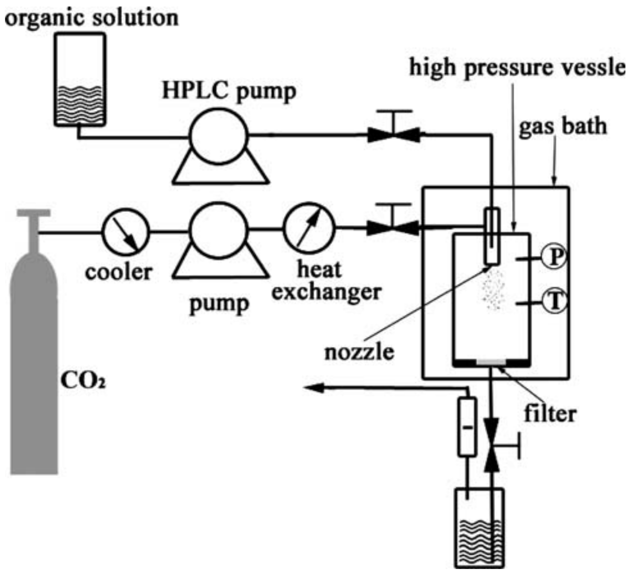


Fig. 1 Schematic diagram of SEDS apparatus

While in a SAS process, the liquid solution and supercritical CO_2 were sprayed through different nozzles respectively.

Surface morphology and particle size distribution characterization

The surface morphological examination of PLLA microparticles was performed using a scanning electron microscope (JSM-5900LV, Japan). The particle size and particle size distribution of PLLA microparticles was analyzed using a zetasizer (Malvern Zetasizer Nano ZS90, Malvern Instrument Co. Ltd., UK).

Results and discussion

Effects of initial drops size and saturation ratio of PLLA solution

Rantakylä [9] studied the effect of the initial droplet size on the final particle size in the semi-continuous SAS technique. The particle formation model was tested with PLLA in DCM and carbon dioxide. However, the droplet and particle formation models did not explain the observed changes of particle sizes with changes in operating conditions. It was suggested that the initial droplet sizes, formed at the nozzle, did not have an effect on the final particle size. While in the report of Choi [10] they calculated theoretical initial droplet size with the equation derived by Lorenzetto and Lefebvre determining the relation between the initial droplet size and particle size. The results showed that there was a linear relationship between the initial droplet size and particle diameter.

In the SEDS process of our study, the supercritical CO_2 also acted as ‘spray enhancer’ by mechanical effect, the initial droplet could be resulted in a smaller size. And we mixed acetone into PLLA DCM solution (1:1, v/v), since PLLA was insoluble in acetone, in a same concentration of PLLA solution, mixing of acetone into DCM generated a higher saturation ratio of PLLA solution for the anti-solvent effect of acetone.

Figure 2 showed the SEM photomicrographs of PLLA microparticles prepared in (a) SAS process, CH_2Cl_2:CH_3COCH_3 = 1:0; (b) SEDS process, CH_2Cl_2:CH_3COCH_3 = 1:0; (c) SAS process, CH_2Cl_2:CH_3COCH_3 = 1:1; and (d) SEDS process, CH_2Cl_2:CH_3COCH_3 = 1:1. And the processing parameters were 12 Mpa, 306 K, 0.5% (w/v), 1.0 ml·min^{-1}, and MW 100 kDa. The corresponding particle size distributions were shown in Fig. 3, and the mean particle sizes were 3340, 2160, 1100 and 1050 nm, respectively. The results indicated that mean particle size of PLLA microparticles prepared in SEDS process was smaller than that of SAS process due to the smaller size of initial drops and more intense transfer rate in SEDS process; and mixing of acetone into PLLA DCM solution would decrease the particle size of PLLA microparticles greatly, it was because that mixing of acetone increased the saturation ratio of PLLA solution, while contacting with supercritical CO_2, it generated a higher super-saturation ratio of the solution, resulting in fast nucleation and growth, and consequently created smaller particles.

It revealed that smaller initial droplet could produce a final particle with smaller size, and the saturation ratio of PLLA solution played an important role on the final particle size.

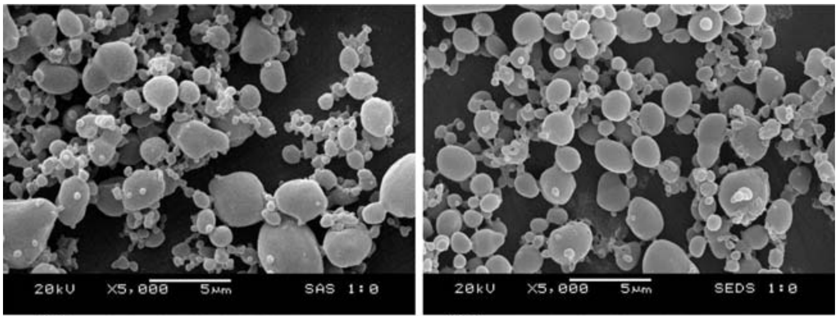
Effect of processing parameters

To optimize the processing parameters, the effects of pressure (P, 8–16 MPa), temperature (T, 306–312 K), PLLA concentration (C, 0.5–1.5%, w/v) and flow rate of PLLA solution (F, 0.5–1.5 ml min^{-1}) on the properties of PLLA microparticles were studied. Table 1 showed the orthogonal design matrix and the experimental results. From the calculation shown in Table 1, the relationship of influencing factors for particle size is C > F > T > P. It is shown that within our experiment conditions, concentration of the organic solution is the greatest factor to determine diameter of microparticles, followed by the flow rate of the solution, temperature and pressure.

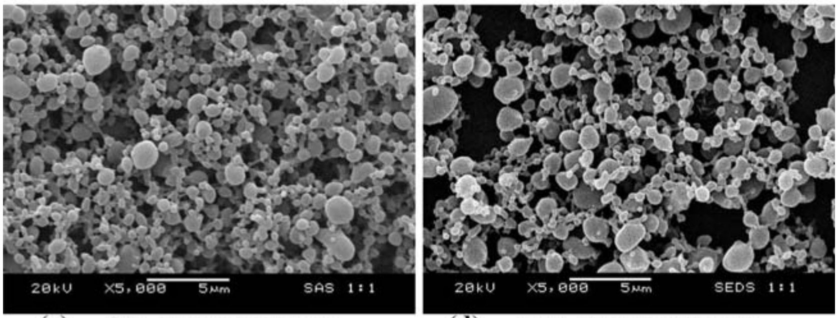
Effect of PLLA concentration

Wang [8] and Reverchon [11] found that the solution concentration affected the morphology and particle size of final product greatly, higher solution concentration resulted in formation of bigger particles and increase of agglomeration; Wu [12] also reported that the lower concentration of solution was favorable to produce smaller particles with narrower particle size distribution.

Figure 4a showed the SEM photomicrograph of microparticles prepared with 1.5% PLLA, comparing to the product prepared with 0.5% PLLA (as shown in Fig. 2b), it was found that the PLLA microparticles prepared of higher PLLA concentration had bigger particle size and heavier agglomeration. It was because that increasing the polymer

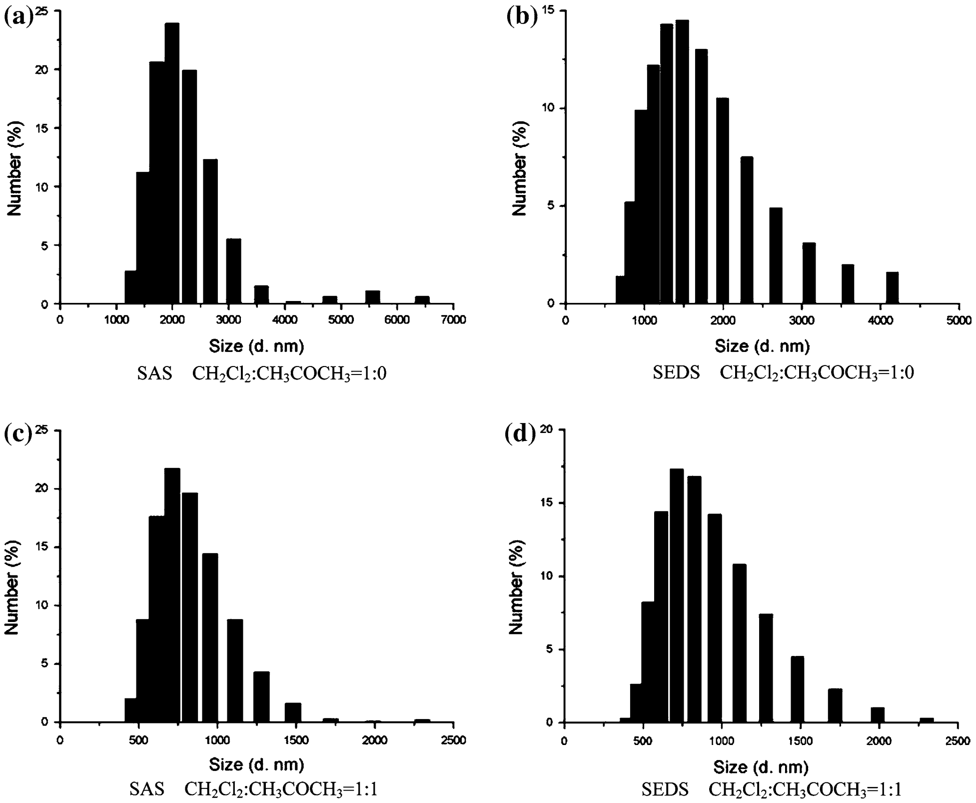


(a) SAS CH_2Cl_2:CH_3COCH_3=1:0 **(b)** SEDS CH_2Cl_2:CH_3COCH_3=1:0



(c) SAS CH_2Cl_2:CH_3COCH_3=1:1 **(d)** SEDS CH_2Cl_2:CH_3COCH_3=1:1

Fig. 2 SEM photomicrographs of PLLA microparticles prepared in different conditions



SAS $CH_2Cl_2:CH_3COCH_3=1:0$ SEDS $CH_2Cl_2:CH_3COCH_3=1:0$

SAS $CH_2Cl_2:CH_3COCH_3=1:1$ SEDS $CH_2Cl_2:CH_3COCH_3=1:1$

Fig. 3 Particle size distributions of PLLA microparticles prepared in different conditions

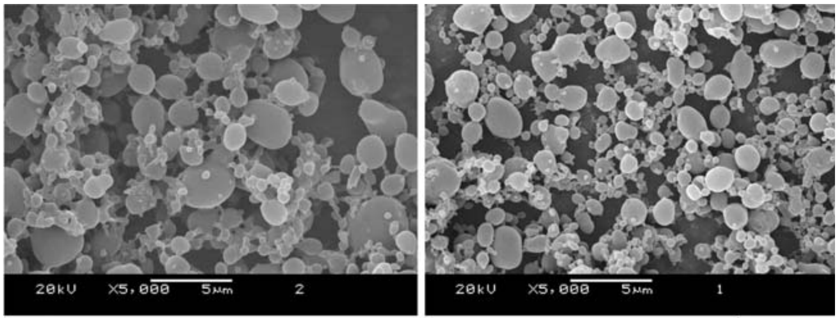
Table 1 Orthogonal design matrix and experimental results

Factors	P (MPa)	T (K)	C (% w/v)	F (ml min^{-1})	Results (d. nm)
Expt. 1	16	306	0.5	0.5	1,100
Expt. 2	16	309	1.0	1.0	1,700
Expt. 3	16	312	1.5	1.5	6,638
Expt. 4	12	306	1.0	1.5	2,160
Expt. 5	12	309	1.5	0.5	2,250
Expt. 6	12	312	0.5	1.0	1,990
Expt. 7	8	306	1.5	1.0	2,459
Expt. 8	8	309	0.5	1.5	1,860
Expt. 9	8	312	1.0	0.5	1,720
Mean 1	3,146.000	1,906.333	1,650.000	1,690.000	
Mean 2	2,133.333	1,936.667	1,860.000	2,049.667	
Mean 3	2,013.000	3,449.333	3,782.333	3,552.667	
Range	1,133.000	1,543.000	2,132.333	1,862.667	

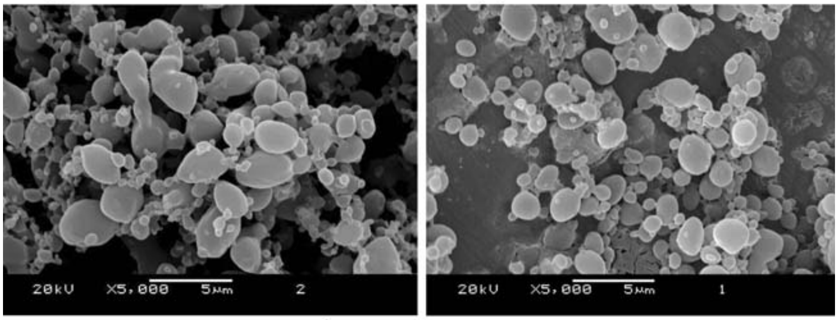
concentration would result in higher viscosities that had a stabilizing effect on the jet and yield bigger droplets, and high polymer concentrations might even reduce the atomization forces to such a low level that they were not sufficient to break up the jet into droplets. In that condition, the precipitation kinetic was much faster than the droplet formation kinetic and fibers were formed rather than microparticles. Taki [13] reported that fibers were yielded while the PLLA concentrations exceed 3.0%.

Effect of flow rate of PLLA solution

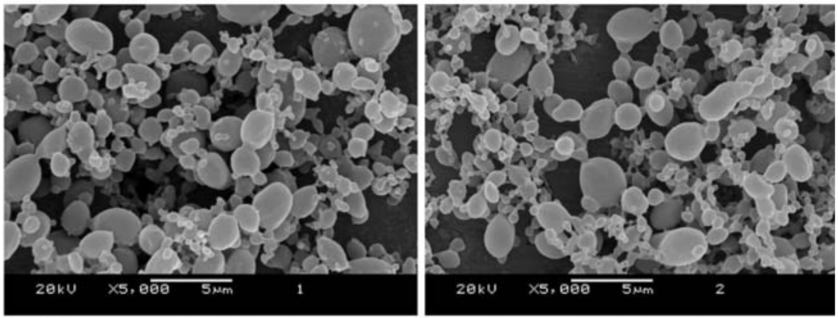
Higher flow rates decreased the mass-transfer rates of organic solvent out of the droplets, thus reducing the achievable super-saturation ratio. Lower super-saturation ratios engendered fewer nuclei, which in turn yield larger particles. [14] Figures 4b, 2b and 4c showed the SEM photomicrographs of microparticles prepared in a flow rate of 0.5, 1.0 and 1.5 ml·min^{-1}, respectively. With the



(a) PLLA concentration: 1.5% (w/v) **(b)** flow rate: 0.5 ml·min^{-1}



(c) flow rate: 1.5 ml·min^{-1} **(d)** temperature: 312 K



(e) pressure: 8 MPa **(f)** pressure: 16 MPa

Fig. 4 SEM photomicrographs of PLLA microparticles prepared in different conditions

increasing of flow rate, the particle size of PLLA microparticles and the agglomeration were increased.

Effect of temperature

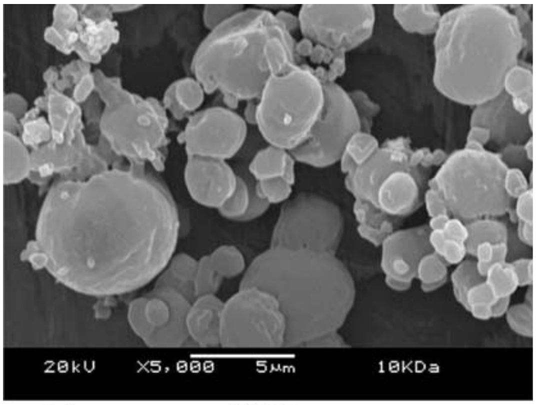
The change of temperature affected the density of CO_2 and therefore also the mass transfer between liquid and CO_2. With increasing of temperature, the CO_2 density was decreased, and consequently the decrease of the expansion ability to organic solvent, meanwhile, the molecular kinetic energy of solute was increased, the droplets were possibly colliding with each other and combining or some droplets were breaking up, it resulted in bigger particle size and heavier agglomeration.

Figure 4d showed the SEM photomicrograph of microparticles prepared at 312 K, obviously, this product had bigger particle size and heavier agglomeration than that of the samples prepared at 306 K (as shown in Fig. 2b). Rantakylä [9], Cocero [15] and Ghaderi [16] also reported the similar results.

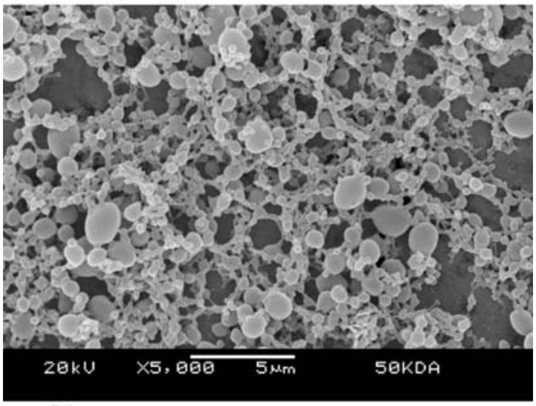
Effect of pressure

The PLLA microparticles prepared at the pressure of 8, 12 and 16 MPa were shown in Fig. 4e, 2b and 4f correspondingly, it was found that there was no significant difference between the PLLA microparticles prepared at 8 and 16 MPa, however, these two products had bigger particle size and heavier agglomeration than that of the samples prepared at 12 MPa. The possible reason was as following:

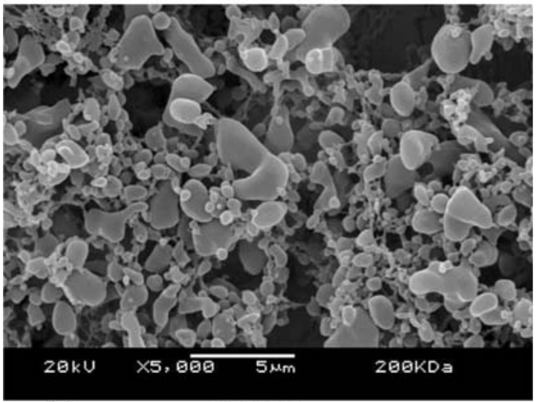
The droplet size depended either on the interfacial tension between the organic solution and the supercritical phase or on the density of CO_2. Increasing the pressure resulted in an increase in the CO_2 density, it induced a better dispersion of PLLA solution in supercritical CO_2 and hence a decrease in the droplet size, and as a consequence the acceleration of the



(a) 10 kDa



(b) 50 kDa



(c) 200 kDa

Fig. 5 SEM photomicrographs of PLLA microparticles with different MW

mass transfer phenomena. Thereby, super-saturation within the liquid phase is reached more quickly what prevented the crystals from growing. [17] While with the increasing of pressure, the diffusion coefficient of the supercritical CO_2 was comparable to the high volumetric expansion of DCM, causing a fast diffusion of CO_2 into the solvent and vice versa and a fast precipitation. As a result, particles were not precipitated individually, it led to bigger particle size and heavier agglomeration [18].

Effect of PLLA MW

Figures 5a, b, 2b and 5c showed the SEM photomicrographs of microparticles with MW of 10, 50, 100 and 200 kDa, correspondingly. Among them, the PLLA microparticles with MW of 10 kDa had the biggest particle size, with increasing of the PLLA MW, the particle size of product decreased first and then increased, and the product with MW of 200 kDa shifted to be irregular in shape and gave rise to partially bridged particles and agglomerates. It was probably because that the PLLA with smaller MW had a stronger chain stretching effect, during the precipitation of PLLA in supercritical CO_2, it generated heavier intertwine between molecular chains and thus resulted in bigger particles. Meanwhile, at the same concentration, the higher MW of PLLA was, the higher viscosity of PLLA solution was, the PLLA solution with higher viscosity was easier to reach the supersaturation and it generated a faster precipitation of PLLA in supercritical CO_2, however, the increasing of viscosity of PLLA solution also generated a bigger size of droplet and decreased the mass transfer performance.

Conclusions

Based on supercritical CO_2 technologies including SAS process and SEDS process, PLLA microparticles were studied. It was found that the saturation ratio of PLLA solution, concentration of the organic solution and flow rate of the solution could affect the surface morphology, particle size and particle size distribution of PLLA microparticles greatly. By adjusting the experimental parameters, various PLLA microparticles with different mean particle size would be produced for different purposes. Fine particles would be resulted in SEDS process by using dichloromethane/acetone mixture as solution; the resulting PLLA microparticles had a rather spherical shape, smooth surface and a narrow particle size distribution. The study of PLLA microparticles in supercritical CO_2 made it feasible to reach the goal of co-precipitation of drug and PLLA as composite microparticles for controlled drug delivery.

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