COMPARISON BETWEEN SC-CO_2 EXTRACTION AND SOLVENT EVAPORATION OF O/W EMULSIONS FOR DRUG-POLYMER MICROSPHERES PRODUCTION

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Abstract

Supercritical fluids extraction of oil-in-water (O/W) emulsions is a new technique for the production of drug/polymer microspheres with controlled size and distribution. This process uses supercritical carbon dioxide (SC-CO_2) to extract the oily phase of an O/W emulsion producing a microspheres suspension. A systematic study on the characteristics of the microspheres obtained by SC-CO_2 extraction and by conventional solvent evaporation technique was proposed starting from the same emulsion, to explore the benefits of the proposed process. For all the systems studied, the microspheres obtained by solvent evaporation (SE) included at least the 10% of particles larger than the original droplets and their sizes and distribution was strictly related to the temperatures used. On the contrary, the microspheres produced by SC-CO_2 showed particles size distributions (PSDs) always smaller than the ones of the droplets from which they were generated. The observed behavior was justified considering the faster precipitation route performed during the SC-CO_2 processing that may prevent droplet coalescence or aggregation phenomena typically occurring during the solvent evaporation process. These results suggest that, starting from a given emulsion, the PSD of the produced particles is not only related to the emulsion droplet size but also the solvent elimination process that can play a relevant role in determining the final size of the product.

Introduction

Drug/polymer microspheres are used in advanced drug formulations to assure an accurate active principle release during a given period of time. Microspheres are conventionally prepared using various techniques as: solvent evaporation or extraction, phase separation and spray-drying. The solvent evaporation/extraction technology is widely used because easy to be implemented by a simple beaker/stirrer setup. However, solvent evaporation technique requires relatively high temperatures or reduced pressures and, in several cases, can be inappropriate for producing large amounts of microspheres in a robust and well-controlled manner **[1].** Coacervation is frequently impaired by residual solvent or coacervating agents found in the produced microspheres. Spray-drying is relatively simple and of high throughput, but, cannot be used for highly temperature sensitive compounds. Moreover, both technologies are not well suited for producing microspheres in the low micrometer size range because the particle size control is very difficult and yields for small batches can be moderate. As a consequence, the production of microspheres with a controlled and narrow particle size, in several cases, is still a challenge **[2-3].**

It is well know that close to the critical point, small changes in temperature or pressure can produce large changes in the density/solvation ability of SC-CO_2. This property is currently used in a wide range of extraction applications and, therefore, it can be also used for the extraction of organic solvent, from emulsions, to produce microspheres. In addition, lower viscosity and higher diffusivity of SC-CO_2 with respect to the liquid solvent can improve mass transfer, which is often a limiting factor for the solvent elimination. As a consequence, the proposed technology can overcome several disadvantages of the conventional methods for the production of drug/polymer microspheres. In this work a comparative study between the characteristics of the microspheres obtained by SC-CO_2 extraction and of the ones produced by conventional solvent evaporation technique is proposed to

explore the benefits of the supercritical process. Chemical and physical analyses on the produced microspheres were also performed to characterize the produced devices.

Experimental Methods

Materials

CO_2 (99.9% SON Naples, Italy), polyvinyl alcohol (PVA, Mol wt: 30000–55000, Aldrich Chemical Co.), ethyl acetate (EA, 99.9% pure, Aldrich Chemical Co.), piroxicam (PX) and vancomicyn hydrochloride (VM) (Sigma-Aldrich Co.), poly (lactic/glycolic) acid (PLGA, 85:15 Aldrich Chemical Co) were used as received.

Emulsion Preparation

For single O/W emulsion preparation, a known amount of PX/PLGA mixture was dissolved into water-saturated EA to form an organic solution. This solution was then added into a known amount of the EA-saturated aqueous PVA solution (0.8% w/w) to form an emulsion (ratio 20:80) using a high-speed stirrer for 2–3 min at 2800 rpm. For the double W/O/W emulsion preparation, a known amount of VM was dissolved in 1 ml of PVA-water solution and then emulsified in EA using an ultrasonic homogenizer. The primary emulsion was then added into a known amount of the EA-saturated aqueous PVA solution (0.8 %w/w) to form the double emulsion (ratio 20: 80).

SC-CO_2 Apparatus

In a typical experiment, 50 g of emulsion were placed into a 0.25 dm^3 cylindrical stainless steel vessel. SC-CO_2 was delivered using a high pressure diaphragm pump (Milton Roy, model Milroyal B) and was bubbled into the extraction vessel at a constant flow rate, between 0.1-0.5 kg/h, through a cylindrical stainless steel dispenser put on the bottom of the extractor. Temperature was maintained constant using an air-heated thermostated oven. A separator located downstream the micrometering valve was used to recover the liquid solvent extracted. At the exit of the separator a rotameter and a wet test meter were used to measure the CO_2 flow rate and the total quantity of CO_2 delivered, respectively. A schematic representation of the apparatus used for microspheres preparation is proposed elsewhere **[4-5].**

Solvent Evaporation

EA was evaporated from the different emulsions under controlled and mild vacuum (170 mmHg, rotating evaporator) for 8h at 38°C. During the evaporation, the emulsion was swept by a continuous nitrogen flow at constant flow rate (70 L/h).

Solvent Residue Analysis

The microsphere suspension, collected at the end of each run, was analyzed to determine the efficiency of the solvent removal from the emulsion. The EA residue was measured using a head space sampler coupled to a gas chromatograph interfaced with a flame ionization detector (GC-FID). EA was separated using a fused-silica capillary column 30 m length, 0.25 mm internal diameter, 0.25 μm film thickness. GC conditions were: oven temperature at 40°C for 8 min. The injector was maintained at 180°C (split mode, ratio 1:1) and helium was used as the carrier gas (7 ml/min). Head space conditions were: equilibration time 60 min at 100 °C, pressurization time 2 min, loop fill time 1 min. Head space samples were prepared in 10 mL vials filled with 4 mL of microsphere suspension.

Size and Morphology Characterization

Droplet size distributions (DSD) and particle size distributions (PSD) were measured by dynamic light scattering (mod. Mastersizer S, Malvern Instruments Ltd., Worcherstershire, UK). Analyses were performed just after the preparation of emulsions and of microsphere suspensions using several milligrams of each sample (corresponding to more than one million of droplets or particles) and repeated ten times. Microspheres morphology characterization was performed using a Scanning Electron Microscope (SEM, mod. 420, LEO); powders were dispersed on a carbon tab previously stuck to an aluminium stub (Agar Scientific) and coated with gold-palladium (layer thickness 250Å) using a sputter coater (mod. 108A, Agar Scientific).

Drug loading

Drug loading was determined by dissolving a known mass (2.5 mg) of microspheres in 1 mL of 0.25 M sodium hydroxide. Samples were rotated for at least 24 h at 10 rpm to ensure the complete

dissolution of the polymer. Blank (PX free) microspheres of the same size were treated identically. The concentration of PX and VM in the resulting solutions was measured by an UVvis spectrophotometer (mod. Cary 50, Varian, California, USA) measuring the absorbance at 276 nm and 254 nm, respectively, in a quartz cuvette and then subtracting absorbance values obtained for the blank microspheres.

Results and Discussion

Operating pressure and temperature conditions were selected to facilitate the extraction of the oily dispersed phase of the emulsion, but to avoid also drug or polymer losses due to their dissolution in SC-CO_2 and to avoid the emulsion loss caused by its washing out by the SC-CO_2 stream. Using O/W emulsion (ratio 20:80) the optimized operating conditions in our apparatus were found to be of 80 bar and 38°C, with a flow rate of 0.5 kg/h for 40 min when charging 50 g of emulsion **[4]**.
A systematic comparison between the characteristics of the microspheres obtained by SC-CO_2 extraction at the previously defined process conditions and by conventional solvent evaporation technique was performed, starting from the same O/W emulsion. Particularly, the emulsions were prepared with a different percentage of PLGA from 2.5 to 5% and 7.5% in the oily phase, whereas, the PX was maintained always as the 10% w/w of the charged polymer. Microspheres obtained with SC-CO_2 technology showed always a PSD narrower that the microsphere ones obtained by the conventional evaporation process. The observed behavior is illustrated in **Figures 1, 2 and 3,** where the distributions curves of the PLGA/PX microspheres obtained by SC-CO_2 extraction (dashed line) and by solvent evaporation (continuous line) are plotted together. The mean size (MS), the standard deviation (SD) and the variation coefficient (VC) are also reported. The microspheres distribution curves (SC and SE, compared on each figure) were generated from emulsions prepared at different PLGA concentration in the oily phase of 2.5, 5 and 7.5% w/w, respectively. The distribution data of the related emulsion droplets are reported in **Table 1**, for comparison.

Looking at the **Table 1** and at the **Figures 1-3** is evident that the microsphere mean size is directly related to the droplet mean size present in the starting emulsion. Particularly, it was observed that, fixing all other parameters during the emulsion preparation, when the oily phase viscosity was increased (due to the higher polymer concentration in the solvent), larger droplets were obtained that resulted in the formation of larger microspheres. However, using the supercritical extraction of the emulsion a more accurate microsphere size tailoring can be obtained, because the microsphere produced with this technology exhibited PSDs always narrower with respect to the droplet size distribution of the emulsion from which they were generated. Particularly, the MSs and the SDs of the microspheres produced by SC-CO_2 were always smaller than the ones of the emulsion from which they are generated; the CV was the same of the corresponding emulsion. On the contrary, the PSD curves of the microspheres produced by solvent evaporation showed always particles larger than the starting droplet; i.e., higher MS and SD values than the ones of the emulsion from which they are generated. For example, in the case of microspheres obtained with a PLGA percentage of 5% in the oily phase of the emulsion (see also **Figure 2**), the microspheres obtained by solvent evaporation included a 10% of particles from 3 to 6 microns that are not present in the SC-CO_2 sample. The observed behavior can be justified considering the faster precipitation route obtained in SC-CO_2 processing (only 30 min) that can prevent droplet coalescence or aggregation phenomena that typically occurring during the solvent evaporation process, that takes almost 8 hours to be completed.

Table 1. Distribution data of the droplets in emulsion.

	PLGA concentration in EA droplets of O/W emulsions		
	2.5 % w/w	**5 % w/w**	**7.5 % w/w**
MS (μm)	0.99	2.06	3.54
SD (μm)	0.42	0.89	1.77
VC (%)	42	43	50

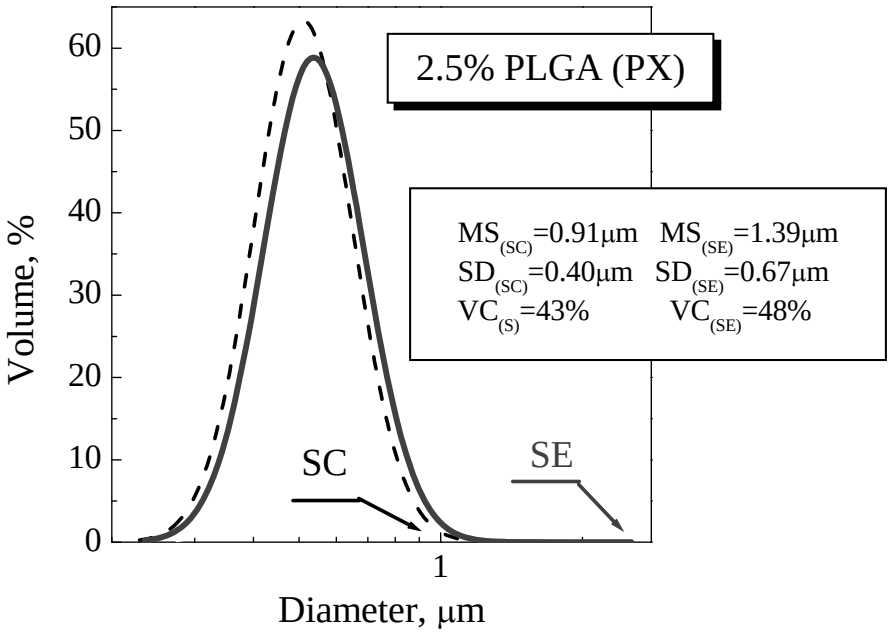

Figure 1. Distribution curves of PLGA/PX microspheres obtained by SC-CO_2 extraction (dashed line) and by solvent evaporation (continuous line).

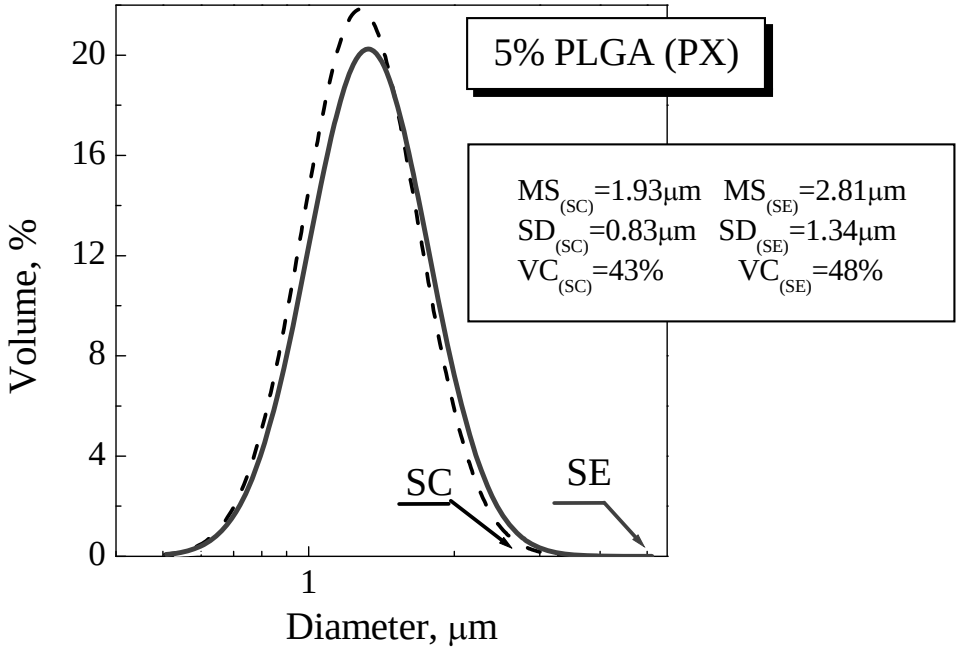

Figure 2. Distribution curves of PLGA/PX microspheres obtained by SC-CO_2 extraction (dashed line) and by solvent evaporation (continuous line).

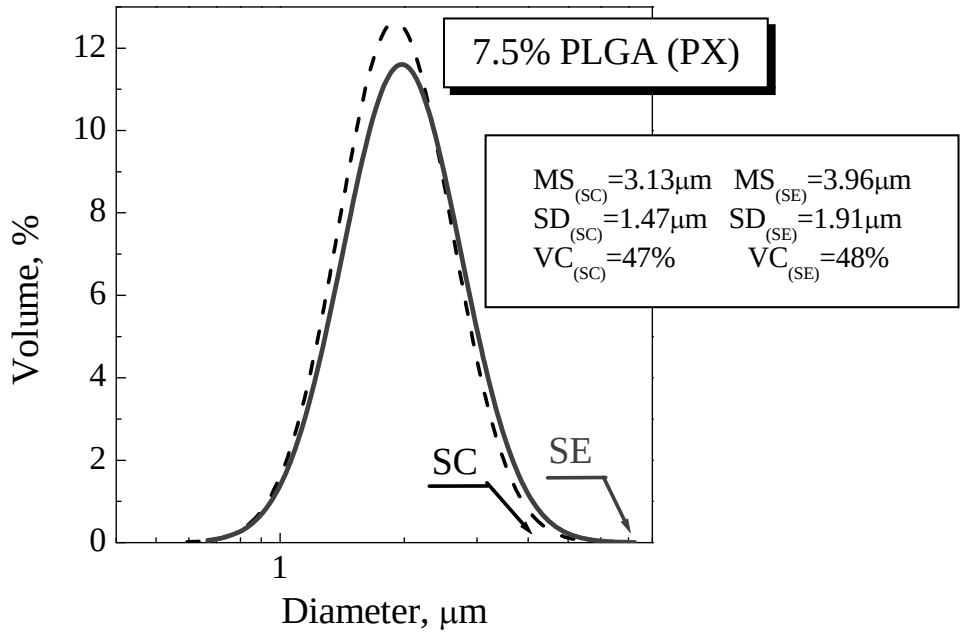

Figure 3. Distribution curves of PLGA/PX microspheres obtained by SC-CO_2 extraction (dashed line) and by solvent evaporation (continuous line).

The same behavior was also observed, starting from the double W/O/W emulsion. For example, in **Figure 4** are reported the distribution curves of the PLGA/VM microspheres obtained by SC-CO_2 extraction (dashed line) and by solvent evaporation (continuous line). The distribution data of the emulsion from which they were generated are: MS_E = 1.75 μm, the SD_E = 0.78 μm and VC_E = 42%. In this case the emulsion contained the 5% of PLGA w/w in the oily phase, whereas, the VM was the 10% of polymer. An example of the particles morphology obtained in this case is illustrated in the SEM image reported in **Figure 5.** Also in this case, the PSD of the microspheres produced by SC-CO_2 extraction showed a smaller MS and almost the same VC with respect to the original droplets in emulsion; whereas, the microspheres produced by solvent evaporation showed again higher MS, SD and VC respect to the same droplets in emulsion.

EA residue in the microspheres produced by SC-CO_2 extraction is lower than 10 ppm. The very low solvent residue obtained is another important advantage of the proposed process with respect to the conventional SE that showed an EA residual content of 500 ppm. The higher solvent residue obtained can be explained considering the Vapor/Liquid behavior of the system water/EA at the operating temperature and pressure. Indeed, in the hypothesis that during the evaporating process, as long as, EA is evaporated from the aqueous phase of the emulsion, a shift in the emulsion equilibrium is generated (leading to the diffusion of the organic solvent from the emulsion droplets to the continuous phase), the maximum amount of EA that can be evaporated from water is related to the miscibility hole of the binary system at given operating conditions. Therefore, a very low solvent residue cannot be obtained for thermodynamic limitations.

The encapsulation efficiency of the microspheres produced by SC-CO_2 extraction was in the range of 80 and 95%. Smaller encapsulation efficiency was measured in the microspheres produced by SE. The observed result was again attributed to the very fast SC process, if compared to the conventional SE; higher encapsulation efficiency can be expected, since the drug has less time to migrate in the continuous phase.

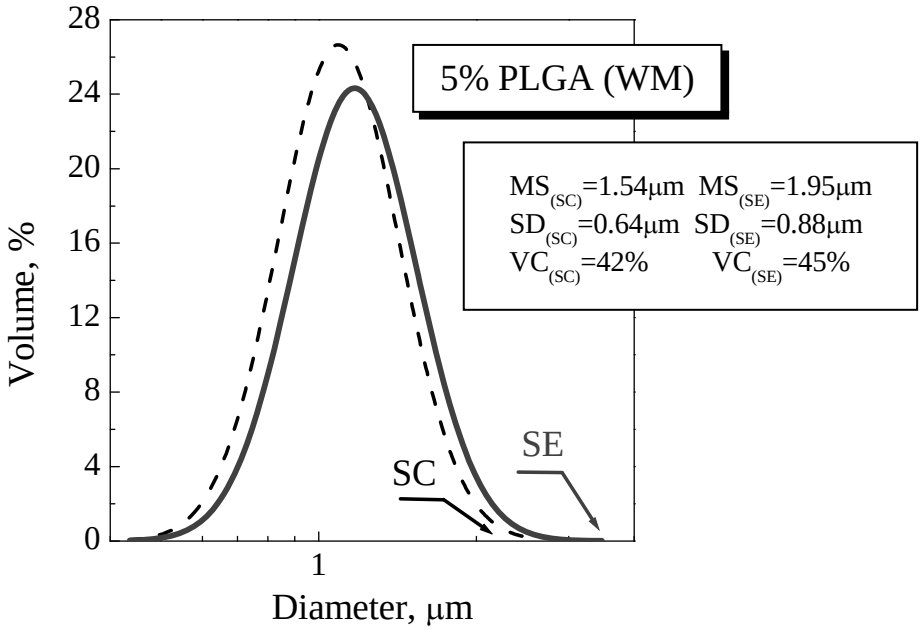

Figure 4. Distribution curves of PLGA/VM microspheres obtained by SC-CO_2 extraction (dashed line) and by solvent evaporation (continuous line).

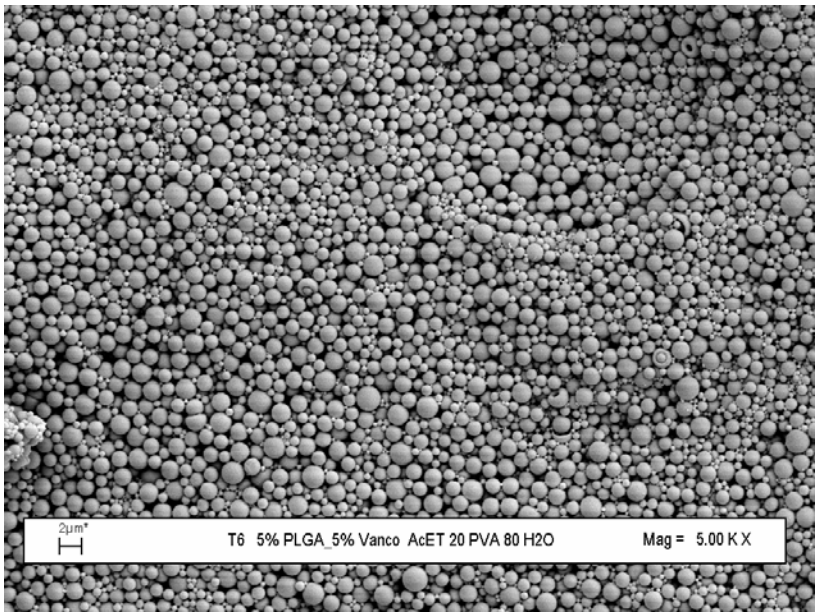

Figure 5. SEM image of the PLGA/VM microspheres obtained by SC extraction starting from W/O/W emulsion.

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