Supercritical Antisolvent Precipitation of Piroxicam Systems: Preliminary Experiments.

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Abstract

The purpose of this study is to investigate the influence of supercritical CO_2 on the physicochemical properties of Piroxicam (PXC), a poorly soluble drug. The supercritical anti-solvent (SAS) technique was used to precipitate the drug at the pressure of 100 and 120 bar from three different solvents (acetone, ethylacetate and dichloromethane) to study how they would affect the final product. The samples were analyzed before and after the treatment to highlight possible changes in the habitus of the crystals. The solid state analysis of both samples untreated and treated with CO_2, showed that the applied method caused a decrease of the crystallite size and transition to the pure α form resulting in needle-shaped crystals, regardless of the chosen solvent. In order to identify which process was responsible for the above results, Piroxicam was further precipitated from the same three solvents by traditional evaporation method (RV-samples). On the basis of this cross-testing, the solvents were found to be responsible for the reorganization into a different polymorphic form, and the potential of the SAS process to produce needle shaped particles in the micron range, with an enhanced dissolution rate compared to the RV-Piroxicam, was ascertained.

Keywords: Supercritical CO_2 ; SAS; Piroxicam; Polymorphism

INTRODUCTION

Supercritical fluids (SCF) processes have been widely used to crystallize various organic and inorganic compounds [1]. Particle formation of pharmaceutical compounds is a particularly promising area in which the SCF process could be partially commercialized.
The use of carbon dioxide (CO_2) is one of the major advantages in the SCF process, as CO_2 is nontoxic and presents mild critical conditions, making it an ideal substitute for organic solvents. Moreover, CO_2 is gaseous at ambient conditions, which simplifies the problem of solvent residues [2]. SCF processes using SC-CO_2 have been widely used in the pharmaceutical arena with two objectives: micronization and modification of solid state characteristics.
The SAS process is a one-step process that facilitates control of particle formation characteristics and direct formation of dry and fine particles through an increased rate of mass transfer.
In recent years, the modification of solid state characteristics such as crystal habit, crystallinity, and polymorphism has gained increasing attention in pharmaceutical research and has been successfully achieved through the recrystallization of drug particles using various SAS processes [3-9]. In particular, SCF process operating parameters can be adjusted to vary supersaturation and conditions for nucleation and crystal growth across a wide range. Accordingly, the different crystalline forms of a compound in which the molecules have

different arrangements and/or conformations can be controlled to exploit compound polymorphism using the SAS process. Polymorphism is important in pharmaceuticals because different polymorphic forms usually exhibit different physicochemical properties, including melting point and solubility [10].
Piroxicam is a nonsteroidal anti-inflammatory drug (NSAID), licensed for acute and long-term use in the relief of signs and symptoms of osteoarthritis and rheumatoid arthritis but not analgesia due to its delayed onset of pain relief. According to the Biopharmaceutical Classification System (BCS), it is a class II drug, characterized by low solubility and high permeability which mainly displays dissolution-dependent oral bioavailability [11]. Indeed, the reported delay in the onset of pain relief is mainly due to the poor water soluble nature of the drug rather than to a particular pharmacological effect.
The aim of this study is to investigate the solid state characteristics of Piroxicam by varying pressure and solvent in SAS operating conditions. We particularly focused our attention on the resulting polymorphisms. The physicochemical characteristics of the final Piroxicam particles obtained through the SAS process were studied and compared with polymorphs already described in the literature [12] and obtained with rotavapor (RV) technique.

MATERIALS AND METHODS

Piroxicam and CO_2 (purity 99.9%) were purchased from Galeno (Italy) and Siad (Italy) respectively. All other chemicals were of reagent grade and used without further purification.

Rotavapor Method

PXC was added to the appropriate solvent in a round bottom flask under stirring. The solvent was then removed under reduced pressure in a rotary evaporator (Buchi R-114, Flawill, Switzerland) at 50±1 °C. Before the characterization, samples were kept for 3 days in a desicator under vacuum at room temperature.

SAS Method

Volumetric expansions of acetone, ethylacetate (EtAc) and dichloromethane (DCM) were checked before using them for further SAS experiments.
A schematic diagram of SAS equipment used in this study is reported in Figure 1.

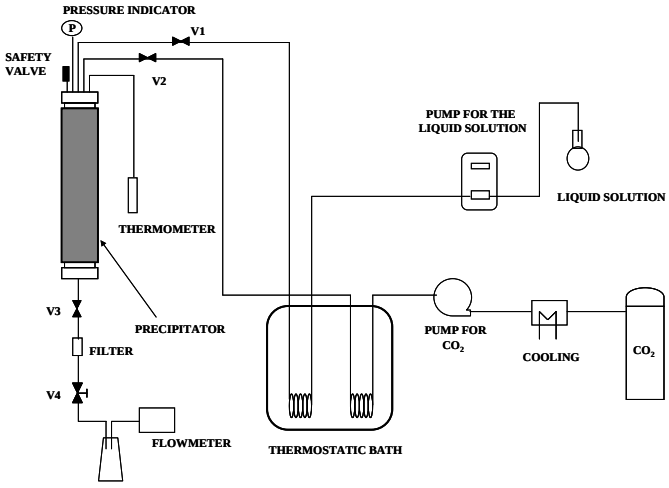

Figure 1: pictorial representation of the SAS apparatus used in the present study

The precipitator (AISI-316 steel, internal diameter and volume of 50 mm and 400 cm^3 respectively) was jacketed ensuring temperature to be kept within 40 ± 0.5 °C. the sample solutions, kept at the precipitator temperature by an electric heat plane, were pumped

(ConstaMetric® 3200 P/F) to the top of the precipitator and then sprayed through a nozzle with a diameter of 100 µm. liquid CO_2 was fed from the top of the precipitator by a high pressure pump (Lewa EK-M-210V1). The outlet flow was then filtered (0.22 µm) to prevent precipitate losses and regulated by a heated metering valve (Whitey SS-21RS4).
Temperature and pressure values in the precipitator were measured by a Delta OHM thermometer (HD 9214, ± 0.1 °C) and a DRUCK pressure transducer (DPI 260, ± 0.1 bar). The precipitator was filled with CO_2 to the experimental pressure; then the solution and CO_2 were pumped to the reactor at constant flow. The experiments were performed using a solution flow rate of 2 mL min^{-1}. The CO_2 and solution flow rate ratio was 10 mL min^{-1}. After spraying the precipitate was washed with approximately 5 l of CO_2 before its collection. The pressure and temperature were 100 and 120 bar at 40 °C. Saturated solutions were used for SAS precipitations.

Characterization of the drug

Differential scanning calorimetry (DSC)

Calorimetric analysis was performed with a DSC mod. TA 4000 (Mettler, Greifensee, Switzerland), equipped with a measuring cell DSC 20. Samples, containing a fixed amount of NMS, were placed in pierced aluminum crucible and heated at a scanning rate of 10 °C per min from 30 to 220 °C under air atmosphere.

Hot stage Microscopy (HSM)

HSM observations of morphological features and changes during heating in the samples were monitored using a hot plate (FP 52 Mettler, Greifensee, Swiss), connected to a temperature controller (FP 5 Mettler). An adequate amount of each sample was placed on a glass slide and heated at 10 °C min^{-1} in the temperature range 30-220 °C.The behaviour of the samples was observed via an optical microscope (Reichert Biovar, Wien, Austria) (magnification 100 x).

Powder X-ray diffraction studies (PXRD)

PXRD studies were done using a STOE D500 (Siemens, Monaco, Germany) diffractometer with Cu Kα radiation (λ = 1.5418 Å), monochromatised by a secondary flat graphite crystal. The scanning angle ranged from 3° to 40° of 2 θ, steps were of 0.05° of 2 θ, and the counting time was of 5 s/step. The current used was 20 mA and the voltage 40 kV.

Diffuse reflectance infrared Fourier transform (*DRIFT*) *spectroscopy*

DRIFT spectra were obtained using a FT-IR spectrometer (FT-IR 300 Jasco, Tokyo, Japan) and dispersed in KBr. An average of 20 scans for each sample were collected at 4 cm^{-1} resolution in the range 4000-400 cm^{-1}.

Determination of drug dissolution

Profiles of Piroxicam release were obtained according to the USP 32 paddle method: 100 rpm, 900 mL of phosphate buffer (pH 1.2 and pH 7.4), T= 37 ± 0.1 °C, sink conditions (C<0.2 Cs). The aqueous solution was filtered (0.45 µm porosity) and continuously pumped to a flow cell in a spectrophotometer and absorbance were recorded at 334 and 354 nm for pH 1.2 and 7.4 respectively. The composition of the dissolution media was 0.2 M NaCl/0.2 M HCl (pH 1.2) or 0.2 M KH_2PO/0.2 M NaOH (pH 7.4) according to USP. Experimental points were the average of at least three replicates, and standard deviations did not exceed 5% of mean value. Dissolution profiles were compared to that of the pure and RV crystallized drug.

RESULTS

Solubility of PXC in supercritical CO_2 at 40 °C ranges between $5 \cdot 10^{-6}$ and $5 \cdot 10^{-5}$ mole fraction at 100 and 200 bar respectively [13]. Solubility of PXC in the different organic solvents was determined using turbidimetric method and is reported in Table 1.

Solvent	mg/ml PXC
Acetone	17
Ethylacetate	11
Dichloromethane	50

Table 1: Solubility of Piroxicam in the considered organic solvents

Rotavapor was used for the recrystallization of PXC from the different saturated solvents.
According to Kordikowski et al. [5], dichloromethane, acetone and ethylacetate show the highest expansion capacity at the tested temperature, and were therefore chosen as solvents for precipitation experiments. The precipitation of PXC from the selected solvents gave a fluffy, voluminous powder and characterized by the presence of long needles, as typical of CO_2-precipitated samples [14-18].
As reported in Figure 2 the thermogram of starting PXC shows two endothermic peaks at about 198 and 202 °C attesting the presence of two crystal forms, reported in literature as α and β respectively [12]. After the rotavapor recrystallization in DCM or Acetone, PXC changes to pure β form, while the drug in EtAc changes to α.
All the SAS precipitation samples show an α form of the drug also characterized by a low enthalpy of fusion (ΔH) than starting drug and RV-samples.

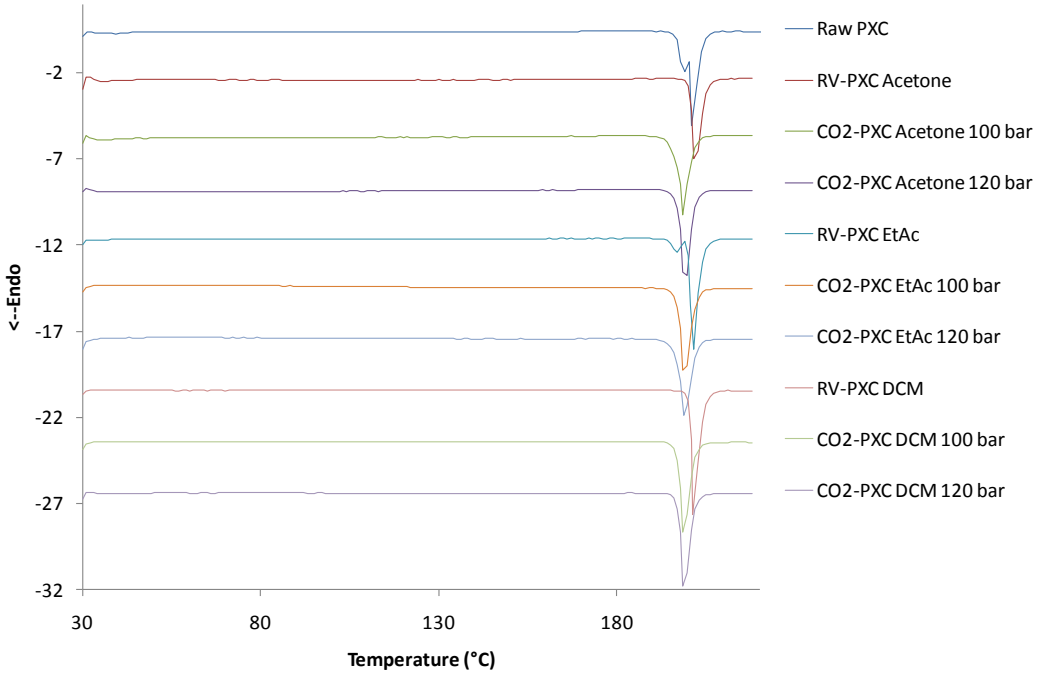

Figure 2: DSC thermograms

These data were confirmed by HSM and Figure 3 shows the different morphology of the drug. For brevity are reported only the β form and α form of PXC obtained with the RV and SAS (at 100 bar) processed with Acetone.

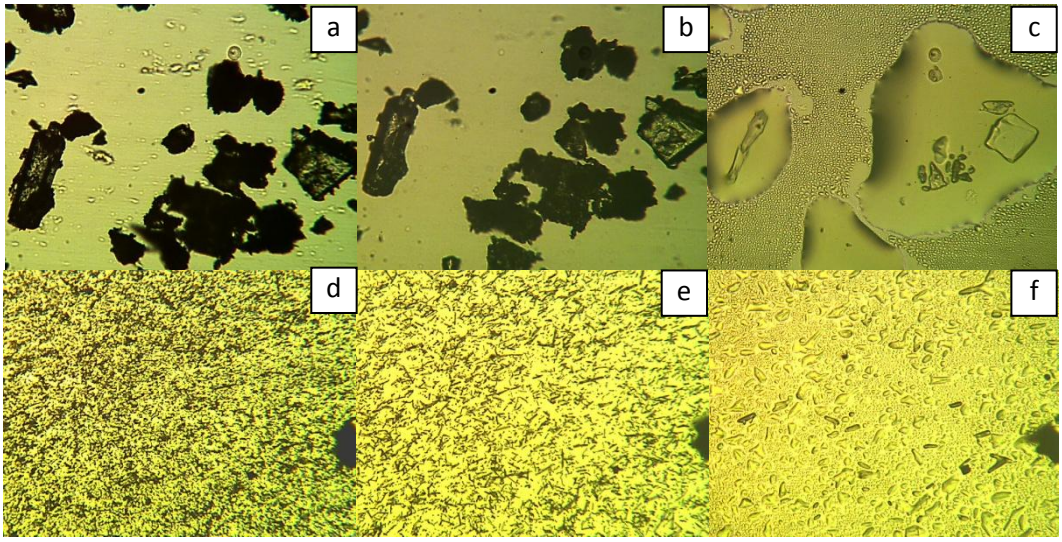

Figure 3: microphotographs of RV-PXC recrystallized from Acetone at 30 °C a),195 °C b), 201 °C; CO_2-PXC processed with Acetone at 100 bar at 30 °C d), 195 °C e), 198 °C f).

The XRD patterns of the treated and untreated samples are reported in Figure 4.
Commercial PXC diffractogram presented high intensity peaks at: 8.96, 10.1, 15.14, 15.75, 16.21, 23, 25.8 of 2Θ.
The XRD patterns of the sample RV-treated with Acetone and DCM could be clearly distinguished from the starting Piroxicam pattern because they appear to be in β form [12] as reported from the previous DSC analysis (Figure 2). Instead, the RV-treated sample prepared with EtAc shows the typical diffractogram of α form [12].
The XRD patterns of the CO_2-treated samples indicate the complete organization into α form and the intensity reduction of the main peaks suggest a decrease in crystallite size with respect to both commercial PXC and the RV-treated.

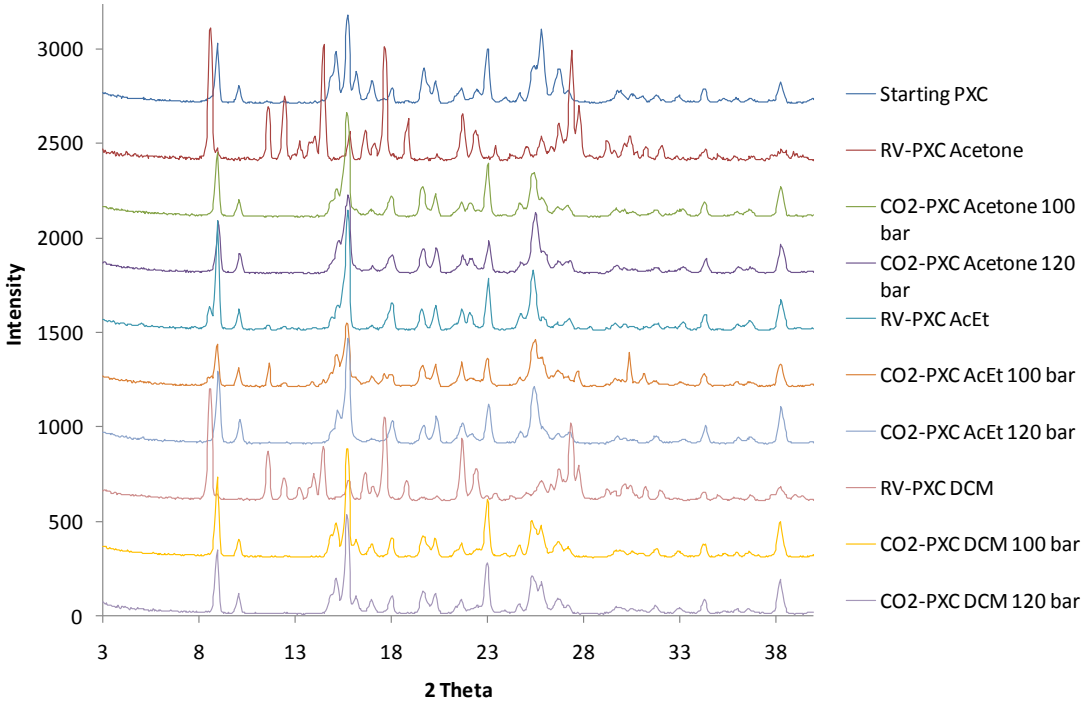

Figure 4: PXRD diffractograms

The main results of the DRIFT analysis on the starting PXC and treated samples showed two different spectra (Figure 5). Clearly, the starting PXC, PXC CO_2-treated and RV-treated with ethylacetate presented the typical band of the α-form, while the drug RV-treated with Acetone and DCM attested the presence of β-form. The –NH and –OH stretching lies at 3389 and 3333 cm^{-1} in α and β forms respectively as reported in literature [12]. For brevity reported only the β form and α form of PXC obtained with the RV and SAS (at 100 bar) processed with Acetone are reported.

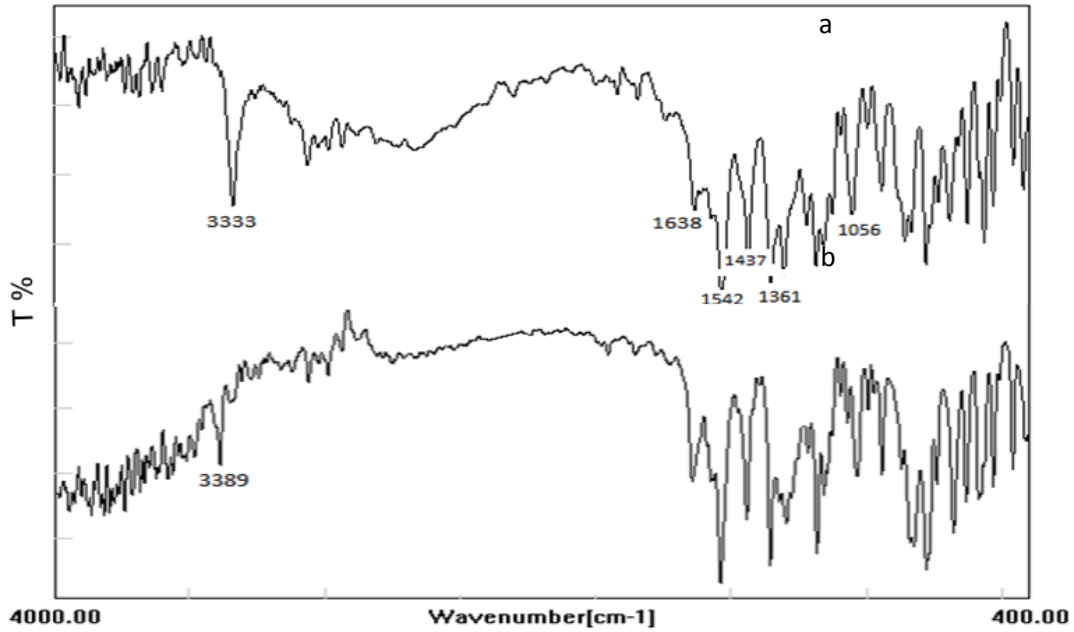

Figure 5: DRIFT spectra of: RV-PXC recrystallized from Acetone a), CO_2-PXC processed with Acetone at 100 bar b).

Even though previous physico-chemical characterizations attested the almost complete transformation into two pure different crystal forms after SAS and RV-processing with the tested solvents, the aim is to test the dissolution performance of the CO_2-treated samples compared to the RV-samples.

The dissolution profiles are reported in Figure 6. A remarkable increase of the PXC dissolution rate was achieved by its processing with supercritical CO_2 as compared to the RV-samples.

These great differences between the dissolution rates were attributable to changes in crystallite size and morphology.

Furthermore, crystallites in the RV-samples seemed to have larger dimensions compared to those treated with supercritical CO_2 and, the β-form of drug, obtained with Acetone and DCM, retard the dissolution.

It must be also pointed out that no significant differences were found between the dissolution performances of the samples obtained with different solvents and pressure in the SAS process. On the other hand, a significant difference was found between the dissolution profiles of the CO_2 samples and the RV-samples in particular for the PXC Acetone and DCM recrystallized.

For brevity CO_2-Piroxicam systems processed at 100 bar are not reported.

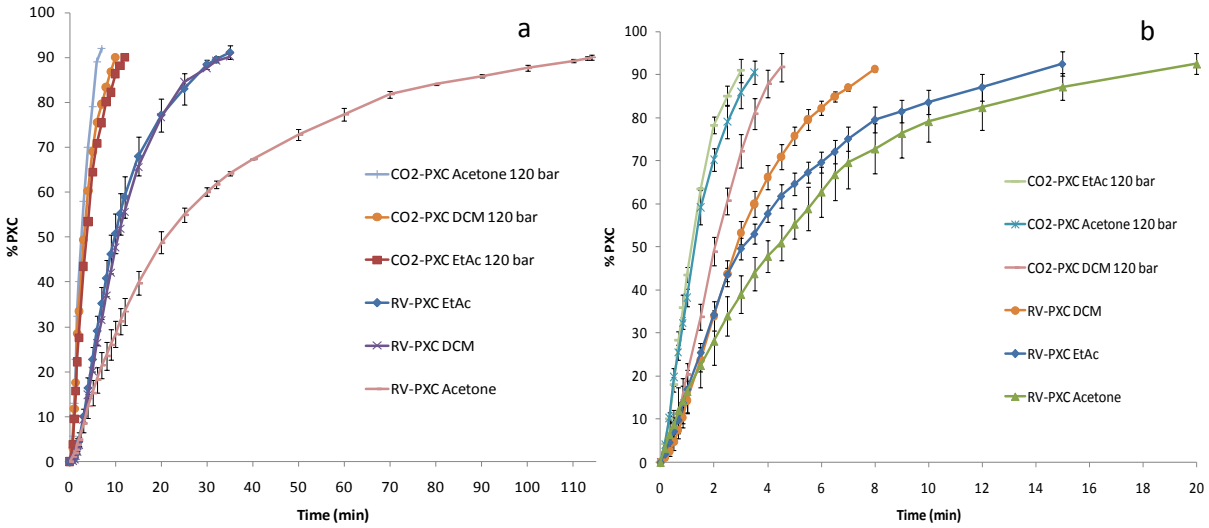

Figure 6: Dissolution profile at pH 1.2 a), and pH 7.4 b).

CONCLUSIONS

The above reported experimental evidences show that by treating solutions of commercial PXC in acetone, ethylacetate or dichloromethane with supercritical CO_2 yields samples mostly composed of α form.

The transition to needle-shape form also occurred by simple recrystallisation in vacuum evaporator when Piroxicam is treated with ethylacetate. It is reasonable to infer that this transformation is due to the solvents rather than to the treatment with supercritical CO_2. The photomicrographs of the samples treated and untreated with CO_2 illustrated that the SAS method changed the appearance of the crystals between the samples.

Furthermore, the CO_2-samples were arranged in particles with small dimensions, thus confirming that SAS technique is a viable mean to produce micron-size particles with narrow particle size distribution.

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