

# Cellulose Acetate Nanocarrier Production by Supercritical Assisted Electrospray

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Supercritical assisted electrospray is an emerging process used for the production of polymeric micro- and nanoparticles of controlled size and distribution. The main innovation consists of the addition of supercritical CO<sub>2</sub> to the polymeric solution, with the aim of obtaining an expanded liquid, characterized by reduced viscosity and surface tension. Cellulose acetate (CA) was selected as the model polymer, thanks to its peculiarities, such as low cost, biodegradability and biocompatibility. The experiments were performed at different pressures (from 80 bar to 140 bar) and polymer concentrations (from 0.2% to 1% w/w). CA nanoparticles were successfully produced; working at 140 bar and 30 kV, particles characterized by a mean diameter in the range from 266.7±145 nm to 343.9±120 nm were obtained, processing 0.2% w/w and 1% w/w CA solutions, respectively. These results open the way to the production of biopolymeric sub-microcarriers that, loaded with active molecules, can be used in nutraceuticals.

## 1. Introduction

Nutraceuticals are substances, food or a part of the food, that can provide medical or health benefits, including the prevention and treatment of diseases (De Felice, 1995; Kitryte et al., 2020). Among nutraceuticals, various bioactive compounds are included, such as: carotenoids, vitamins, dietary fiber, and phenols (Nile and Park, 2014; Uwineza and Waśkiewicz, 2020). However, their clinical efficacy is limited due to the poor aqueous solubility that leads to a poor oral bioavailability (McClements and Xiao, 2014). Therefore, nanocarriers should be developed for nutraceuticals delivery, since they can enhance the drug bioavailability (Cardea et al., 2018). Several conventional micronization processes have been proposed to produce micro- and nanocarriers (Chou et al., 2021); i.e.: spray-drying, emulsification/solvent evaporation, liquid antisolvent precipitation, freeze drying and jet-milling. These processes are characterized by several limitations: mainly, the production of particles with broad size distributions (PSDs), possible degradation of the product due to mechanical or thermal stresses and large use of organic solvents or other toxic substances (Baldino et al., 2016; Reverchon, 1999). In the case of emulsification techniques, nanoparticles are obtained in a liquid state and drying steps are required to obtain nanoencapsulates in a powder form (Ezhilarasi et al., 2013).

Electrospray can represent a simple way to produce micro- and nanoencapsulates in a one-step processing, that can be used in numerous applications from food, pharmaceutical and up to the biomaterials field (Morais et al., 2020). The major advantage of electrospray is the high encapsulation efficiency that can provide a sustained release of the encapsulated material, a great thermal stability and an enhanced protection of the bioactive compounds from chemical degradation (Tapia-Hernández et al., 2015). However, in order to obtain the desired properties of the final products, it is necessary to optimize different experimental parameters, such as polymer/solvent composition, voltage, flow rate, type of collectors, and distance between the collector and needle tip (Morais et al., 2020).

Cellulose acetate (CA) is a polymer derived from cellulose and, due to its low cost, biodegradability and biocompatibility, it represents a promising choice for nanocarriers production and active compounds entrapment (Baldino et al., 2017; Baldino et al., 2019a; Oprea et al., 2020). For example, Kumar et al. (2014) produced glimepiride-loaded CA microparticles by emulsion solvent evaporation method, for pharmaceutical applications. The mean particle size of these microparticles ranged from 145.26 µm to 187.42 µm.

However, the process was complex and required a drying step of 12 h. De Marco et al. (2013) produced CA micrometric particles through a Supercritical AntiSolvent (SAS) process; but they were characterized by a strong coalescence. This result was explained considering that a small quantity of dimethylsulfoxide (DMSO) contained in the starting mixture was retained by microparticles after the process, leading to solid bridges formation. Lee et al. (2018) designed pH-sensitive and muco-adhesive polymeric particles that were prepared by a double-emulsion technique, using CA phthalate, in order to slow down antigen release in the gastrointestinal tract. The process consisted of many steps: the double-emulsion solution was evaporated, then, microparticles were collected by centrifugation, washed with distilled water and lyophilized under vacuum. Microparticles showed an average size ranging from 1.18  $\mu\text{m}$  to 1.53  $\mu\text{m}$  and were characterized by an irregular shape and a reduced control of the final morphology.

To the best of our knowledge, only one attempt has been performed for CA microparticles production, using electrospray (Severgnini et al., 2020). In that study, urea loaded-CA microparticles were produced using a solution flow rate of 0.5 mL/h and a solvent mixture of acetone and DMSO that was able to provide a stability of the jet cone. However, corrugated particles with a mean diameter of  $2.2\pm 0.3 \mu\text{m}$  were produced and the addition of urea did not affect the morphology of CA particles. During the drug release test, a significant burst effect was detected for free urea; whereas entrapped urea was almost completely released in 7 h. Moreover, the configuration of this traditional electrospray showed various limits, such as: low solution flow rates, reduced control of the final morphology, and use of toxic organic solvents.

These drawbacks can be overcome using the supercritical  $\text{CO}_2$ -assisted electrospray, in which  $\text{CO}_2$  is added to the polymeric solution before the spraying process, to form a gas expanded liquid (GXL). GXL is characterized by reduced values of cohesive forces (surface tension and viscosity) (Baldino et al., 2015). Operating in this way, solution flow rates up to hundred times higher than the traditional electrospray can be tested and, varying the main process parameters (applied voltage, pressure, polymer concentration), a good control of particle size and PSDs can be assured (Baldino et al., 2019a; Baldino et al., 2019b). This new process was applied to particle generation of an high molecular weight polyvinylpyrrolidone (PVP) (Baldino et al., 2019a). Microparticles, characterized by diameters ranging between 0.55 and 2.25  $\mu\text{m}$ , were produced, increasing the PVP concentration from 1% to 5% w/w and varying the operative pressure between 80 and 120 bar. Continuing to increase PVP concentration, the solution viscosity increased; therefore, first large and very large particles were formed; then, the process was no more able to produce jet break-up, and microfibers were obtained (Baldino et al., 2019b).

In this study, for the first time, supercritical  $\text{CO}_2$ -assisted electrospray was used for the production of cellulose acetate nanoparticles. The effect of pressure and CA concentration on particles morphology was investigated; whereas the applied voltage was fixed at 30 kV. The aim was the optimization of these biocompatible nanocarriers for the further encapsulation of nutraceuticals.

## 2. Materials and Methods

### 2.1 Materials

Cellulose acetate (average Mn ca. 50 000, with acetyl content of 39.7%) and acetone (purity >99.5%) were bought by Sigma-Aldrich (Milan, Italy).  $\text{CO}_2$  (99.9% purity) was purchased from Morlando Group s.r.l. (Sant'Antimo (NA), Italy).

### 2.2 Apparatus

Cellulose acetate powder was dissolved in acetone at different concentrations by weight (0.2%, 0.5% and 1%), at room temperature and using a magnetic stirrer at 100 rpm. 50 mL of solution were loaded into a stainless-steel high pressure vessel with an internal volume of about 70 mL. This vessel was closed and  $\text{CO}_2$  was pumped from the bottom, up to the desired pressure, using a high-pressure pump (Gilson, mod. 305, Middleton, WI, USA). Three different pressure values were investigated during the experimentation; i.e., 80 bar, 120 bar and 140 bar. Pressure in the vessel was measured using a test gauge (mod. MP1, OMET, Lecco, Italy). Supercritical  $\text{CO}_2$  was added in order to form a GXL. The system was left undisturbed for 10 minutes for equilibration. Then, nitrogen was introduced from the top of the vessel at the same pressure selected for the process, with the aim of maintaining the pressure constant during the discharge of the vessel. The solid-liquid-gas solution at the exit of the vessel was sent to a thin wall injector (100  $\mu\text{m}$  internal diameter), opening an ON/OFF valve (Swagelok ON/OFF, Nordival s.r.l., Rovato (BS), Italy). Temperature was set at 35  $^\circ\text{C}$ ; it was measured by a type J thermocouple and adjusted using a PID controller (mod. 305, Watlow, Corsico (MI), Italy). The applied voltage (30 kV) was ensured using a FUG Elektronik generator (mod. HCP 35-3500, Schechen, Germany).

At the exit of the injector, IR-light was irradiated by two infrared-lamps (Efbe-Schott, 150 W, Germany), with the scope of enhancing the evaporation of the liquid solvent. The collector was located at a 20 cm distance and consisted of two adjacent stainless-steel blocks, covered by an aluminum foil (Baldino et al., 2019b). A schematic representation of the plant layout is reported in Figure 1.

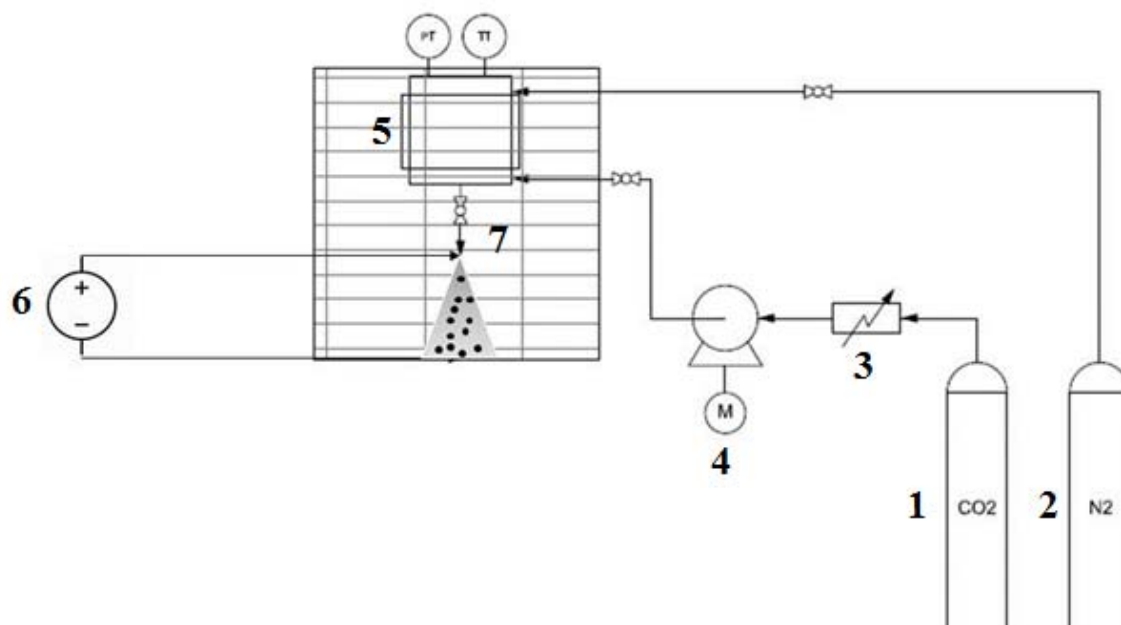


Figure 1: Schematic representation of the electro spray plant: 1. CO<sub>2</sub> storage tank; 2. N<sub>2</sub> storage tank; 3. Cooler; 4. high-pressure pump; 5. high-pressure vessel; 6. generator; 7. injector

### 2.3 Characterizations

Cellulose acetate particles were coated with gold using a sputter coater (Agar Auto Sputter Coater mod. 108 A, Stansted, UK) at 40 mA for 120 s; then, particles morphology was observed by a field emission scanning electron microscope, FESEM (mod. LEO 1525, Carl Zeiss SMT AG, Oberkochen, Germany).

Mean diameter, standard deviation and particle size distributions (PSDs) were measured by an image analysis software (Sigma Scan Pro 5.0, Aspire Software International Ashburn, VA) using FESEM images (Reverchon et al., 2002). The elaboration of PSDs was carried out using Microcal Origin Software (release 8.0, Microcal Software, Inc., Northampton, MA).

### 3. Results and discussion

The key point of the supercritical CO<sub>2</sub> (SC-CO<sub>2</sub>) assisted electro spray is the solubilization of SC-CO<sub>2</sub> in a polymeric solution, before the spraying process, to produce a GXL, characterized by a reduced viscosity and surface tension. This solution is, then, atomized by the force related to reciprocal repulsion between electric charges that opposes the solution surface tension and viscosity (Baldino et al., 2019a; Baldino et al., 2019b).

In this study, the experiments were performed setting the applied voltage at 30 kV; the operative pressure was varied between 80 bar and 140 bar, and CA concentration from 0.2% to 1% w/w.

Operating at 0.2% w/w CA, the solution produced nanoparticles at all operative pressures. PSDs of these samples, obtained at different working pressure, are shown in Figure 2. Mean particles size ranged from 525.1±272 nm to 266.7±145 nm, working at 80 bar and 140 bar, respectively. The increase of pressure contributed to the production of particles characterized by smaller diameters and sharper PSDs, since a larger disruptive force was exerted on the liquid jet coming out from the injector.

The same effect of pressure was observed operating at larger values of CA concentration, as reported in Figure 3. In Figure 3a, PSDs of nanoparticles produced using a 0.5% w/w CA solution are shown; particle mean diameters decreased from 586.8±167 nm to 280±43 nm, increasing pressure from 80 bar to 140 bar. In Figure 3b, PSDs, related to particles produced using a 1% w/w CA solution, are reported; the increase in pressure led to a reduction of particle mean diameters that varied from 589.4±226 nm to 343.9±120 nm.

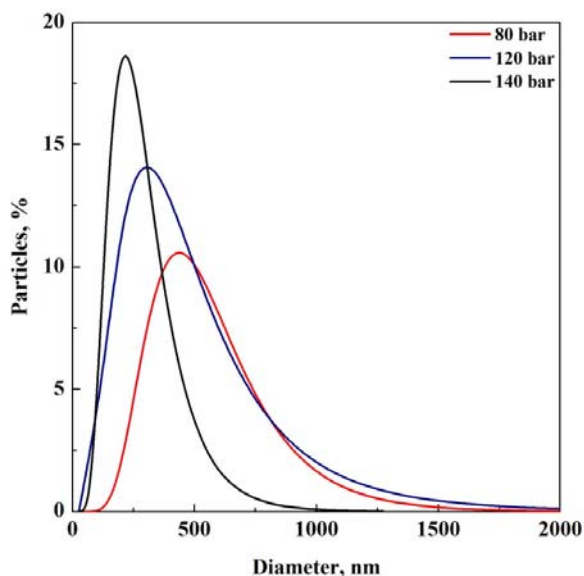


Figure 2: PSDs of samples produced by supercritical electro spray at  $P=80$  bar,  $P=120$  bar and  $P=160$  bar;  $E=30$  kV and  $CA=0.2\%$  w/w

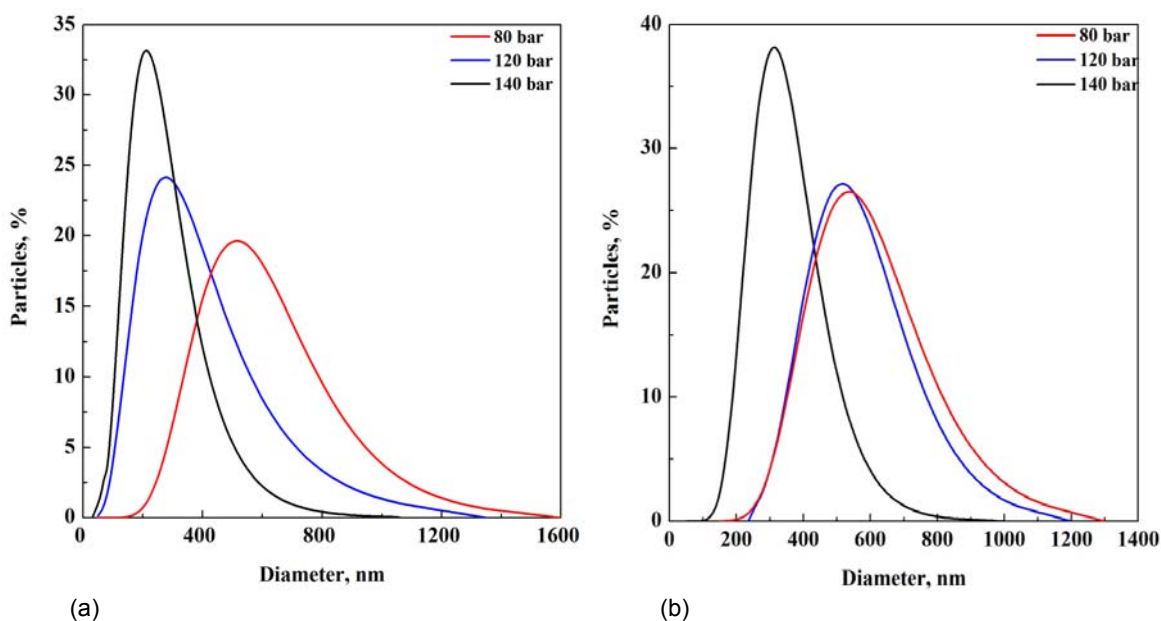


Figure 3: PSDs of samples produced by supercritical electro spray at  $P=80$  bar,  $P=120$  bar and  $P=140$  bar;  $E=30$  kV and  $CA=0.5\%$  w/w in (a), and PSDs of samples produced by supercritical electro spray at  $P=80$  bar,  $P=120$  bar and  $P=140$  bar;  $E=30$  kV and  $CA=1\%$  w/w in (b)

The increase in CA concentration from 0.2% to 1% w/w range did not affect in a significant way the viscosity of the solution: consequently, only a slight increase in the particles mean diameter was measured working at a 1% w/w CA concentration. However, the increase in CA concentration contributed to improve the final particle sphericity. In particular, two other mechanisms, apart from solvent evaporation and polymer diffusion, can affect particle shape: chain entanglement and Coulomb fission. Chain entanglements are the physical interlocking among the polymer chains; whereas, Coulomb fission refers to the explosion of droplets that occurs when charged droplets reach the Reyleigh limit. Therefore, if during evaporation entanglements are present before the Coulomb fission occurs, particles will remain spherical and cannot be deformed by electrical forces; on the contrary, if Coulomb fission and evaporation occur simultaneously, the fission may induce deformation of the particle into smaller particles characterized by irregular shapes (Almería et al.,

2010). Therefore, spherical particles are generated when a sufficiently entangled network is formed prior to the onset of the Reyleigh limit. In this case, larger CA concentrations in the starting solution could favor this phenomenon, as it can be observed comparing Figure 4a, Figure 4b and Figure 4c.

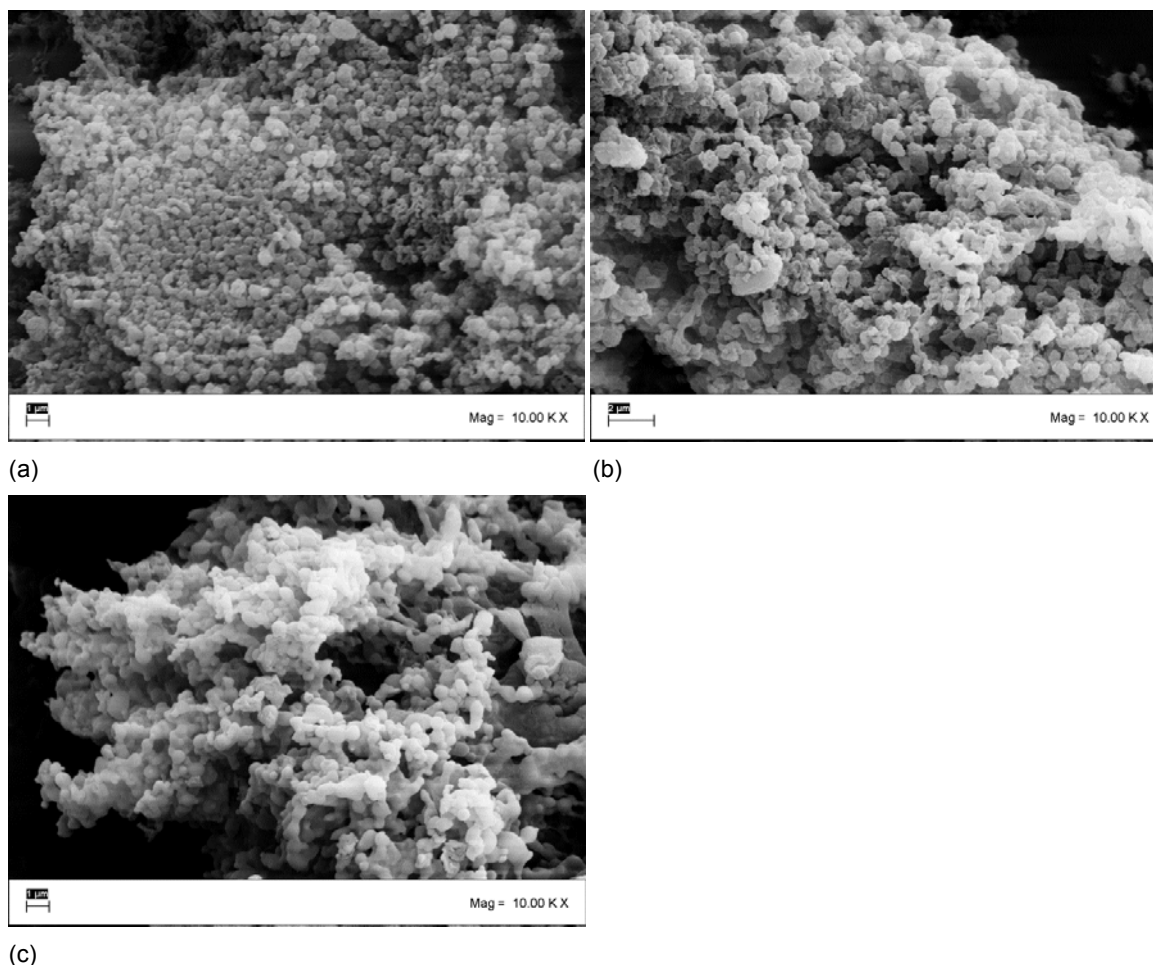


Figure 4: SEM images of particles obtained at 140 bar from: (a) 0.2% w/w CA solution; (b) 0.5% w/w CA solution; (c) 1% w/w CA solution

#### 4. Conclusions

Biocompatible nanoparticles of cellulose acetate were successfully produced by supercritical CO<sub>2</sub> assisted electro spray. The increase in pressure led to a reduction of particle mean diameter accompanied by a sharpening of PSDs. The increase in CA concentration promoted the production of particles characterized by a more regular shape.

In the future, rutin-loaded particles, for nutraceutical applications, will be produced to verify the enhancement of the bioavailability of poorly-water soluble active compounds.

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