SUPERCRITICAL ANTISOLVENT MICRONIZATION OF NALMEFENE HYDROCHLORIDE

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Supercritical AntiSolvent precipitation (SAS) has been used to produce micronized particles of Nalmefene Hydrocloride, a selective narcotic antagonist and a promising adjunctive for the treatment of ethanol dependence.

Experiments have been performed using ethyl alcohol (EtOH) and dimethyl sulfoxide (DMSO) as liquid solvents. The effect of SAS process parameters on the morphology, particle size and particle size distribution have been studied for the system Nalmefene HCl/EtOH, using SEM images of the processed material. The influence of the concentration of liquid solution on the dimensions of Nalmefene particles and the effect of the ratio of CO_2/liquid solution flow rates have been studied. Various morphologies have also been observed varying the precipitation pressure: irregular crystals, coalesced nanoparticles and spherical microparticles. When operating at temperatures of 40 and 60°C and in the pressure range between 120 and 150 bar, we have obtained nano-particles with a mean diameter ranging from 200 to 300 nm, that could be used for injectable suspensions and micro-particles with mean diameters ranging from 0.5 to 2 μm, that can be used for inhalation delivery.

INTRODUCTION

In the pharmaceutical field it is important to obtain micronized particles of controlled dimensions, without loosing the purity and therapeutical characteristics of the starting compound. Dimensions of poorly water soluble drug particles are strictly correlated with the bioavalibility of pharmaceutical principles. The bioavalibility is the percentage of the drug absorbed by the body that reaches the general blood circulation in relation to its initial dosage. Traditional micronization techniques are often characterized by the use of organic solvents, which are difficult to eliminate from the product completely. In addition, mechanical devices are also used, that can damage the product and long processing periods with high costs are needed. The increasing interest of pharmaceutical industry in particle formation processes using Supercritical Fluids has three aims: increase bio-availability, design sustained-release formulations and the use of active agents for new types of drug delivery, less invasive than parenteral delivery (oral, pulmonary, transdermal).

Nalmefene Hydrochloride is a selective narcotic antagonist, it prevents or reverses the effects of opioids, including respiratory depression, sedation and hypotension. It is used to reverse the effects of opioids after general anesthesia and in the treatment of overdose and the systemic effects of intrathecal opioids [1]. It is completely bioavailable with intramuscular or subcutaneous injection administration, and can be administrated by oral treatment. When delivered in aerosol formulation, the pharmaceutical principle is more effective because the pulmonary delivery bypasses adsorption and metabolic processes that may limit its efficiency.

For this purpose, it is desirable to have particles less than 5 μm diameter, which are suitable for inhalation.

The aim of this work is to ascertain the feasibility of SAS processing for the Nalmefene HCl to produce micro- or submicrometric powders.

APPARATUSES AND PROCEDURE FOR SAS PRECIPITATION

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 diameter 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.

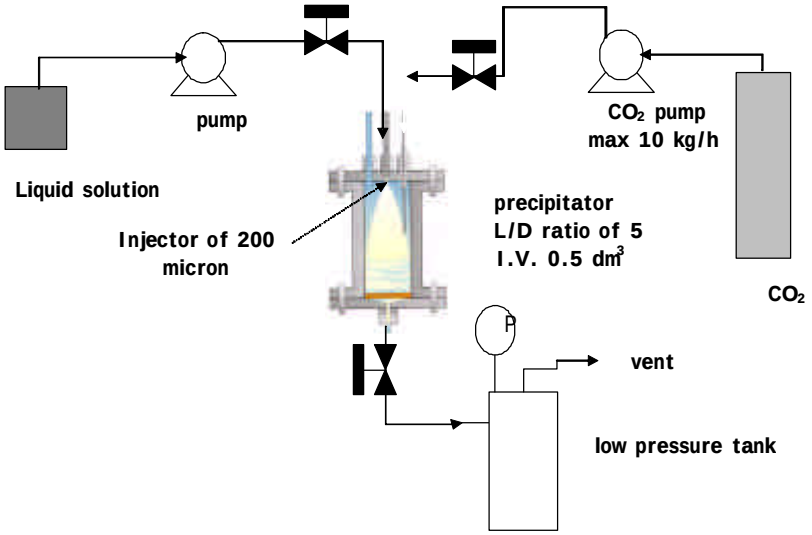

fig. 1. SAS laboratory apparatus

Materials

Nalmefene HCl with a purity of 99.9% (Mw=375.9 MP=203÷206°C) was supplied by Contral Pharma Ltd (Finland). Ethyl alcohol (EtOH) with a purity of 99.9% was supplied by Sigma-Aldrich (Italy). CO_2 99.9% was given by SON (Naples, Italy).

The solubility profile of Nalmefene HCl in liquid solvents was obtained from Contral Pharma Ltd. (Finland). The approximate solubility in EtOH was measured by our research group and was 26 mg/ml at room temperature. Untreated material was crystalline.

Details of the procedure of a SAS experiments have been given elsewhere [2,3,4].

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). Particle size and particle size distributions (PSD) were measured from SEM images using the Sigma Scan Pro image analysis software (Jandel Scientific); about 800 particle diameters were measured in the elaboration of each PSD.

RESULTS AND DISCUSSION

From a thermodynamic point of view, to perform a successful micronization the solute has to be insoluble in the antisolvent and the liquid solvent has to be completely miscible in the supercritical antisolvent at the process conditions. This last condition has been verified for various pairs of liquid solvent-supercritical antisolvent [5] using the expansion curves of the liquid solvent in contact with the supercritical antisolvent at a fixed temperature. By increasing the pressure, the liquid solvent expands due to the increasing quantity of CO_2 that is solubilized in it. In order to verify the feasibility of the supercritical antisolvent precipitation on Nalmefene HCl, to find the best solvent and the approximate operative conditions for the process, preliminary phase separation studies was performed at VTT Processes (Finland) using a static Variable Volume View-cell. When 1.2 weight-% Nalmefene HCl in EtOH was pressurized with CO_2 in the cell, a solid precipitate appeared from the liquid phase when CO_2 pressure reached 85 bar at 45 °C. Complete dissolution of the liquid ethanol in CO_2 occurred at a pressure higher than 100 bar leaving the solid nalmefene precipitate. Looking at the general considerations, and from the results obtained by phase separation studies we deduced that Nalmefene HCl could be tested with SAS technique, using EtOH as the solvent at pressures higher than about 100 bar.

We decided to test Nalmefene HCl using an EtOH solution in a concentration relatively far from the saturation value. We prepared a solution of 15 mg/mL and we performed the first test at 40 °C and 150 bar, delivering the solution at 2 mL/min and the CO_2 at 29.7 g/min, with a mass ratio CO_2/solution of 18.8. We produced partially coalescent particles with a mean dimension of about 200 nm (**figure 2**).

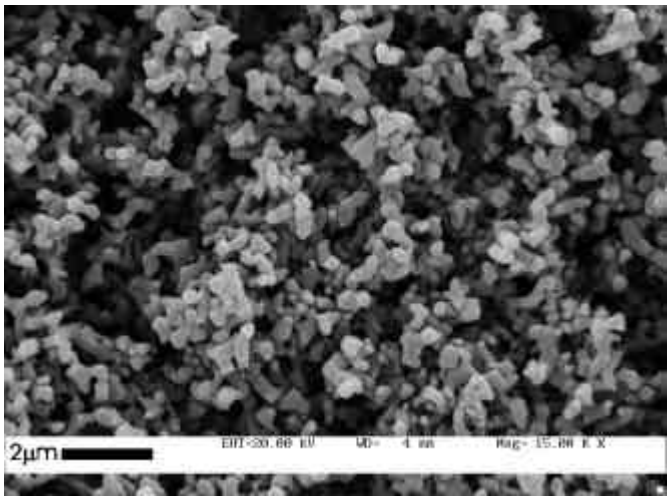

fig. 2.SEM image of Nalmefene HCl particles precipitated from EtOH at 150 bar, 40°C, 15 mg/mL, R=18.8.

The coalescence may be also controlled by the precipitation pressure, as in the case of Tetracycline [6], where the coalescence decreased with increasing pressure and in the case of

Rifampicin [7], where the coalescence decreased with decreasing pressure. Therefore, we performed other experiments varying the operative pressure. However, increasing the operative pressure we obtained particles with the same morphology observed in the first test.

We also tested the effect of temperature. We performed experiments at 60 °C at the same concentration of Nalmefene HCl as in the previous experiments. At the pressure of 150 bar we obtained the same results of the previous tests. Then, we decided to work in a different operative point changing the ratio SC CO_2/solvent. We increased the mass ratio CO_2/solution decreasing the solvent flow rate to 1.5 mL/min, with a mass ratio CO_2/solution of 25.06 and we performed various tests at 40°C and 60°C, and pressures ranging between 120 and 150 bar. At 40°C we obtained again partly coalescent particles, at 60°C we obtained material macroscopically less expanded then the other tests (**figure 3**) and the SEM images show that the resulting particles were spherical and well separated (**figure 4)**. At these operative conditions we found an increase of the mean particle size of Nalmefene HCl with increasing the pressure.

fig. 3. Images of the bottom of the Precipitation Vessel.

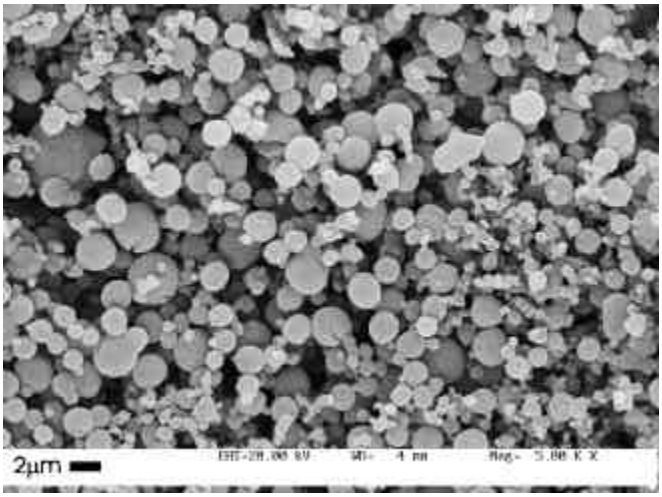

fig. 4.SEM image of Nalmefene HCl particles precipitated from EtOH at 130 bar, 60°C, 15 mg/mL R=25.06.

The different morphologies obtained changing the operative conditions could be explained by the experimental observations performed in previous works [2,16] prevalently in terms of high pressure vapour-liquid equilibria.

The particle size distribution of the powders obtained at different pressures is usually presented in terms of percentages calculated on particle number. In **figure 5** it is possible to observe that all distributions are approximately log-normal and the mode of all the particle

size distributions ranges between 1-1.5 μm. If particle size distributions are calculated on the basis of particles volume, G-Cas distributions are approached and the mean of the particle size distributions moves from 2 μm to 3.5 μm with increasing of the pressure. Indeed, when volume is considered the distribution takes into account the relative weight of small and large particles and larger particles prevail in the distribution (see **figure 5**). In particular, increasing the pressure we obtained particles ranges of 0.5-3 μm, 1-5 μm, and 1.5-5.5 μm respectively, suitable for inhalation purposes.

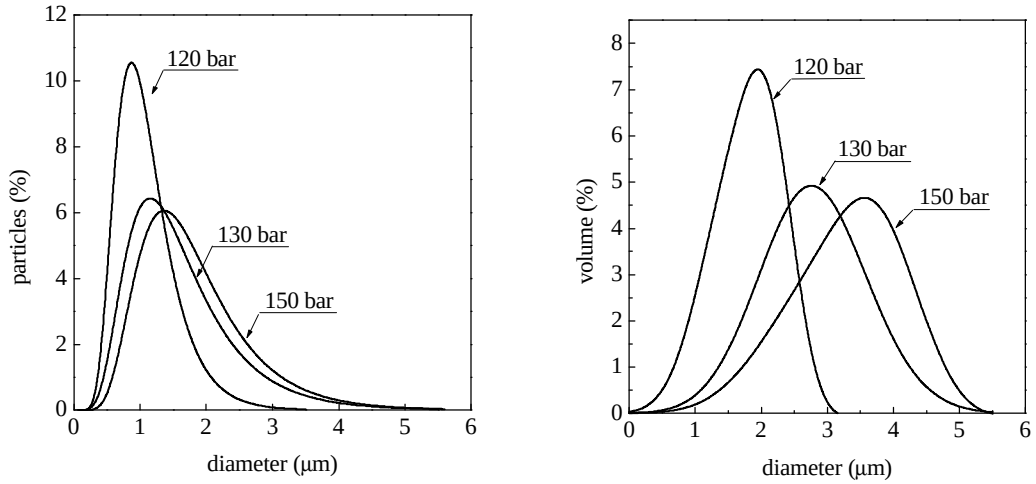

fig. 5. PSDs of Nalmefene HCl powders from EtOH. Calculations in terms of particle number percentages (left side) and volume percentages (right side).

We also studied the influence of the solvent, performing experiments with DMSO at low ratio mass rate CO_2/solution, 40°C and 150 bar, the same pressure as in previous experiments performed using EtOH. We obtained spherical particles with the mode at 1.5 μm (**figure 6**). This result demonstrates that the solvent has an important role in the process and, in particular for the system Nalmefene-SC CO_2-DMSO we can obtain particles with the desired size distribution using lower temperatures.

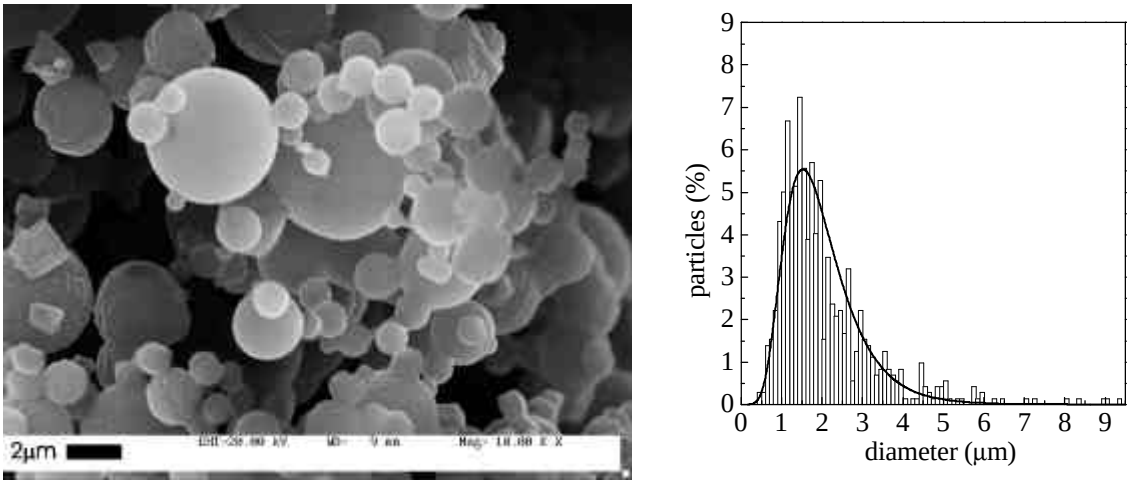

fig. 6. SEM image and PSDs of Nalmefene HCl powders from DMSO at 150 bar, 40°C, 50 mg/mL.

CONCLUSIONS

Using SAS technique Nalmefene HCl was successfully micronized. Varying the operative conditions we obtained different kind of particles. At low CO_2/solution ratio we obtained connected particles with a mean dimension of about 200 nm, the product being perhaps useful for and injection purposes. At higher ratio we obtained particles with a mean diameter of about 1.5 μm and a strict limited range, which are suitable for inhalation (**figure 3**). In particular we obtained the best result at 60 °C and 130 bar, delivering the solution at 2 mL/min and the CO_2 at 29.7 g/min, with a mass ratio CO_2/solution of 25.06; in this case the mean diameter was of about 3 μm and the 91.4% particles was ranged between 1 μm and 5 μm, the particle size useful for aerosol formulation.

Acknowledgments

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