Supercritical Antisolvent Micronization of Minocycline Hydrochloride
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Abstract

Micronization of minocycline hydrochloride dissolved in ethanol and with supercritical carbon dioxide as antisolvent was successfully performed using a recently built SAS apparatus. Amorphous particles of minocycline ranging from 100 to 1000 nm (depending on the operating conditions) were obtained. The mean particle size and the particle size distribution were determined by Dynamic Light Scattering. Images were obtained by Scanning Electron Microscopy (SEM) to verify the shape and the size of the micronized particles. The quality of the micronized minocycline was analyzed by HPLC. Experiments were carried out in order to study the effects of the pressure (75–130 bar), temperature (35-50 ºC) and concentration of the liquid solution (1-20 mg.mL^{-1}). Furthermore, the effect of antisolvent/solvent flow ratio on the mean particle size and particle size distribution of the obtained final product was also analyzed.

Keywords: supercritical antisolvent, micronization, minocycline, ethanol, antibiotic.

1. Introduction

Minocycline (Mcc) [1] is a second-generation long-acting tetracycline that penetrates well into the central nervous system (CNS) via blood-brain barrier. In addition to its actions as antibiotic, Mcc has other biologic effects, recently discovered, such as affecting inflammation, proteolysis, angiogenesis, apoptosis, metal chelation, ionophoresis and bone metabolism [2,3]. In all biological applications of Mcc, particle size and particle size distribution are important parameters that influence the bioavailability, the delivery route and the pharmacokinetics of this drug.

The utilization of supercritical CO_2 (SC-CO_2) to micronize pharmaceutical compounds has become an important subject in recent years [4]. Advantages, such as high purity and low content of residual solvent in final products, environmental protection and experimental versatility justify the large application of SC-CO_2 to pharmaceutical compounds [5].

Among the several micronization techniques based on SC-CO_2, the supercritical antisolvent process (SAS) is the most suitable to bioactive substances. This technique uses both the high power of supercritical fluids to dissolve the organic solvents and the low solubility of the pharmaceutical compounds in supercritical fluids [6] to induce their precipitation from the initial liquid phase.

The need to study a particular system with industrial interest (antisolvent / solvent / substance to be micronized) is due to the lack of information about the phase diagram of the ternary system in question, type of morphology that will be obtained and size of micronized particles.

In the present work, the micronization of minocycline hydrochloride solubilized in ethanol and with supercritical carbon dioxide as antisolvent was successfully performed using a recently built SAS apparatus. Since the SAS micronization was achieved, a study was carried out to analyze the effects of pressure (75 - 130 bar), temperature (35 – 50ºC) and concentration of the liquid solution (1 - 20 $mg.mL^{-1}$) in the particle size and particle size distribution. The effect of antisolvent/solvent flow ratio on the mean particle size and particle size distribution of the micronized powder was also analyzed, with antisolvent flow/ solvent flow ratios of 5, 15 and 50 on a mass basis.

2. Experimental Section

2.1. Materials

Ethanol (EtOH) (purity ≥ 99.8%) was purchased from Riedel-de Haën (Germany) and carbon dioxide (99.998%) from Air Liquide (Portugal). Minocycline hydrochloride was gently offered by CIPAN (Portugal) and was classified as acceptable according with USP 29.

Mcc was observed by a scanning electron microscope (SEM) Philips XL 30 FEG SEM before and after the micronization process. Samples were covered with approximately 250 Å of gold using a sputter coater ((Jeol, model JFC-1100). Particle Size (PS) and Particle Size Distribution (PSD) were determined by dynamic light scattering using a Brookhaven Instruments (BI) equipment (BI-200SM Goniometer and BI-9000AT correlator) with a He-Ne laser (632.8 nm, 35 mW) from Spectra Physics (model 127) as light source. The results were analyzed using the BI-ZP software package from Brookhaven. The quality of the micronized Mcc was assessed by HPLC (Agilent, model 1100), column (Thermo, Hypersil BDS C18 5μm, dimensions 250x 4.6 mm) and following the method described in the minocycline hydrochloride monograph (USP 29).

2.2. Equipment and experimental procedures

The recently built SAS apparatus is represented on Figure 1.

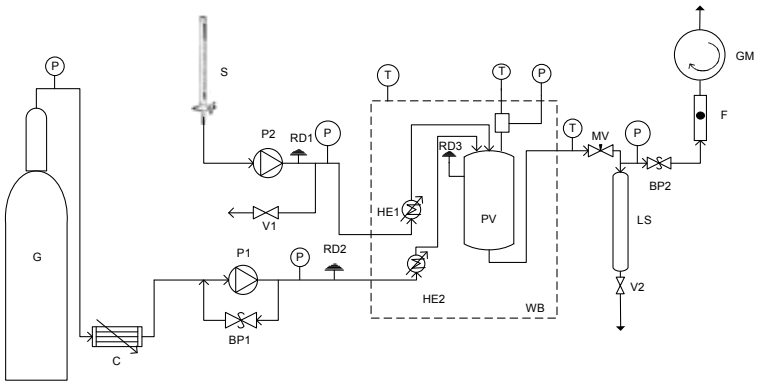

Fig. 1: Schematic diagram of the SAS apparatus. BP1 and BP2 = back pressure regulators; C = CO_2 cooler; F = calibrated flow meter; G = CO_2 cylinder; GM = dry test meter; HE1 and HE2 = heat exchangers; LS = liquid solvent recover vessel; MV = micrometering valve; P1 and P2 = high-pressure pumps; PV = precipitation vessel; RD1, RD2 and RD3 = rupture disks; S = liquid solution supply; WB = thermostatic water bath.

The CO_2 is cooled with an ice bath, C, before be compressed by a pump, P1, and the pressure is controlled by a back pressure regulator, BP1. Afterwards, the CO_2 is heated, HE2, in the water bath and enters into the precipitation vessel, PV. Simultaneously, the solution, S, is pumped, P2, also heated in the water bath, HE1, and fed to the precipitation vessel through a nozzle, (125 μm ID, 1 cm length, stainless steel) located in a distinct inlet point from the CO_2, but also in the top of the precipitation vessel. A stainless steel frit was put on the bottom of the PV to collect the micronized powder and to let the SC-CO_2 – organic solvent mixture pass through. The flow rate of the mixture that leaves PV is controlled by a micrometering valve, MV, located between the PV and the liquid solvent recover vessel, LS, were the mixture suffers a decompression (pressure <30 bar) to induce the separation of the CO_2 from the organic solvent. The pressure in the vessel, LS, is controlled by a back pressure regulator, BP2. After leaving the vessel LS, the CO_2 passes through a calibrated flow meter, F, and a dry test meter, GM, to be quantified.
All the experiments were performed following the same procedure and started by pumping the CO_2 to the precipitation vessel until it reached the desired pressure. Afterwards, a previous calculated amount of organic solvent is injected into this vessel to ensure that all the operation will be carried out in steady state. When the organic solvent concentration inside the vessel reaches the fed concentration, the micrometering valve, MV, is regulated to establish the flow rate at the exit (bottom) of the precipitation vessel and it is given some time for the system to stabilize. In that point, the solution is injected and the micronization takes place. At the end of the solution injection, SC-CO_2 passes during approximately 75 minutes to remove all the organic solvent from the precipitation vessel.

3. **Results and Discussion**

To analyze the viability of EtOH as organic solvent, a preliminary experiment was carried out. The conditions for this experiment were chosen taking into account the phase diagram of the system SC-CO_2 + EtOH [8]. The comparison between Mcc before and after micronization can be observed in Figure 2, where the SEM images are represented. It is important to note that practically all Mcc has precipitated against the wall of the precipitation vessel, in the upper part.

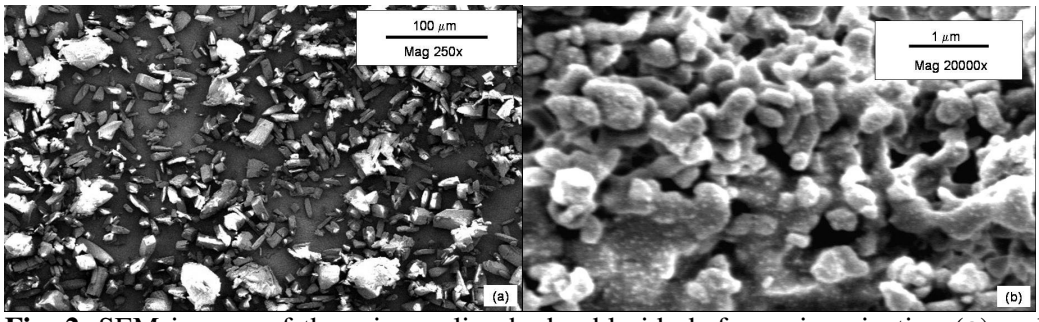

Fig. 2. SEM images of the minocycline hydrochloride before micronization (a) and after micronization (b). The Mcc was precipitated from ethanol at 100 bar, 40º C, 10 mg.mL^{-1} and with a SC-CO_2 /Solution flow rate of 15 on a mass basis.

3.1. Effect of the process parameters

The experiments carried out to study the effect of the process parameters in the obtained powder are summarized in Table 1.

Table 1: Summary of the experiments performed and mean particle diameter (MPD) obtained

Experiment	P (bar)	T (ºC)	Co (mg.mL^{-1})	Solution flow (mL.min^{-1})	CO_2 flow (L.min^{-1})	M.P.D (nm)
1	75	40	10	1	6.56	-
2	90	40	10	1	6.56	306
3	100	40	10	1	6.56	313
4	110	40	10	1	6.56	324
5	130	40	10	1	6.56	253
6	130	40	1	1	6.56	277
7	130	40	3	1	6.56	274
8	130	40	5	1	6.56	293
9	130	40	7	1	6.56	294
10	130	40	20	1	6.56	293
11	130	35	10	1	6.56	329
12	130	50	10	1	6.56	139
13	130	40	10	0.3	6.56	176
14	130	40	10	3	6.56	203

This table shows that neither the pressure nor the initial concentration of the liquid solution (Co), in the studied ranges, affect the obtained mean particle diameter (MPD)

and the differences are within the standard deviation. On the other hand, temperature is the parameter that shows more influence on particle size and on particle size distribution, as presented in Figures 3 and 4, respectively.

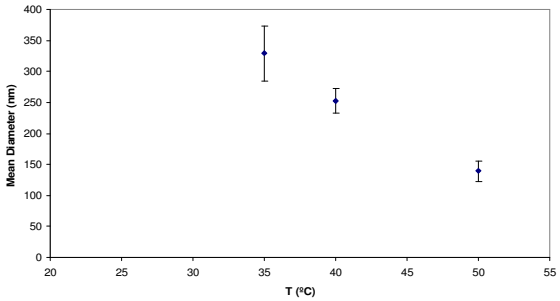

Fig. 3. Mean particle diameter of minocycline hydrochloride aggregates precipitated from EtOH (at 130 bar, Co= 10 mg.min^{-1}, $F_{solution}$ = 1mL.min^{-1}, $F_{SC\text{-}CO2}$ = 6.56 L.min^{-1}) versus temperature (ºC).

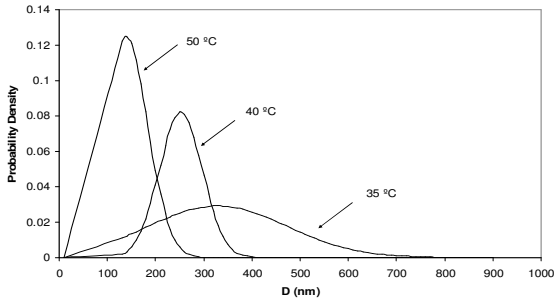

Fig. 4. Particle size distribution (represented as probability density functions) of minocycline hydrochloride aggregates precipitated from EtOH (at 130 bar, Co= 10 mg.min^{-1}, $F_{solution}$ = 1mL.min^{-1}, $F_{SC\text{-}CO2}$ = 6.56 L.min^{-1} and at three different temperatures) versus particles diameter (nm).

The MPD reduces with the increase in temperature and PSD becomes narrower. This effect has already been observed in the micronization of other compounds [9] and was demonstrated that this behavior is directly related to the solubility of the compound in the supercritical phase.

The effect of the solution flow rate is related to the composition of the mixture, operating point in the isothermic (p, x) diagram and to the fluid dynamics of the system. An option was made to keep the SC-CO_2 flow rate constant and to test different solution flow rates. The values tested in this work (0.3; 1 and 3 mL.min^{-1}) correspond to a SC-CO_2 / EtOH ratio of 50, 15 and 5, respectively, on a mass basis.

The solution flow rate does not present a significant influence on the MPD as it was expected, since it depends on the supersaturation of Mcc in the supercritical phase and it was already seen that the initial concentration does not have influence on the MPD. On the other hand, particle size distribution (Figure 5) is more dependent on the fluid dynamics features and it results from a balance between micromixing and nucleation/growth kinetics (assuming that surface tension is practically inexistent and, consequently, no droplets are formed) [10, 11].

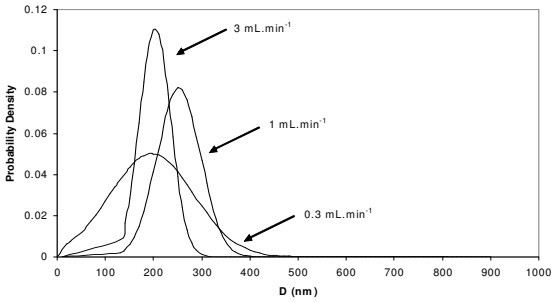

Fig. 5. Particle size distributions (represented as probability density functions) of minocycline hydrochloride aggregates precipitated from EtOH (at 130 bar, 40 ºC, Co= 10 $mg.min^{-1}$, $F_{SC\text{-}CO2}$ = 6.56 $L.min^{-1}$ and at three different solution flow rates) versus particles diameter (nm).

Furthermore, Figure 5 shows that the solution flow rate is not an important parameter to control MPD, but is very important to narrow particle size distribution.

4. Conclusions

This work demonstrates that it is possible and viable to micronize minocycline hydrochloride by the SAS process, using ethanol as solvent and SC-CO_2 as antisolvent. In all the experiments carried out, Mcc always precipitates with the same amorphous morphology.

Although several studies on micronization of pharmaceutical compounds were published, in recent years, it is very difficult to predict the effect that a supercritical micronization will have on the bioavailability and on the bioactivity of the drug. Therefore, more studies are necessary to characterize the micronized powder and to evaluate its biological effects, as antibiotic as well as all the other possible applications of Mcc.

This work is part of a more extensive study, in which the authors wish to characterize the effect of supercritical micronization on the properties of the pharmaceutical compound.

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