Nanoparticle fabrication of biodegradable polymers using supercritical antisolvent: Effects of mixing and thermodynamic properties

Lai Yeng Lee
MEBCS Program, Singapore-MIT Alliance, 4 Engineering Dr 3, Singapore 117576, Singapore

Kenneth A. Smith
Massachusetts Institute of Technology, Department of Chemical Engineering, Cambridge MA 02139

Chi-Hwa Wang
MEBCS Program, Singapore-MIT Alliance, 4 Engineering Dr 3, Singapore 117576, Singapore

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Introduction

Particle formation is an important application of supercritical fluid technology [1]. The common methods for particle formation using supercritical fluids include the rapid expansion of supercritical solutions (RESS) [1-3] and the supercritical antisolvent (SAS) processes [1, 2, 4-10]. The most commonly used supercritical fluid in pharmaceutical applications is CO_2 due to its relatively accessible critical point, abundance and its low toxicity [1-2]. Since most organic solvents are miscible with supercritical CO_2, a low residual solvent content can be easily achieved in the final product without extensive downstream purification to remove excess solvent. Several factors may affect the particle size and properties achieved from SAS process. This includes the phase behavior of the ternary mixture, the hydrodynamics of the solution injected into the supercritical phase, as well as the thermodynamic conditions of the supercritical fluid. Considerable literature suggests that the controlling parameter for particle size in the SAS process is the rate of mass transfer [11]. This may be influenced by both the spray hydrodynamics and thermodynamic properties of the supercritical fluid phase. Chattopadhyay and Gupta [6-10] used an ultrasonic vibrating surface to break up the solution jet into smaller droplets and also increase the mass transfer rates between the supercritical CO_2 and the solvent. This has been termed the supercritical antisolvent with enhanced mass transfer process (SASEM).

In this study, biodegradable and biocompatible Poly $_L$ lactide (PLA) was used to fabricate microparticles and nanoparticles using a SAS and a modified SASEM process. The main difference between this modified process and the usual SASEM process is that the organic solution spray was directed away from the ultrasonic vibrating surface. The atomization of the inlet solution was achieved by jet breakup at supercritical pressures and not due to ultrasonic liquid atomization. The ultrasonic probe is fitted into the high pressure vessel to create extensive mixing and turbulence within the vessel during the modified SASEM process. The organic solution was introduced into the high pressure vessel through a 0.5 mm ID stainless steel capillary. The high pressure vessel has borosilicate glass windows which allow observation of the SAS and the modified SASEM process.

Materials and methods

Materials

Poly (L-lactic acid) (PLA, Product Number P1566, MW = 85,000 – 160,000Da) was purchased from Sigma Aldrich. Paclitaxel was a generous gift from Bristol Myers Squibb. Dichloromethane (DCM, Product Number DS1432, HPLC/Spectro Grade) was purchased from Tedia (Tritech Scientific Pte Ltd, Singapore). Compressed CO_2 was purchased from Soxal (Singapore Oxygen Air Liquide Pte Ltd).

Microparticles and nanoparticles preparation

The experimental equipment for the SAS and modified SASEM processes is shown in Figure 1. PLA and paclitaxel were first dissolved in DCM. The high pressure vessel, which has a volume of 59 cm^3, was first filled with compressed CO_2. Liquefied CO_2 was subsequently pumped into the vessel using a high pressure pump to the required pressure. The temperature in the vessel was controlled by use of a water bath. The organic solution was pumped into the high pressure vessel at a flow rate of 2ml/min

through a capillary tubing of ID 0.5mm. After the batch precipitation process, the DCM-CO_2 mixture was vented off to a fume cupboard. Fresh CO_2 was introduced into the vessel at 50 bars for 3 times to remove any residual DCM in the particles. The particles were collected at the bottom of the vessel on a 0.22 micron cellulose acetate filter during the venting process.

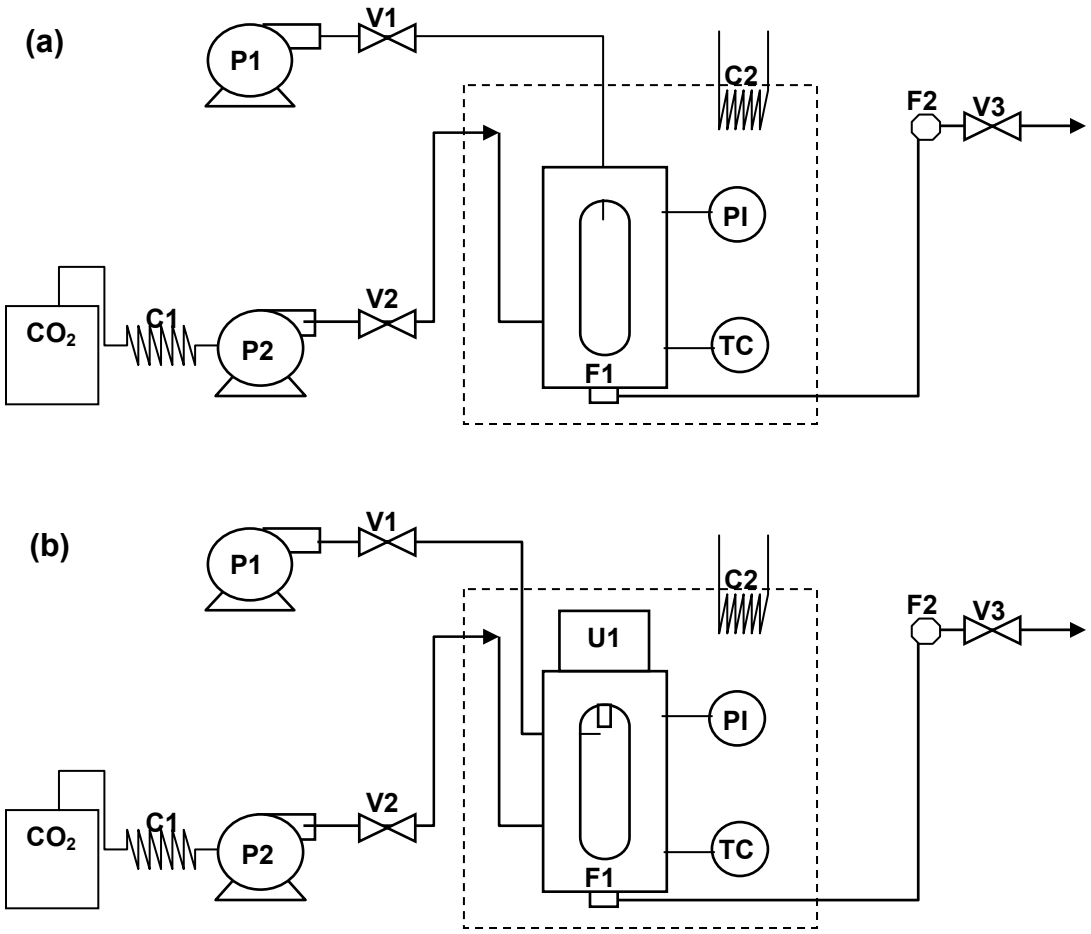


Figure 1. Experimental setup for (a) SAS production of micro and nanoparticles of PLA (b) SAS (with ultrasonication) production of micro and nanoparticles of PLA.
HP: Jerguson 12-T-32 high pressure vessel (For supercritical antisolvent process); U1: Ultrasonic system; Branson sonifier and converter, Sonics and Materials probe (3/8" probe tip diameter); C1: Polyscience 912 refrigerating circulator (for liquefying CO_2); C2: Polyscience 712 circulator with temperature control (Water bath); P1: Eldex B-100-S HP series pump (for solution injection); P2: Jasco HPLC pump (for pumping liquefied CO_2 into HP vessel); F1: 0.22 micron filter membrane (to collect particles from bottom of vessel); F2: stainless steel filter (0.22 micron, for filtering the contents leaving the vessel); V1: Swagelok 1/16" tube connection ball valve (solution line);V2: Swagelok 1/16" tube connection ball valve (CO_2 line); V3: Swagelok 1/8" tube connection ball valve (to vent); TC: Thermocouple connected to Thermometer read out; PI: Swagelok Pressure gauge

Size and Surface morphology analysis

Qualitative observation of the size and surface morphology of the particles was achieved by scanning electron microscopy (SEM, JEOL JSM-5600 LV). Platinum coating (Autofine Coater, JEOL JFC-1300) of the samples was required before SEM analysis.

Thermogram properties analysis

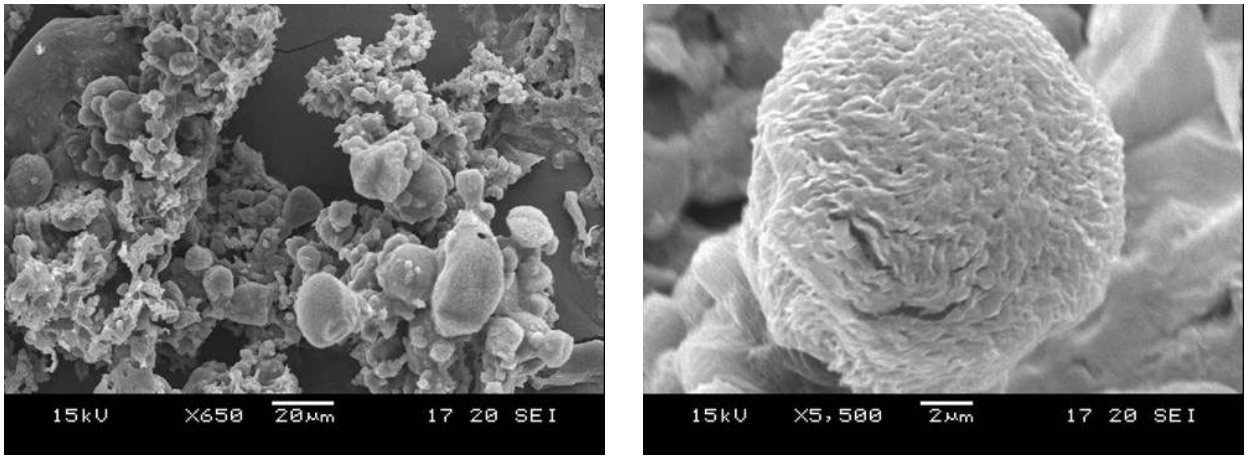
Phase behavior of the particles was studied by differential scanning calorimetry (DSC, 2920 modulated, Universal V2.6D TA instruments). Approximately 2-10 mg of particles was loaded onto standard aluminum pans (40mg) with lids. The samples were purged with pure dry nitrogen at flow rate of 5 ml/min. A blank aluminum pan was used as reference in all the experiments. The analysis was carried out using a temperature ramp of 10 ^{o}C/min from 20 – 280 ^{o}C.

Results and Discussion

Size and surface morphology

The effects of hydrodynamic and thermodynamic properties of the organic and supercritical fluid phases on the resultant particle sizes were investigated. Paclitaxel-loaded PLA particles were also produced to explore the encapsulation of drug molecules in the matrix of PLA micro- and nanoparticles.

Figure 2 shows PLA particles obtained from SAS at supercritical conditions of 80 bars and 35 Celsius. Figure 3 shows PLA particles obtained from SAS at supercritical conditions of 90 bars and 35 ^{o}C at different solution inlet flow rate. From Figures 2 and 3, it can be observed that the particles achieved from the SAS process with no external mixing have wide particle size distributions.



(a) **(b)**

Figure 2. SEM pictures of PLA particles fabricated at solution flow rate of 4 ml/min at 80 bars and 35 Celsius. (a) Particles taken at 650 x magnification; (b) Surface morphology of particles taken at 5,500 x magnification

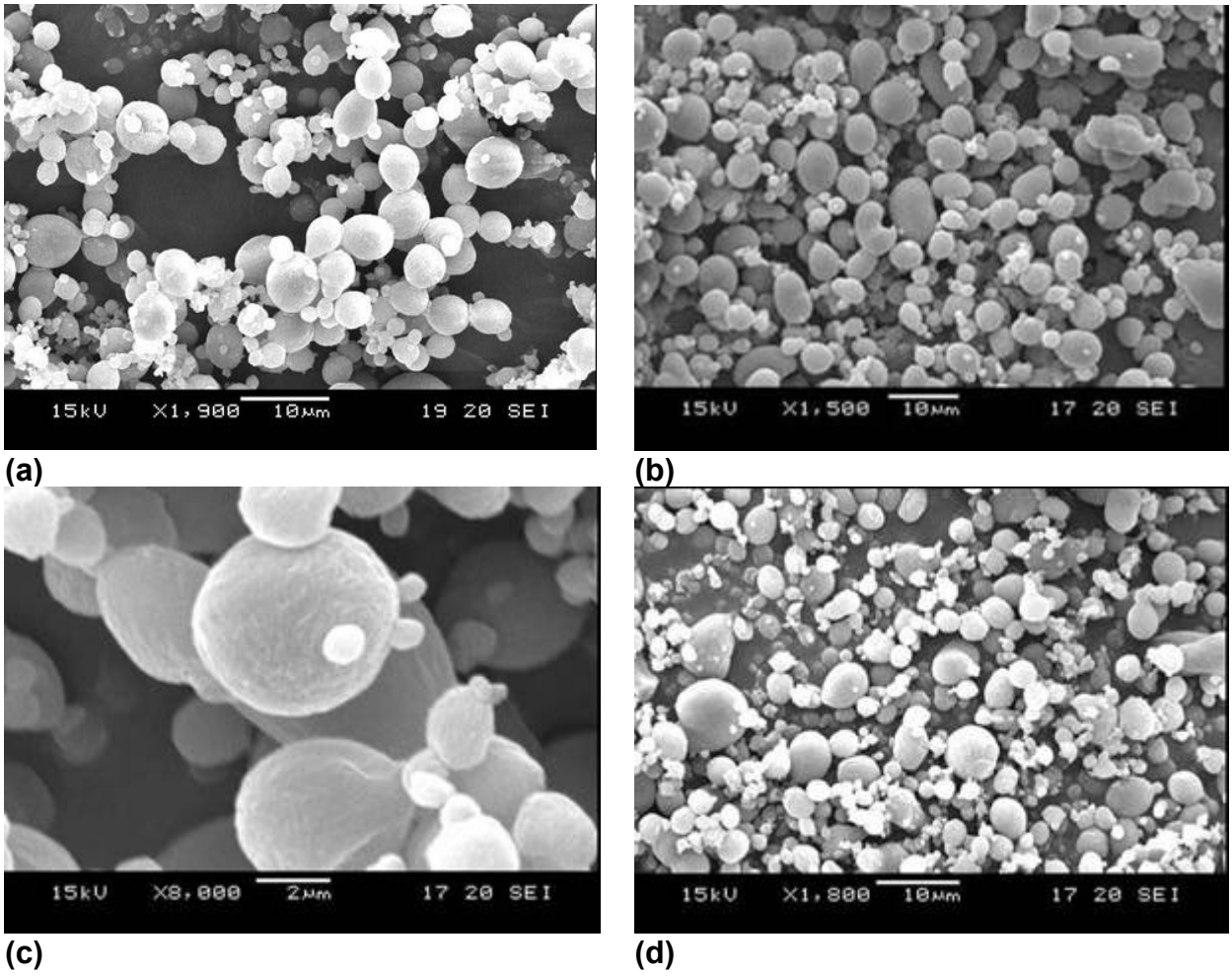
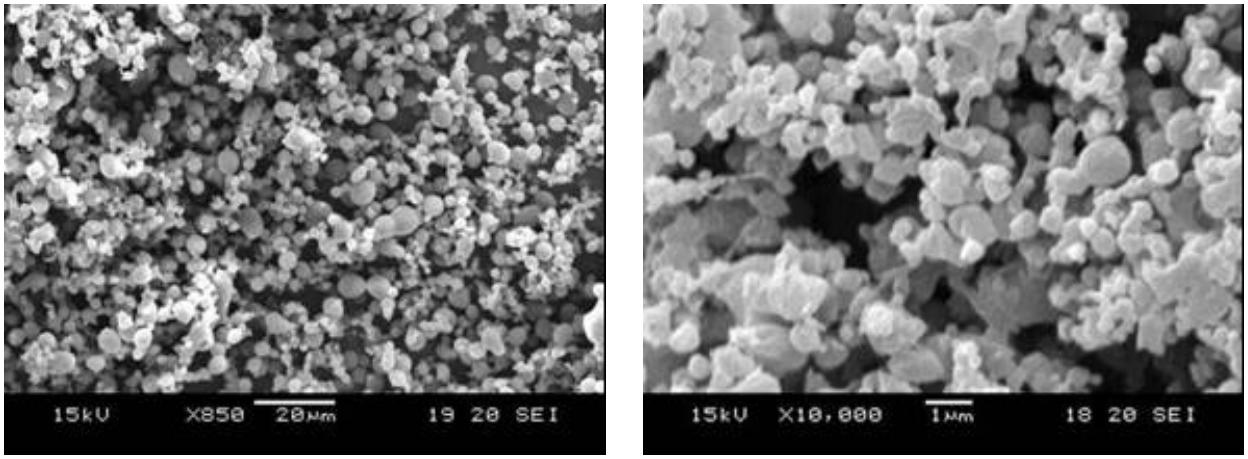


Figure 3. SEM pictures of PLA particles fabricated 90 bars and 35 Celsius. (a) Solution flow rate at 2 ml/min, 1,900 x magnification; (b) Solution flow rate at 4 ml/min, 1,500 x magnification; (c) Solution flow rate at 4 ml/min, Surface morphology taken at 8,000 x magnification; (d) Solution flow rate at 6.3 ml/min, 1,800 x magnification

Furthermore, particles obtained at 80 bar (Figure.2) were much more agglomerated than those obtained at 90 bars (Figure. 3). In Figure 3, microparticles of approximately 5-10 micron and smaller particles of 0.5 – 1.0 micron can be observed. No significant difference was observed in particles obtained at different solution flow rates. From Figure 3c, it can be seen that the particles have quite smooth surface morphology. Figure 2b shows rougher particles than those achieved using similar flow rate at 90 bars. This implies a difference in the mass transfer rate between the solvent-antisolvent phases at different supercritical pressures.

Paclitaxel-loaded particles at 10% drug loading were also fabricated for controlled release applications. Conditions were similar to those corresponding to Figure 3a. These are illustrated in figure 5a. The figure shows that the particle sizes and morphologies obtained were similar to those for the blank PLA particles. The effect of using ultrasonic vibration in a modified SASEM process was also studied. Figure 54b shows paclitaxel-loaded PLA particles obtained at 60 micron vibration amplitude at 20 kHz. It can be observed that application of ultrasonication significantly decreases the

size of the particles obtained. Nanoparticles of approximately 200 to 500 nm were achieved.



(a) **(b)**

Figure 4. SEM pictures of PLA particles fabricated at solution flow rate of 2 ml/min at 90 bars and 35 ^{o}C. (a) No ultrasonication, 850 x magnifications; (b) Ultrasonic vibration amplitude of 60 μm, 10,000 x magnifications.

Thermogram properties

The thermogram analysis of the drug-encapsulated microparticles and nanoparticles allow us to determine whether most of the paclitaxel was molecularly dispersed in the polymer matrix or crystallized out as needles during the precipitation process [12, 13]. The exotherms for the paclitaxel and PLA used in this study are shown in Figure 5. Pure paclitaxel has a characteristic endothermic peak at approximately 223.0 ^{o}C [14] and the DSC analysis shows paclitaxel with an endothermic peak at 224.5 ^{o}C which is in close agreement with literature value. The melting peak for pure PLA before processing was 178.7 ^{o}C. The exotherms for blank PLA (without paclitaxel) and PLA (with 10% paclitaxel) fabricated using the supercritical antisolvent process are illustrated In Figure 5. The endothermic melting peak for the blank PLA particles was 175.8 ^{o}C which was approximately 3 ^{o}C lower then the untreated PLA particles. It was also observed that with paclitaxel-loaded particles, the endothermic peak is reduced by approximately another 2 ^{o}C. The supercritical antisolvent process with ultrasonication did not significantly alter the polymeric structure for PLA. There was also no endothermic peak observed for all the paclitaxel-loaded particles exotherm in the vicinity of the 220-230 ^{o}C near the melting point of paclitaxel. Therefore, we can deduce that in the fabrication of the paclitaxel-loaded PLA particles, there was no significant phase separation and crystalline paclitaxel formed at 10% drug loading.

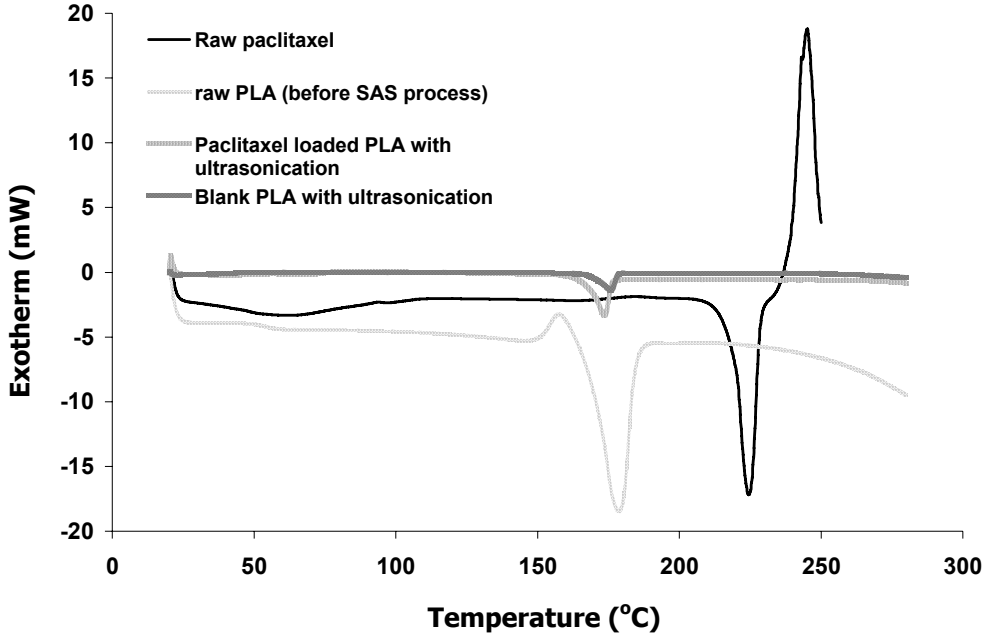


Figure 5. Thermogram properties of raw paclitaxel, raw PLA, Blank PLA particles using modified SASEM and paclitaxel loaded PLA particles.

Conclusions

The effects of ultrasonic vibration amplitude on the extent of mixing in the high pressure vessel and the resulting effects on particle sizes have been investigated. Studies with PLA have shown that, without ultrasonication, highly polydisperse particles are obtained. When ultrasonication is applied during the SAS process, more monodisperse powders are obtained. Varying solution flow rate showed little effect on the resultant particle sizes. Varying the supercritical pressure affects the mass transfer between the solvent–antisolvent phases and hence influences the size and morphology of the particles. Effects of varying ultrasonic vibration amplitude were also studied (not shown here). It was found that the particle size generally decreases as the ultrasonic vibration amplitude increases. Paclitaxel-loaded particles could also be fabricated by use of the modified SASEM method.

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