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 supercritical antisolvent (SAS) apparatus. Amorphous particles of minocycline ranging from 0.1 to 1 μm (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 confirm the morphology and the size of the powders before and after the material is processed. The quality of the micronized minocycline was analyzed by HPLC and was compared with that obtained before micronization.

Experiments were carried out in order to study the effects of the pressure (75–130 bar), temperature (35–50 °C) and concentration of the liquid solution (1–20 mg mL−1) on the mean particle size and particle size distribution of the final product obtained. The effect of the antisolvent flow/liquid solution flow (AS/L) ratio was also analyzed. Experiments were performed with (AS/L) ratios equal to 5, 15 and 50 on a mass basis.

The experimental results clearly indicate that SAS micronization of minocycline hydrochloride with ethanol as solvent and SC-CO2 as antisolvent, above approximately 80 bar (the only region where it occurs), takes place in a homogeneous supercritical phase formed in the precipitator and particle formation results from gas-phase nucleation. This conclusion is consistent with results mentioned in the literature for similar systems.

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1. Introduction

Minocycline (Mcc) is a second-generation long-acting tetracycline that penetrates well into the central nervous system (CNS) via blood–brain barrier [1]. In addition to its action as antibiotic Mcc has other biologic effects, recently studied, such as affecting inflammation, proteolysis, angiogenesis [2], apoptosis, metal chelation, ionophoresis and bone metabolism [3,4]. In all biological applications of Mcc, mainly directed to the CNS, particle size and particle size distribution are important parameters that influence the bioavailability, delivery route and pharmacokinetics of the drug.

The utilization of supercritical CO2 (SC-CO2) to micronize pharmaceutical compounds has become an important subject of research in recent years [5]. This fact could be explained taking into account the several advantages that it presents for the pharmaceutical industry, namely the efficient separation, by decompression, from both organic co-solvents and solid products. On the other hand, the high purity and low (or none) content of residual solvent in final products, environmental protection and experimental versatility justify the current interest in this new technique [6–32].

Among all the micronization techniques involving supercritical fluids [33], the supercritical antisolvent process (SAS) can be considered the most suitable one for pharmaceutical compounds, since it combines the high power of supercritical fluids to dissolve the organic solvents and the low solubility of these therapeutics in supercritical fluids [34], to determine their precipitation from the initial liquid solution.

The type of particles that can be obtained with a SAS apparatus is, among others, function of the operating point in the phase diagram of the ternary system (antisolvent/organic sol-
vent/compound to be micronized), as discussed by Reverchon et al. [35]. If micronization occurs above the mixture critical point (MCP), where only a homogeneous supercritical phase is formed in the precipitator (Q-type mixtures), particle formation results from gas-phase nucleation [36]. If the operating point is below the MCP, several processes can occur, namely, gas-phase nucleation, liquid phase nucleation and/or crystal growth or both simultaneously, depending on the composition of the phases and on the supersaturation achieved. This behavior can explain the discrepancies observed in the effects of the parameters (pressure, temperature, flow ratios, initial concentration, etc.) on mean particle size and particle size distributions obtained by several authors [37]. To predict the morphology and the mean particle size, it is necessary to know the effect that the compound to be micronized has in the phase diagram of the binary system, involving the supercritical antisolvent and the organic solvent. Only after the clarification of this operating point, it will be possible to make considerations about fluid dynamics, nucleation or mass transfer to a certain ternary system.

The absence of information about phase diagrams of ternary systems leads to the need of study each particular system (antisolvent/organic solvent/compound to be micronized), to evaluate if this compound precipitates and (if so) to analyze the effect of the several parameters on the morphology, mean particle size and particle size distribution of the formulation obtained. For this reason, in this work, the micronization of minocycline hydrochloride was evaluated with two different organic solvents, dimethyl sulfoxide (DMSO) and ethyl alcohol (EtOH), using supercritical carbon dioxide as antisolvent.

Once precipitation only occurs with ethanol, experiments were performed with this solvent to study the effects of pressure (75–130 bar), temperature (35–50 °C) and concentration of the liquid solution (1–20 mg mL⁻¹). The effect of antisolvent flow/liquid solution flow (AS/L) ratio on the mean particle size and particle size distribution was also analyzed. Experiments were carried out with (AS/L) ratios equal to 5, 15 and 50 on a mass basis.

2. Experimental

2.1. Materials

Ethanol (EtOH) (purity ≥ 99.8%) and dimethyl sulfoxide (DMSO) (purity ≥ 99%) were purchased from Riedel-de Haën (Germany) and carbon dioxide (99.998%) from Air Liquide (Portugal). Minocycline hydrochloride was gently supplied by CIPAN (Portugal) and was classified as acceptable according with USP 29.

2.2. SAS apparatus

The micronization experiments were performed in a recently built SAS apparatus schematically represented in Fig. 1. The CO₂ is cooled with an ice bath, C, before being compressed by a HPLC pump, P₁, (Gilson, model 308) and the pressure is controlled by a back pressure regulator, BP₁, (Tescom, model 26-1722-44-043). Afterwards, the CO₂ is pre-heated in a heat exchanger, HE₁, and enters into the precipitation vessel, PV, (HIP, 300 mL I.V.). Simultaneously, the solution, S, is pumped, P₂, (Gilson, model 305), also heated, HE₂, and fed to the precipitation vessel through a nozzle (125 μm ID, 1 cm length, stainless steel). This nozzle is located in a distinct inlet point from the CO₂, but also in the top of the precipitation vessel. A stainless steel frit was put on the bottom of the precipitator to collect the micronized compounds and to let the SC-CO₂/organic solvent mixture pass through. The flow rate of the mixture that leaves the precipitator is controlled by a micrometering valve, MV, (Hoke, model 1315G4Y) located between the pre-
cipitator and the liquid solvent recover vessel, LS, (Swagelok, 300 mL I:V.). Here the mixture suffers a decompression (pressure < 30 bar) to induce the separation of the CO2 from the organic solvent. The pressure in the vessel, LS, is controlled by a back pressure regulator, BP2, (Tescom Europe, model 26-1763-24-043). After leaving the vessel LS, the CO2 passes through a calibrated flow meter, F, (Omega, model FL-1468-S) and a dry test meter, GM, (AMC, model DTM-200A) to be quantified.

After reaching the target pressure, by pumping CO2, 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 micromterming 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-CO2 will clean the micronized powder until practically all the organic solvent leaves the precipitator. This washing time with pure SC-CO2 was calculated following the method proposed by Reverchon [5,38] and consists in considering the precipitation vessel as a continuous stirred tank reactor (CSTR). In the case of this high pressure vessel, with an internal volume of 300 mL and with the SC-CO2 flow rate of 6.56 L min\(^{-1}\) (PTN), the necessary time for the concentration of the organic solvent to decrease to 0.01% of its initial value is approximately 75 min.

2.3. Analytical methods and particle characterization

2.3.1. Chromatography

Mcc was analyzed by HPLC (Agilent, model 1100), column (Thermo, Hypersil BDS C18 5 μm, dimensions 250 mm × 4.6 mm), following the method described in the minocycline hydrochloride monograph (USP 29).

2.3.2. Scanning electron microscope (SEM)

Samples of the powder (before and after micronization) were observed by SEM, (Philips XL 30 FEG). The samples were covered with 250 Å of gold using a sputter coater (Jeol, model JFC-1100).

2.3.3. Dynamic light scattering

Particle size and particle size distribution 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) (Spectra Physics, model 127) as light source. The results were analyzed using the BI-ZP software package from Brookhaven.

Mcc was suspended in filtered pure water and special cares were taken to eliminate the dust and to avoid the aggregation of particles. Every measurement was repeated, at least, five times.

The mean particle sizes and standard deviations obtained were used to fit the particle size distribution to a log-normal distribution.

3. Results and discussion

The first step in supercritical antisolvent micronization studies is the choice of the organic solvent. This choice must take into account the solubility of the compound to be micronized in the organic solvent and the phase diagram of the binary system SC-CO2 plus organic solvent. The best option is always the possibility to work with a single phase [35]. Accordingly, two possibilities were available: dimethyl sulfoxide (DMSO) and ethanol (EtOH) [11,39,40].

Experiments were carried out with DMSO, with pressures up to 130 bar, temperature up to 50 °C and concentrations of the liquid solution up to 57 mg mL\(^{-1}\). All the tests lead to the same result; Mcc was recovered in the LS vessel, together with the DMSO and the precipitation vessel was completely empty. This observation leads to the conclusion that DMSO acts as entrainer and increases the solubility of Mcc (\(\lambda_{Mcc} \approx 10^{-7}\) in a binary mixture with pure SC-CO2, according with our observations) in the supercritical phase and in the studied ranges of temperature and pressure.

To analyze the viability of EtOH as organic solvent, a preliminary experiment was carried out taking into account the phase diagram of the SC-CO2 + EtOH system [40]. Since the objective was to work above the mixture critical point (MCP), a pressure of 100 bar (approximately 20 bar above the MCP for the mentioned binary system at 40 °C) was selected. This choice increases the possibility to work in a single phase region, even if the addition of Mcc changes significantly the phase diagram. The comparison between Mcc before and after micronization can be observed in Fig. 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. That fact, together with the morphology of the micronized Mcc (Fig. 2(b)), suggests the possibility of particle formation from gas-phase nucleation [35].

3.1. Effect of the process parameters

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

3.1.1. Effect of the pressure

One of the issues that arise from the possibility of the gas-phase nucleation is the need to know if precipitation will occur in a two phase region of the phase diagram. The MCP (40 °C) of the system CO2 + EtOH is approximately 80 bar [40] and one experiment was carried out at 75 bar in which no precipitation occurred. In fact, Mcc remained inside the precipitation vessel, dissolved in EtOH, after the established washing time with SC-CO2. This behavior shows that, to have precipitation, there cannot be any liquid phase inside the precipitation vessel or Mcc will be dissolved again.

The experiments carried out at pressures between 90 and 130 bar produced particles with the same morphology represented in Fig. 2(b). The mean particle diameters (MPD) obtained are shown in Fig. 3.
The tendency to a decrease of the MPD with an increase of the pressure has already been observed by other authors [8, 28, 32, 41], although in this case only a slight tendency was observed. The explanation for this effect, proposed by Reverchon and Della Porta [8] is related to the enhancement in solvent power of SC-CO₂ with increasing pressure that will reduce the solvation sphere of the EtOH molecules and, consequently, decreases the possibility of interaction between EtOH and Mcc. In this study, the pressure does not affect significantly the particle size distribution.

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

<table>
<thead>
<tr>
<th>Experiment</th>
<th>P (bar)</th>
<th>T (°C)</th>
<th>Co (mg mL⁻¹)</th>
<th>Solution flow (mL min⁻¹)</th>
<th>CO₂ flow (L min⁻¹)</th>
<th>MPD (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>40</td>
<td>10</td>
<td>1</td>
<td>6.56</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>40</td>
<td>10</td>
<td>1</td>
<td>6.56</td>
<td>306</td>
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<tr>
<td>3</td>
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<td>10</td>
<td>1</td>
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<td>1</td>
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</tr>
<tr>
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<td>130</td>
<td>40</td>
<td>10</td>
<td>1</td>
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</tr>
<tr>
<td>6</td>
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<td>277</td>
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<td>40</td>
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<td>1</td>
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<td>274</td>
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<tr>
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<td>40</td>
<td>7</td>
<td>1</td>
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<td>293</td>
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<td>40</td>
<td>10</td>
<td>0.3</td>
<td>6.56</td>
<td>176</td>
</tr>
<tr>
<td>13</td>
<td>130</td>
<td>40</td>
<td>10</td>
<td>3</td>
<td>6.56</td>
<td>203</td>
</tr>
</tbody>
</table>

As the pressure does not show a significant effect on the MPD, the following experiments were performed at 130 bar.

3.1.2. Effect of concentration
The initial concentration of the liquid solution does not influences the particle size and particle size distribution, as shown in Fig. 4. One possible reason for this behavior can be given
Fig. 5. Mean particle diameter of minocycline hydrochloride aggregates precipitated from EtOH (at 130 bar, Co = 10 mg min$^{-1}$, $F_{\text{solution}} = 1 \text{ mL min}^{-1}$, $F_{\text{SC-CO}_2} = 6.56 \text{ L min}^{-1}$) versus temperature.

Fig. 7. Mean particle diameter of minocycline hydrochloride aggregates precipitated from EtOH (at 130 bar, 40°C, Co = 10 mg min$^{-1}$, $F_{\text{SC-CO}_2} = 6.56 \text{ L min}^{-1}$) versus solution flow rate.

3.1.3. Effect of the temperature

Figs. 5 and 6 show that the temperature has a major effect on particle size and particle size distribution, respectively. The MPD reduces with the increase in temperature, while particle size distribution becomes narrower. This effect has already been observed by Rehman et al. [42] for the micronization of nicotinic acid. These authors demonstrated that this behavior is directly related to the solubility of the compound in the supercritical phase. Near the MCP an increase in temperature at constant pressure leads to a decrease in the density of the SC-CO$_2$ and, consequently, a reduction in the solubility of EtOH and Mcc in the supercritical phase. A lower solubility will allow a higher degree of supersaturation and particles with a smaller MPD.

3.1.4. Effect of the solution flow rate

This parameter 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 evaluate different solution flow rates. The values studied 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 (Fig. 7) as it was expected, since this parameter depends on the supersaturation of Mcc in the supercritical phase (once again, admitting that precipitation occurs above the MCP) and it was already seen in Section 3.1.2 that the initial concentration does not affect MPD. On the other hand, particle size distribution (Fig. 8) 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) [36,43].

The observation of Figs. 7 and 8 shows that the solution flow rate is not a relevant parameter to control MPD, but is very important to narrow particle size distribution.
Table 2
Comparison of HPLC analysis of Mcc, before and after micronization, humidity in the samples and corresponding limits to the several items

<table>
<thead>
<tr>
<th>Related substances</th>
<th>Before micronization</th>
<th>After micronization</th>
<th>Limits USP</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Epiminocycline (%)</td>
<td>0.62</td>
<td>2.4–6.0</td>
<td>≤1.2</td>
</tr>
<tr>
<td>Other impurities (%)</td>
<td>1.3</td>
<td>1.3–1.5</td>
<td>≤2.0</td>
</tr>
<tr>
<td>Dosage (µg/mg)</td>
<td>918</td>
<td>809–878</td>
<td>890–950</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>7.4</td>
<td>6.3–7.8</td>
<td>4.3–8.0</td>
</tr>
</tbody>
</table>

3.2. Characterization of minocycline hydrochloride

Minocycline hydrochloride was analyzed according to the method described in the U.S. Pharmacopeia (USP 29), which determines the dosage or the potency of the antibiotic, as well as related substances and corresponding limits. Table 2 shows the results from HPLC analysis of Mcc, before and after the micronization, as well as the limits imposed by the U.S. Pharmacopeia.

In the column corresponding to the micronized Mcc, the represented range contains the values obtained in all the micronization experiments that were carried out.

The parameters that cannot be classified as acceptable are the percentage of epiminocycline and the assay. The results of the analysis show that neither of these parameters is related to the experimental conditions of the micronization and that they are interrelated. The apparently random degree of epimerization of the micronized Mcc suggests that it can be a problem for the solution of Mcc in EtOH, before micronization takes place. More studies are necessary to minimize or to avoid the epimerization.

Besides this analysis, another parameter that is regulated by USP is the morphology of the particles that should be crystalline; however, micronized Mcc presents an amorphous structure. These USP specifications are for the use of Mcc as antibiotic. For all the other applications of Mcc mentioned in this work, additional studies of bioactivity and bioavailability of micronized Mcc are necessary.

4. Conclusions

This work demonstrates that minocycline hydrochloride can be micronized by the SAS process, using ethanol as solvent and SC-CO₂ as antisolvent. On the other hand, the use of dimethyl sulfoxide as solvent is not possible, at least in the tested conditions.

The effect of the process parameters on the particle size and particle size distribution, together with the Mcc morphology obtained, allows a better understanding of the type of micronization that occurs with this system, showing that it can only occur in the supercritical phase. This result could be anticipated if the phase behavior of the ternary system was known.

Although several studies of micronization of pharmaceutical compounds have been 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. The fact that micronized Mcc does not meet USP specifications, as antibiotic, requires more studies about its effects in all the other possible therapeutic applications.

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

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