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Mangiferin nanoparticles precipitation by supercritical antisolvent process

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ABSTRACT

Nano- and microparticles of mangiferin were precipitated by a supercritical antisolvent (SAS) process. A preliminary study of typical solvents used in SAS processes was carried out in order to select a solvent (or solvent mixture) that had good miscibility with supercritical CO_2. Mixtures of acetone with dimethyl sulfoxide or 1-methyl-2-pyrrolidone led to submicron particles with a spherical morphology. The main parameters that could affect the nano- or micro-precipitation were studied using mixtures of acetone and dimethyl sulfoxide rather than 1-methyl-2-pyrrolidone due to the low toxicity of DMSO. Higher CO_2 and liquid solution flow rates, higher pressures, lower temperatures and lower nozzle diameter are recommended for the formation of nanoparticles. The morphologies of the precipitates were analyzed by scanning electron microscopy (SEM). X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) were carried out in an effort to assess the possible loss of crystallinity and activity of the precipitates. The dissolution profiles of mangiferin were obtained in simulated fluids and processed mangiferin dissolved faster and had a higher solubility than the raw material. This situation would enable the use of mangiferin in pharmaceutical, cosmetic or nutraceutical applications.

1. Introduction

Mangiferin (MNG) is an active polyphenol that is present in various plants such as *Mangifera indica* L. The growing interest in this xanthone C-glucoside is due to its strong antioxidant, analgesic and antitumor activities and its anti-allergic, antibacterial and antiviral properties [1–3]. Moreover, mangiferin has been tested in diabetes treatment and it has been associated with the prevention of periodontitis, neurodegenerative disease and aging symptoms [4–6].

However, the antioxidant activity outlined above does not necessarily lead to an in vivo biological effect and this implies poor bioavailability due to the low solubility of mangiferin in water [7]. This problem can be minimized by reducing the particle size or by creating microcapsules of MNG with carriers that have the ability to complex with a variety of guest molecules, thus improving the solubility, bioavailability and stability and protecting the compound from degradation [8,9].

Micronization of MNG improves its water solubility and thus increases the bioavailability and biological activity of this compound.

Supercritical fluids technology is achieving prominence in the pharmaceutical, cosmetic and agrifood fields as it is able to produce, in an environmentally friendly way, high quality added value products without solvent residues and with tunable particle size distributions. Carbon dioxide (CO_2) has been widely used in such processes due to its low toxicity, low cost and its relatively low critical parameters such as temperature (31.1 °C) and pressure (73.8 bar), which are sufficiently mild to allow the particle size reduction of thermolabile solutes such as nutraceuticals and pharmaceuticals.

A SAS process is characterized by the organic solvent being almost completely removed from the process and by the one-step processing achieved by removing CO_2 with a simple pressure change. It is a requirement in this process that the solute to be micronized is not soluble in CO_2 but is soluble in a solvent that is miscible with CO_2. MNG – as with other polyphenols [10] – has a low solubility in supercritical CO_2 and is relatively soluble in organic solvents such as DMSO and NMP, which are widely used in these antisolvent processes. In such processes the organic solution containing the solute of interest is sprayed through a nozzle to generate drops of solution. The diffusion of the supercritical CO_2 into these drops is favored and subsequent expansion of the solvent leads to a high supersaturation of solute solution and the precipitation of a powder.

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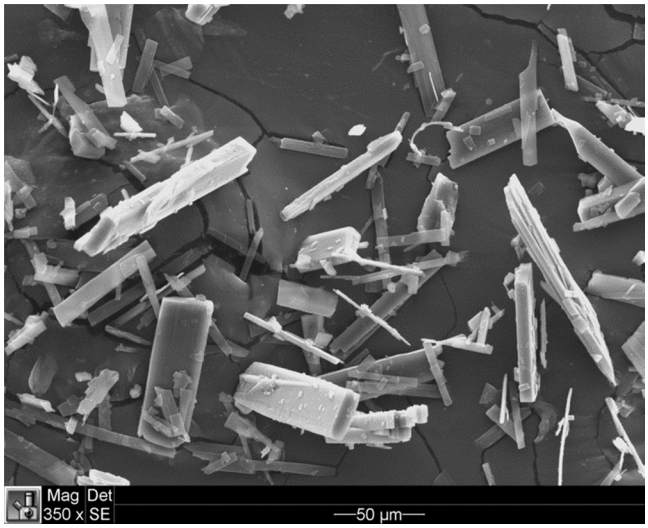

Fig. 1. SEM image of unprocessed mangiferin.

In the work described here, several solvents for use in an SAS process with MNG were evaluated in order to select the best solvent for a subsequent extensive study of the influence that the operating conditions have on morphology and particle size distribution. MNG dissolution experiments were carried out in simulated fluids.

2. Materials and methods

2.1. Materials and analytical methods

Mangiferin ($C_{19}H_{18}O_{11}$) was purchased from Glentham Life Science. Acetone (99.9%), absolute ethanol, dimethyl sulfoxide (DMSO) (99.9%) and 1-methyl-2-pyrrolidone (NMP) (99.5%) 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 mangiferin was 53.20 ± 28.33 μm (Fig. 1).

2.2. SAS process

A preliminary study was carried out to identify the most suitable organic solvent for the SAS process discussed here. The solvents were chosen on the basis that they were good solvents for MNG and have been used in SAS processes. The experimental conditions for solvent screening experiments are detailed in Table 1. The operating conditions were pressure (P) 100 bar; temperature (T) 40 °C; initial concentration of the solution (cc) 20 mg/mL; CO_2 flow rate (Q_{CO_2}) 10 g/min; liquid solution flow rate (Q_L) 3 mL/min; nozzle diameter (θ_n) 100 μm and washing time 60 min.

Once the best solvent had been identified, the main parameters that could influence the particle size, particle size distribution and morphology of precipitates in the SAS process were studied. All of the experiments were performed in a pilot plant developed by Thar Technologies® (model SAS 200). A schematic diagram of the equipment is shown in Fig. 2 and the system was described in detail in a previous publication [11]. The SAS 200 system comprises two high-pressure pumps in order to pump the CO_2 (P1) and the solution (P2); a stainless steel precipitator vessel (V1) (2 L volume) in which the powder is collected, consisting of two main parts, a cylinder body and the frit, all surrounded by an electrical heating jacket (V1-HJ1); an automated high-precision back-pressure regulator (ABPR1) attached to a motor controller with a position indicator; a jacketed (CS1-HJ1) stainless steel cyclone separator (CS1) (0.5 L volume) to separate the solvent (ethanol) and CO_2.

All of the experiments were carried out according to the same experimental procedure. First, CO_2 was pumped into the vessel and it was held until supercritical conditions of pressure and temperature were achieved. A solution of MNG in the organic solvent was pumped into the precipitator vessel and sprayed through a nozzle. The atomization of the organic solution led to the formation of small droplets of solvent containing the MNG. A rapid mass transfer occurred between these phases and CO_2 was dissolved in the solvent, which in turn caused supersaturation of the liquid solution and the consequent precipitation of the MNG as a powder, which accumulated on the internal wall of the vessel and/or on the frit.

2.3. Sample characterization

Scanning electron microscopy (SEM) images of the different precipitated MNG samples were obtained using a PHILIPS XL30 scanning electron microscope. Prior to analysis the samples were placed on carbon tape and then coated with gold using a sputter coater. The SEM images were processed using the image analysis software Scion Image (Scion Corporation) to obtain the particle size and particle size distribution. The diameters of 300 particles were counted to perform the analysis in each experiment. The mean particle size (PS) and standard deviation – as a measure of the distribution width – were calculated using StatGraphics Plus 5.1 software with fitting to lognormal distributions.

Fourier transform infrared spectroscopy (FTIR) was performed on a Bruker Tensor 37 FTIR spectrophotometer with a spectral resolution of 0.6 cm^{-1} in order to analyze possible structural changes in MNG after the SAS process. The transmittance measurements were carried out by the KBr technique with potassium bromide pellets containing 1% by weight of MNG. The spectra were measured in the 4000–400 cm^{-1} range.

X-ray diffraction (XRD) analysis was performed on a Bruker D8 Advance diffractometer in order to determine the amorphous or crystalline nature of the precipitates obtained in the SAS process. The analysis was carried out at a fixed time after production in order to confirm the amorphous or crystalline character of the samples. All diffraction patterns were scanned from 2° to 50° in 2θ angles with a step size of 0.02° and 1 s as the step time.

2.4. Dissolution profiles

MNG dissolution profiles were obtained for the pure drug and for the precipitated nano- and microparticles. A simulated gastric fluid (SGF) and a simulated intestinal fluid (SIF), i.e., phosphate buffer solutions at pH 1.2 and 6.8 ± 0.1, respectively, were prepared in our

Table 1
Preliminary SAS precipitation of mangiferin (experimental conditions: (P) 100 bar; (T) 40 °C; (cc) 20 mg/mL; (Q_{CO_2}) 10 g/min; (Q_L) 3 mL/min; (θ_n) 100 μm and (t_w) 60 min).

Run	Solvent	Success	Macrostructure	Microstructure
1	DMSO	–	Solvent on the frit	–
2	NMP	–	Solvent on the frit	–
3	Acetone: DMSO (3:1) (v: v)	+	Fine precipitate on the wall	Spherical microparticles
4	Ethanol: DMSO (3:1) (v: v)	+	Fine precipitate on the upper side of vessel and solvent on the frit	Interconnected stick like microparticles
5	NMP: DMSO (3:1) (v: v)	+	Fine precipitate on the upper side of vessel and solvent on the frit	Stick like microparticles
6	Acetone: NMP (3:1) (v: v)	+	Fine precipitate on the wall	Spherical microparticles
7	Ethanol: NMP (3:1) (v: v)	+	Fine precipitate on the wall	Interconnected stick like microparticles