Drug Polymorphs by Supercritical AntiSolvent Micronization

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ABSTRACT
Rifampicin, an antibacterial drug, has been used to investigate the influence of Supercritical AntiSolvent (SAS) process on the solid-state properties, crystal habit and polymorphism of pharmaceutical compounds.
Dimethyl sulfoxide (DMSO), dichloromethane (DCM), acetone, ethyl alcohol (EtOH), methyl alcohol (MeOH), and ethyl acetate (EtAc) have been employed as liquid solvents, and carbon dioxide has been used as an antisolvent.
The effect of the different solvents on the crystal habit has been studied using SEM (Scan Electron Microscopy) images of both processed compounds, the differences in the crystallinity and polymorphism have been analysed by differential scanning calorimetry (DSC). The x-ray diffraction patterns of rifampicin revealed variations of crystallinity and crystal orientations depending upon the solvent used. The effects of SAS process parameters such as temperature and pressure on the precipitated crystals were investigated for the system rifampicin/EtOH. Pressure did not affect the morphology of the precipitated rfampicin, nevertheless varying the temperature to 40°C and 60°C the micronized powder was modified from hydrate to anhydrous. Anyway, the x-ray analysis showed that there was no primary polymorphic modification varying the operating conditions. In all the cases SAS processed crystals show more ordered appearances with clean surfaces compared with the unprocessed particles.
Keywords: Supercritical, polymorphism,SAS

1 INTRODUCTION

A solid compound can exist in several crystalline forms. Gross structural modifications such as polymorphism and solvate formation is notably common for certain groups of drugs. Polymorphs are crystalline phases containing the same molecules or ions but having different conformations and/or packing arrangements in the solid-state. Solvates are also crystalline phases and are formed when solvent molecules are present in the crystal lattice, leading to molecular adducts with the host molecules. If the solvent is water, the molecular adducts are termed hydrates [1].
Crystal habit is the external shape of a crystal. An identical compound can have many crystal habits due to the different growth rate of each crystal surface. The habit does not reflect the internal structure of a crystal. A crystal that has an identical internal space lattice can show different crystal habits. However, if a pharmaceutical compound that has a single crystalline behaviour undergoes a habit modification, its bioavailability may be changed.
Medicinally active substances exhibit only rarely a single crystalline structure. Polymorphic forms can have remarkably different physical properties including solubility and melting point resulting in different stability and bioavailability of drug products. If a mixture of polymorphs occurs in a pharmaceutical formulation, quantitative control of crystallization is needed to ensure a fixed proportion of forms. In addition, solid-state recrystallization phenomena, which may have conversion times of between seconds and years, will have to be suppressed to maintain integrity during product shelf-life.
Due to its complex structure, rifampicin exhibits polymorphism and exists in two polymorphic forms, i.e. form I and form II and an amorphous form. Morphology and crystal habit of different rifampicin samples were studied by different authors [2,3]. Samples of form I and form II have shown uniform rod like particles, whereas amorphous form was a devoid of long range crystal lattice. Rifampicin also exists as hydrates and various solvates, which eventually convert into amorphous form at room temperature or after desolvation [4].
Rifampicin has been already micronized by SAS technique [5] using different solvents for the drug: Dimethyl sulfoxide (DMSO), dichloromethane (DCM), acetone, ethyl alcohol (EtOH), methyl alcohol (MeOH), and ethyl acetate (EtAc).
In the experiments performed using NMP, MeOH and DCM, rifampicin was precipitated in form of tightly networked nanoparticles, while using EtAc some millimeters long needlelike crystals were mostly obtained. Using DMSO a powder formed by small particles precipitated in the collection vessel. The comparison of Rifampicin XRD patterns showed that Rifampicin was crystalline before processing and amorphous after SAS. This difference has been explained by the authors considering the precipitation speed that can characterize the SAS. The anstisolvent process is very fast compared to the crystal formation and does not allow the organization of the compound in a crystalline form.

The aim of the work is to obtain crystals of different morphologies using the SAS technique,then the effect of SAS micronization technique on the crystalline structure of rifampicin in terms of-crystal habit and polymorphisms will be studied.

2 APPARATUS AND PROCEDURE

2.1 Apparatus

The SAS laboratory apparatus (figure 1) is located at the University of Salerno (Italy) and consists of an HPLC pump equipped with a pulse dampener (Gilson, model 805) used to deliver the liquid solution, and a diaphragm high-pressure pump (Milton Roy, model Milroyal B) to deliver supercritical CO_2. A cylindrical vessel with an internal volume of 500 cm^3 and an internal diameter of 5 cm was used as precipitation chamber.

The liquid mixture was delivered to the precipitator through a 200 μm i.d. stainless steel nozzle. Supercritical CO_2 was delivered through another inlet port located on the top of the chamber. Before entering the precipitator, CO_2 was heated to the process temperature. The precipitation chamber was electrically heated using thin band heaters. A stainless steel frit located at the bottom of the chamber was used to collect the produced powder, but it allows the CO_2-organic solvent solution to pass through. A second collection chamber located downstream and the micrometering valve was used to recover the liquid solvent. A backpressure valve regulated the pressure in this chamber. At the exit of the second chamber a rotameter and a dry test meter are used to measure the CO_2 flow rate and the total quantity of antisolvent delivered, respectively.

Details of the procedure of a SAS experiments have been given elsewhere [6,7].

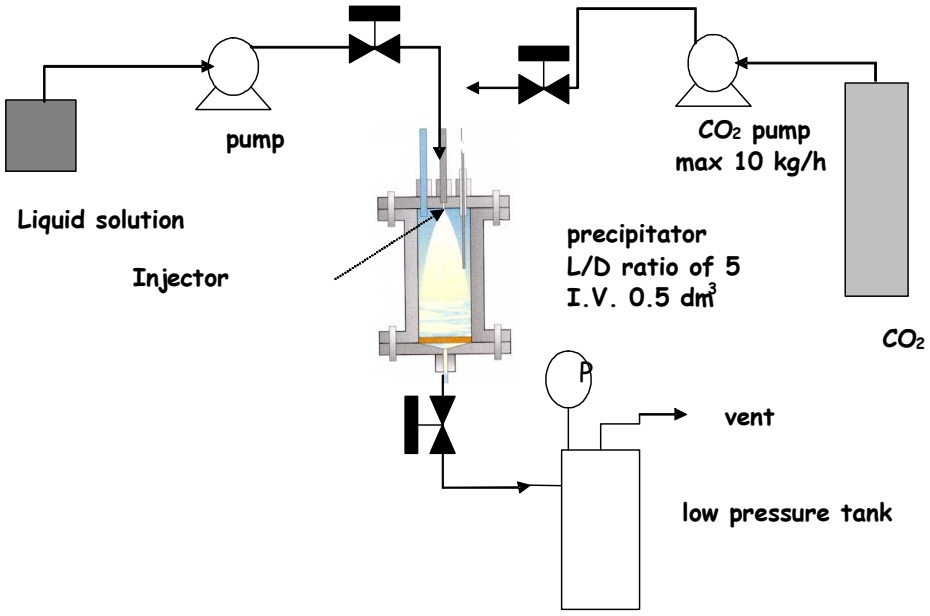

figure. 1. SAS laboratory apparatus

2.2 Materials

Rifampicin with a purity of 99.9% (Mw=822.94) was supplied by Sigma-Aldrich (Italy). Ethyl alcohol (EtOH) with a purity of 99.9%, methyl alcohol (MeOH) with a purity of 99.9%, ethyl acetate (EtAc) with a purity of 99.9% and dichloromethane (DCM) with a purity of 99.9% were supplied by Sigma-Aldrich (Italy). CO_2 99.9% was given by SON (Naples, Italy). The approximate solubility in the different solvents was measured by our research group and is 7 mg/mL in EtOH, 60 mg/mL in MeOH, 40 mg/mL in EtAc and 60 mg/mL in DCM. Untreated material was crystalline.

2.3 Analytical methods

Samples of the powder precipitated on the metallic frit were observed by a Scanning Electron Microscope (SEM) mod. LEO 420. The SEM samples were covered with 250Å of gold using a sputter coater (Agar model 108A).

The X-ray diffractogram of the powder sample was recorded on a BRUCKER D8 ADVANCE diffractometer (BRUKER AXS) operating at 40 kV and 20 mA with Ni-filtered Cu Ka radiation operating at room temperature. The range of 2θ diffraction angle examined was 5-45°, the count time for each step was equal to 2 s/step, and the step width was 0.05° (2θ).

Thermograms of rifampicin were recorded on a Differential Scanning Calorimeter (Mettler Toledo, DSC TC11, USA) using Mettler Stare system. The temperature axis and cell constant of DSC were previously calibrated with indium standard materials (melting point 156.6°C). A heating rate of 10°C/min was employed over a temperature range of 25-300°C with nitrogen purging (50 mL/min). Rifampicin powder sample (5±0.5 mg) was accurately weighed into an aluminum pan and an empty aluminum pan was used as a reference.

3 RESULTS AND DISCUSSION

In a recent work [8] it has been evidenced a relationship between high pressure phase equilibria and the morphology of SAS precipitated materials. Correlations between the observed morphologies and the position of the process operating point with respect to the mixture critical point (MCP) have been established [9,10]. The morphology and the dimensions of SAS precipitates substantially can be correlated to high pressure VLEs of the ternary (liquid+solute+supercritical fluid) system and the correlation between the ternary system and the corresponding binary system solute/CO_2 has been proved in may cases. The most effective way to precipitate crystals is to choose the operative conditions to perform the precipitation inside the miscibility hole of the binary system solvent-CO_2 (point **L** in figure 2).

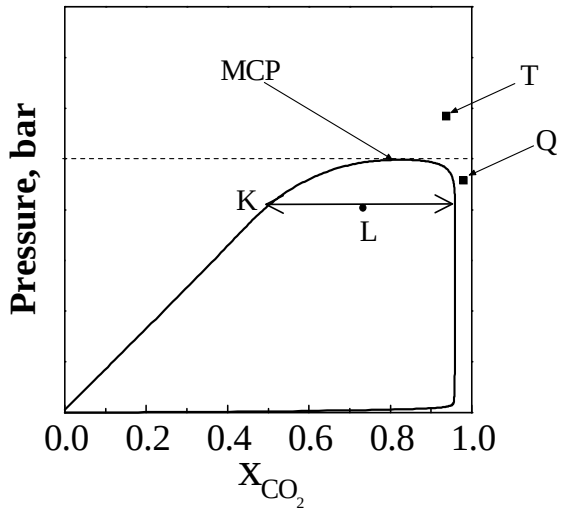

figure 2. Position of the operating points with respect to the miscibility hole at a fixed concentration of solute

The aim of these preliminary experiments was the determination of different crystal habit and polymorphic forms. The untreated rifampicin has a crystal shape, with the an irregular morphology (figure 3).

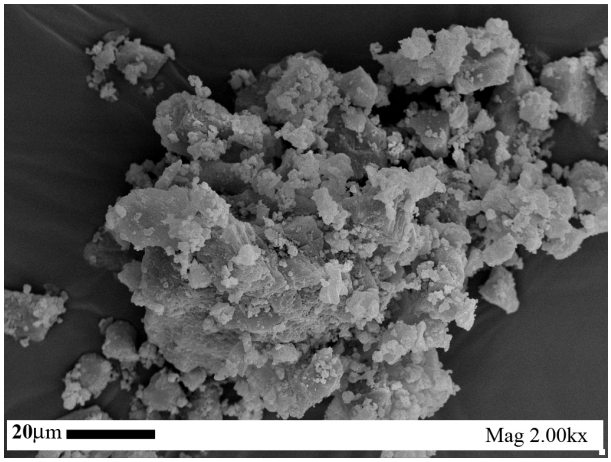

figure 3. SEM image of untreated rifampicin

Experiments using EtOH as solvent have been performed varying the operative conditions. The concentration of rifampicin in EtOH has been 5 mg/mL (71% of the saturation value).
The effect of the operative pressure has been studied at 60°C and the experiments have been performed at 125, 130, 150 bar. Pressure affected the crystal habit of the product, in particular at 130 bar spherical particles have been obtained (figure 4b), at 150 bar two different crystal habits coexisting in the same precipitate powder were produced (figure 4c). From the DSC and the x-ray analysis, no differences have been noticed in the micronized powder, therefore the pressure did no affect the polymorphism of the micronized rifampicin.

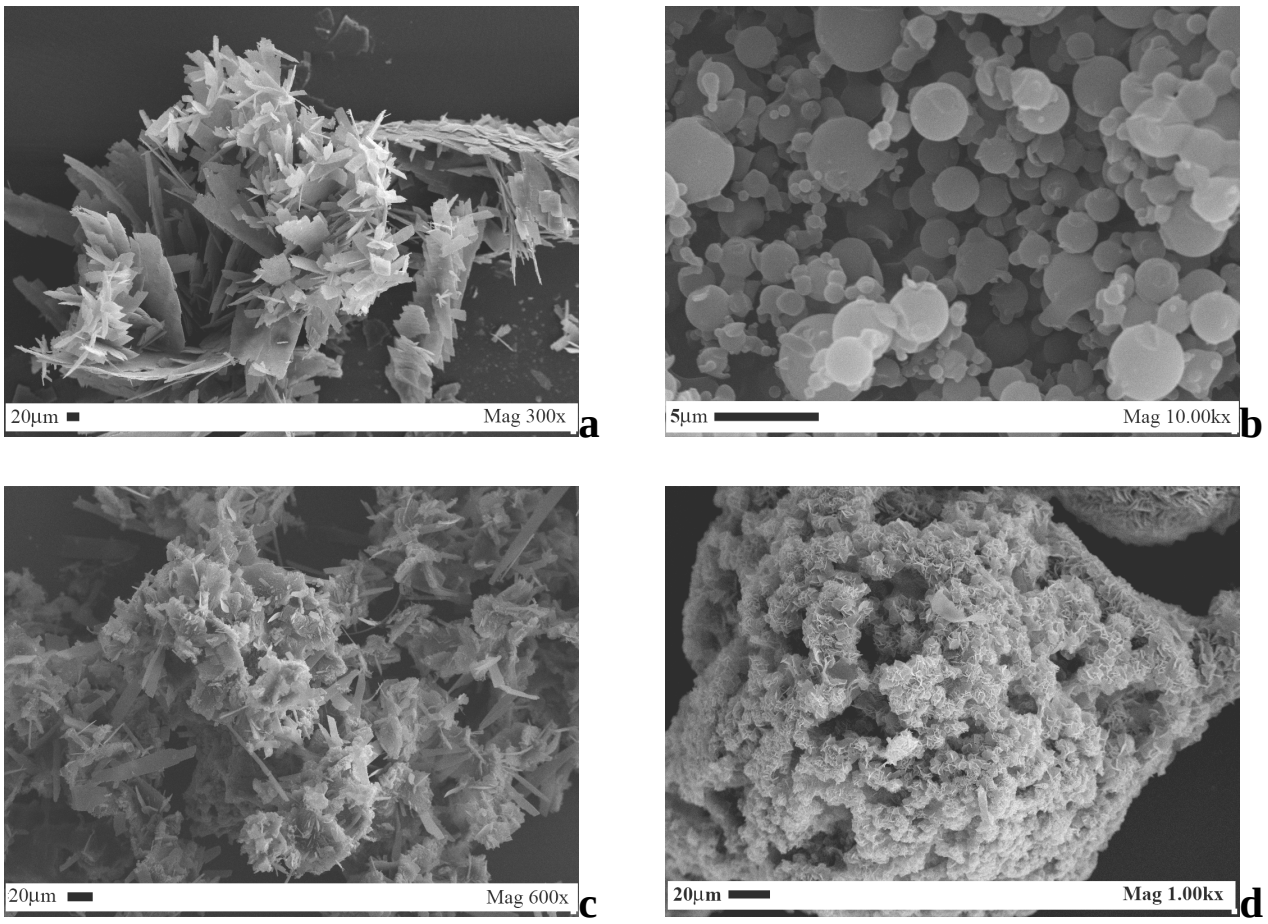

figure 4. SEM image of rifampicin precipitated from EtOH at 60°C, 20 mg/ml and (a) 125 bar, (b) 130 bar, (c) 150 bar, (d) 150 bar 40°C

The effect of operating temperature has been studied at 150 bar performing the experiments at 40°C and 60°C. By decreasing the temperature, different crystals have been obtained (figure 4d), they are very thin lamellar crystals joined together in a rose-like shape. The DSC thermograms show that the micronized powder has a different form compared to the untreated and that at 40°C the hydrated rifampicin has been produced. This result has been confirmed by x-ray analysis, indeed, the diffractograms in figure 5 show that there is no difference in the crystalline structure of the particles precipitated by EtOH as solvent, nevertheless there are differences between the SAS micronized powder and the untreated drug.

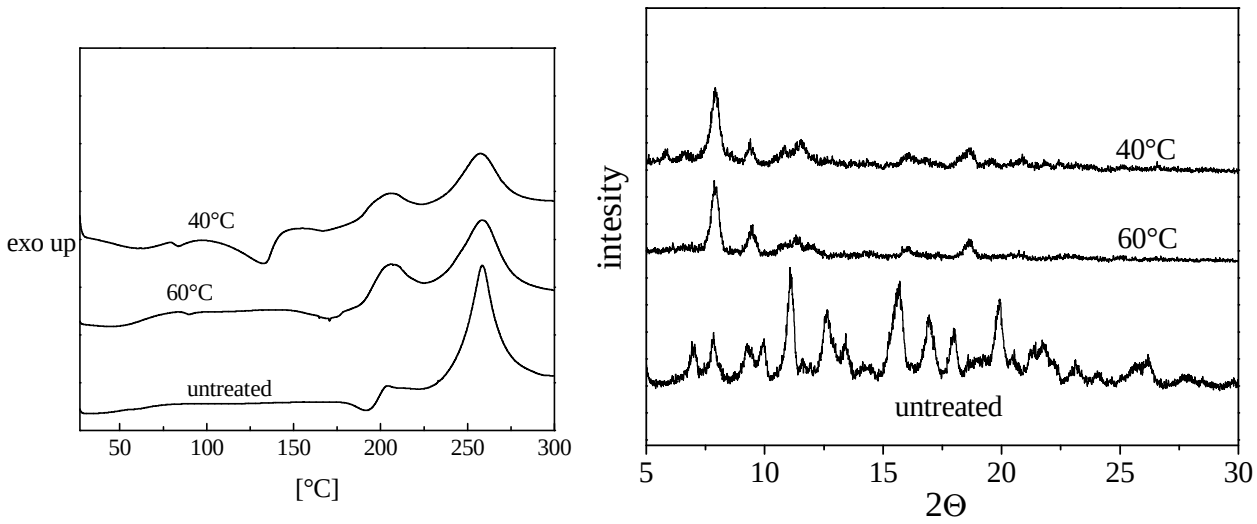

figure 5. DSC thermograms (left) and x-ray diffractograms (right) of rifampicin powders precipitated from EtOH at different operative temperature

In all the experiments performed using DCM as solvent, the material has been extracted by CO_2 and no precipitates have been collected.

From literature [5] we know that performing SAS precipitation using MeOH as solvent at 150 bar, 40°C coalescent nanoparticles have been obtained, therefore the experiments have been performed in MeOH at 150 bar, 60°C, 20 mg/mL (rifampicin/MeOH). The particles obtained have the shape of lamellar crystals combined in long needles of more than 200 μm in length, as shown in figure 6a.

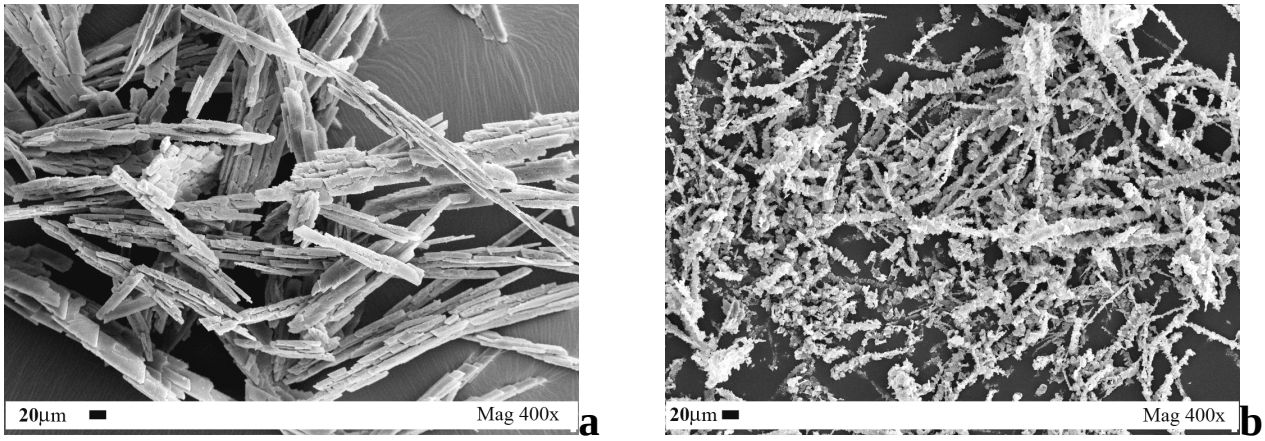

figure 6. SEM image of rifampicin precipitated from (a) MeOH at 150 bar, 60°C, 20 mg/mL, (b) EtAc at 150 bar, 40°C, 20 mg/mL

The experiments in EtAc performed at 150 bar, 40°C, 20 mg/mL (rifampicin/EtAc) produced particles of different crystal habit compared to the one obtained using MeOH: rhomboidal flat crystals connected in long chains.

In the figure 7 the DSC thermograms and x-ray diffractograms of the powder precipitated by SAS using different solvents and the untreated rifampicin are presented. The micronized powder has a different crystalline form when compared to the untreated; indeed the x-ray patterns have neither the characteristic peak of the untreated form II at 9,93° and 11,10° 2θ (figure 7) nor of the form I observed at 13.65 and 14.35° 2θ [2,3]. The thermogram of the untreated form II shows melting endotherm at 180-197°C, immediately followed by recrystallization exotherm to form I at 197-223°C, that is a characteristic of solid-liquid-solid transition. Finally, rifampicin decomposes at 247-266°C. This behaviour is also noticed in the micronized rifampicin with some differences in the pattern below 180°C. We hypothesize that the form II was modified to the corresponding hydrated and solvate forms. Rifampicin precipitated from EtOH shows a dehydration endotherm at 102-150°C, which is characteristic of the monohydrate form [4]; instead, rifampicin precipitated from MeOH shows the dehydration endotherm at 50-113°C. The latter peak might be attributed to a dehydrate form, this interpretation is or supported by the peak at 5.78° 2θ in the diffractogram in figure 7.

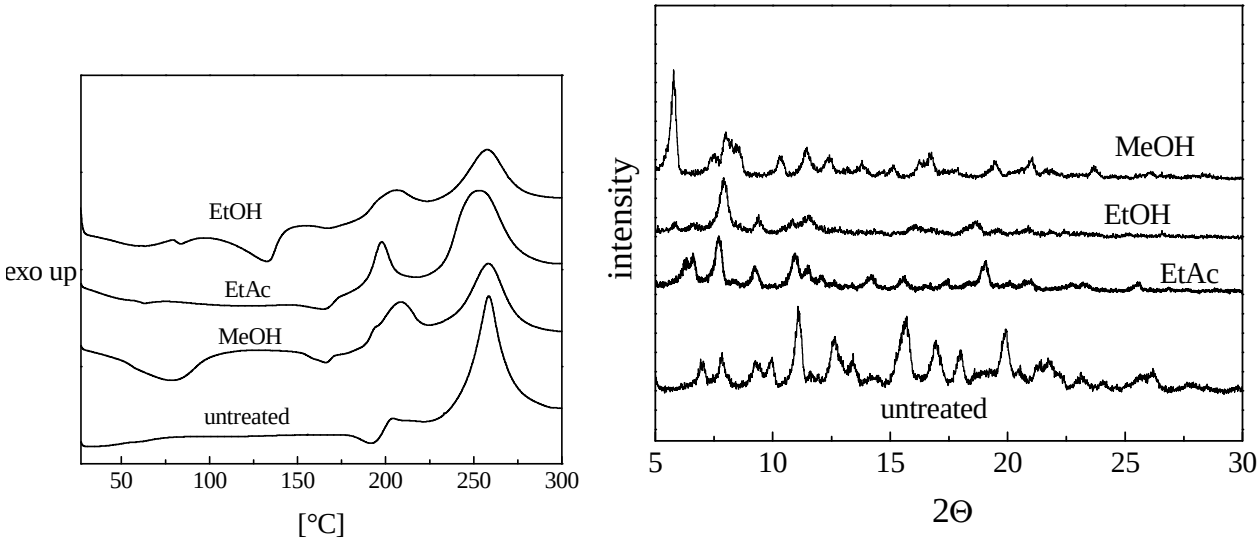

figure 7. DSC thermograms (left) and x-ray analysis (right) of rifampicin powders precipitated from different solvents

4 CONCLUSIONS

Using SAS technique and performing the precipitation from liquid phase different forms of rifampicin have been obtained. They were achieved by varying the solvent and the operative conditions of the process. Different solvents have affected the crystal habit of the precipitated particles, moreover secondary polymorphic variation have been noticed. The DSC and x-ray analysis confirmed that using EtOH and MeOH as solvents hydrated forms have been obtained, maybe due to the tendency of the alcohols to absorb humidity. The dehydration endotherm of the DSC thermograms showed that water is linked in a different way, in the case of EtOH it is a monohydrate and in the case of MeOH a dihydrate. This observation was confirmed by the x-ray diffractograms.

Using EtOH as solvent, no effect of pressure on the final morphology has been noticed, nevertheless increasing the temperature, rifampicin was not precipitated in the hydrated form, but the precipitate was anhydrous. In all cases the crystals obtained showed a clean surface and the structure is revealed as a defined shape, whereas untreated material is characterized by irregular crystals of undefined shape. The results obtained are promising for further investigation on the possibility to produce different polymorphic forms using SAS technique.

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