

Precipitation of fluticasone propionate microparticles using supercritical antisolvent

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ABSTRACT

Background: The ability of supercritical fluids (SCFs), such as carbon dioxide, to dissolve and expand or extract organic solvents and as result lower their solvation power, makes it possible the use of SCFs for the precipitation of solids from organic solutions. The process could be the injection of a solution of the substrate in an organic solvent into a vessel which is swept by a supercritical fluid. The aim of this study was to ascertain the feasibility of supercritical processing to prepare different particulate forms of fluticasone propionate (FP), and to evaluate the influence of different liquid solvents and precipitation temperatures on the morphology, size and crystal habit of particles.

Method: The solution of FP in organic solvents, was precipitated by supercritical carbon dioxide (SCCO₂) at two pressure and temperature levels. Effects of process parameters on the physicochemical characteristics of harvested microparticles were evaluated.

Results: Particle formation was observed only at the lower selected pressure, whilst at the higher pressure, no precipitation of particles was occurred due to dissolution of FP in supercritical antisolvent. The micrographs of the produced particles showed different morphologies for FP obtained from different conditions. The results of thermal analysis of the resulted particles showed that changes in the processing conditions didn't influence thermal behavior of the precipitated particles.

Evaluation of the effect of temperature on the size distribution of particles showed that increase in the temperature from 40 °C to 50 °C, resulted in reduction of the mean particle size from about 30 μm to about 12 μm.

Conclusion: From the results of this study it may be concluded that, processing of FP by supercritical antisolvent could be an approach for production of diverse forms of the drug and drastic changes in the physical characteristics of microparticles could be achieved by changing the type of solvent and temperature of operation.

Keywords: Supercritical fluids, Fluticasone, Precipitation, Microparticles.

INTRODUCTION

A successful formulation of particulate products depends on the physicochemical properties of the constituents, where powder flow, compression characteristics, physical stability and bioavailability are affected by physical properties of drug substances and formulation additives (1). Processes that allow particle engineering for pharmaceutical materials will enable scientists to design solid dosage forms tailored to possess optimal physicochemical attributes (2).

In the past decade, particle formation processes based on the use of SCFs, have been introduced as a viable means for control of crystal formation. The SCF state is the state attained by gases and liquids when subjected to temperatures and pressures above their critical parameters (T_c and

P_c). The SCF state exists as a single phase exhibiting low viscosity, low surface tension and high diffusivity, facilitating high mass transfer rates in comparison to liquids. Also SCF is highly compressible and as a result its density and thereby its solvation power are altered by careful control of temperature and pressure (3). Of many possible SCFs, CO₂ is the most widely used. It has low critical points ($T_c = 304.1$ K and $P_c = 7.38$ MPa), and as a process solvent offers additional benefits of being non-toxic, non-flammable, environmentally acceptable, inexpensive, and can be used at a mild critical temperature suitable for the processing of thermally labile compounds (4). The first known precipitation process based on supercritical technique is known as Rapid Expansion of Supercritical Solutions (RESS).

A component is dissolved in a supercritical fluid which expands rapidly through a nozzle. Due to this strong pressure and temperature drop, fast nucleation and growth of the crystalline particle occur (5).

In the case of polar compounds which are not soluble in supercritical fluids (particularly CO₂), SCFs could be used as antisolvent and in this process, a solution consisting of an organic solvent which has to be completely miscible within the SCF and a solid material dissolved in this solvent, is sprayed into a high-pressure vessel filled with SCF. This mixing process brings about an increase in the molar volume of the solution, and hence the solubility power towards the solute decreases and precipitation occurs (6). In a specific implementation of this process, the substrate solution and a stream of SCCO₂ can be introduced through a cocentric nozzle into the vessel for better dispersion of solution (7).

Fluticasone propionate (FP) is a potent glucocorticoid with a high topical anti-inflammatory activity and low systemic effects, widely used in the treatment of asthma by pulmonary delivery (8). The low solubility of FP in the SCCO₂ (9) and also formation of FP microparticles using supercritical antisolvent which in addition to the formation of "a non-homogenous mixture of some spherical particles" resulted in the formation of "small ribbons" (10,11), was reported previously.

On the line of assessment of potentials of supercritical processing for production of different forms of drugs, this study focused on the precipitation of FP microparticles from some ordinary organic solvents by supercritical antisolvent. Dichloromethan (DCM), methanol (MeOH) and acetone (ACE) were selected as solvents and in the presence of each solvent, particle formation process was performed at two pressure and temperature levels and the effects of operation parameters on the solid characteristics of FP were investigated. These specific characteristics are important factors to control technological and biopharmaceutical properties of drug products, particularly in the case of inhalation and intravenous dosage forms that require precise control of the particle size, shape, density and surface properties.

MATERIAL AND METHODS

Material

Micronized FP was kindly gifted by Cipla, India (purity of 99% and particle sizes below 5 µm). Carbon dioxide (purity of 99.5%) was supplied by

Daga, Iran. Organic solvents: DCM, purity of 99.5%; MeOH, purity of 99.5%; ACE, purity of 99.0% were purchased from Merck, Germany.

Methods

Particle production

A schematic diagram of the apparatus, used in this study, is shown in figure 1 and the details are presented elsewhere (12).

Particle formation process began by delivering CO₂ to the precipitation chamber to attain desired conditions which are listed in Table 1. Then in each experiment, pure solvent was delivered through the nozzle to the chamber for 5 min. At this time, solution of FP dissolved in DCM, MeOH or ACE in concentration of 5mg/mL was delivered through the nozzle at flow rates of 1 mL/min and CO₂ flow of 20 mL/min. During this process, particles were precipitated on a filter located at the bottom of the chamber and on the walls of the chamber. This stage was allowed to take place about 60 min or higher, to allow collection of adequate solid particles. The experiment was complete when the delivery of the drug solution to the chamber was interrupted. However, SCCO₂ continued to flow for 20 min to wash the chamber for the residual content of organic solvents. When the washing process was over, the CO₂ flow was stopped and the chamber was depressurized down to atmospheric pressure.

Particle size analysis

A small amount (about 10 mg) of each FP sample was dispersed in 5 mL of water with the aid of tween 80 and sonication in water bath (Starsonic 60, Italy) for 2 min. The size of the particle powders were measured by laser diffraction method (Mastersizer X, Malvern Instruments, UK) at obscuration between 0.18 and 0.20. Each sample was measured in triplicates. The size distribution was expressed by equivalent volume diameters at 10 (d_{10%}), 50 (d_{50%}) and 90% (d_{90%}) cumulative volume.

Scanning electron microscopy (SEM)

The morphology of unprocessed and processed particles was examined using SEM (CamScan MV 2300, England). Particles of representative samples were coated with gold-palladium at room temperature before any examination. The accelerator voltage for scanning was 25.0 kV.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra (KBr) were recorded with a spectrophotometer (Mega-IR, 550, Nicolet, USA) in the range 400-4000 cm⁻¹, using a resolution of 4.000 cm⁻¹ and 4 scans.

High-pressure liquid chromatography (HPLC)

The amount of FP in the samples was determined by a modified HPLC assay based on the European Pharmacopoeia monograph (13).

Thermal analysis

Differential scanning calorimetry (DSC) and Thermogravimetric analysis (TGA) were accomplished using a differential scanning calorimeter (Polymer laboratories, USA). Approximately 10 mg of the materials was placed in aluminum pans and analyzed under dry nitrogen purge. The temperature range was 25-400 °C. Calibrations in energy and temperature of the calorimeter were achieved by measurement of the fusion of pure indium.

RESULTS AND DISCUSSION

Crystallization of FP by supercritical antisolvent was performed in conditions created by changes in some experimental variables such as the type of solvent, the pressure and temperature. Generally, the success of supercritical antisolvent precipitation process depends on the solubility of the liquid solvent in the supercritical antisolvent and based on the fact that the solute is not soluble in the antisolvent. It also depends on the fast solubilization of the liquid due to the gas-like diffusion characteristic of SCF. This last characteristic is fundamental for assurance that small particles are obtained (14).

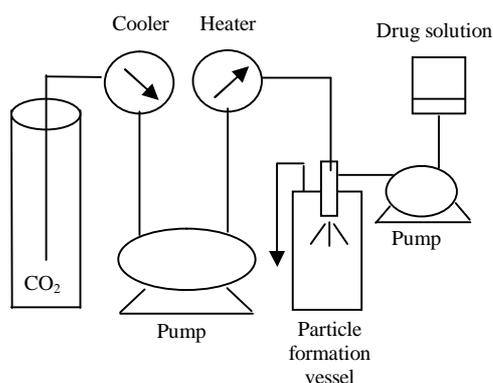


Figure 1. Schematic diagram of the apparatus

The solubility of FP in SCCO₂ is very low (9) and all of the organic solvents applied in this study are miscible with SCCO₂ (15). However, in the case of precipitation of FP by SCCO₂, at lower pressure (100 bar), the process was successful for particle formation when ACE and DCM were used as solvents. In contrast, injection of methanolic solutions of FP resulted in the

formation of little amounts of cubic particles that was not adequate for physicochemical characterization. The low yield of FP in this case could be referred to the increase of the drug solubility in the SCCO₂ - MeOH mixture due to the polar nature of MeOH and its greater potential for hydrogen bonding and dipole-dipole interactions (16).

As it has been presented in Table 1, no particle precipitation in the vessel was observed when the operations were accomplished at higher pressure (200 bar). The change in type of the solvent did not alter this finding. Dissolution of FP in continuous circulation of high pressure SCCO₂ could be the reason for this phenomenon.

Table 1. Summary of particle formation conditions

Run	Solvent	Temp. (°C)	Pressure (bar)	Yield (%)
1	ACE	40	100	43%
2	ACE	40	120	NP*
3	ACE	50	100	37%
4	DCM	40	100	46%
5	DCM	40	120	NP
6	DCM	50	100	40%
7	MeOH	40	100	<5%

*NP: No Particle Precipitation

Table 2. Particle size distributions of FP samples

Run	D _{10%} (µm)	D _{50%} (µm)	D _{90%} (µm)	Mode (µm)
1	9.13	32.76	186.31	31.45
3	4.63	12.51	85.37	12.22
4	7.97	31.98	119.86	24.38
6	3.22	13.60	80.54	10.94

Fig. 2 shows selected SEM images of FP before and after precipitation from different solvents. It is clear that the type of solvent strongly affected the morphology of particles strongly. The image of unprocessed FP showed aggregation of fine particles with irregular shapes and sizes less than 5 µm (Fig. 2A). When ACE was used as the solvent, the processed particles consistently exhibited tubular habit in a regular shape (Fig 2B) and FP precipitated from DCM resulted in the formation of flake-like particles (Fig 2C). Different particulate habits could be the result of interaction between supercritical antisolvent and organic solvent which are different in isothermal compressibility. This may lead to variation in mechanism of supersaturation, nucleation, and

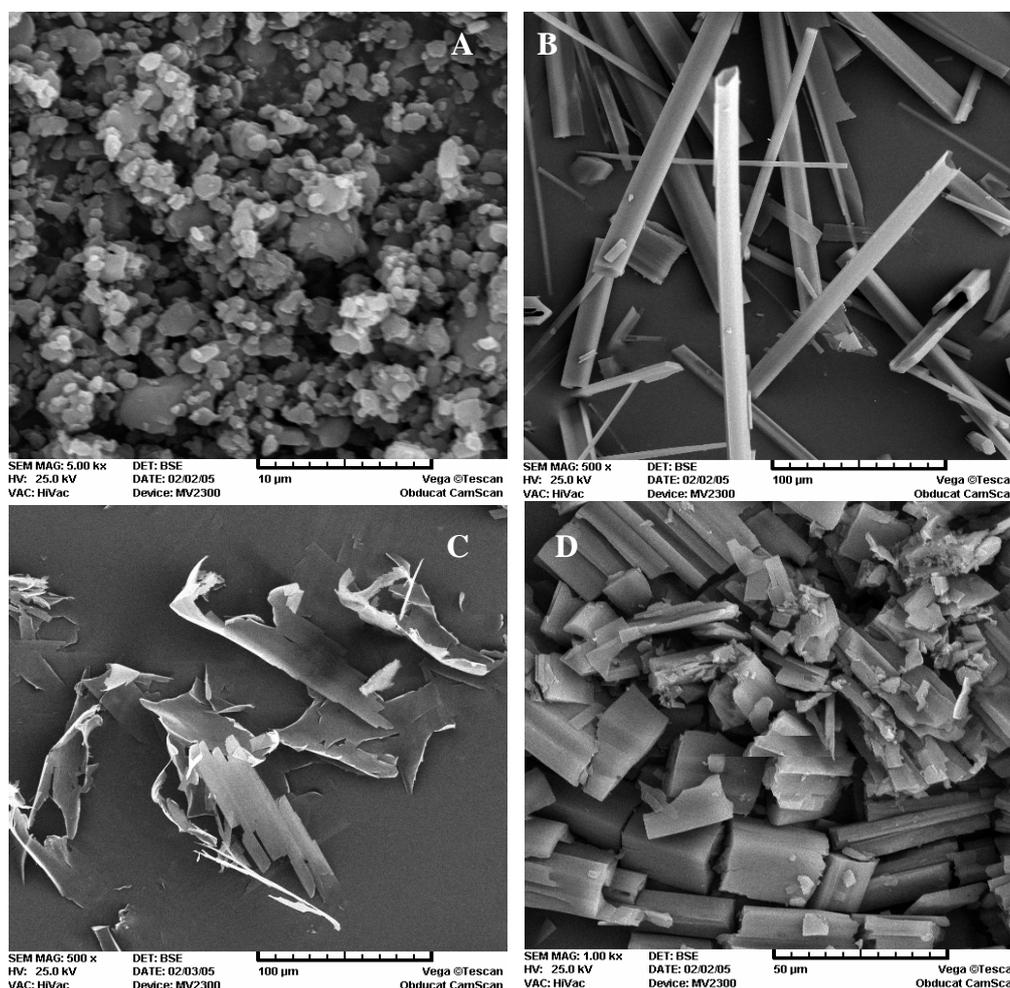


Figure 2. SEM images of FP: A) unprocessed FP, B) processed in the presence of acetone (run 1), C) processed in the presence of dichloromethane (run 4), D) processed in the presence of MeOH (run 7)

crystal growth during the mixing process of solutions and antisolvents. Therefore, production of crystals of different morphologies and physical properties can be made possible by change in the type of solvents and antisolvent (17,18). ACE and DCM are completely miscible with SCCO_2 , but there are different vapor-liquid equilibria (VLE) and volumetric expansion for these solvents in the presence of SCCO_2 and variation in particulate habits of processed FP could be related to differences in behavior of two solvents (19,20). While the use of non-spherical particles have been traditionally avoided due to the potential problems of powder flow, preparation of FP powders with elongated particles may provides a means to achieve selective delivery such as respiratory routes (21,22). SEM images indicate that FP crystals have better appearances with clean surfaces when compared with the unprocessed materials. This means that the process provides suitable environment for the

solid growth of a single crystal, where the conditions for growth-related imperfections and solvent occlusion into the crystal faces are minimized.

On the other hand, the state of the crystal surface may be an important factor influencing the rate of drug release (23). The smooth surfaces of processed particles show that the processed crystals may provide the possibility of improving the drug dissolution properties. However, the possibility of using the FP microparticles for drug delivery requires further studies.

The physical appearance of particles does not reflect the internal structure of a crystal. A crystal that has an identical internal space lattice may show different crystal habits (17). The DSC is a technique which is used to measure the temperature and energy variation involved in the phase transitions, and reflects the degree of crystallinity and stability of the solid state of pharmaceutical compounds (24). Fig. 3 shows the DSC and TGA

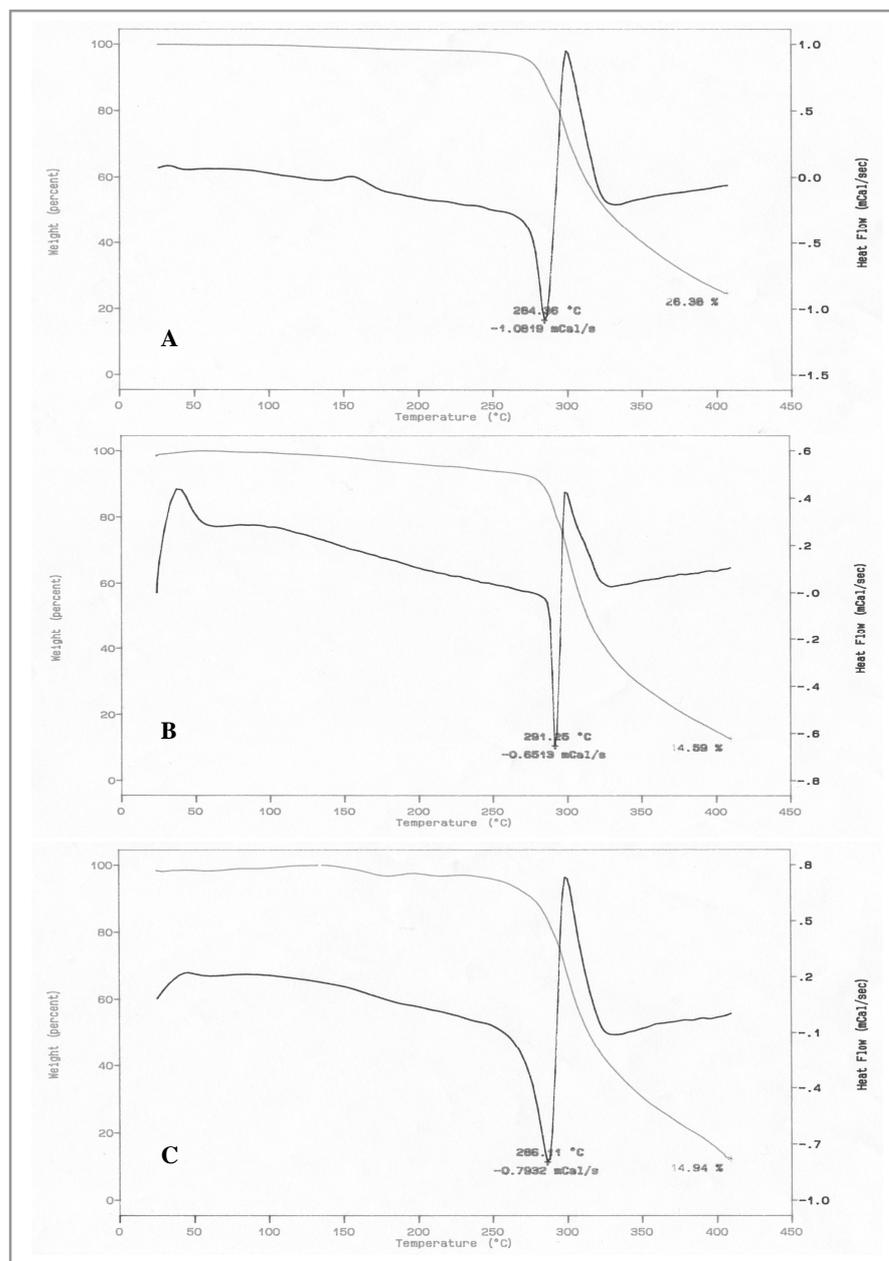


Figure 3. DSC and TGA thermograms of FP: A) unprocessed FP, B) processed in the presence of dichloromethane (run 4), C) processed in the presence of acetone (run 1).

curves for the unprocessed and processed FP particles. The result showed that variation in the processing conditions apparently didn't influence the thermal behavior of the precipitated particles. All three samples exhibited endothermic behavior at 285-290 °C when melting took place. Also the curve of the unprocessed FP showed an exothermic transition around 150 °C, which could be as a result of crystallization of amorphous particles which are formed during the mechanical milling process (25).

The crystallization temperature had practically no effect on the morphology of FP particles within the temperature ranges which was investigated. However, particle size of the processed samples was considerably dependent on the temperature. Evaluation of the size distribution of particles by laser light scattering (Table 2) showed that increasing temperature from 40°C to 50°C, reduced the mean particle size from about 30 µm to about 12 µm. Although particle size analysis by laser raction could be ideally used for spherical

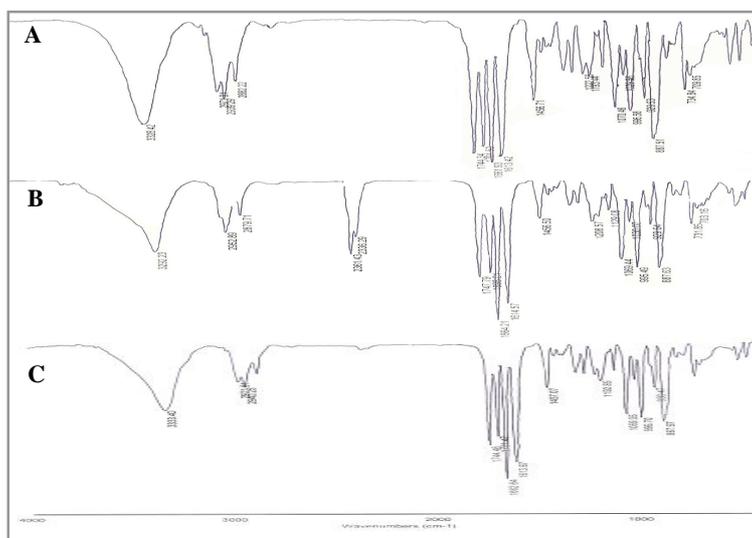


Figure 4. FT-IR spectra of FP: A) unprocessed FP, B) processed in the presence of acetone (run 1), C) processed in the presence of dichloromethane (run 4).

particles, assessment of this results and SEM images would be an acceptable estimation about the effect of process temperature on the size of produced particles. Possibly, it could be supposed that the rate of supersaturation and crystal growth, which is directly related to the temperature of operation, have a great impact on the control of the size of FP particles (18).

In the IR spectra which have been presented in Fig. 4, similar absorption peaks could be in the finger print regions (right side of graphs) with comparable peak numbers. Only an absorption peak in the graph B around 2300 cm^{-1} is different which could be related to the presence of excess air (containing CO_2) in the IR chamber. In general, it could be supposed that the precipitation process has not affected the chemical structure of FP. Quantification of more than 96% of the drug by HPLC analysis in processed samples confirmed this finding.

CONCLUSION

Particle engineering by supercritical carbon dioxide and numerous operative parameters in these processes provides an attractive and efficient procedure to design a variety of particulate and had considerable effects on characteristics of the precipitated particles. Changing the physical characteristics of FP particles could be a preliminary study to find more efficient particles for drug delivery. In this way, evaluation of other operative parameters such as flow rate and concentration of drug in solution, flow rate of SCCO_2 and nozzle properties could be proposed.

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