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Precipitation of Pioglitazone Hydrochloride Microparticles by Supercritical Anti-Solvent (SAS)

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Authors' contributions

This work was carried out in collaboration between all authors. Author SMR performed the study. Author KK wrote the protocol and wrote the first draft of the manuscript. Author AV designed and managed the experimental process and analyses of the study. Author MH performed bibliography. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The purpose of this research was to evaluate the effects of supercritical precipitation on the physicochemical characteristics of Pioglitazone hydrochloride (PG). Therefore, PG powder was dissolved in methanol, ethanol, acetone, dichloromethane and binary mixtures of these solvents and the solutions were sprayed into the supercritical carbon dioxide. The processed powders were harvested then analyzed by using scanning electron microscopy (SEM), differential scanning calorimetery (DSC) and Infrared Spectroscopy (IR) methods. Different morphologies of PG particles were observed in the SEM micrographs and the particle sizes varied between 37.1 µm and 95.1 µm. The IR spectroscopy showed no changes in molecular structure of drug during the process. Also, the DSC thermograms confirmed the similar thermal behavior of PG before and

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after processing. The dissolution rate of unprocessed and processed powders was compared and showed enhancement in the dissolution rate of processed powders up to 2 times.

Keywords: Supercritical fluid; microparticles; pioglitazone hydrochloride; SAS process.

1. INTRODUCTION

The solubility is a fundamental challenge for bioavailability enhancement and normal absorption pattern in pharmaceutical research. According to the statistics, about 30% of the drugs listed in the United States Pharmacopeia are poorly water-soluble or insoluble and a majority of new drug developments have been failed because of poor biopharmaceutical properties and solubility [1,2].

In addition to the manufacturability of solid dosages, the dissolution rate which is proportional to surface area of drugs, is largely dependent on particle size distribution of drug and is improved by the reduction of particle size. This is particularly important for drugs in Biopharmaceutical Classification System Class II (low solubility; high permeability) as the bioavailability is typically governed by drug dissolution in this category [3,4]

Numerous conventional techniques such as crushing, grinding, milling spray drying, freeze-drying and recrystallization of the solute particles from solutions using liquid anti-solvents have been used for particle size reduction. But there are a number of obstacles including instability under milling conditions, residual solvents of recrystallization process, thermal and chemical degradation of products due to high temperatures, high-energy requirements, large amounts of solvent, solvent-disposal problems and broad particle size distributions [5]. To overcome the mentioned drawbacks, the use of supercritical fluids (SCFs) has increased rapidly over the last two decades and several processes for particle formation have been studied due to the flexible operating condition for controlled particle size and size distribution as well as mild operating temperature [6]. Among different possible compounds, carbon dioxide is most widely used SCF because it is inexpensive, nonflammable, chemically stable, nontoxic and can be completely removed from any extract [7]. By changing pressure and/or temperature above the critical point of carbon dioxide (T_c = 31.3ºC, P_c = 72.8 bar) solvation power of supercritical CO_2 (sc-CO_2) can be adjusted by any changes in the density and dielectric constant [8,9].

There are several sc-CO_2 processes for particle design that can be classified into two major categories depending on whether the sc-CO_2 is used as a solvent or an anti-solvent. Anti-solvent precipitation processes generally include the dispersion of drug solution that was dissolved in an organic solvent, through a nozzle and into a continuous supercritical anti-solvent (SAS) phase which generally sweeps the vessel [10].

Pioglitazone hydrochloride (PG) is an oral anti-hyperglycemic agent uses in the treatment of type 2 diabetes (Fig. 1). PG exerts its glucose-lowering effects by binding to peroxisome proliferator activated receptors gamma (PPARγ) and increasing the receptor sensitivity to insulin [11].

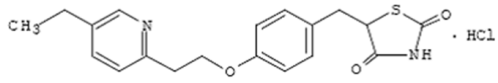

Fig. 1. Chemical structure of PG

The aqueous solubility of PG free base was investigated by Seedher and Kanojia in water, surfactant containing solutions and as a function of pH in buffer solutions. They reported low solubility of drug in water and aqueous solutions of sodium dodecyl sulfate, cetyltrimethylammonium bromide and polysorbate [12].

On the line of supercritical processing for fine particles production, this study focused on the precipitation of PG microparticles from some ordinary organic solvents using Solution Enhanced Dispersion by Supercritical Fluids (SEDS) method as an anti-solvent technique. Therefore, dichloromethane (DCM), methanol (MeOH), acetone (ACE) and ethanol (EtOH were selected as solvents and the effects of operation parameters on the solid characteristics were analyzed.

2. MATERIALS AND METHODS

2.1 Material

Pioglitazone hydrochloride was kindly gifted by Osveh Co. Iran. Methanol, ethanol, dichloromethane and acetone of analytical grades were purchased from Merck Co., Germany. CO_2 of high purity (>99.9%) was purchased from Sabalan Co. Iran. All chemicals were used without further purification.

2.2 SEDS Particle Formation Method

A schematic diagram of the apparatus, used in this study and the details were presented elsewhere [12]. The experiments began by delivering CO_2 after passing it through a cooling device to the precipitator (vessel with 0.4 L internal volume) by a syringe pump until the desired pressure was achieved. The precipitator was placed in an equipped oven for exact temperature regulation. The gas entered the precipitation vessel through a concentric nozzle placed in the upper side of the precipitator. This nozzle led to co-introduction of sc-CO_2 and drug solution into the precipitator and improvement the dispersion of droplets. The experiments started by entering CO_2 to the precipitator until the desired pressure (250 bar) was attained. Then pure solvent was delivered through the nozzle to the chamber for 2 min. At this point, PG solution was dissolved in DCM, MeOH, EtOH or ACE in concentrations of 20 mg/mL and was delivered through the nozzle at flow rates of 0.5 and CO_2 flow of 20 mL/min. Precipitated particles were collected on a filter located at the bottom of the chamber and on the walls of the chamber. This stage was allowed to take place in about 30 min or higher, in order to collect of adequate solid particles. The end point of the experiment pertained to the interruption of the drug solution to the chamber. However, sc-CO_2 continued to flow for 5 min to wash the chamber from residual methanol. When the washing process was completed, the CO_2 flow was stopped and the chamber depressurized down to atmospheric pressure. The effects of variables including type of solvent and temperature on particle characteristics were evaluated. Table 1 presents the varying parameters and their bonds.

2.3 Scanning Electron Microscopy (SEM)

The morphology characterization of unprocessed and processed particles was analyzed using SEM (Hitachi, S-4160, Japan). The samples were coated with gold in an argon atmosphere using a sputter coater. The accelerator voltage for scanning was 25.0 kV.

2.4 Particle Size Measurement

Approximately, 5 mg of each PG sample was dispersed in 5 ml water by the aid of tween® 80 and bath sonication (Starsonic 60, Liarre, Italy) for 2 min. The volumetric equivalent diameter of particles was measured by laser diffraction method (Mastersizer X, Malvern Instruments, U.K.) at obscuration between 0.18 and 0.20. Each sample was measured in triplicate. The size distribution was expressed by equivalent volume diameters at 10 ($d_{10\%}$), 50 ($d_{50\%}$) and 90% ($d_{90\%}$) cumulative volume.

2.5 Fourier Transforms Infrared Spectroscopy (FT-IR)

FT-IR spectra were recorded with a spectrophotometer (Mega-IR, 550, Nicolet, USA) in the range of 400-4000 cm^{-1}, using a resolution of 4.000 cm^{-1} and 4 scans. Samples were diluted with KBr at concentration of 1% and pressed to obtain self-supporting disks.

2.6 Thermal Analysis

Differential scanning calorimetery (DSC) was accomplished using a differential scanning calorimeter (Polymer laboratories, USA). Approximately 5 mg of the materials were placed in aluminum pans and analyzed under dry nitrogen purge. The temperature rang was 25ºC to 400ºC. Calibrations in energy and temperature of the calorimeter were achieved by measurement of the fusion of pure indium.

2.7 Dissolution Studies

Dissolution rate of PG was assessed in distillated water (pH 7.0, 500 mL) using a flask with stirring speed of 100 rpm at temperature of 37±0.5ºC.

Aliquots of 4 mL were withdrawn manually at 10, 15, 20 and 30 min, filtered (0.45 µm syringe filter, Sartorius Corp. Germany), suitably diluted (add 4 mL of methanol). The same volume of medium at 37±0.5ºC was replaced for constant volume. Samples were analyzed by spectrophotometry at 235 nm. The UV spectrophotometric method was developed and validated in our laboratory.

Table 1. Levels of variables in the formation of PG microparticles using SEDS

Run number	Solvents	Solvent ratio	Temperature (°C)
F1	DCM-MeOH	50-50	45
F2	DCM-MeOH	50-50	55
F3	DCM-EtOH	50-50	45
F4	DCM-EtOH	50-50	55
F5	ACE-MeOH	50-50	45
F6	ACE-MeOH	50-50	55

3. RESULTS AND DISCUSSION

PG was recrystallized using SAS method and the effects of experimental variables on improvement of the physical characteristics of the particles were studied. Generally, the success of supercritical precipitation process relies on the solubility of the liquid solvent in the SAS and the fact that the solute is not soluble in the anti-solvent [13]. In addition, it depends on the fast solubilization of the liquid due to the gas-like diffusion characteristic of SCF. This last characteristic is a fundamental feature for assurance that small particles are obtained. It should be mentioned that all of the organic solvents applied in this study were miscible with sc-CO_2 [14].

Due to the relatively low solubility of PG in acetone (ACE), dichloromethane (DCM), methanol (MeOH) and ethanol (EtOH), binary combination of these solvents were applied for higher solute solubility. The drug was dissolved in the mixture of solvents and then the solution was continuously sprayed through a nozzle into the sc-CO_2. As a preliminary test, it was realized that when the flow rate was set at 5 mL/min, obtained particles were too large (approximately millimeter). Thus, the flow rate was reduced and fixed at 0.5 mL/min for all of experiments. In addition, the concentration was increased to 20 mg/mL, assuring that experiment yield was relatively acceptable.

Fig. 2 shows SEM images of unprocessed PG and selected samples of processed particles in runs F_5 and F_6. According to the micrograph of unprocessed PG, there was a mixture of particles with different sizes that bridged together and aggregation of fine particles was considered. It can be observed that the SEM photographs of the F_5 represented relatively irregular and semi spherical morphology, while this was changed to tabular particles in F_6. So by altering the solvent mixture from ACE/MeOH to DCM/MeOH, morphology was changed and produced particles appeared to be aggregated. The diffusion coefficient of the sc-CO_2 at this pressure is comparable to the high volumetric expansion of solvents, causing a fast diffusion of CO_2 into the solvent and vice versa and a fast precipitation. As a result, particles were not precipitated individually and a partial bridging of particles occurred [15].

The results of the particle size analysis were presented in Table 2. It clearly indicated a reduction in particle size of PG after precipitation by sc-CO_2 and the mean particle size ranged from 37.1 μm to 95.1 μm. ACE and DCM were the solvents with the higher solubility in sc-CO_2 compared with MeOH and EtOH. This difference in the solvation power could be the reason for the changes in the particle size. The level of supersaturation in the mixture of MeOH-ACE was less than MeOH-DCM. Higher vapor pressure of DCM led to faster diffusion of solvent and sc-CO_2 in comparison with ACE consequently higher nucleation rate and finer particles were produced [16,17]. On the other hand, it was assumed that two competing phenomena exist in the particle formation as temperature changes. Temperature enhancement causes to lower sc-CO_2 density and higher diffusivity. Therefore, solute solubility in sc-CO_2 decreases and supersaturation will be reached faster. These phenomena lead to particle size reduction [18]. On the other hand, solubility of drugs in organic solvents increases by temperature enhancement and consequently, the supersaturation will be attained slower and the particle sizes will growth [19]. In the case of PG, enhancement in operating temperature from 45ºC to 55ºC, led to mean particle size reduction that could be attributed to the solute solubility reduction in sc-CO_2, while particle size distribution became narrower. Similar results have been published for salbutamol sulphate particles [14] and minocycline hydrochloride [20].

DSC is a technique applies to measure energy and variation involved in phase change. It also reflects the degree of crystallinity and solid state stability of pharmaceutical compounds [21]. Fig. 3 illustrated the DSC curves for the unprocessed (A) and processed (B) PG particles. It showed the heat flow with temperature plot of PG particles and the diagrams were almost similar, suggesting that the solid states of particle crystals did not change dramatically after the supercritical processing. The mild shift between two peaks could be related to the particle size.

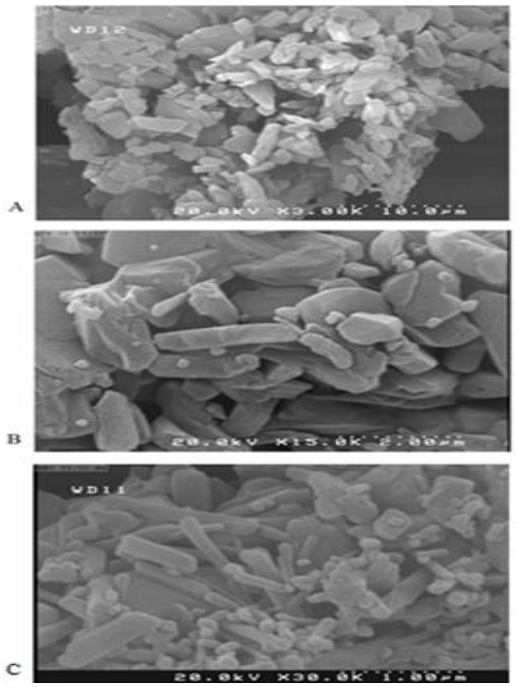

Fig. 2. SEM images of PG: A) unprocessed particles, B) processed at the conditions of run (F_5), C) processed at the conditions of run (F_6)

FT-IR analysis was performed to determine whether there was any difference in the structures of the unprocessed PG and that obtained from the precipitation process. In the IR spectra which were presented in Fig. 4, similar absorption peaks could be observed in the finger print regions (right side of graphs) with comparable peak numbers. Thus, it could be deduced that there was no variation in the molecular structure of the PG before and after processing.

The dissolution profiles of the F_4 sample as processed particles along with unprocessed one were shown (Fig. 5). Processed PG dissolution rate was higher than unprocessed PG that showed a poor dissolution profile and only 30% of drug was released at first 30 min while 55% of processed one was released at the same time. The enhanced dissolution rates of processed PG may be due to many factors such as decreased particle size of drug [22].

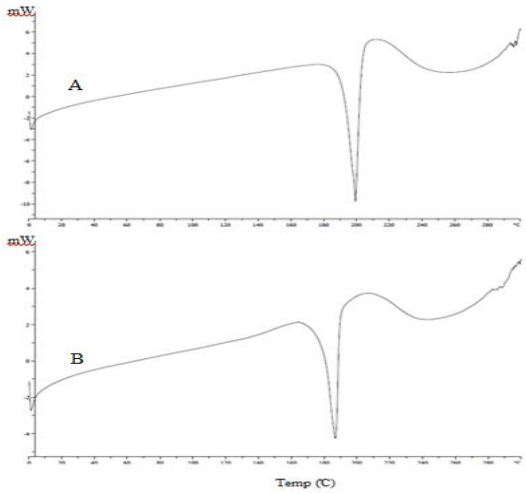

Fig. 3. DSC thermograms of PG: A) unprocessed PG, B) processed at the conditions of run F_1

Table 2. Particle size distribution of PG samples

Sample	$D_{10\%}$ (µm)	$D_{50\%}$ (µm)	$D_{90\%}$ (µm)	Mean particle size (µm)
unprocessed	20.58	156.05	450.05	195.74
F1	9.06	57.36	321.13	39.94
F2	8.72	48.23	231.32	63.83
F3	4.97	33.59	193.55	63.62
F4	3.03	24.94	75.64	37.11
F5	8.72	48.23	231.32	95.15
F6	13.11	71.48	198.54	91.45

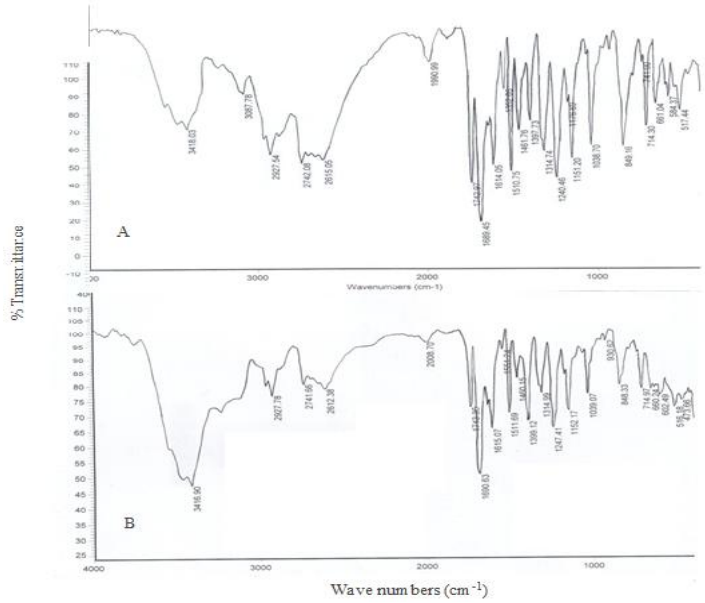

Fig. 4. FT-IR spectra of PG: A) unprocessed, B) processed at conditions of run F_4

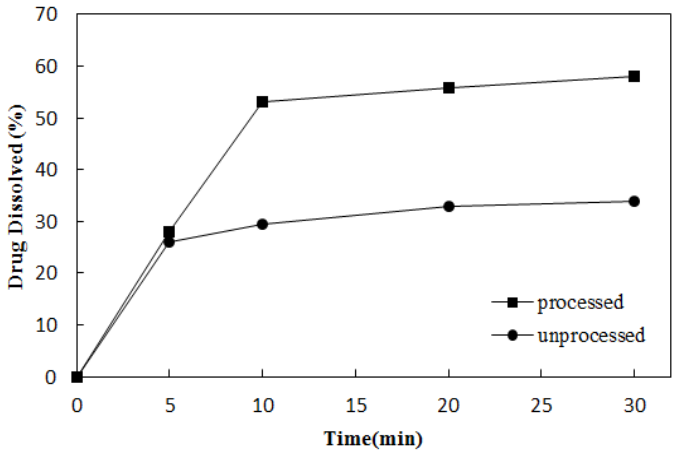

Fig. 5. Dissolution profiles of processed and unprocessed pioglitazone

4. CONCLUSION

It was demonstrated that size and surface properties of particles play a critical role in the behavior of active ingredients in a variety of pharmaceutical solid dosage forms. Considering the results from analyzing the particle size by scanning electron microscopy and physicochemical characterization by FTIR and differential scanning calorimetry, it appeared that this supercritical processing method was influential in the preparation of PG microparticles. The results depicted a significant change in morphology and particle size at various solvent mixtures and different temperatures. Solvation power of the solvent with higher solubility in sc-CO_2 decreased faster in the presence of sc-CO_2, therefore, faster supersaturation and subsequently smaller particles were formed.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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