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Precipitation of submicron particles of rutin using supercritical antisolvent process

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ABSTRACT

Spherical submicron particles of rutin were prepared by a supercritical antisolvent process (SAS). Selection of the appropriate solvent determined the success of the precipitation process. A mixture of acetone and DMSO in a 9:1 ratio was selected in order to study the main parameters that influence the SAS process in terms of particle size, size distribution and particle morphology. Smaller particles were produced at higher temperature and pressure, and with a lower initial concentration of the solution. A lower liquid solution flow rate is recommended to obtain submicron particles but the CO_2 flow rate and nozzle diameter had a negligible effect on particle size, at least at the levels evaluated. The precipitated powders were analysed by Scanning Electron Microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-Ray Diffraction (XRD) and Differential Scanning Calorimetry (DSC). The SAS process contributed to the dehydration of rutin, thus conferring higher added value. Moreover, amorphization of the processed samples was produced during the process. Processed rutin particles dissolved more rapidly than commercial rutin in simulated fluids and this is related to the particle size reduction and the loss of crystallinity due to amorphization. This effect was more pronounced in simulated intestinal fluids than in gastric fluids.

1. Introduction

Polyphenols from the flavonoid family are attracting increasing interest for use in the treatment of many diseases. There is a trend to use natural flavonoids rather than the synthetic compound in order to prevent adverse side effects in humans [1]. Phenolic compounds from several waste plant sources have been the focus of numerous studies [2] because of their anticancer, antioxidant, anti-pyretic, analgesic, anti-inflammatory and antiviral activities, amongst others [3–6].

Rutin (quercetin-3-rhamnosylglucoside) is a natural flavone derivative composed of the flavonol, quercetin, and the disaccharide rutinose [7]. Rutin is a polyphenolic compound found in fruit and vegetables and it has potential applications in the food [8], cosmetic [9] and pharmaceutical industries [10–14]. However, the low aqueous solubility of rutin can lead to poor bioavailability, variability and lack of dose proportionality, all of which limit its clinical applications. Therefore, the production of suitable formulations is essential to improve the solubility and bioavailability of such polyphenols [9]. One way to improve water solubility over a short time, i.e., its dissolution rate, is to reduce the particle size. In this way the contact surface of the polyphenol with the solvent is increased and this leads to enhanced bioavailability and biological activity [15]. Thus, the generation of rutin nanoparticles and the identification of formulations containing rutin have been the focus of numerous studies [16–19]. Not only the reduction of particle size but also the amorphization of the processed particles would provide additional advantages such as better compressibility and higher dissolution rate [20].

Most methods for the production of submicron particles and encapsulation techniques involve excessive amounts of organic solvent, high temperatures, and post-processing steps and, furthermore, residues are present in the final product. These drawbacks could be avoided by using supercritical CO_2 ($scCO_2$) technologies due to the low toxicity of this compound and the elimination or reduction of organic solvent in the processes. This approach would contribute to a substantial improvement in human health and lessen the environmental impact. Moreover, the properties of CO_2 can also be adjusted continuously by altering the experimental conditions and this leads to a defined high product quality in terms of purity and uniform dimensional characteristics [21,22].

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Very few studies on rutin supercritical precipitation have been reported in the literature. The rapid expansion of supercritical solutions (RESS) using ethanol as cosolvent was employed by Santos and Meireles to encapsulate rutin [23]. The use of a cosolvent in the RESS process indicates the low solubility of rutin in supercritical CO_2, as with many other flavonoids [24], and this is therefore an excellent candidate solvent for micronization by a supercritical antisolvent processes (SAS). In a previous study [25], quercetin, another flavonoid similar to rutin, was successfully precipitated by a SAS process to give particles nearly in the nanometer range. In the SAS process an organic solution containing the solute is sprayed through a nozzle into a vessel in which CO_2 is flowing at supercritical conditions, i.e., it is completely miscible with the organic solvents and mixes without interface limitations. The mixing between these streams causes the precipitation of the solute due to the anti-solvent effect of CO_2 [26].

The requisites for a successful SAS process a priori are the complete miscibility between the organic solvent and the antisolvent (CO_2) and the insolubility of the solute in CO_2, conditions that are achieved on working above the mixture critical point (MCP) of the binary solvent-CO_2 mixture. The presence of a solute at quite low concentration does not modify the solvent-CO_2 equilibria [27] but at higher concentration the presence of a solute could modify the binary equilibria by moving the MCP to higher pressures. So, in order to ensure a successful precipitation of micro- or nanoparticles, the SAS operating conditions of pressure and temperature should be far above the mixture critical point (MCP) of the binary systems in order to avoid the risk of working at subcritical conditions. Moreover, the jet break-up mode of the solution should be achieved by tuning the parameters that influence the hydrodynamics of the SAS, such as liquid solution flow rate and nozzle diameter. The mass transfer of the three components involved in a typical SAS experiment also plays an important role as long as an interface exists between the injected solution and $scCO_2$, with mass transport taking place across a phase boundary [28]. It is known that the choice of the organic solvent is one of the key factors that influences morphology, particle size distribution and even the success of powder precipitation [28,29]. In this way, Liu et al. suggested the use of solvents with a higher ratio of density and viscosity, lower surface tension, and lower solvation power in order to form smaller camptothecin microparticles [30]. The work reported here concerned a preliminary study of solvents and mixtures with the aim of identifying the best option to precipitate submicron rutin particles. The main parameters of the SAS process, namely pressure, temperature, CO_2 and liquid solution flow rates and nozzle diameter, were evaluated in terms of their effect on particle size and size distribution. Morphology changes in the precipitates were also evaluated. The dissolution profiles of submicron rutin particles were carried out in simulated fluids.

2. Materials and methods

Rutin hydrate ($C_{27}H_{30}O_{16}{\cdot}xH_2O$), dimethyl sulfoxide (DMSO), absolute ethanol and acetone (99.9%) were purchased from Sigma-Aldrich (Spain). CO_2 with a minimum purity of 99.8% was supplied by Linde (Spain). The mean particle size of commercial rutin hydrate was around 25 μm, as can be seen in Fig. 1.

2.1. SAS process

The experiments were carried out in an automatic semicontinuous pilot plant, model SAS 200, developed by Thar Technologies®. This pilot plant was described in a previous publication [31]. The flow diagram is provided in Fig. 2.

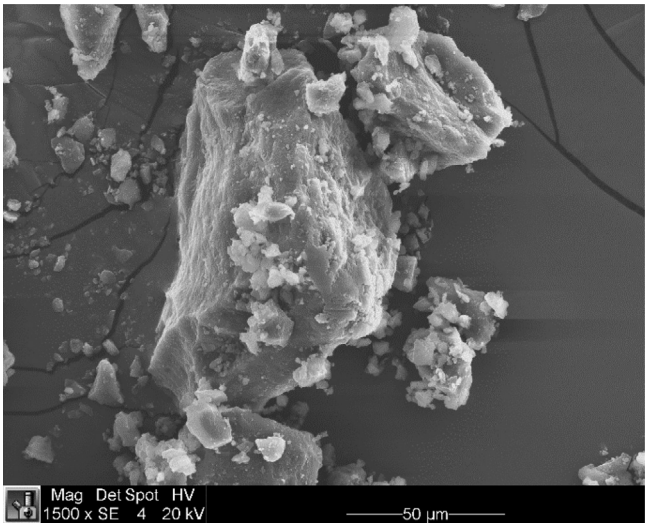

Fig. 1. SEM image of commercial rutin hydrate.

The SAS 200 system comprises a high-pressure pump for CO_2 pumping (P1) and another one for solution pumping (P2). The powder is collected in a stainless steel precipitator vessel (V1) (2 L volume) surrounded by an electrical heating jacket (V1-HJ1) to keep the set temperature inside. This vessel is composed of two main parts, a cylinder body and a frit at the bottom. The system also has an automated high-precision back-pressure regulator (ABPR1) in order to keep the pressure constant and a jacketed (CS1-HJ1) stainless steel cyclone separator (CS1) (0.5 L volume) to separate the solvent and CO_2.

In this semicontinous process the CO_2 is always flowing into the vessel in order to achieve the supercritical conditions of pressure and temperature. The rutin solution was pumped into the vessel through a nozzle, which enhances the mass transfer between this solution and supercritical CO_2 ($scCO_2$). If the time for the disappearance of interfacial tension is lower than the time for jet break up, single phase mixing will be achieved and nanoparticles are produced due to a nucleation mechanism as in the gas phase. However, if the time for the disappearance of interfacial tension is higher a multiphase mixing regime will be formed and this will evolve to a single phase mixing. Depending on the nucleation kinetics, the precipitation of spherical microparticles (from a multiphase jet region) or nanoparticles (from a single phase jet region) will be generated [32]. In any case, powder was accumulated on the internal wall and on the frit at the bottom of the vessel.

Several mixtures of solvents were tested in order to obtain a clear rutin solution and fine powder precipitation. The solvent mixtures and selected results are listed in Table 1. DMSO was chosen as the starting point for the mixtures because it is a good solvent for rutin. Moreover, this solvent is widely used in SAS processes due to its miscibility with CO_2.

Once the solvent mixture had been selected, a study of the main SAS process parameters that could have an influence on particle size and size distribution, e.g., pressure, temperature, concentration, CO_2 and liquid solution flow rates, and nozzle diameter, was carried out. A considerable washing time of 60 min was set in all experiments. The experimental conditions for SAS experiments are listed in Table 2.

2.2. Sample characterization

The morphology of the precipitated powders was analyzed by Scanning Electron Microscopy (SEM) using a QUANTA scanning electron microscope. Prior to analysis the samples were placed on