Control of the Fine Cholesterol Particles Using Supercritical Compressed Antisolvent

Yousef Bakhbakhi and Meilana Putra

***Abstract*—**In contrast with customary unit operations, techniques based on supercritical fluids show unique unique chemical, physical and mechanical characteristics that make them suitable for specialized applications. One such technique is synthesizing with supercritical fluids, where the unique fluid characteristics and solvent properties of supercritical fluids are utilized. In this research, Compressed antisolvent (PCA) method has been employed to produce fine particles of some pharmaceuticals. Crystal particles of Cholesterol with uniform morphology have been obtained at all successful PCA conditions. However, particles generally tend to coalesce in fine aggregate-gathering assemblage. The effect of the PCA process parameters on morphology, particle size and particle size distribution have been investigated. The ongoing study highlights the potential of a gaseous antisolvent based process as an attractive and scalable technology for the manufacturing of fine particles for pharmaceutical applications.

***Keywords*—**pharmaceutical, antisolvent, supercritical fluid, cholesterol particles.

I. Introduction

APPLICATIONS of supercritical fluids (SCFs) are becoming more numerous and commercially attractive. SCFs can afford the peculiar features of the dense gases, such as high compressibility and diffusivity, very high evaporation rate and the possibility of fine tuning the solvent power through density modulation. The utilization of SCFs for the processing of pharmaceuticals, nutraceuticals and other products has attracted considerable interest in recent years as an emerging "green" technology [1], [2]. Crystallization using SCFs have several advantages over conventional liquid solvents/antisolvents crystallization as their physical properties such as density and solubility can be "tuned" within a wide range of processing conditions by varying both temperature and pressure. Supercritical antisolvent techniques are considered highly effective for producing superior products of fine and uniform particles [2].

Moreover, SCFs can be easily separated from both organic cosolvents and solid products, providing a potentially clean, recyclable, and environmentally friendly technology [1].

Supercritical fluid technology opens up a new perspective in particle design providing an enabling technology for superior particle design and for reducing manufacturing complexity.

This is particularly important for drugs formulation in which particle morphology, size, surface and thermodynamic properties of drugs and excipients are necessary for reliable and efficient controlled drug delivery

Antisolvent techniques such as the Compressed antisolvent (PCA) process exploit the low solubility of most compounds in the antisolvent, in particular CO_2, which has to be miscible with the organic solvent. PCA precipitation can potentially overcome the limitations of liquid antisolvent processing, since very small micronic or submicronic particles can be obtained with narrow particle size distributions and with the complete elimination of the solvents. In the PCA 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 PCA crystallization process lies in the possibility of obtaining solvent free, micrometer and submicrometer particles with a narrow size distribution [3]. 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 PCA technique attractive for the manufacturing of high-valued products, such as pharmaceuticals [4].

The scientific literature shows that PCA treated materials can range from nanoparticles to microparticles to large empty particles [1–6]. The products can be amorphous or semi-crystalline; but, crystalline particulates have also been reported [1, 2]. Many of the PCA produced powders range in the micron-size region that has been the target of several studies: many industrial applications require these particle dimensions to obtain the best process performance. For example, small particles in the 1–5 μm range with a narrow particle size distribution are needed for applications in pulmonary delivery and controlled release systems [7].

To contribute at a better knowledge of PCA applicability to nanosized materials, the scope of this work is to demonstrate that the capability of producing fine particles is a general feature of the PCA process and that it is possible to describe conditions of the PCA parameters at which nanoparticles of controlled size and distributions can be obtained. Literature data together with an extensive PCA experimentation have been performed to assess the possibility of obtaining general

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validity rules for fine particles production. The mechanism that can produce fine particles during PCA has also been investigated.

II. Experimental

A. Materials

Precipitation with Compressed Antisolvent was carried out using one solute-solvent model systems: Cholesterol with a minimum purity of 99.8% purchased from Sigma-Aldrich. Absolute DCM with a minimum purity of 99%, acquired from King saud university (KSU) central storages, was used to prepare the Cholesterol solutions sprayed into the precipitator. Carbon dioxide of an instrument grade (99.99 % purity) was used as an antisolvent and further purified by passage through columns containing molecular sieves (Aldrich) to remove excess water and oxygen, respectively.

B. Procedure

PCA crystallization of Cholesterol with compressed CO_2 was performed by preparing a predetermined volume of Cholesterol solution at a saturation concentration, for the given operating temperature, and injected into the 50 ml crystallization vessel. When the system equilibrated thermally, the pressurization step by the injection of CO_2 was initiated, while the liquid phase mixing was taking place. A controlled CO_2 flow rate was maintained until the full liquid volumetric expansion of was achieved. Consequently, the CO_2 supply feed was stopped, while mixing for the system to equilibrate was continued for approximately one hour. Then, a washing/drying step was performed by flushing the expanded liquid phase with an injected CO_2 at a constant flow rate for a minimum time of five hours. Finally, the crystallization vessel was depressurized by venting the entire fluid mixture of the vessel, and the dry solid powder was collected for off-line particle size analysis. A schematic representation of the PCA experimental apparatus is shown in Figure 1.

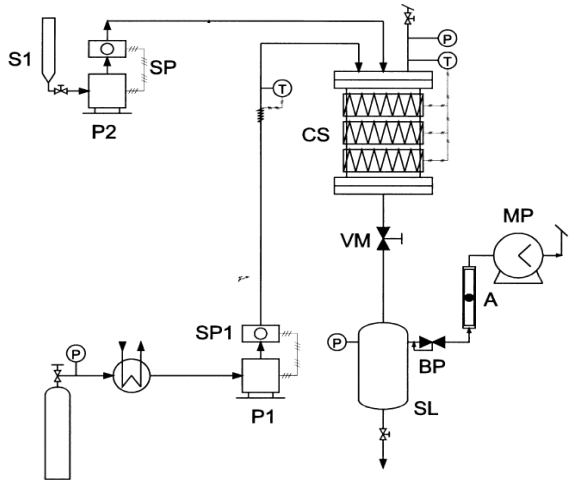

Fig. 1. Schematic diagram of the of the PCA apparatus: P1 and P2, high pressure pumps; SP1 and SP2, pressure dampeners; S1, liquid solution supplier; CS, precipitation vessel; VM, micrometering valve; BP, backpressure valve; SL, liquid separator; A, calibrated rotameter; and MP, wet test meter.

C. Characterization

Quantitative analysis of the precipitated particles was carried out using the following instruments: scanning electron microscopy (SEM, JEOL JSM-6610LV) provided particle morphology, size, size distribution (PSD), and degree of agglomeration. In order to achieve the best possible statistical representation of the formed particles in terms of particle size and size distribution, analysis of the photomicrographs were taken in several different regions of the collected sample, with a minimum of 1000 particles being used for each measurement.

III. Results And Discussion

In this research, the workability of the PCA precipitation process to produce fine particles with controlled particle size distribution and product quality under mild and inert conditions was investigated, using Cholesterol -DCM as the model system. The volumetric expansion, the pressure-temperature-volume behavior of the investigated organic solvent, toluene, with the antisolvent, carbon dioxide, has already been studied [8]. The literature indicates that large liquid volumetric expansions have been achieved at both subcritical and supercritical conditions. In this work, the effect of the process parameters such as the antisolvent addition rate, temperature, solute concentration and agitation rate on Cholesterol particle size, size distribution morphology, and crystallinity was investigated.

The prerequisites for successful PCA process are the complete miscibility between the liquid solvent and the antisolvent and the insolubility of the solute in the antisolvent (or, rather, in the solution solvent–antisolvent formed in the precipitator). In general, Cholesterol particles produced using the PCA process had a needle like structure with a high aspect ratio, necessitating the careful analysis of particle size. As a check on the SEM photomicrograph based measurements, the particle size distributions of several samples were also evaluated using laser diffraction.

A. Effect of Antisolvent Addition Rate

In this set of experiments, the effect of antisolvent addition rate was investigated at different levels of carbon dioxide addition rate, namely, 30, 40, and 60 gms/min Figure 2. (c) shows the SEM photomicrograph of the particles generated at the lowest addition rate, i.e., 30 gm/min, where the particle size distribution was multimodal with a large degree of agglomeration, and a mean axial size of 88.6 μm. When the antisolvent addition rate was increased to 40 gm/min, a multimodal particle size distribution persisted with a moderate degree of agglomeration, but with a smaller mean axial size of 57.2 μm (Figure 1. (b)). It can also be observed that the primary particles as shown in Figure 2., have a needle- shaped morphology.

At the highest level of antisolvent addition rate (60 gm/min), the particle size distribution became much smaller, 46.6 μm with a narrower distribution (Figure 2. (a)). It is evident that increasing the antisolvent addition rate directly lowers the mean particle size.

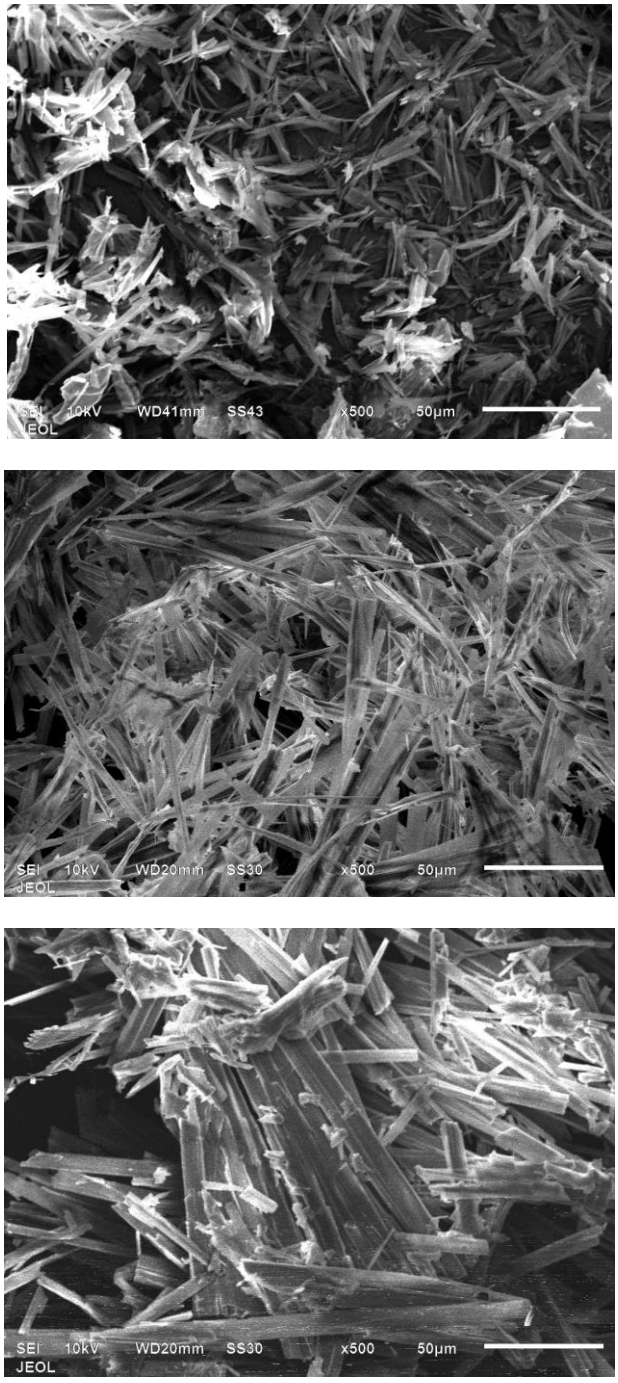

Fig. 2. SEM photomicrographs of Cholesterol produced by PCA showing the effect of increasing antisolvent addition rate: (a) 60 ml/min, (b) 40 ml/min, (c) 30 ml/min.

Theoretically, the magnitude of the supersaturation level is a strong function of the applied volumetric expansion rate. A faster rate of anti-solvent addition will generate higher levels of supersaturation, thus, higher levels of nucleation, and consequently, a larger number of smaller size particles with narrow particle size distribution. The obtained results are in agreement with this framework, as the mean particle size resulting from the fast expansion rate (60 gm/min), is far smaller than that resulting from the slow expansion rate (30 gm/min). In addition, the competition between the nucleation and growth dynamics of the PCA process explain the multiodal nature of the Cholesterol particles. Low addition rates enhance crossing of the critical supersaturation line between the nucleation zone and the metastable zone during volumetric expansion. This results in additional bursts of nucleation and growth.

B. Effect of Temperature

Figure 3. shows the SEM photomicrographs generated during experiments at 35 and 50°C. It is evident that the mean particle size increases and the distribution is multimodal.

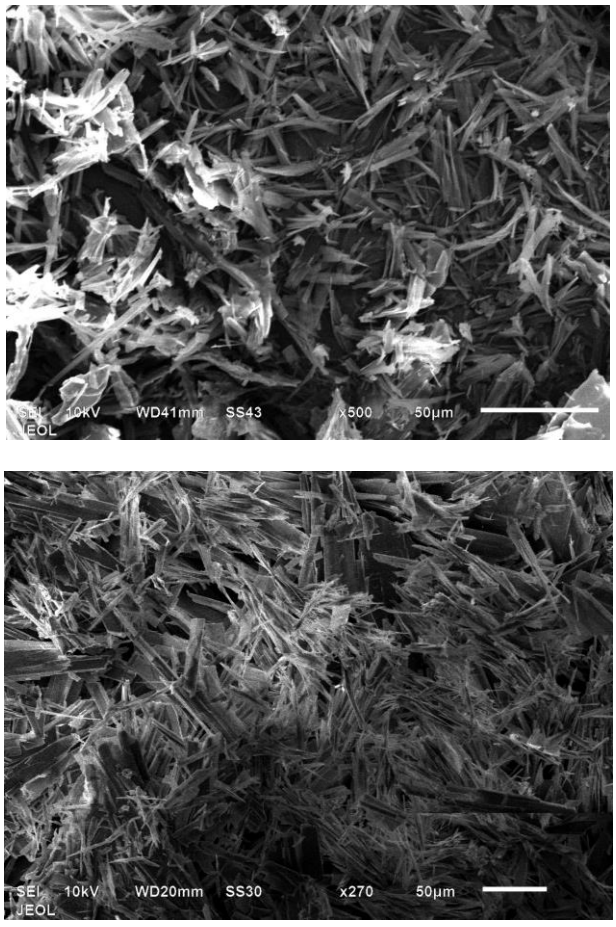

Fig. 3. SEM photomicrographs of Cholesterol produced by PCA showing the effect of increasing temperature: (a) 35 °C, (b) 50°C.

In this set of experiments, the effect of antisolvent addition rate was investigated at different temperature (35, 40, 45 and 50 °C). As illustrated in Figure 3., an increase in the temperature for the recrystallization process results in a direct increase of the Cholesterol mean particle size, and a broadening of the size distribution. It can also be observed

that the primary particles have a needle-shaped morphology with a large degree of agglomeration. Higher temperatures were found to increase the level of agglomeration.

The effect of temperature on the particle size can be explained in terms of the thermodynamic characteristics, and the nucleation-growth dynamics of the PCA process. Increasing temperature will increase the solubility of the pharmaceutical in the organic solvent, hence moving the position of the saturation and critical supersaturation lines upwards in addition to changing their shape [5].

Hence, increasing the temperature lowers the magnitude of the generated supersaturation during the PCA process (analogous to lowering the volumetric expansion rate) as the profile moves closer to the saturation line. This is followed by a gradual decline-depletion in the supersaturation as the nuclei grow, i.e., a high growth rate follows. This may lead to multiple crossing of the critical supersaturation line between the nucleation and metastable zones, resulting in increased multimodal behaviour. Thus, larger particle sizes with broad particle size distributions are expected.

IV. Conclusions

This work showed that the production of fine Cholesterol particles could be achieved by means of the PCA process. It was demonstrated that the particles mean size, and particle size distribution can be strongly controlled using the PCA process through the manipulation of the process parameters; antisolvent addition rate and temperature. The higher the antisolvent addition rate, the smaller the size of the generated particles and the narrower the size distribution. In contrast, the temperature exhibited the opposite effect, as the temperature was reduced the particle size and size distribution were lowered. Higher temperatures were found to increase the particles size and the level of agglomeration. The achieved experimental results necessitate further investigation for a better understanding of the theoretical underpinnings of the dynamics of the PCA process and how the process parameters influence the volumetric expansion profile of the liquid phase, thus the magnitude of supersaturation, and consequently the characteristics of the final product.

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