

Supercritical-CO₂ Assisted Electro spray to Produce Cellulose Acetate+Rutin Micro-Carriers

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Supercritical-CO₂ assisted electro spray is a new process used to produce polymeric micro- and nanoparticles characterized by tunable and regular morphologies. The major innovation consists of the addition of supercritical CO₂ to the polymeric solution, obtaining a gas expanded liquid having reduced values of viscosity and surface tension.

Due to its biodegradability and biocompatibility, cellulose acetate (CA) was selected as polymeric carrier for microparticles production, that can be used for drug delivery applications. Indeed, CA solutions were loaded with a poorly-water soluble compound, rutin (RUT), to improve its bioavailability. The experiments were performed at different CA concentrations (0.5 and 1 wt%) and different RUT concentration, that was varied from 2.5 to 7.5 wt% with respect to CA; the applied voltage was set at 30 kV. CA/RUT microparticles were successfully produced; working at 140 bar and 30 kV, particles characterized by an average diameter of 980±120 nm and networked fibers were obtained, processing 1 wt% CA solution and using a RUT concentration of 7.5 wt% with respect to CA. IR spectroscopy revealed the physical dispersion of RUT into CA particles.

1. Introduction

Micro- and nanoparticles allow sustainable application of solids on large research areas, such as: chemically inert additives (polymer fillers, pigments, dye and UV protection), chemically active particles (catalysts, biomaterials, antimicrobial additives and nutraceuticals), regulatory compliance, inorganic solar cell constituents, molecular diagnostic devices and multiphase systems (batteries, fuel cells and flow through reactors) (Stark et al., 2015).

Cellulose acetate (CA) is the acetate ester of cellulose, a natural polysaccharide derived from cellular walls of plants, and it is often used as polymeric matrix for chemically active particles production. Some advantages of using this polymer to encapsulate active compounds consist of its non-toxicity and biodegradable features. Therefore, cellulose acetate-based materials can be used in the health sector, such as nutraceutical, pharmaceutical and biomedical applications, to protect the bioactivity and to increase the bioavailability of poorly-water drugs. In particular, it is used for drug delivery applications within the gastro-intestinal tract, due to its resistance to pH of gastric media (Mazumder et al., 2017).

Loaded CA-based particles have been produced using different techniques, such as: oil-water (o/w) solvent evaporation, emulsification-solvent evaporation, and spray-drying, with the aim to encapsulate drugs for oral delivery. Chaturvedi et al. (2011) prepared microspheres of poly(3-hydroxybutyrate) and CA for 5-fluorouracil delivery, a chemotherapeutic drug. The microparticles, whose diameters ranged from 29 to 67 µm, were produced by solvent evaporation of a water-in oil emulsion. Drug encapsulation efficiency was low (ranging from 42 to 57%); a toxic solvent, dichloromethane, was used as oil phase, and consequently, long time processes were required to obtain completely dried particles. Remuñán-López et al. (1998) produced chitosan base particles to be used as controlled release systems. The particles consisted of chitosan microcores entrapped in hydrophobic CA butyrate, with the aim of delaying chitosan dissolution kinetic and, consequently, ensuring a sustained drug release. The composite particles were prepared by water-in oil-in water (w/o/w) solvent evaporation technique combined with freeze-drying. The size of microparticles varied from around 50 to 70 µm. Chandiran Irisappan et al. (2013) formulated glibenclamide loaded CA microparticles produced by

emulsification-solvent evaporation technique. The microparticles resulting from emulsion preparation were separated by filtration, washed with petroleum ether and, then, air dried for 12 hours. The average size of particles ranged from 132 to 178 μm and encapsulation efficiency varied from 90 to 98%. Lauro et al. (2005) obtained rutin and quercetin gastro-resistant microparticles by spray-drying using CA trimellitate or CA phthalate as coating polymers. After spraying, microparticles were stored under vacuum for 48 hours, at room temperature. Scanning Electron Microscope (SEM) images showed that particles were not spherical, aggregated and showed the presence of needle shaped drug crystals outside microsphere surfaces.

To overcome the previous limitations mainly related to the use of toxic organic solvents, low drug encapsulation efficiency, difficulty to obtain nanoparticles characterized by regular spherical shape and sharp particle size distribution (PSD), supercritical- CO_2 (SC- CO_2) has been used due to its low toxicity, low cost, its capability to produce high product quality in terms of purity and uniform particle size. In particular, De Marco et al. (2013) verified the possibility to use solvent mixtures of dimethyl-sulfoxide (DMSO) and acetone to produce microparticles, with tunable morphology, of CA, using Supercritical Antisolvent (SAS) process. The authors found out the possibility to produce non-coalescing CA microparticles characterized by an average diameter of $0.42 \mu\text{m} \pm 0.15 \mu\text{m}$, using a 50/50 (v/v) mixture of DMSO/Acetone. García-Casas et al. (2017) produced quercetin (QT)/cellulose acetate phthalate (CAP) particles by SAS process. Nanoparticles of CAP around 110-145 nm were achieved varying the pressure from 80 to 90 bar. When coprecipitation was carried out, the average size of QT/CAP nanoparticles was almost the same with respect to only CAP and also X-Ray Diffraction (XRD) analysis confirmed that QT was not entrapped into the polymeric structure.

The electrospray-based technique is largely used to produce polymeric particles. This process is characterized by the application of an electric potential difference between injector and collector, to obtain jet-break up, that lead to particles formation. Indeed, compared with other microencapsulation/nanoencapsulation processes, electrospray has several advantages, such as: high encapsulation efficiency and effective protection of drugs bioactivity. Moreover, it is simple to use, low cost, it uses a low amount of solvent and electrosprayed particles are obtained in one-step. However, this process requires very small solution flow rates (in the order of $\mu\text{L/h}$), and toxic solvent residues can contaminate the electrosprayed particles (Severgnini et al., 2020).

To overcome these limitations, Baldino et al. (2019) realized a new process arrangement, in which CO_2 was added to the solution before spraying process, with the aim of reducing solution viscosity and surface tension, forming a gas expanded liquid (GXL). The authors were able to obtain polyvinyl-pyrrolidone (PVP) sub-microparticles at high production rate, up to 1000 times higher than traditional one, and with a good control over average particle diameters. Using the same process, Guastafarro et al. (2021) performed the Supercritical-Assisted Electrospray (SA-Electrospray) of CA, at three different pressure (80, 120 and 140 bar) and polymer concentrations (0.2, 0.5 and 1 wt%). CA nanoparticles, having an average diameter of $343.9 \pm 120 \text{ nm}$, were obtained, working at 140 bar, 30 kV and using 1% w/w CA solutions.

The goal of this work is the production of CA sub-microparticles loaded with rutin (RUT), using SA-Electrospray process, with the aim of improving its oral bioavailability for nutraceutical applications and understanding how the addition of a guest molecule in CA/acetone system can modify the process behavior and the operability range for electrospray. RUT belongs to flavonoid class, with significant antioxidant, chelating and antimicrobial properties. In this work, the experiments will be performed keeping fixed the applied voltage at 30 kV and varying the pressure from 80 to 140 bar, the CA concentrations (0.5 and 1 wt%) and the RUT content in the starting solutions. The effects related to the change of these parameters on the final particles morphology will be investigated by SEM. Fourier-transform Infrared (FT-IR) analysis will be used to ascertain that no chemical interactions are present between RUT and CA.

2. Materials and methods

2.1 Materials

Cellulose acetate (CA, average Mn ca. 50 000 with acetyl content of 39.7%), rutin hydrate (RUT, purity 95%) and acetone (purity >99.5%) were bought by Sigma-Aldrich. CO_2 (99.9% purity) was purchased from Morlando Group s.r.l. (Sant'Antimo (NA), Italy).

2.2 Apparatus

CA powder was dissolved in acetone using two concentrations by weight (0.5 and 1%), at room temperature and using a magnetic stirrer, at 100 rpm; RUT concentration was varied from 2.5 wt% to 7.5 wt% with respect to CA. 50 mL of solution were loaded into a stainless-steel high-pressure vessel that had a volume of about 70 mL. The CO_2 was pumped through a pump (Gilson, mod. 305, Middleton, WI, USA) from the bottom of the vessel, up to the desired pressure value. 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). SC-CO₂ was added in to form a GXL and after 9 minutes of equilibration, nitrogen was introduced to ensure the same value of pressure drop during the solution discharge. The GXL was sent to an injector (140 μm internal diameter), opening an ON/OFF valve (Swagelok ON/OFF, Nordival s.r.l., Rovato (BS), Italy). Temperature, that was set at 35 $^{\circ}\text{C}$, was measured by a thermocouple and adjusted using a PID controller (mod. 305, Watlow, Corsico (MI), Italy). To ensure the applied voltage, that was set at 30 kV, a FUG Elektronik generator (mod. HCP 35-3500, Schechen, Germany) was used. At the exit of the injector, two infrared-lamps (Efbe-Schott, 150 W, Germany) enhanced the evaporation of the liquid solvent. The collector consisted of an aluminium foil and was located at a 20 cm distance.

2.3 Characterizations

Gold was used to coat cellulose acetate particles, using a sputter coater (Agar Auto Sputter Coater mod. 108 A, Stansted, UK), at 40 mA for 180 s; then, particles morphology was imaged 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 using an image analysis software (Sigma Scan Pro 5.0, Aspire Software International Ashburn, VA) in combination with Microcal Origin Software (release 8.0, Microcal Software, Inc., Northampton, MA).

FT-IR spectra were realized using a Shimadzu FT-IR spectrophotometer (mod. IRTracer-100, MIDAC Co, Costa Mesa, CA). Pellets were prepared mixing the produced materials and KBr (1:100 by weight). Scans were performed at a resolution of 32 cm^{-1} .

3. Results and discussion

The first set of experiments was performed fixing CA and RUT concentrations at 1 wt% and 7.5 wt% with respect to CA, respectively. The pressure ranged from 80 bar up to 140 bar. Performing the experiments at 80 bar, the solution discharge led to injector blockage. Increasing the operative pressure, the jet resulted unstable, and a pulsed electro spray was obtained. According to the literature (Juraschek and Röllgen, 1998), electro spraying pulsations are caused by an imbalance between the emission rate of the liquid and its supply rate to the apex of the cone.

In this case, the produced imbalance led to a continuous change of solution viscosity coming out from the injector that caused a morphology variation of the electro sprayed products. In particular, particles and networked fibers were clearly observed in Figure 1.

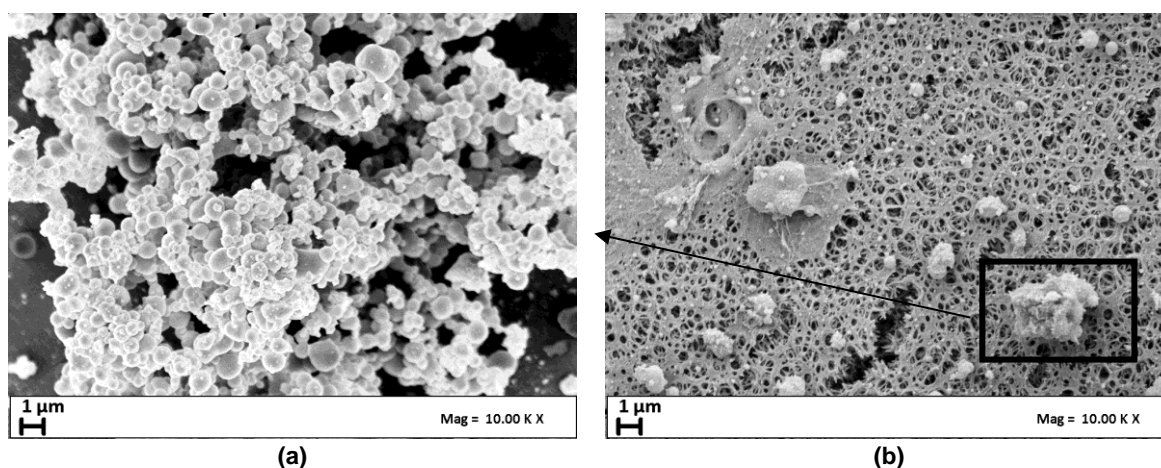


Figure 1: a-b) Particles and networked fibers produced using 1 wt% CA concentration, RUT concentration of 7.5 wt% with respect to CA, 30 kV and a pressure of 140 bar.

To obtain particles alone and to eventually avoid the transition zone (Baldino et al., 2019; Guastaferrero et al., 2020), the pressure value was fixed at its highest previously tested value (i.e., 140 bar) and the viscosity of the solution was reduced. In particular, CA concentration was reduced down to 0.5 wt% and RUT concentration was varied from 2.5 to 7.5 wt% with respect to CA. In this case, the electro spray was performed in a continuous mode and the effect of RUT content variation on the final particle morphology is reported in Figure 2. The addition of RUT led to an increase in the CA particle average diameter: the average diameter of particles produced using only 0.5 wt% CA concentration was 280 ± 43 nm (Guastaferrero et al., 2021), whereas the CA/RUT particles showed an average diameter value ranging from 709 ± 287 nm, for RUT concentration

equal to 7.5 wt% with respect to CA, up to 410 ± 231 nm, for RUT concentration equal to 2.5 wt% with respect to CA.

The increase in solution viscosity generally lead to an increased resistance of the solution to be separated into droplets and, subsequently, to a more stable operation and to an increase in the droplet diameter. Moreover, in this work the increase of solution viscosity, upon the addition of RUT, also led to the formation of particles characterized by more spherical shape (as can be seen comparing Figure 3 with Figure 4b in (Guastaferrero et al., 2021)). Indeed, particles are not spherical when the viscosity solution is not high enough to provide a sufficient cohesive force to form perfectly spherical aggregates and when the formed droplets of the jet are very small and, subsequently, the evaporation time is short.

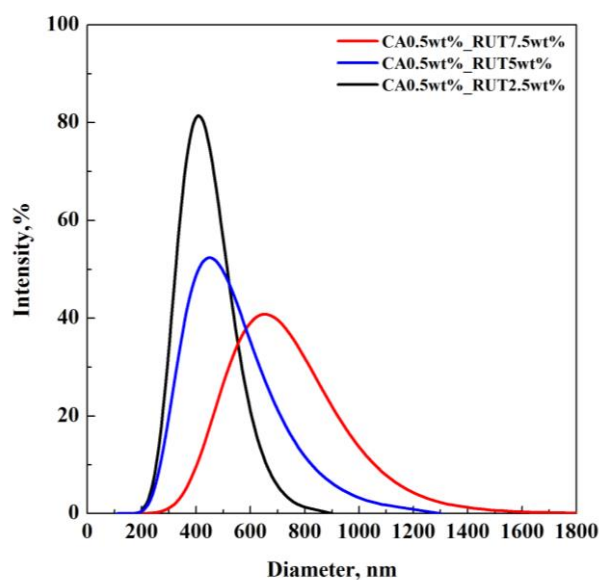


Figure 2: PSDs of CA/RUT particles produced using 0.5 wt% CA concentration, $P=140$ bar, $E=30$ kV.

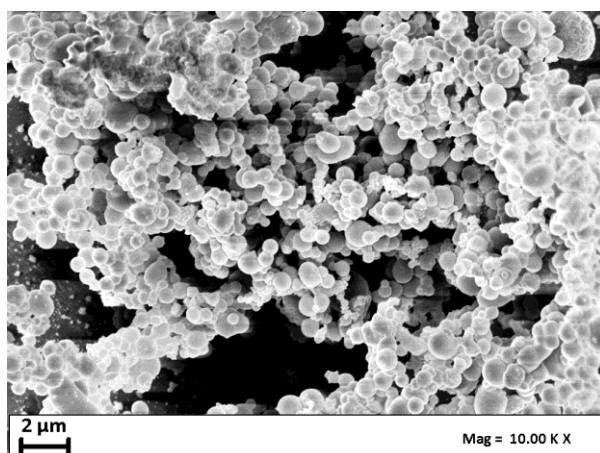


Figure 3: Particles of CA/RUT particles produced using 0.5 wt% CA concentration, RUT concentration equal to 5 wt% with respect to CA, $P=140$ bar, $E=30$ kV.

With the aim of providing the effective encapsulation of RUT in CA particles and giving information about the chemical/physical interactions between RUT and CA, the IR spectra are reported in Figure 4.

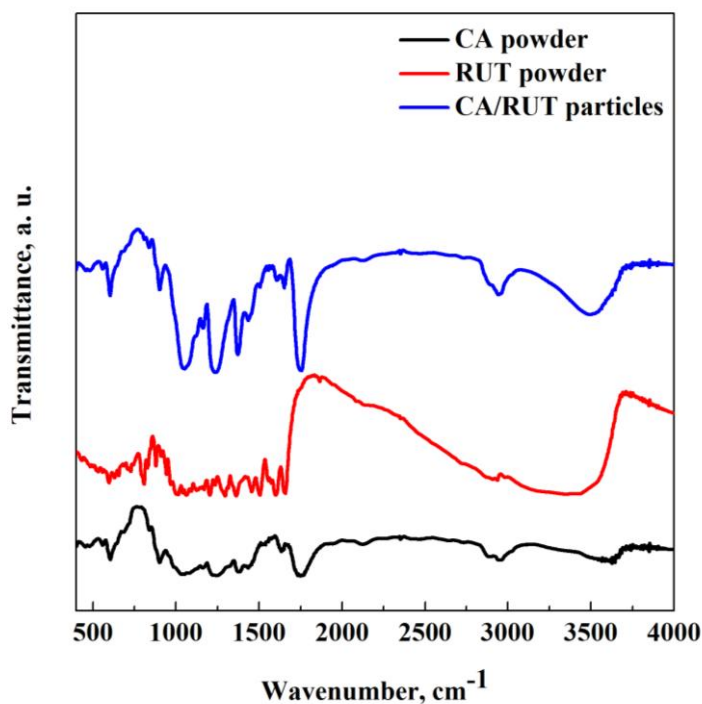


Figure 4: IR spectra of CA powder (black line); RUT powder (red line); CA/RUT particles (blue line).

The CA powder characteristic peaks presented at 607, 900 and 3626 cm^{-1} are attributed to stretching, rocking and wagging of -OH groups, respectively. The IR spectrum of CA powder also presented peaks at 1251 cm^{-1} (ether group stretching), at 1435 cm^{-1} (CH_2 vibration), at 1633 cm^{-1} ($\text{C}=\text{C}$ stretching), at 1755 cm^{-1} (ester group stretching), at 2139 and 2933 cm^{-1} that are attributed to the stretching of CH bond (Milovanovic et al., 2016). The RUT powder showed characteristic peaks at 596, 808 and 3419 cm^{-1} (stretching, rocking and wagging of -OH groups), at 2933 cm^{-1} (CH_2 stretching), in the range from 1060 to 1294 cm^{-1} (bending of CH bonds), at 1359 cm^{-1} (COH vibrations), at 1504 cm^{-1} (CO stretching groups) (Sinduja and Abraham John, 2018). The spectrum of CA/RUT particles showed the appearance of flavonoid characteristic peak related to COH vibrations (at 1369 cm^{-1}), that suggests the simultaneous presence of RUT and CA into the composite particles.

4. Conclusions

The SC- CO_2 assisted electrospray revealed successful in the production of cellulose acetate sub-microcarriers loaded with rutin. Several experiments have been performed (varying the operative pressure, CA concentrations and RUT content) with the aim of understanding the process parameters, that allowed to obtain only nanoparticles. With respect to the previous work performed with only CA, it was possible to observe a narrower range of operating conditions in which electrospray could be performed, due to RUT addition. IR spectra showed that RUT was effectively encapsulated into CA matrix.

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