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Crystallization of silibinin from organic solutions using supercritical and aqueous antisolvents

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ABSTRACT

Silibinin, an anticancer drug, was crystallized from organic solutions using supercritical and aqueous antisolvents. Silibinin was dissolved in acetone and ethanol at concentration range of 0.01–0.04 g/mL, and the drug solutions were placed in contact with two different antisolvents, carbon dioxide and water. The mixing of the drug solutions and antisolvents led to the prompt precipitation of silibinin in a solid crystal form. The experimental variables, such as temperature, solution concentration, mixing rate and solution/antisolvent volume ratio were manipulated. When the experiments were conducted with a supercritical antisolvent, the effects of external additives on the crystal habit were examined. α-D-Glucose penta acetate, triton X-100 and urea were added to the solution at concentration range of 0.001–0.003 g/mL as external additives. The temperature increase of 20 °C induced 25% increase in particle size. As the solution concentration was increased from 0.01 to 0.04 g/mL, the average particle size decreased from 35.5 to 22.0 μm in supercritical antisolvent experiments, while the particle size increased from 8.9 to 30.4 μm in aqueous antisolvent experiments. The use of different kinds of external additives resulted in different modifications of the particle shape and structures.

1. Introduction

Antisolvent crystallization is a useful technology for the production of fine solid particles when a target crystallizing substance is moderately soluble in a particular solvent. In order to achieve the precipitation of a target substance, another solvent (antisolvent) is added, normally under ambient conditions, to decrease the solubility of the crystallizing substance and promote precipitation. Pharmaceutical and fine chemical manufacturers frequently rely on antisolvent crystallization because the heating and cooling of solutions are not involved. In the operation of antisolvent crystallization, a solvent and antisolvent must be chosen so they do not interact with the target substance or do not form a co-crystal or a solvated crystal [1,2].

Antisolvent crystallization can provide a large supersaturation of the solution leading to an extremely high nucleation rate. In the crystallization process, the high nucleation rate brings about the generation of small size particles and increases the amorphous domain inside the crystalline structure. Because of these effects, antisolvent crystallization can be used in the production of submicronic particles of pharmaceutical compounds as well as in the manufacture of crystals that require an enhanced drug release rate. Indeed, the polycrystalline drug particles with higher amorphous portions exhibit a faster dissolution rate in solutions.

In general, three types of fluids: gas, liquid, and supercritical fluids can be employed as antisolvents. Crystallization processes that use gas- or supercritical fluid antisolvents have been studied widely to produce particles of polymers and pharmaceuticals [3–5]. In these processes, different methods of mixing and flow configurations of solutions and antisolvents have been adopted to optimize the properties of the resulting crystals. The operations were performed in either a batch- or continuous-type, and sometimes the antisolvent acted as a dispersion media to improve the micronization of the precipitated particles. The use of a gas- or supercritical fluid antisolvent eliminates the concerns regarding residual antisolvent remaining on the crystal surface and the antisolvent can be separated easily from the solution. In fact, the residues of solvent used as an antisolvent could be not only found on the crystal surface, but also entrapped inside the crystals, where it is even more difficult to remove them. In addition, the solvent can be recycled by a simple mechanical separation from the products. In a gas- or supercritical fluid antisolvent process, however, the crystallization process should be performed in a high pressure apparatus to maintain the antisolvents under a high pressure or in

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a supercritical state. Therefore, the expensive installation cost is the major drawback for the gas- and supercritical fluid antisolvent process. In this respect, the use of a liquid, such as water, as an antisolvent might be another choice because the crystallization system does not require the use of heavy equipment and can be operated without the need of pressurization. Indeed, the use of an aqueous antisolvent in addition to a gas- or supercritical antisolvent can demonstrates the influence of the kind of antisolvents on the properties of precipitated crystals.

In the antisolvent crystallization processes, several experimental variables, such as temperature, solution concentration, type of organic solvent, mixing rate, and the solution/antisolvent volume ratio can affect the solid-state properties of the crystals. In addition to these experimental variables, additional tools such as external additives and ultrasonic waves can be applied to the crystallizing system to manipulate the crystal properties. The addition of external additives can modify the crystal habit which can improve the properties of the produced powder such as filterability, flowability, compactability and dissolution profile. The additives adsorb or interact with a particular growing crystal surface in such a way to alter the grow rate and its relative area [6–8]. The presence of ultrasonic waves bring about considerable benefits such as the induction of primary nucleation, inhibition of agglomeration, manipulation of crystal size distribution and modification of polymorphism [9–11]. Therefore, external additives and ultrasonic waves are frequently used in crystallization processes, in which particle size reduction, habit modification and inhibition of agglomeration are essential.

Silibinin, a major active constituent of silymarin, is considered to be a promising new treating agent for cancer. The compound is extracted from milk thistle which is traditionally used for the treatment of liver diseases. Recently, this agent has been reported to have significant anti-neoplastic effects in a variety of in vitro and in vivo cancer models including skin, breast, lung, prostate, and kidney carcinomas [12].

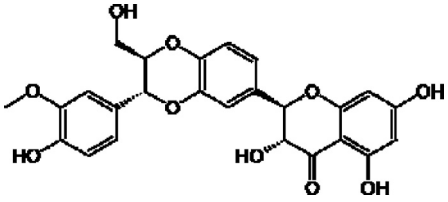
In this study, silibinin was crystallized from organic solutions using carbon dioxide and water as a supercritical- and an aqueous-antisolvent, respectively. Silibinin was dissolved in acetone and the drug solutions were mixed with carbon dioxide and water leading to particle precipitation. Two sets of silibinin crystals produced by using the two different antisolvents were compared. Indeed, the supercritical- and aqueous-antisolvent experiments are different in the respect of two points, which are the type of antisolvent (carbon dioxide and water) and the mixing method of the drug solution and antisolvent. In supercritical experiments, antisolvent is introduced into the solution and in aqueous experiments, solution is injected into the antisolvent. Therefore, the different properties of the two types of antisolvent (gaseous and aqueous) and the different mixing mechanism in crystallization processes may bring about the significant modifications of the silibinin crystals.

2. Experimental methods

2.1. Materials

Silibinin (CAS 22888-70-6) was purchased from Sigma-Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). Acetone (Aldrich, 99.5%) and ethanol (Aldrich, 99.5%) were used as the organic solvents for silibinin, and carbon dioxide (Daesung gas, 99.5%) and distilled water were used as the antisolvents. α-D-Glucose penta acetate (CAS 604-68-2), triton X-100 (CAS 9002-93-1) and urea (CAS 57-13-6) were used as external additives and were obtained from Sigma Co. All chemicals were used as received. Table 1 lists the physico-chemical properties of silibinin.

Table 1
Physico-chemical properties of silibinin.

Properties	
Chemical formula	$C_{25}H_{22}O_{10}$
Molecular weight	482.44 g/mol
Solubility	Soluble in acetone, ethyl acetate and alcohols Practically insoluble in water
Melting point	158 °C
Usage	Antihepatotoxic properties that protect liver cells against toxins, anticancer effect against human prostate cells.
Chemical structure	

2.2. Apparatus and experimental procedure

The first step of the crystallization experiment was the preparation of silibinin solutions in the organic solvents. Silibinin was dissolved in acetone and ethanol at concentrations ranging from 0.01 to 0.04 g/mL. At these concentrations, the silibinin was completely dissolved in the solvents. These silibinin solutions were used in both the supercritical and aqueous antisolvent experiments. In particular, when external additives were used in the supercritical antisolvent experiments, a selected additive was dissolved in the silibinin solution at concentrations of 0.001 (α-D-glucose penta acetate), 0.003 (triton X-100) and 0.001 (urea) g/mL.

The supercritical and aqueous antisolvent experiments were conducted using two different experimental apparatus as shown in Fig. 1. The supercritical antisolvent experiments were carried out using high-pressure antisolvent crystallization equipment and the aqueous antisolvent experiments were performed in a crystallizing vessel operated under ambient conditions. The supercritical antisolvent equipment (Fig. 1(a)) consisted of a carbon dioxide supplying part, crystallizing chamber (Jerguson Gauge, Model 19-T-40, 1/2(W), 1/2(L) $12^{2}/_{3}$(H)), and a depressurizing section. The crystallizing chamber was placed in an air bath to maintain a constant temperature during the experiments. The crystallizing chamber had dual windows through which one could observe the phenomenon of particle precipitation and crystal growth. For the supercritical antisolvent experiments, 10 mL of a prepared silibinin solution was loaded into the crystallizing chamber. The temperature of the air bath was increased as desired. Normally, the experiments were conducted at temperatures of 25, 35 and 45 °C. After the solution was loaded, carbon dioxide was injected into the crystallizing chamber at a controlled rate and the mixing of the drug solution and antisolvent was achieved. This process was carried out in batch conditions after the inlet of carbon dioxide. When the carbon dioxide injection was carried out, three different injection rates were used: slow, medium and rapid. In these three injection modes, the pressure of the crystallizing chamber increased at rates of 0.4, 1.7 and 10.3 bar/min, respectively. Carbon dioxide was injected at a constant rate and as a result, the pressure increase occurred at a steady state. The injection of carbon dioxide and subsequent pressure increase caused an expansion of the solution and the nucleation of silibinin. During the carbon dioxide injection, the nucleation of silibinin particles was observed in the pressure range of 42–65 bar, depending on the solution concentration and temperature. After nucleation, the system was further pressurized up to 95 bar until the carbon dioxide and the solution phases almost merged. In fact, at 95 bar, the upper level of the