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Original Research Paper

Effect of process parameters on the recrystallization and size control of puerarin using the supercritical fluid antisolvent process

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ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form 26 December 2015
Accepted 28 December 2015
Available online 26 January 2016

Keywords:
Puerarin
Microparticles
Supercritical fluid
GAS
Crystallization
Particle size

ABSTRACT

The purpose of this study was to investigate the influence of the supercritical CO_2 processing on the particle size and morphology of puerarin crystals. The process parameters included solvents, temperature, pressures, antisolvent times, addition volumes, antisolvent addition rates and solute concentrations. After being processed, the dramatic reduction of the dimensions and the change of the crystal shape were noticed. Decreasing the antisolvent addition rate, increasing the temperature and the addition volume below 50 ml led to a decrease in size. The new crystal of puerarin generated at the optimal conditions was 30.34 μm. The solvent of methanol and the concentration of 60 mg/ml were found to determine the type and degree of crystallinity of particles. These results showed that this process has the potential to produce a drug recrystallization product with newly generated crystal forms and the size of drug particles could be controlled through the tuning of various experimental conditions.

1. Introduction

For most orally administered poorly-soluble compounds, the bio-absorption process is rate-limited by dissolution in gastrointestinal fluids; in the case of parenteral administration, the effective bio-availability of compounds is limited by solubility issues. As for the crystal drug, two key characteristics of crystalline solid dosage forms are crystal habit and the crystal size distribution [1].

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Peer review under responsibility of Shenyang Pharmaceutical University.
http://dx.doi.org/10.1016/j.ajps.2015.12.001

The conventional techniques for reduction of particle size include mechanical comminution (through milling, crushing and grinding), lyophilization and recrystallization of the solute particles from solution (through solvent-antisolvent techniques, spray drying and freeze-drying). All these techniques suffer from one or more disadvantages, such as thermal and/or chemical degradation, high solvent requirements or difficult removal of solvent traces from the final product. Besides, the classical crystallization techniques usually lead to a mixture of polymorphs because of the multi-step process used. Therefore, there is increasing interest in developing technologies which, particularly in the case of pharmaceuticals, allow one to produce microparticles with controlled particle size distribution and product quality (crystallinity, purity, morphology) under mild and inert conditions. Since the mid-1980s, a new method of powder generation has appeared involving crystallization with supercritical fluids. CO_2 is the most widely used solvent and its innocuity and "green" characteristics make it the best candidate for the pharmaceutical industry. Supercritical fluid technology, particularly when using CO_2, offers different possibilities to tackle the above-mentioned challenges [2,3]. And it is also interesting to check if the supercritical crystallization (a single-step unit) may give different results. These include the processes called rapid expansion of supercritical solutions (RESS), precipitation with compressed antisolvent (PCA, sometimes referred to as SAS, i.e., supercritical antisolvent process, or ASES, i.e., aerosol spray extraction system) and gas antisolvent recrystallization (GAS) [4–7]. In the GAS process, high pressure CO_2 is injected into the liquid phase solution, which causes a sharp reduction of the solute solubility in the expanded liquid phase. As a result, precipitation of the dissolved compound occurs. The potential advantages of the GAS recrystallization process lie in the possibility of obtaining micron and submicron particles with a narrow size distribution and lower residual solvent. By varying the process parameters, the particle size, size distribution and morphology can be "tuned" to produce a product with desirable qualities. This makes the GAS technique attractive for the micronization of high-valued products such as pharmaceuticals [8,9].

Adopting a GAS process to recrystallize pharmaceutical compounds will provide highly versatile methodology to generate new polymorphs of drugs. Many researchers have employed the GAS process for micronization and recrystallization of various pharmaceutical substances [10–12]. They have concentrated on the size reduction of pharmaceutical compounds and they observed changes in the external shape and size distribution of the resulting particles. The diversity of experimental parameters of the GAS process can vary the conditions for nucleation and crystal growth steps in a wide range. It is possible to produce drug particles with different crystalline arrangements but identical chemical compositions. Such behavior is called polymorphism, meaning the ability of any compound to crystallize into more than one distinct crystalline state [13]. This can be important to the quality of a given product. In the pharmaceutical field, an active substance may exhibit different activities and shelf life depending on the polymorph. Properties such as solubility, dissolution rate, density, physical stability and melting point change depending on the type of crystalline forms. Therefore, the various polymorphs of a given pharmaceutical compound will exhibit different drug release characteristics and biological activity.

With the application of modern isolation technology and high throughput biological screen capability, more and more natural compounds with biological activity are being isolated and identified. However, many of these compounds with potent activity *in vitro* fail to generate good activity *in vivo* owing to their poor water-solubility, poor permeability and/or poor stability [14]. Puerarin (Pur) was such a drug, chemically designated as 8-C-β-D-Glucopyranosyl-4′, 7-hydroxy-Isoflavone, and was one of the main active constituents of *Pueraria lobata (wild.) ohwi*, a famous Chinese traditional medicine. Pur has many types of beneficial effects on cardiovascular, neurological and hyperglycemic disorders. However, its poor solubility in water limits its absorption *in vivo* [15–17]. Solubilizer is often added to the injection formulation used clinically to increase its solubility. Research on the polymorphism of Pur was quite few. There was only related literature which reported that Pur did have various crystalline forms depending on experimental conditions adopted in the conventional solution crystallization techniques [18]. The objective of this research was to investigate the feasibility of the GAS recrystallization technique to generate small particles of Pur with a high degree of purity and narrow size distribution. By surveying the production of different crystalline forms of Pur using the GAS process, we focused on how the solid-state properties of the recrystallized particles including particle size, morphology and crystal form varied with the process parameters such as the antisolvent addition rates, the pressures, the addition volumes, the concentrations, the antisolvent times, the types of solvent and temperatures. And we hope to find new polymorphs with better physico-chemical properties such as higher solubilities, dissolution rate and smaller particle size. The newly generated polymorph of Pur could be used for the injection or oral formulation with better bioactivity. So, here we recrystallized Pur by using CO_2 as an antisolvent and examined the characteristics of the GAS processed crystals by using various analytical instruments.

2. Materials and methods

2.1. *Materials*

Absolute ethanol, acetone and methanol, analytical grade, purity of 99.7%, were bought from Guangzhou Chemical Reagent 2 Factory (Guangzhou, China). Pur, purity of 99.9%, was obtained from Beijing Union Pharmaceutical Factory (Beijing, China). Liquid CO_2, instrument grade, purity of 99.5%, was purchased from Zhonghong Industrial Gas (Shenzhen) Co. Ltd. (Shenzhen, China).

2.2. *Experimental procedure*

A schematic diagram of the GAS apparatus is shown in a previous paper [19]. The unit consists of three sections: a CO_2 supplying system controlled by Isco Series D pump power controller (Teledyne Isco, Inc., Lincoln, NE), a crystallizing chamber (Thar Designs Inc., Pittsburgh, PA), and a depressurizing section.