

## Poli (L-lactide) Micronization by Supercritical Fluids

Antonio Montes <sup>a,\*</sup>, María Dolores Gordillo<sup>a</sup>, Clara María Pereyra<sup>a</sup>, Gabriele Di Giacomo<sup>b</sup>, Enrique José Martínez de la Ossa<sup>a</sup>

<sup>a</sup>Department of Chemical Engineering and Food Technology, Faculty of Sciences, University of Cádiz, International Excellence Agrifood Campus (CeIA3), 11510 Puerto Real (Cádiz), Spain.

<sup>b</sup> Department of Chemistry, Chemical Engineering and Materials, University of L'Aquila, 67100 L'Aquila, Italy.  
antonio.montes@uca.es

Polymer microparticles are useful for several applications like stationary phases in chromatography, adsorbents and catalyst supports, as well as drug delivery systems. The application of supercritical fluids for particle precipitation has been developed an ideal alternative to conventional processes in the past decades. In this work Poly(L-lactic acid) (PLLA), a thermoplastic aliphatic polyester derived from renewable resources, such as corn starch, tapioca products or sugarcane has been processed by supercritical fluids, particularly with rapid expansion of supercritical solutions (RESS) and supercritical antisolvent (SAS) processes. RESS process experiments were failed but the morphology was greatly improved from irregular blocks to spherical microparticles in the SAS process. The polymer concentration and pressure effect have been too evaluated. At higher concentration of the initial solution the particle size was decreased. As well when pressure is increased the particle size is decreased

### 1. Introduction

Polymers play an essential role in daily life because of the extraordinary range of properties (Painter and Coleman, 1997) being used as rubbers, synthetic plastics, fibers in the textile industry and biopolymer in biological structures and functions, among others. Moreover polymer microparticles are useful for several applications like stationary phases in chromatography, adsorbents and catalyst supports, as well as drug delivery systems (Dixon et al., 1993).

Supercritical fluids have larger diffusivities than those of typical liquids, resulting in higher mass-transfer rates, and solvent power and selectivity can be also adjusted continuously by altering the experimental conditions (temperature and pressure). So, the application of supercritical fluids has been developed for particle precipitation as an ideal alternative to conventional processes in the past decades. Among most commonly used supercritical fluids, supercritical CO<sub>2</sub> (sc-CO<sub>2</sub>) has replaced conventional organic solvents, dealing to implementation of environmentally friendly chemical processes. SC-CO<sub>2</sub> is inexpensive, non-toxic, non-flammable, readily available, easily recycled and it has good properties as a solvent (Garay et al., 2010). Processing polymer with supercritical fluids can offer several advantages as particle size reduction and plasticization. The plasticization of polymers is characterized by increased segmental and chain mobility and by an increase in interchain distance. The plasticization of polymers induced by scCO<sub>2</sub> has an impact on many polymer-processing operations, as viscosity reduction for polymer extrusion and blending, enhancement of the diffusion of additives through polymer matrices for impregnation and extraction, enhancement of monomer diffusion for polymer synthesis, foaming of polymers, and changes in polymer morphology due to induced crystallization (Kazarian et al., 2000).

Poly(L-lactic acid) (PLLA) a thermoplastic aliphatic polyester derived from renewable resources (Gwon et al., 2012) have been widely studied in drug delivery systems (Campardelli et al., 2012) because of their excellent biocompatibility and biodegradability. PLLA is also used as bioabsorbable polymer as bone fixation devices, owing to its mechanical property profile, thermoplastic possibility and biological properties

(Lopes et al., 2012). In this work has been tried the polymer precipitation by scCO<sub>2</sub>, particularly with RESS and SAS processes. In the RESS method, the sudden expansion of the supercritical solution (polymer dissolved in scCO<sub>2</sub>) through a nozzle and the rapid phase change at the exit of the nozzle lead to a high level of supersaturation, thus causing very rapid nucleation of the substrate in the form of very small particles, which are collected from the gas stream. However, in the SAS process diffusion of the supercritical fluid into the drops of solvent is generated by spraying the solution through a nozzle, a process that solubilizes the CO<sub>2</sub> in the solvent and vice versa. This process leads to a high supersaturation of the drug solution and powder precipitation.

## 2. Experimental section

PLLA (viscosity 0.8-1.2 dL/g), dichloromethane (DCM) (purity 99.5%) and methanol (99.9 %) were purchased from Sigma-Aldrich (Spain). CO<sub>2</sub> with a minimum purity of 99.8% was supplied by Linde (Spain). The mean particle size of commercial PLLA was about 1 mm as can be shown in the Figure 1.

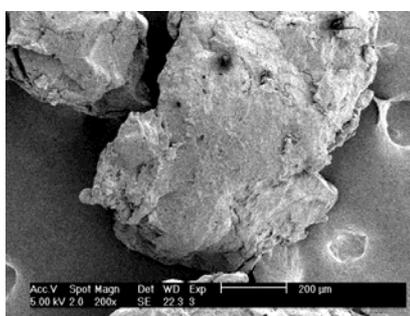


Figure 1: SEM image of unprocessed PLLA

The experiments 1-6 shown in Table 1 were carried out in a pilot plant developed by Thar Technologies® (model RESS250). Pressures of 200-300 bar and temperatures of 60-115 °C were assayed into the vessel. The rest of operating conditions were held constant.

A schematic diagram of this equipment is shown in Figure 2. The RESS250 system comprises the following main components: a high-pressure pump for the CO<sub>2</sub> (P1); a 250 mL stainless steel extractor vessel (V1) surrounded by an electrical heating jacket (V1-HJ1); a magnetic stirrer (maximum speed 2500 rpm) to hold the solute and supercritical CO<sub>2</sub> in continuous mixing and a stainless steel collection vessel (V2) where particles are precipitated.

The following auxiliary elements were also necessary: a low pressure heat exchanger (HE1), cooling lines and a cooling bath (CWB1) to keep the CO<sub>2</sub> inlet pump cold and to chill the pump heads; an electric high-pressure heat exchanger (HE2) to preheat rapidly the CO<sub>2</sub> in the extractor vessel to the required temperature; safety devices (rupture discs and safety valves MV1 and MV2); pressure gauges for measuring the pump outlet pressure (P1, PG1) and the extractor vessel pressure (V1, PG1); thermocouples placed inside (V1-TS2) and outside (V1-TS1) the extractor vessel, on the nozzle (N1-TS3), and on the electric high pressure heat exchanger to obtain continuous temperature measurements; and a FlexCOR coriolis mass flowmeter (FM1) to measure the CO<sub>2</sub> mass flow rate. A 5 μm filter (F1) was fitted at the exit of the extraction vessel to ensure that during expansion of the solution undissolved PLLA particles will not be carried over with the scCO<sub>2</sub> flow.

All factors that have an influence on the precipitation process (temperature, flow rate, pressure, etc.) were controlled automatically (using ICM software). A particularly important component of the RESS 250 system is the nozzle that sprays the supercritical solution inside the precipitator vessel (V2). A stainless steel nozzle from Thar Technologies, with an inner diameter of 100 μm and length of 1170 μm, was used in this work.

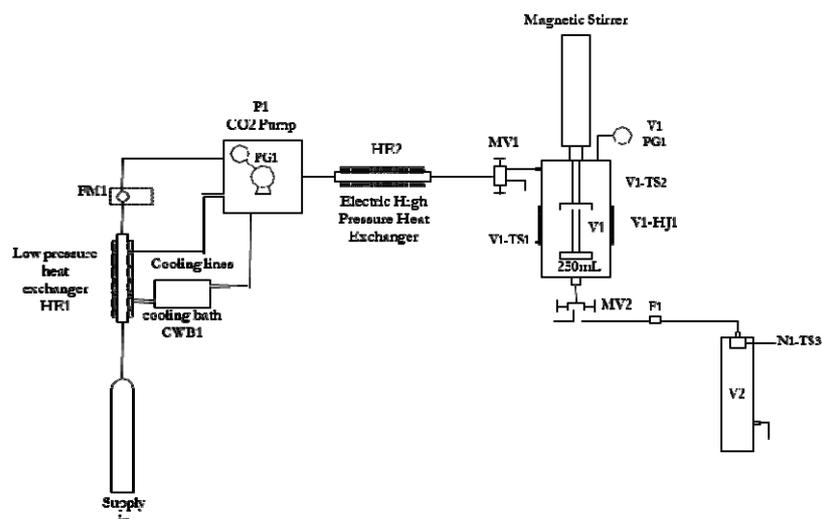


Figure 2: Schematic diagram of RESS250 pilot plant

The equipment was modified by heating the tubing between the extractor vessel (V1) and the collection vessel (V2) up to the extraction temperature in order to avoid solute precipitation caused by a fall in temperature lowering the solubility. The glass collection vessel had to be replaced by stainless steel because freezing associated with the decompression led to the glass vessel breaking.

Table 1: Experimental design and quantitative results of particle size (PS)

RESS process							
run	P (bar)	T <sub>extraction</sub> (°C)	T <sub>preexpansion</sub> (°C)	%Methanol	Particle size (µm)	success	
1	150	45	80		----	-	
2	200	60	80	0	----	-	
3	300	100	100		----	-	
4	150	45	80		----	-	
5	200	60	80	10	----	-	
6	300	100	100		----	-	
SAS process							
run	P (bar)	T (°C)	Q <sub>CO2</sub> (g/min)	Q <sub>L</sub> (mL/min)	cc (mg/mL)	Particle size (µm)	success
7					5	5.29±1.97	+
8					10	3.04±0.99	+
9	100				15	2.07±1.51	+
10		35	11	4	30	1.42±0.72	+
11	150				15	1.69±0.56	+
12	200					1.32±0.59	+

The experiments 1-6 were performed by following the same procedure. Firstly, the commercial PLLA powder was placed in the extraction vessel. The vessel was closed and CO<sub>2</sub> was pumped into the extractor vessel at the same time as the electrical heater and heat exchanger were switched on. Once supercritical conditions for CO<sub>2</sub> (pressure and temperature) had been achieved, the system was held for 1 hour to ensure complete equilibrium. Valve MV2 was then opened and the supercritical solution was

expanded through a pre-heated nozzle. This nozzle was pre-heated in order to compensate for the heat loss and to prevent the nozzle from clogging during expansion.

The rest of the experiments shown in Table 1 (runs 7–12) were carried out in another pilot plant developed by Thar Technologies® (model SAS 200). Pressures of 100-250 bar at temperatures of 35 °C and polymer concentration between 5-30 mg/mL were assayed. A schematic diagram of this equipment is shown in Figure 3 and the system was described in detail in a previous publication (Tenorio et al., 2007).

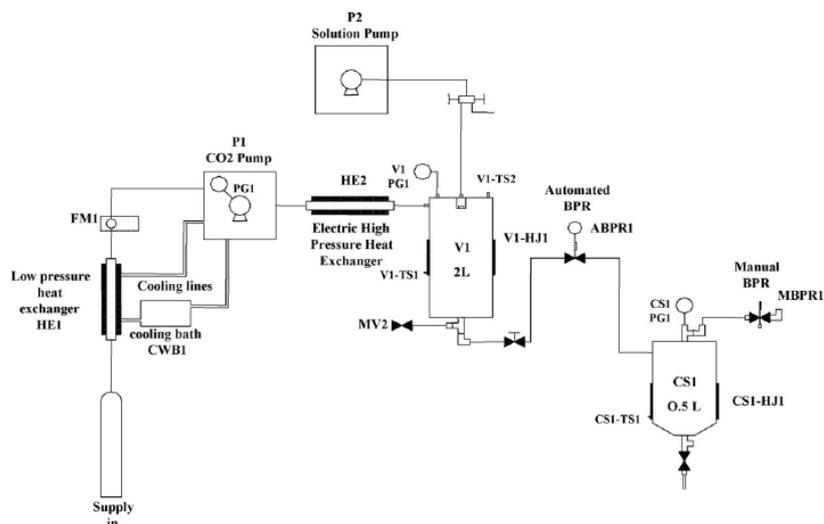


Figure 3: Schematic diagram of SAS 200 pilot plant

The experiments 7-12 were performed following the same procedure. First, CO<sub>2</sub> was pumped into the vessel at the same time as the electrical heater, heat exchanger, and automatic backpressure regulator were switched on. When CO<sub>2</sub> supercritical conditions (pressure and temperature) had been achieved and the solution pump had been primed, the liquid solution was pumped to the precipitator vessel and sprayed inside the vessel itself by means of the nozzle. The small drops of solvent were dissolved by the supercritical CO<sub>2</sub>, causing supersaturation of the liquid solution and consequent precipitation of the PLLA in the form of a powder that accumulated on the internal wall of the vessel.

### 3. Results and Discussion

First of all, RESS process was tried using different operating conditions (Table 1) and all these experiments with and without cosolvent were failed. Although several authors (Kim et al., 1996; Gaderman et al., 2009) have achieved to precipitate PLLA with this technique other ones had to use cosolvents like tetrahydrofuran (Tom and Debenedetti, 1994) or chlorodifluoromethane (Sane and Thies, 2007) to obtain microparticles of this polymer. This can be due to different type of PLLA used with different molecular weight and viscosity. PLLA used in this work was not enough soluble to be precipitated by this technique. So due to the low solubility of PLLA in supercritical CO<sub>2</sub> SAS process was carried out and successfully polymer precipitation was achieved.

Since SAS process seemed to be a good technique to precipitate PLLA the influence of initial concentration of the solution and pressure, two of the key of SAS precipitation, were studied. Pressures between 100-200 bar, at 35 °C into the vessel and polymer concentration between 5-30 mg/mL were assayed. The morphology was greatly improved from irregular blocks to spherical microparticles as can be seen in the experiment shown in Figures 4 and 5 with regard to Figure 1.

The initial concentration of the solution had a marked effect on particle size. When this concentration was increased the particle size of the polymer was decreased. An increase in the initial concentration of the

solution has two opposing effects: on the one hand, higher supersaturations from higher concentration tend to decrease the particle size; and on the other hand, higher condensation rate from higher concentration tend to increase the particle size (Martin and Cocero, 2004). In our case, an increase in the initial concentration of the solution leads to smaller particles sizes with a narrower distribution. Thus, the first effect prevails under the operating conditions used in this work. However Sacchetin et al. found the opposite effect in the PLLA precipitation (Sacchetin et al., 2013).

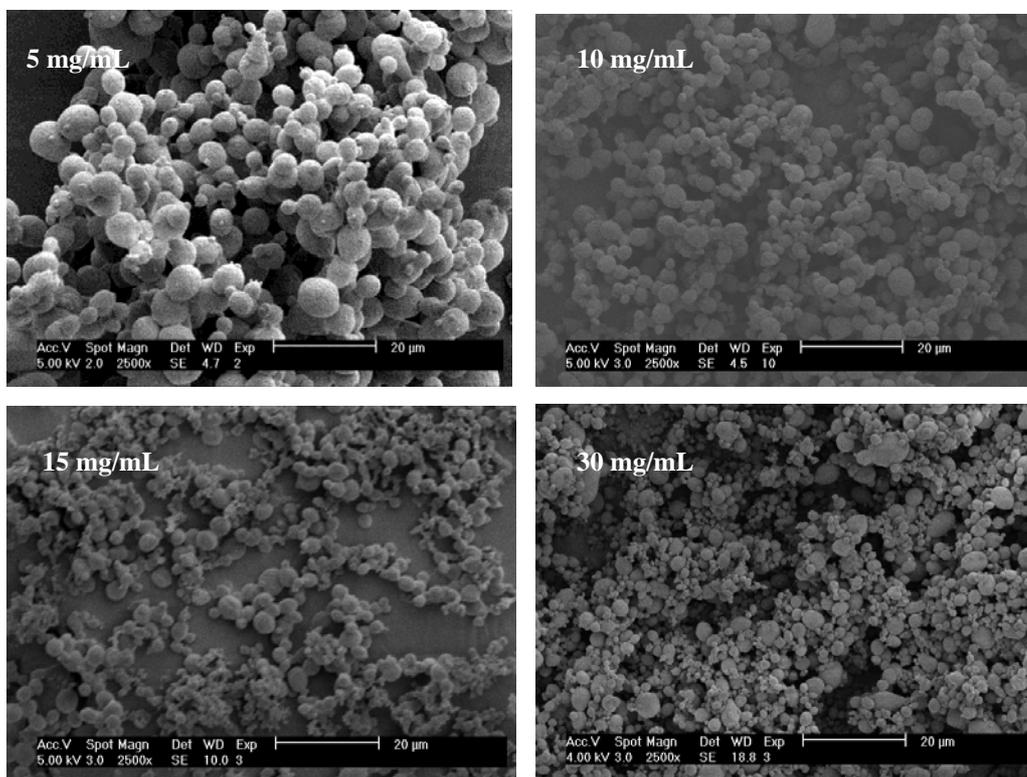


Figure 4. SEM images of PLLA precipitated particles at 100 bar and 35°C into the vessel by SAS process

Pressure effect can be observed in the Figure 5. At higher operating pressure smaller particle size and particle size distribution narrower were produced. This fact is in agreement with PLLA precipitation carried out by Sacchetin et al, recently (Sacchetin et al., 2013). This result can be explained by considering that an increase in pressure at constant temperature enhances the solvent power of supercritical CO<sub>2</sub> towards DCM, thus the liquid solvent molecules are more strongly captured by the CO<sub>2</sub>. In contrast, the possibility of interaction between DCM and PLLA is reduced (Snaveley et al., 2002).

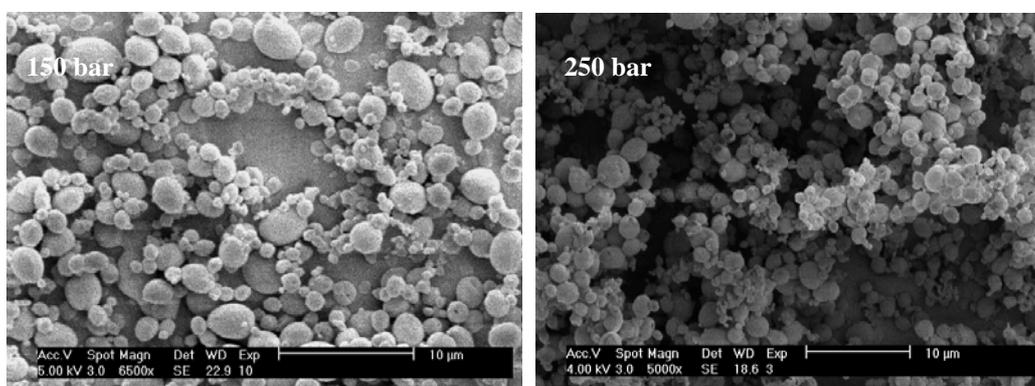


Figure 5. SEM images of PLLA precipitated particles at 15 mg/mL and 35°C into the vessel by SAS process

#### 4. Conclusions

Processing PLLA with supercritical CO<sub>2</sub> has been carried out. RESS experiments with and without cosolvent were failed in the used operating conditions range of this work. However PLLA SAS processing has been carried successfully out. The morphology of the polymeric particles has been improved from irregular blocks of millimetres to spherical particle in the micrometer range. The initial concentration of the solution had a marked effect on particle size. When this concentration was increased the particle size of the polymer was decreased. At higher operating pressure smaller particle size and particle size distribution narrower were produced.

#### References

- Campardelli, R. , Reverchon, E. , Della Porta, G.,2012, Biopolymer particles for proteins and peptides sustained release produced by supercritical emulsion extraction, 20<sup>th</sup> International Congress and Process Engineering CHISA, 25-29 August 2012,Praha, ISBN:978-80-905035-1-9.
- Dixon, D.J., Johnston, K.P., Bodmeier, R.A., 1993, Polymeric materials formed by precipitation with a compressed fluid antisolvent, *AIChE Journal*, 39 (1),127–139.
- Garay, I., Pocheville, A., Madariaga. L., 2010, Polymeric microparticles prepared by supercritical antisolvent precipitation, *Powder Technology*, 197, 211–217
- Gadermann, M., Kular, S., Al-Marzouqi, A. H., Signorell, R., 2009, Formation of naproxen–polylactic acid nanoparticles from supercritical solutions and their characterization in the aerosol phase, *Physical Chemistry Chemical Physics*, 11, 7861–7868.
- Gwon, J., Cho, D. W., Kim, S. H., Shin H. Y., Kim. H., 2012, Phase behaviour of the ternary mixture system of poly(L-lactic acid), dichloromethane and carbon dioxide. *J. Chem. Thermodynamics*, 55, 37–41.
- Kazarian, S. G., 2000, Polymer Processing with Supercritical Fluids, *Polymer Science*, 42 (1), 78–101.
- Kim, J.-H., Paxton, T. E., Tomasko, D. L., Microencapsulation of Naproxen Using Rapid Expansion of Supercritical Solutions, *Biotechnology Progress*, 12, 650-661.
- Lopes, M. S. Jardini, A. L. Filho, R. M., 2012, Poly (lactic acid) production for tissue engineering applications, 20<sup>th</sup> International Congress and Process Engineering CHISA, 25-29 August 2012,Praha, ISBN:978-80-905035-1-9.
- Martin, A., Cocero, M. J., 2004, Numerical modeling of jet hydrodynamics, mass transfer, and crystallization kinetics in the supercritical antisolvent (SAS) process, *J. Supercritical Fluids*, 32, 203-219.
- Painter, C., Coleman, M. M., Eds., 1997, *Fundamentals of polymer science an introductory text*. Technomic Publishing Company, Inc., Lancaster, PA., USA.
- Sacchetin, P. S. C., Morales, A.R., Moraes, Â. M., de Tarso Vieira e Rosa, P., 2013, Formation of PLA particles incorporating 17  $\alpha$ -methyltestosterone by supercritical fluid technology, *J. Supercritical Fluids*, 77, 52– 62.
- Sane, A., Thies, M. C., 2007, Effect of material properties and processing conditions on RESS of poly(l-lactide), *J. Supercritical Fluids*, 40 , 134–143.
- Snavely, WK, Subramaniam, B, Rajewski, RA, Defelippis, MR., 2002, Micronization of insulin from halogenated alcohol solution using supercritical carbon dioxide as an antisolvent, *J. Pharmaceutical Sciences*, 91, 2026–2039.
- Tenorio, A., Gordillo, M. D., Pereyra, C. M., Martínez de la Ossa, E. M., 2007, Relative Importance of the Operating Conditions Involved in the Formation of Nanoparticles of Ampicillin by Supercritical Antisolvent Precipitation, *Ind. Eng. Chem. Res.*, 46,114-123.
- Tom, J.W., Debenedetti, P.G., 1994, Precipitation of poly(l-lactic acid) and composite poly(l-lactic acid)–pyrene particles by rapid expansion of supercritical solutions, *J. Supercritical Fluids*, 7, 9–29.