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Particle design applied to quercetin using supercritical anti-solvent techniques

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ABSTRACT

Quercetin is a strong naturally occurring antioxidant that is exploited in pharmaceutical and cosmetics applications. Unfortunately, quercetin is highly susceptible to oxidation. Besides, its poor solubility in water and low bioavailability upon oral administration limit the use in drug formulations for the treatment of human diseases. In an effort to overcome these drawbacks, the micronization and coprecipitation of quercetin particles with a low-cost biocompatible polymer (ethyl cellulose, EC) was studied by using supercritical anti-solvent process (SAS) with a non-toxic solvent ethyl acetate. The results showed that SAS micronization of quercetin led to a reduction in the quercetin particle size and crystallinity without a change in the needle-like habit. SAS coprecipitation of quercetin with EC at moderate pressure and temperature (10 MPa and 35 °C) led to obtaining quasi-spherical particles. The coated polymer avoid the growth of quercetin crystals, thus amorphous particles in the submicron range (mean size ranging between 150 and 350 nm) were formed. Promising coprecipitation results were reached with quite high process yields (above 85%) and encapsulation efficiencies up to 99% that provided a high stability to the coated quercetin with EC against oxidation.

1. Introduction

Quercetin is one of the major dietary antioxidants and it is widely distributed in fruits and vegetables, particularly onions, apples, cherries, citrus fruits, broccoli and tea. This flavonoid has scavenging properties towards radical oxygen species and such characteristics are related to the ability of this compound to reduce the effects of aging and the risk of chronic diseases such as certain forms of cancer, pulmonary, cardiovascular and neurodegenerative diseases [1,2].

Unfortunately, the complex chemical structure and the antioxidant properties of this drug are also responsible for its lack of stability during distribution, storage or in the gastro-intestinal tract due to its easy degradation by oxidant factors such as temperature, visible light and oxygen. Moreover, quercetin has a bitter taste, poor permeability and poor bioavailability (less than 17% in rats and even as low as 1% in human) [3,4] due to its high hydrophobicity and low solubility in water (<0.01 mg/mL) [5]. These characteristics lead to a low absorption upon oral administration [2]. As a result, the use of this flavonoid in functional foods or in clinical applications is severely limited. Improved formulations with enhanced dissolution rates and bioavailability are required before quercetin can be used as a therapeutic agent for the treatment of human diseases.

Micronization and encapsulation with biopolymers form part of the strategies used to overcome the challenges outlined above. The dissolution rate of such materials increases as the particle size is decreased, corresponding to a high specific surface area, and encapsulation with polymers leads to an enhancement in either the bioavailability or the dissolution kinetics [4,6]. In addition, encapsulation protects sensitive substances against degradation factors, enhances absorption through the cells and allows the design of controlled delivery systems [7]. Numerous approaches have been used to produce quercetin nano-crystals. Conventional processes such as high-pressure homogenization, cavi-precipitation, bead-milling and anti-solvent precipitation have been used to precipitate quercetin. The latter two techniques produced the smallest quercetin nanocrystals (270 and 170 nm, respectively) [4,6]. Encapsulation techniques have also been studied. Lucas-Abellan et al. successfully used the cyclodextrin complexation method to

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encapsulate quercetin [8]. Costa-Silva et al. fabricated quercetin-loaded solid lipid microparticles with glyceryl trimyristate, soy lecithin and/or glycerol behenate for pulmonary administration by hot solvent diffusion and hot emulsion [9]. Moreover, the emulsification/solvent evaporation method in conjunction with freeze drying was explored to nano-encapsulate quercetin with PLA [10].

Nevertheless, the aforementioned techniques do have some disadvantages. For example, bioactive substances are degraded due to chemical, shear stress or heat sensitivity, the control of particle size and morphology is poor and the encapsulation efficiencies and precipitation yields are low. Moreover, the processes require large amounts of organic solvents, surfactants and other additives, all of which may contaminate the environment and products with toxic residues [11–15].

In contrast to the above, in supercritical processes the use of harmful solvents is limited, product degradation is avoided as the processes are carried out at mild temperatures and the need for post-treatment steps is removed [7,11–15]. Furthermore, it is quite easy to control the micronized particle characteristics (habit, particle size distribution, crystalline structure) by changing the process parameters [11,15]. Hence, supercritical precipitation techniques may overcome the drawbacks associated with conventional processes for particle design.

Carbon dioxide (CO_2) is the most commonly used supercritical fluid for pharmaceutical applications because it has low toxicity, an achievable critical point (31.06 °C and 7.38 MPa), the physical properties (i.e. viscosity, density and diffusivity) can be controlled by tuning on the temperature or pressure and the spontaneous separation of CO_2 from the product can be easily achieved by pressure reduction–thus avoiding the need for drying steps [13,14]. Moreover, CO_2 is considered safe by the US Food and Drug Administration (FDA). Carbon dioxide is non-flammable, abundant and recyclable. In supercritical precipitation processes, the supercritical CO_2 (SC-CO_2) can be used as a dissolution medium, as an anti-solvent or as a solute. When the solute is poorly soluble in the supercritical fluid, the latter is used as an anti-solvent. Based on the poor solubility of quercetin in SC-CO_2, different supercritical anti-solvent techniques (SAS) such as SAS [15], enhanced dispersion by supercritical fluid (SEDS) [11,13,14] and SAS with enhanced mass transfer (SAS-EM) [6] have been studied for the micronization of quercetin.

In previous studies, dimethyl sulfoxide (DMSO), methanol (MeOH), ethanol (EtOH), acetone, acetonitrile, IPA and ethyl acetate were explored as organic solvents for the micronization of quercetin by SAS [14,15]. The use of alcohols (MeOH/EtOH) as organic solvents gave the best results. MeOH produced micron-sized quercetin particles (1–10 μm in size) and high yields (75–80%) [14]. Needle-like or flake-like quercetin particles have been obtained using the aforementioned techniques. Nevertheless, quercetin particles with spherical habit were obtained by an SAS process on using ethanol as the organic solvent [15].

In SEDS, a coaxial nozzle is used to introduce the SC-CO_2 and the solution of the target substances, with this approach favouring the formation of tiny droplets and enhancing the mass transfer rates [11]. Submicron particles, which are 3–4 times smaller than the raw quercetin, were obtained by SEDS, whereas through conventional crystallization particle, particle sizes were doubly increased comparing to unprocessed quercetin [11,13]. In the precipitation of quercetin by SEDS, an increase in temperature led to an increase in the particle size [11] whereas an increase in pressure had the positive effects of reducing the particle size and increasing the yield [14]. Moreover, the use of low concentrations of quercetin in the organic solution led to the formation of small particles whereas higher concentrations led to an increase in the yield [14]. Subsequently, Kakran et al. [6] modified the SAS process by applying ultrasound in order to enhance mass transfer between the solution and the supercritical phase. The SAS-EM led to a significant reduction in the particle size of quercetin to the nanoscale (120–450 nm). In addition, the introduction of ultrasound energy prevented agglomeration of the particles.

In contrast, to the best of our knowledge very little has been reported on the encapsulation of quercetin by SAS techniques. The polymers polylactic acid (PLA) and poloxamers have been evaluated to encapsulated quercetin by SAS using methanol and acetone as organic solvents [16,17]. However, PLA is an expensive polymer whose use has remained essentially limited to high-priced special applications. Furthermore, methanol is toxic and not allowed for food or pharmaceutical applications.

In the work described here, ethyl cellulose (EC) was used as encapsulant agent for coating quercetin particles. EC is a biocompatible polymer that is widely used to prepare controlled delivery systems and to stabilize pharmaceuticals against active interactions, hydrolysis and oxidation. It is appreciated for its low toxicity, good film forming abilities and relatively low cost [18]. Numerous drugs and some natural products, such as ethenzamide, propranolol hydrochloride Centchroman, *Garcinia mangostana* extract and squalene, have been encapsulated with EC by conventional and non-conventional methods [19–23].

According to supercritical techniques, the studies have been focused mainly on the precipitation of EC [24–29]. There are few studies about the coprecipitation of EC with drugs or active compounds by SAS. Amoxicillin and ampicillin are among the drugs that have been coprecipitate with this polymer [30–32]. Naproxen has been also coprecipitated by SAS, but in this case a mixture of EC and methylcellulose was used as coating agent [33].

Therefore, the work described here proposes a green process for the micronization of quercetin particles and coprecipitation of quercetin with EC by SAS technique using ethyl acetate as organic solvent which is generally recognized as safe (GRAS). The aim of the study was to determine good operating conditions for the production of quercetin particles efficiently encapsulated in ethyl cellulose in order to avoid thermal/light degradation of the active compound. Different process parameters, such as the quercetin/polymer mass ratio, the organic solution velocity, the solvent/CO_2 molar ratio and the capillary diameter were studied. Results were analyzed according to the process yield, quercetin loading, encapsulation efficiency and powder characteristics (morphology, particle size and crystallinity) of the resulting particles. The habit, size and crystallinity of the quercetin particles and coprecipitates were characterized by SEM, light scattering and X-ray diffraction. Quercetin content of coprecipitates was quantified by UV spectrophotometry. In addition, the stability of the quercetin particles and coprecipitates was analyzed by the DPPH antioxidant assay.

2. Materials and methods

2.1. Materials

Quercetin aglycone (98%) was purchased from Sigma-Aldrich, Germany. Ethyl cellulose (9004-57-3) was obtained from Sigma-Aldrich, France. HPLC grade ethyl acetate was provided by CARLO ERBA Reagents, Italy. Carbon dioxide (99.7%) was purchased from Air Liquide, France. Sodium n-dodecyl sulfate (99%) was purchased from Alfa Aesar GmbH & Co, Germany. Ultrapure water (Milli-Q) was used.

2.2. Supercritical anti-solvent process (SAS)

The experimental set-up used for the SAS process is illustrated in Fig. 1. The equipment consisted of a high pressure precipitation vessel with a capacity of 1 l (Top Industrie S. A., France) equipped with