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Enhancing the solubility and bioavailability of isoflavone by particle size reduction using a supercritical carbon dioxide-based precipitation process

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ABSTRACT

Isoflavones are a group of small molecular compounds found in many plants. Genistein is the most well studied isoflavones because of its beneficial effects in reducing menopausal symptoms, anti-oxidant and anti-cancer. The major difficulty in developing isoflavone-based healthcare products is their low water solubility. In this study, the solubility and oral bioavailability of genistein were increased by reducing its particle size using supercritical CO_2 as an antisolvent in the precipitation process. The effects of various process parameters including type of solvent, pressure of precipitation, and concentration of genistein solution on particle formation were evaluated. We found that under optimized conditions: dissolving 4 mg/mL genistein in acetone and precipitating them with supercritical CO_2 under 100 bar at 40 °C, the size of genistein particles was reduced from its original width of 10–50 μm to ~254 nm. The reduction of genistein particle size not only increased its water solubility by 2 fold but more importantly increased its 24 h-plasma concentration by 2.6 fold after orally administrated to rats. These results proof the concept of using supercritical CO_2 as an antisolvent in the precipitation process to reduce particle size of water insoluble compounds such as genistein and to improve its oral bioavailability.

Keywords: Bioavailability; Solubility; Isoflavone; Supercritical carbon dioxide; Particle size; Precipitation process

1. Introduction

Peroral route is the most preferred route of drug administration. The drug, in the form of powder or tablet, first dissolves in the gastrointestinal fluid and the dissolved drug subsequently permeates through the gastrointestinal membrane. However, this route is not suitable for many drug molecules because they have poor oral bioavailability caused by various biopharmaceutical hurdles. These hurdles include low water solubility, poor permeability of the gastrointestinal membrane, first pass metabolism, and instability in the gastrointestinal environment. Since up to 40% of the new chemical entities are poorly water-soluble (Lipinski, 2002), increasing the solubility of those drugs is an important task in pharmaceutical development.

Genistein is a major component of soy isoflavones which exhibits a variety of biological effects relevant to human health. Specifically, genistein is known to play a role in the treatment and prevention of various disorders, including cancer (Li et al., 2008; Sarkar and Li, 2002; Suthar et al., 2001a), cardiovascular diseases (Altavilla et al., 2004) and osteoporosis (Suthar et al., 2001b). Despite its multiple health benefits,

Abbreviations: PCA, precipitation with compressed antisolvent; XRD, X-ray diffraction; DSC, differential scanning calorimetry.

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the therapeutic application of genistein can be limited as it is a hydrophobic compound, which belongs to class II compounds in the biopharmaceutical classification system with low solubility and high permeability (Motlekar et al., 2006). To increase the oral bioavailability of genistein, some research groups have tried to form complexes between genistein and different carriers such as cyclodextrins (Daruhazi et al., 2008; Lee et al., 2007; Stancanelli et al., 2007) and poly(ethylene glycol) (Motlekar et al., 2006). However, these methods have limitations such as high residual organic solvent and challenges in process scale-up.

Another approach to improve the dissolution rate of poorly water-soluble drugs is to increase the total surface area by reducing the drug particle size (Noyes, 1897). Generally speaking, a number of techniques can be applied to produce fine particles, including spray drying, freeze-drying, milling, liquid antisolvent crystallization and precipitation with supercritical fluid. Compared to the aforementioned techniques, supercritical fluid-based technology has the following four major advantages: (1) less contamination due to the significantly reduced usage of organic solvents, (2) low cost and environmentally benign properties, (3) no degradation of the product because of the mild operating conditions ($T_c = 31.1\,^{\circ}C$, $P_c = 73.8$ bar), (4) controlled particle size and distribution. A particular advantage of using the precipitation with compressed antisolvent (PCA) technique is that the organic solvent-free particles can be formed with no need of further purification and solvent removal. In the PCA process, the compound solution is sprayed through an atomization co-axial nozzle into the compressed carbon dioxide fluid. The dissolution of the supercritical fluid into the liquid droplets is accompanied by a large volume expansion and a reduction in the liquid solvent power. This causes a sharp rise in the supersaturation of the drug molecules within the liquid mixture, and the formation of small, solid drug particles (Dixon et al., 1993; Lin et al., 2007; Wu and Li, 2008). As such, the PCA process was selected in the present study to form nano- and micron-sized particles for increasing the dissolution rate of genistein.

The objective of the present study is to improve the oral bioavailability of genistein by particle size reduction using PCA process. Process variables such as type of solvent, precipitation pressure and genistein solution concentration, which could affect particle size and particle size distribution of genistein were investigated. The physicochemical properties of particles in the solid state before and after the PCA process were characterized by powder X-ray diffraction (XRD) and differential scanning calorimetry (DSC). *In vitro* dissolution rate analysis and *in vivo* oral bioavailability studies in rats were performed to compare the absorption of processed and unprocessed genistein particles.

2. Materials and methods

2.1. Materials

Genistein with a minimum purity of 98% was obtained from Allway Minerals and Science Technology Co., Ltd. (Xi'an, China). Carbon dioxide was supplied by Hong Kong Specialty Gases with a purity of 99.99% (Hong Kong). Acetone with a minimum purity of 99.5% was obtained from Scharlau (Germany). Ethanol and methanol (≥99.9%) were from Merck (Germany). Ethyl acetate (≥99.5%) was obtained from VWR (UK). Tween 80 and sulfatase enzyme from Helix pomatia Type H-1 (sulfatase ≥10,000 units/g solid, β-glucuronidas ≥300 units/mg solid) were purchased from Sigma (USA).

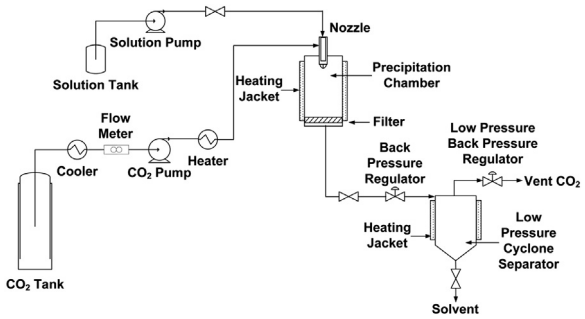

Fig. 1 – Schematic diagram of PCA process.

2.2. PCA process

The experimental setup is shown in Fig. 1 (Lin et al., 2007). The CO_2 tank was connected to a water/ethylene glycol circulating cooler at $-4\,^{\circ}C$ in order to maintain the CO_2 at a low temperature prior to raising it to the desired temperature in a heater. A flow meter was used to monitor the CO_2 flow rate. A high-performance pump was used to deliver the CO_2 fluid into the precipitation chamber (100 mL) which was equipped with a heating jacket and a top-mounted nozzle. A metal filter with a pore size of 5 μm was placed at the bottom of the vessel for collecting the generated particles. This was followed by a back-pressure regulator and a cyclone separator where the remaining solvents were collected while the vapor was vented out of the system. Pressure, temperature and CO_2 flow rate were all controlled by a computerized system governing the whole PCA system.

In a typical experiment, supercritical CO_2 was first pumped into the precipitation chamber. Once the preset conditions were reached, the acetone solution with the dissolved genistein and CO_2 were introduced into the precipitation chamber through the co-axial nozzle. Mixing of the two fluids caused a sharp rise in the supersaturation within the liquid mixture leading to the formation of fine solid particles. The particles collected at the metal filter were thoroughly washed and the solvent remained in the vessel was removed by pumping additional supercritical CO_2 into the precipitation chamber for another 30 min. Finally, the precipitation chamber was slowly depressurized to atmospheric pressure and the particles were collected for characterization and evaluation.

2.3. Morphology

Two high-resolution scanning electron microscopes JSM-6300F and JSM-6700 F (Jeol) were used for particle morphology and size characterization. Gold sputter coater, supplied by Emitech (K575X Turbo sputter coater), was used in sample preparation to prevent the accumulation of static electric charges on the specimen during electron irradiation. For each sample, both width and length of 300 particles were measured to produce a more complete picture of particle size distribution.

2.4. Evaluation of thermal property

Differential scanning calorimetry measurements were carried out with DSCQ1000 (TA instruments, USA). The measurements

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