PARTICLE DESIGN APPLIED TO QUERCETIN USING SUPERCRITICAL ANTISOLVENT TECHNIQUES

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ABSTRACT

Quercetin is an appreciated flavonoid in pharmaceutical applications thanks to its potent antioxidant properties. However, it presents especially the handicap of its low solubility in water which limits its absorption upon oral administration. In order to overcome this drawback, supercritical anti-solvent (SAS) process was applied to micronize quercetin particles as well as to coprecipitate quercetin with a biocompatible polymer (ethyl cellulose). Results showed that SAS micronization of quercetin using ethyl acetate as organic solvent reduces quercetin particle size but without changing their original needle-like morphology. Whereas, SAS coprecipitation of quercetin with ethyl cellulose led to spherical coprecipitate particles in the submicron range (200-400 nm). Quercetin coprecipitation exhibits quite high process yields (above 85 %) and encapsulation efficiency around 99 %.

INTRODUCTION

Quercetin, a natural flavonoid present in a wide variety of fruits and vegetables, has been focusing great attention due to its potent antioxidant properties that reduce the risk of chronic diseases. Nevertheless, its poor solubility in water limits its absorption upon oral administration [1]. In this way, micronization and encapsulation with biopolymers are part of the strategies used to remedy this drawback. The dissolution rate is more rapid when the particle size is small, corresponding to a high specific surface area, and encapsulation with polymers leads to the enhancement of either the solubility or the dissolution kinetics. The encapsulation presents also the advantage of protecting quercetin against degradation factors (light and O_2).

Supercritical processes are alternative methods to traditional techniques, limiting the use of harmful solvents, avoiding product degradation, and controlling distribution, morphology and particle size. In this work, supercritical anti-solvent (SAS) techniques were used to obtain micronized quercetin particles as well as coprecipitated quercetin with ethyl cellulose in order to enhance quercetin bioavailability. Morphology, particle size, encapsulation efficiency, and process yield were also characterized to evaluate the product obtained.

MATERIALS AND METHODS

Materials

Quercetin aglycone with a minimum purity of 98% was purchased from Sigma–Aldrich Germany, ethyl cellulose (9004-57-3) was from Sigma–Aldrich France and the organic solvent ethyl acetate with HPLC grade was provided by CARLO ERBA Reagents, Italy. Carbon dioxide (99.7%) was provided by Air Liquide France.

Supercritical Anti-solvent process (SAS)

The experimental set-up used for the SAS process is illustrated in **Figure 1**. It is mainly composed of a 1 litre high pressure precipitation vessel (Top Industrie S. A., France) equipped with a double jacket connected to a thermostated bath. This autoclave is fed with CO_2 through a regulated high pressure piston pump (LGP50, Separex, France) and with the organic solution through a HPLC pump (Gilson 307, France). The outlet of the autoclave is equipped with a back pressure regulator (Tescom, Germany) to control the pressure during the experiments.

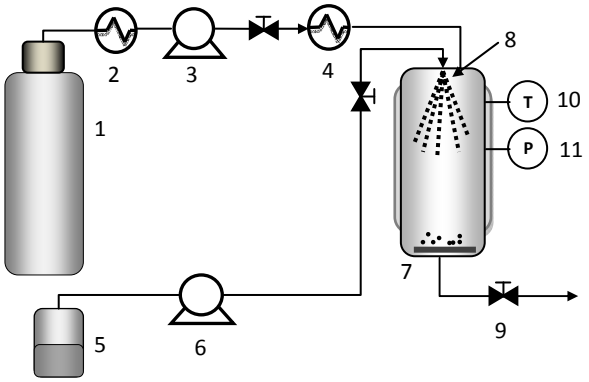

Figure 1: Scheme of the equipment for SAS experiment: 1, CO_2 cylinder; 2, cooling system; 3, piston pump; 4, heat exchanger; 5, organic solution; 6, HPLC pump; 7, vessel (precipitator); 8, nozzle; 9, back pressure regulator; 10, temperature controller; 11, pressure sensor

In a typical SAS experiment, the autoclave is first set to the desired temperature and then fed with preheated CO_2 with a regulated mass flow rate. The pressure inside the autoclave is controlled by adjusting the aperture of the back pressure regulator. When the operating parameters, i.e. pressure, temperature and CO_2 flow rate are reached, the organic solvent was dispersed through a capillary nozzle (inner diameter of 127 µm) in a co-current SC-CO_2 stream to reach the desired solvent/CO_2 molar ratio in the vessel. The organic solvent is then replaced with the organic solution. Precipitation process is then carried out for a fixed time defined by the final weight of product to collect. Finally, a washing step is realized by flowing pure CO_2 in the autoclave in order to remove the remaining solvent. Once the washing time is completed, the autoclave is slowly depressurized and micronized particles or coprecipitates are recovered.

Micronization of quercetin

Quercetin micronization from ethyl acetate solutions was carried out using the SAS process previously described. A quercetin solution in ethyl acetate with a mass fraction of 0.15 % was prepared, kept frozen and protected from light. SAS precipitation experiments were carried out at a pressure of 10 MPa, a temperature of 35 ° C with a capillary diameter of 127 µm, a solvent velocity of 4 m/s and a solvent/CO_2 molar ratio of 5 %.

Coprecipitation of quercetin with ethyl cellulose

Quercetin coprecipitation experiments were carried out with ethyl cellulose as a coating polymer through SAS process. A solution of quercetin and ethyl cellulose in ethyl acetate was prepared with a polymer mass fraction of 1 %, and a quercetin/polymer mass ratio of 10%. Coprecipitation experiments were carried out at the same experimental conditions than micronization ones.

Scanning electron microscopy (SEM)
Morphology and particle size of micronized quercetin and coprecipitates were observed by scanning electron microscopy (SEM). SEM micrographs were taken using Hitachi TM 3000 Environmental Scanning Electron Microscope.

Encapsulation efficiency (EE)
Encapsulation efficiency (EE) was evaluated by comparing the mass of quercetin present at the powder surface of coprecipitates M_s, expressed as mg quercetin/mg coprecipitate, and the total amount of quercetin embedded in the polymer M_q, expressed as mg quercetin/mg coprecipitate. Equation (1) was used for calculation of EE.

$$EE = \left(1 - \frac{M_s}{M_q}\right) x100\% \quad (1)$$

In order to evaluate M_q, the total amount of quercetin present in the coprecipitate, particles were dissolved in ethyl acetate where both polymer and quercetin are soluble. The absorbance was determined at 390 nm since ethyl cellulose does not absorb at this wavelength. While for determining M_s, the mass of quercetin present at the surface of the polymer (not embedded), coprecipitates were put in suspension in a NaOH 1M solution in which only quercetin is soluble. The absorbance of the solution was evaluated at 340 nm. M_q and M_s were calculated according to the respective quercetin calibration curves in ethyl acetate and the alkaline solution which are shown in **Figure 2.**

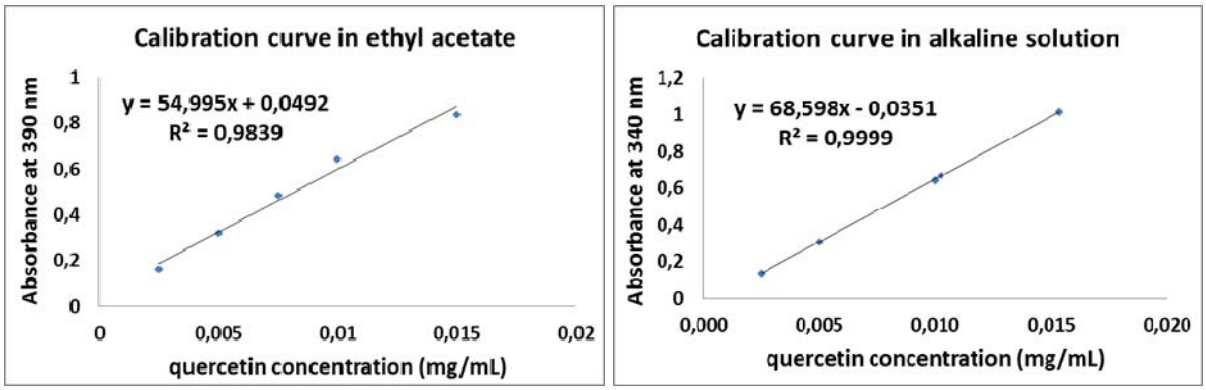

Figure 2: Calibration curve of quercetin A: in ethyl acetate at 390 nm; B: in alkaline solution at 340 nm

Experimental quercetin/polymer mass ratio
The experimental quercetin/polymer mass ratio in the formed coprecipitates was expressed as mg of quercetin/mg of polymer. The ratio was calculated as the relation between the total mass of quercetin M_q (mg quercetin/mg coprecipitate) and the total mass of polymer in coprecipitates. The Equation (2) was used for determining the quercetin/polymer mass ratio.

$$Quercetin/polymer\ mass\ ratio = \frac{Mq}{1-Mq} * 100 \quad (2)$$

Process yield

SAS process yield was defined as the ratio between the final mass of coating particles obtained (M_p) and the initial mass of raw material injected into the autoclave (M_i) as shown in Equation (3).

$$Yield = \frac{M_p}{M_i} x100\% \quad (3)$$

RESULTS

Micronization of quercetin

Needle-like quercetin particles with a mean length of 800-900 nm and wide of 70-140 nm were obtained by SAS micronization process with ethyl acetate at 10 MPa and 35 ºC. **Figure 3** shows SEM micrograph of quercetin particles obtained by SAS at the tested conditions. A reduction of the initial particle size in comparison with original quercetin particles was observed without any change in morphology and habit. Similar results have been also reported by other authors using the same organic solvent [2]. While particles with a roundness of 0.802 were obtained by SAS process using ethanol as organic solvent [3].

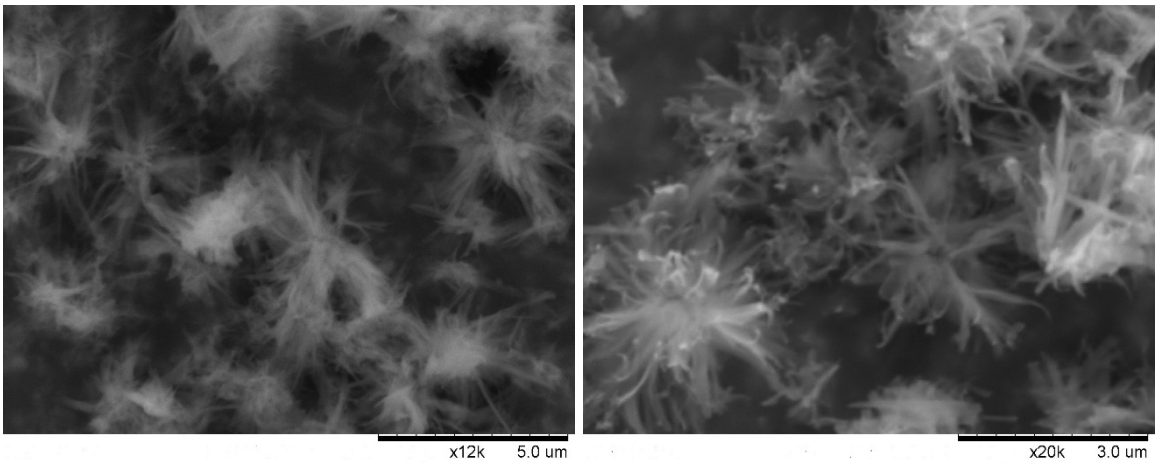

Figure 3: SEM images of micronized quercetin particles collected at 10 MPa and 35 ºC by SAS with ethyl acetate

Quercetin particle size obtained in this work was smaller than those obtained by Santos and Meireles (length 1.87 μm) using the same organic solvent at 10 MPa and 40 ºC, with a lower solvent feed velocity (0.13 m/s), a lower solvent/CO_2 molar ratio (0.9 %) and a higher capillary diameter (177.8 μm) [2]. The conditions tested in the present work were more favorable for micronization of quercetin, since a lower capillary diameter and a higher solvent velocity promote particle size reduction.

Coprecipitation of quercetin with ethyl cellulose

In a second part of this work, quercetin was coprecipitated with ethyl cellulose through SAS process using ethyl acetate as organic solvent. **Figure 4** shows SEM images of the obtained coprecipitates. The formed particles present a spherical shape and a submicron size ranging between 200 to 400 nm.

The morphology and particle size of quercetin/ethyl cellulose coprecipitates were similar to those of micronized ethyl cellulose particles obtained in other work by SAS process with ethyl acetate at the same conditions tested [4].

The yield of coprecipitation process and the encapsulation efficiency of quercetin by SAS process were evaluated according to the methodology above described. A quite high yield of 85 % and encapsulation efficiency close to 99 % were obtained for the experimental coprecipitation conditions tested in this work. In addition, the quercetin/polymer mass ratio of the formed coprecipitates was 10.7 %, similar to the initial mass ratio of the organic solution used for SAS experiment (10 %). Results showed the efficiency of SAS process to obtain coating quercetin particles with ethyl cellulose. Moreover, the growth of the quercetin crystals is avoided.

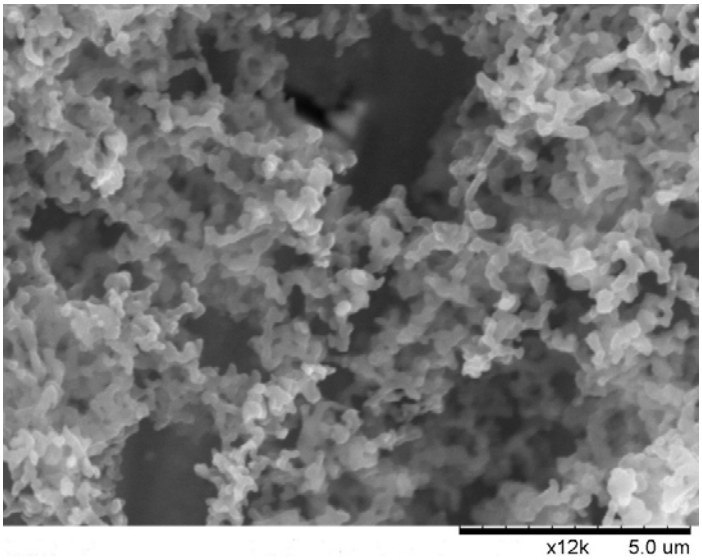

Figure 4: SEM image of quercetin-ethyl cellulose coprecipitates collected at 10 MPa and 35 ºC by SAS with ethyl acetate

CONCLUSION

SAS process was applied to micronize quercetin as well as to coprecipitate it with a biocompatible polymer. Particles with needle like habit and length of 800-900 nm were obtained for micronized quercetin in the tested conditions. SAS coprecipitation results of quercetin with ethyl cellulose were very promising. Indeed coprecipitates with spherical shape and submicron size ranging between 200 to 400 nm were obtained. Furthermore, quite high precipitation yields (above 85 %) and great encapsulation efficiencies (up to 99 %) were reached. However, more studies are required to define the influence of experimental conditions on quercetin micronization, as well as on quercetin coprecipitation with ethyl cellulose by supercritical anti-solvent techniques.

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