Supercritical Assisted Atomization: A Novel Technology for Microparticles Preparation of an Asthma-controlling Drug

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ABSTRACT

The objective of this study was to produce microparticles of a new asthma-controlling drug by supercritical assisted atomization (SAA), proposed as an alternative to conventional jet-milling process. SAA is based on the solubilization of supercritical carbon dioxide in a liquid solution containing the drug; the ternary mixture is then sprayed through a nozzle, and microparticles are formed as a consequence of the enhanced atomization. SAA process parameters studied were precipitator temperature, nozzle diameter, and drug concentration in the liquid solution. Their influence was evaluated on morphology and size of precipitated particles. Spherical particles with mean particle size ranging from 1 to 3 μm of the new anti-asthma drug were produced by SAA. The mass median aerodynamic diameter (MMAD) of the SAA micronized particles and of the conventional jet-milled drug was used to compare the results obtainable using the 2 techniques. Particularly, MMADs from 1.6 to 4.0 μm were obtained by SAA at the optimum operating conditions and by varying the concentration of the solution injected. MMAD of 6.0 μm was calculated for the jet-milled drug. SAA samples also exhibited narrower particle size distribution (PSD). A good control of particle size and distribution together with no drug degradation was obtained by SAA process.

KEYWORDS: supercritical fluid, microparticles, carbon dioxide, asthma-controlling drug.

INTRODUCTION

Pharmaceutical aerosol delivery is undergoing dramatic changes in both inhaler device and formulation aspects. Particularly, there is a rapid move from traditional propellant-driven metered-dose inhalers (MDIs) to the dry powder inhalers (DPIs). Powder drug inhalation does not depend on the use of chlorofluorocarbons as propellants, is less expensive, and does not require coordination between inhalation and device actuation. Recent improvements in the design, ease of use, and multidose capability, make DPIs attractive alternatives to pressurized MDIs (pMDIs) for aerosol therapy in ambulatory patients, especially for the therapeutic treatment of asthma and other bronchial diseases. Dispersed dry powders also generally have greater chemical stability than liquids used in atomizers.

Dry powders for aerosol delivery require a particle size distribution ranging between 1 and 5 μm to avoid impaction and/or sedimentation in the upper airways and with an optimum Mass Median Aerodynamic Diameter (MMAD) of 3 μm.[1] Therefore, particle engineering using appropriate processes and excipients is required to produce particles of optimal size, morphology, and surface properties that would enhance aerosol efficiency. As a consequence, the preparation of drug microparticles with a controlled particle size distribution has become an important step for the development of aerosol delivery formulations.

Conventional methods used are jet-milling, spray-drying, and recrystallization. Several problems are associated with these processes. Some drugs are unstable under conventional jet-milling conditions; the particles would be jagged due to the jet-milling process and could hold electrostatic charges that impair their dispersion in an aerosol device. Thermally sensitive product can be degraded during the drying or can be contaminated by the solvent used during the crystallization process. Moreover, these methods would not provide an efficient control of the particle size; a broad particle size distribution is normally obtained.

Supercritical Fluids (SFs) can take advantage of some specific properties of gases at supercritical conditions: a continuous adjustable solvent power/selectivity obtained varying pressure and temperature; diffusivities of 2 orders of magnitude larger than those of liquids are also obtainable. As a consequence, SFs can show very fast mass transfer and performances that cannot be obtained by conventional solvents. Mild operating conditions and solventless or organic solvent reduced operation are other advantages. Among all the possible SFs, carbon dioxide (CO_2) is largely used. It performs as a lipophilic solvent; it is nontoxic, nonflammable,

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and cheap; and its critical parameters are readily accessible on the industrial scale (T_c = 37.1°C; p_c = 73.8 bar). For example, critical temperature is very near to the room temperature allowing the treatment of thermolabile compounds. Therefore, SFs have been proposed to develop improved and flexible micronization processes.

The most studied SF-based micronization techniques are the following: (1) the rapid expansion of supercritical solutions (RESS)[2,3]; (2) the particles generation from gas saturated solutions (PGSS)[4,5]; (3) the supercritical antisolvent precipitation (SAS)[6-9]; and the supercritical carbon dioxide assisted nebulization (CAN-BD).[10,11] Supercritical fluids assisted atomization (SAA) is a process recently developed by our research group and proposed in this field.[12,13] These techniques use different mechanisms to produce microparticles. For example, in the RESS process the solute to be micronized is dissolved in the SF; then, a fast depressurization produces drug precipitation, and solid microparticles can be obtained. SAS process is based on drug solubilization in a liquid solvent; the solution is then sprayed in a high-pressure vessel containing the SF. The liquid solvent and the SF form a solution, and the drug precipitates as microparticles. In PGSS, the drug is melted and saturated by SF, and then atomized obtaining microparticles.

CAN-BD process, proposed by Sievers and coworkers, produces an aerosol from the mixing (not solubilization) of SC-CO_2 and the liquid solvent, using a near zero volume tee and a capillary injector. On the contrary, SAA process is based on the solubilization of a given percentage of supercritical CO_2 in a liquid solution in which was previously dissolved the solute to be micronized. The solution is obtained in a high pressure vessel loaded with stainless steel perforated saddles that assures a large contact surface between liquid solution and SC-CO_2. Then, the solution is atomized through a nozzle, and drug microparticles are obtained after the droplets are evaporated using warm nitrogen. SAA process was successfully tested on various drugs such as erytromycin,[14] rifampicin,[15] tetracycline,[15] terbutaline,[16] and griseofulvin,[17] and micronic particles with controlled size distributions were produced using water, methanol, or acetone, as liquid solvents. SAA process scale-up is now in progress.

SAA process performance is particularly good in the particle size range appropriate for inhalable powders. Pharmaceutical companies therefore have shown interest in the preparation by SAA of new chemical entities, which are not easily prepared by traditional micronization techniques. The aim of this work is to illustrate the results obtained micronizing by SAA the new chemical entity, HMR1031. This new drug was synthesized by Aventis Pharma[18] and revealed a strong action as an asthma controlling drug during the preliminary in vitro and in vivo tests; however, owing to its low decomposition temperature (~100°C), a partial drug degradation could be observed when micronization was attempted by conventional jet-milling and spray-drying. Moreover, particle size distributions outside the requested ranges were obtained by conventional milling.

The influence of some SAA process parameters on HMR1031 particles was also studied to evaluate the possibility of particle size tailoring. Micronized powders were characterized with respect to morphology, particle size, and particle size distribution. Drug degradation, solvent residue, and drug solid state were also monitored after SAA processing.

Materials and Methods

Materials

HMR1031 (purity 99.9%) was produced by Aventis Pharma. The drug is a white powder and has a molecular weight of 627.75 Daltons, a formula of $C_{35}H_{41}N_5O_6$, and a decomposition temperature of 102°C. The approximate solubility of the HMR1031 at room temperature is of 250 mg/mL in methanol, less than 5 mg/mL in water, and 110 mg/mL in ethanol. Other details about the HMR1031 chemical structure can be found in the related patent registration document.[18] A sample of jet-milled HMR1031 was supplied by Microgrinding SA (Lugano, Switzerland). Carbon Dioxide and Nitrogen (CO_2 and N_2 99.9%) were purchased from SON (Naples, Italy).

SAA Apparatus

SAA apparatus consisted of 2 high-pressure pumps (model 305, Gilson, Middleton, WI) that deliver the liquid solution and CO_2 to a heated bath (Forlab model TR12, Carlo Erba, Milan, Italy) and then to the saturator. The saturator was a high pressure vessel (intense volume [IV] 50 cm^3) loaded with stainless steel perforated saddles (specific surface area of ~10 m^2/m^3), which assures a large contact surface between liquid solution and CO_2, allowing the dissolution of the gaseous stream in the liquid solution. Residence times in the saturator can vary from several seconds to minutes at the commonly adopted process conditions. The solution obtained in the saturator was sprayed through a 100-μm injector into the precipitator. Nitrogen was taken from a cylinder, heated in an electric heat exchanger (model CBEN 24G6, Watlow, St Louis, MO), and sent to the precipitator to assist liquid droplet evaporation. The precipitator was a stainless steel vessel (IV 3 dm^3) operating at atmospheric pressure. The saturator and the precipitator were electrically heated using thin band heaters. A stainless steel frit at the bottom of the precipitator allowed the powder collection and the gaseous stream flow out. A condenser located after the precipitator was used to recover the liquid solvent. Manometers, temperature controllers, and thermocouples

complete the apparatus. The SAA layout is schematically reported in Figure 1, and further details were published elsewhere.[12,13] All the SAA experiments were performed in 2 replicates.

Powder Morphology by Scanning Electronic Microscopy

The micronized powder was observed by a Scanning Electron Microscope (SEM) coupled with a field emission source (FE-SEM, LEO model 1525, Cambridge, UK). Powders were dispersed on a carbon tab previously stuck to an aluminum stub (Agar Scientific, Essex, UK). Samples were coated with chromium (layer thickness 250Å) using a turbo sputter coater (EMITECH model K575X, Houston, TX). Several SEM images were taken for each run at different heights along the precipitator to verify the powder uniformity.

Particle Size Distribution

Particle size distribution (PSD) was measured by laser diffraction using a MasterSizer S (Malvern Instruments Ltd, Malvern, UK) coupled with the Mastersizer S long bed software (Malvern Instruments, rel. version 2.19) that controls the collection, manipulation, and the presentation of the data. The instrument covers a particle size range between 0.05 and 900 μm, with an active beam length of 2.4 mm. The particles were suspended in liquid paraffin with a refractive index of 1.5 and sonicated for 5 minutes before the analysis. The output signal was converted into a PSD by the polydisperse analysis model and the Mie theory, using a drug refractive index of 1.54. The drug was considered partly opaque. Each SAA run was performed in 2 replicates, and the PSD curves obtained from the replicated runs substantially overlapped, confirming a fairly good SAA process reproducibility.

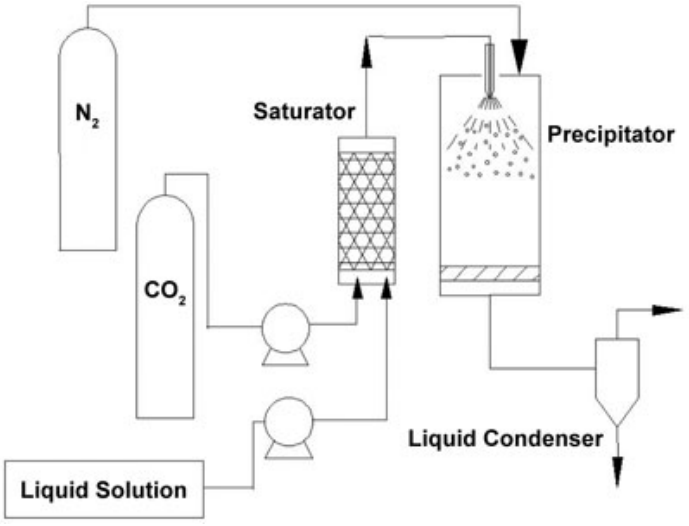

Figure 1. Schematic representation of the SAA apparatus.

Density Measurement and Theoretical Aerodynamic Diameter

The density of the different drug samples was measured with a helium pycnometer (Multivolume Pycnometer 1 model 1305, Micromeritics, Norcross, GA) with 3.5 cm^3 sample cup. Mean values were obtained from 3 measurements of every sample. Density values were used to calculate the MMAD (see Introduction) of the drug microparticles using the following equation[1]:

$$MMAD = (\rho/\chi)1/2 * d_{wg}, \quad (1)$$

where ρ is the sample density expressed as g/cm^3; χ is the dynamic shape factor; d_{wg} is the mean particle diameter.

The dynamic shape factor (χ) is defined as the ratio of resistance force (typically the drag force) on a nonspherical particle to the resistance force on its volume equivalent sphere, when both move at the same relative velocity with respect to gas. The dynamic shape factor is greater than 1 for irregular particles and equal to 1 for spheres.[19] The shape factor of the irregular jet-milled particles was evaluated considering 2 characteristic mean dimensions: width and length. These mean values were measured from SEM images using the Sigma Scan Pro image analysis Software (Version 5.0, Systat Software Inc, Point Richmond, CA); ~1000 particles were considered in each calculation. The shape factor value calculated by static measurement also agreed with the "dynamic one" calculated and reported by De Carlo et al, for particles with same shape and densities.[19]

Drug Degradation

Drug degradation was evaluated performing high-performance liquid chromatography (HPLC)-UV visible (model G131-132, Hewlett-Packard Co, Palo Alto, CA) analysis on untreated and SAA-processed HMR1031. The drug elution was obtained using a reverse phase C_{18} column (4.6 × 250 mm; 5 μm particle size; 80 Å pore size, E7679 Kromasil 100-5C18 5 mm, Bohus, Sweden). The column was equilibrated at a flow rate of 0.8 mL/min with a mobile phase consisting of tetrahydrofuran and water (20:80, analytical grade). Operating at these conditions HMR1031 was monitored at 254 nm with a retention time of 14.1 minutes. All chromatographic analyses were performed at room temperature. The average column backpressure was of ~150 bar. For quantitative analysis, a calibration curve was obtained by successive dilution (10 and 1 ppm) of a drug solution initially prepared at a concentration of 100 ppm. A good linearity was observed in the concentrations explored (R^2 = 0.999). All analyses were performed in triplicate.

Solvent Residue

Methanol residue was measured by a head space sampler (model 7694E, Hewlett- Packard) coupled to a gas chromatograph (GC) interfaced with a flame ionization detector (GC-FID, model 6890 GC-SYSTEM, Hewlett-Packard). Methanol was separated using a fused-silica capillary column (model DB-1, J&W, Folsom, CA); 30-m length, 0.25-mm internal diameter, 0.25-μm film thickness. GC conditions were as follows: oven temperature at 40°C for 8 minutes. The injector was maintained at 180°C (split mode, ratio 1:1), and helium was used as the carrier gas (7 mL/min). Head space conditions were as follows: equilibration time, 60 minutes at 100°C; pressurization time, 2 minutes; and loop fill time, 1 minute. Head space samples were prepared in 10 mL vials loaded with 50 mg of drug. Analyses were performed on each batch of processed drug in 3 replicates.

Drug Solid State

Solid state analyses of HMR1031 samples were performed using an X-ray powder diffractometer (model D8 Discover, Bruker, AXS Inc, Madison, WI). Samples were placed in the holder and flattened with a glass slide to ensure a good surface texture. All samples were evaluated in the 2θ angle range between 20° and 70° with a scan rate of 3 seconds/step and a step size of 0.2°. Analyses were performed on each batch in 2 replicates.

RESULTS AND DISCUSSION

Optimization of the SAA Operating Pressure, Temperature, and Flow Rates

Methanol was selected as the liquid solvent for 2 reasons: it was successfully tested in some previous SAA studies[15,16] and HMR1031 is largely soluble in this solvent.

The solubilization of supercritical CO_2 in the methanol solution is one of the key parameters controlling the efficiency of the SAA process. Particularly, CO_2 solubility in methanol depends on temperature, pressure, and residence time in the saturator, and it is related to high pressure vapor liquid equilibria (VLEs) of the CO_2/methanol/solute ternary system. Data on high pressure VLEs for the binary system methanol/CO_2 were in the literature,[20] whereas quantitative VLE data at high pressure on ternary systems were not. Therefore, pressure and temperature conditions were chosen to ensure the complete miscibility for the binary system solvent-CO_2, making the rough approximation that the modifications of the binary system VLE due to the presence of solute can only consist of the shift of the mixture critical point (MCP) to larger pressures.

Taking into account the VLE data, pressure and temperature in the saturator were explored in the pressure range between 70 and 90 bar and between 70°C and 90°C with the aim of obtaining a single phase. The best results in terms of the processing of HMR1031 were observed operating at a pressure of 80 bar and a temperature of 80°C in the saturator.

Fixed pressure and temperature, the mass flow ratio between CO_2 and liquid solution (R) defines the operating point for SAA into the ternary VLE diagram of the system drug/CO_2/liquid solvent. Since methanol was used as the liquid solvent in some previous studies,[15,16] and an R value of 1.8 allowed a good process reproducibility, this R value was tested also in this work. It corresponds to a molar fraction of CO_2 in the liquid solution of 0.57, calculated in the hypothesis that all CO_2 sent to the saturator dissolves in the methanol solution. HMR1031 micronization tests confirmed this trend: R = 1.8 (CO_2 flow rate = 18 g/min; liquid flow rate = 10 g/min) produced good results in terms of drug particles morphology. When higher R values were used, the injector blocked during the process and SAA failed. This problem occurred because of drug precipitation inside the saturator owing to the formation of 2 or more phases. In this case, a gas phase, containing part of the solvent and of the solute, was formed and induced a partial solute precipitation in the saturator.[14]

Optimization of the SAA Precipitation Temperature

Temperature optimization in the precipitation chamber is required to assist droplet evaporation and to minimize the stress on the drug particles. Preheated Nitrogen with a flow rate of 800 Ndm^3/h was used to set the precipitation chamber at the desired temperature. A precipitation temperature of 40°C was first tested, but particle coalescence was observed. This behavior can be explained considering that too low precipitation temperatures induce a partial solvent recondensation on the precipitated particles. Even in small proportions, the presence of solvent on the particle surface affects the interparticle forces, reducing the interparticle distance. Strong boundary forces, resulting from the surface tension of the solvent, can draw the particles together generating coalescence.[21] Better process performance was obtained by maintaining the precipitation chamber at 50°C; well-separated particles were produced.

Influence of the Drug Concentration and of the Nozzle Diameter on PSDs

Systematic experiments were performed varying HMR1031 concentration in the methanol solution operating at the above reported optimized conditions. Particularly, HMR1031 concentration was varied from 50 to 150 mg/mL to explore

the effect of this process parameter on the size and distribution of the precipitated powders. The morphology of HMR1031 particles obtained in all these runs was always spherical and noncoalescing. Some examples of the particles produced are shown in the SEM images reported in Figure 2 in which the experiments were performed at solute concentrations of 50 and 100 mg/mL, respectively. These SEM images were obtained with the same enlargement (20 K), therefore it is possible to make a qualitative evaluation of the broadening of particle size when the HMR1031 concentration in methanol is increased.

PSDs of the SAA micronized drug were measured by laser diffraction and are reported in Figure 3, in terms of volumetric distributions in a cumulative form. The volume-based particle size distributions enhance the contribution of the larger particles since the volume and not the diameter is

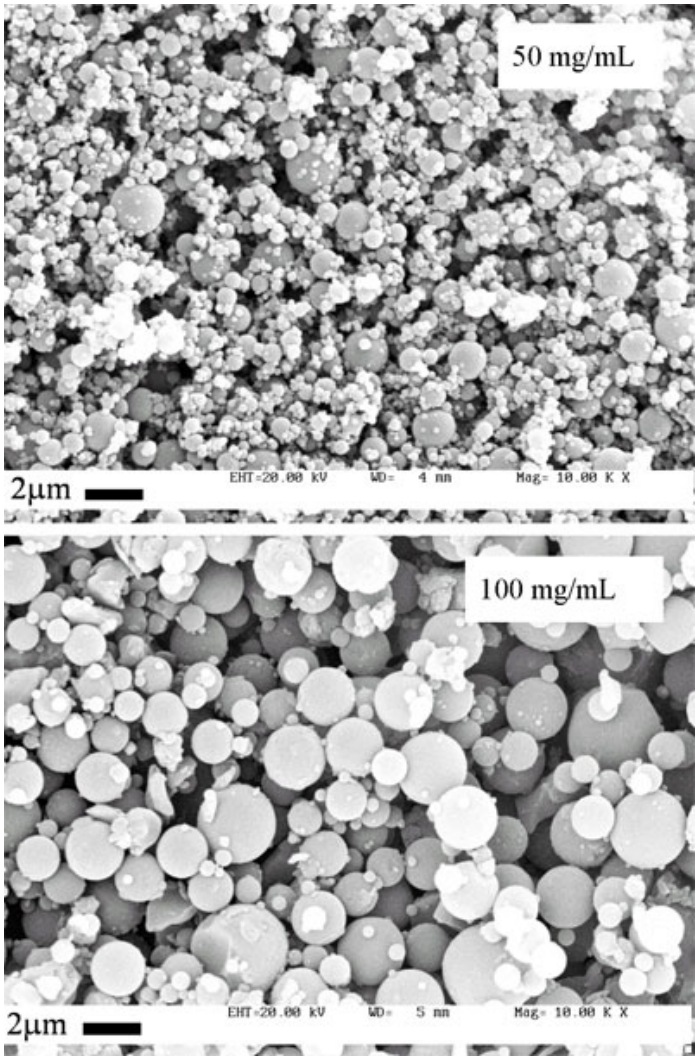

Figure 2. SEM images of HMR1031 precipitated by SAA from methanol operating at 80 bar, 80°C in the saturator and at a temperature of 50°C in the precipitator. The concentration of HMR1031 in the solution was 50 and 100 mg/mL, respectively. Both images are reported with a magnification of 10 K.

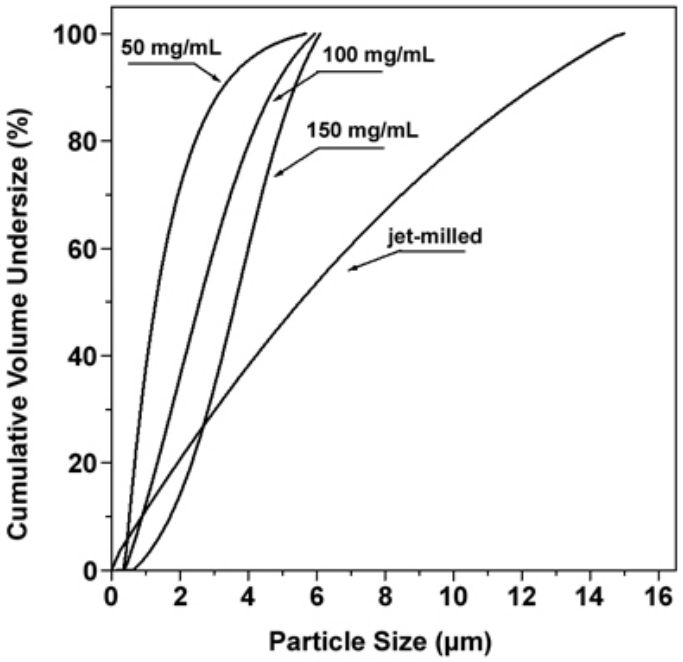

Figure 3. PSD curves of HMR1031 produced by SAA from methanol operating at 80 bar, 80°C in the saturator and at a temperature of 50°C in the precipitator. HMR1031 concentrations of 50, 100, and 150 mg/mL are reported. The PSD of the jet-milled drug is also reported for comparison purposes.

the reported parameter. These distributions are the most relevant when a pharmaceutical compound is described, since the weight of the drug with a given particle size is the key parameter with respect to its therapeutic performance. The PSDs in Figure 3 indicate that HMR1031 microparticles produced at 50 mg/mL exhibit a D_{50} of 1.4 (± 0.1) μm and a D_{90} of 3.2 (± 0.2) μm; particles produced at 100 mg/mL have a D_{50} of 2.6 (± 0.1) μm and a D_{90} of 4.9 (± 0.2) μm, whereas microparticles produced at 150 mg/mL show a D_{50} of 3.6 (± 0.2) μm and a D_{90} of 5.4 (± 0.3) μm.

The PSD of a commercial jet-milled sample is also reported in Figure 3, for comparison purposes; an example of its morphology is illustrated in the SEM image reported in Figure 4. The size distribution of the commercial HMR1031 is wider with respect to the SAA micronized drug and nearly 50% of the milled powder is outside of the aerosol size range, as discussed in the Introduction. In particular, a D_{50} of 5.6 (± 0.2) μm and a D_{90} of 12.4 (± 0.4) μm was measured.

The PSDs were evaluated by laser diffraction method and were always in good agreement with the indications given by SEM images of the SAA micronized and jet-milled particles. However, SEM observations revealed that SAA micronized particles are spherical (see Figure 2) and can be hollow, as demonstrated in a previous work,[17] whereas the jet-milled particles showed an irregular shape (see Figure 4).

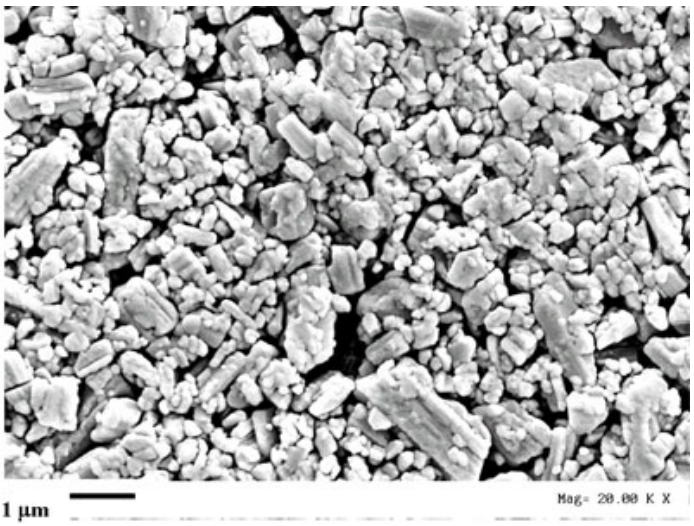

Figure 4. SEM image of jet-milled HMR1031. Magnification 20 K.

As a consequence, these powders can show different aerodynamic behaviors when delivered by aerosol. For this reason, MMADs of drug particles were calculated from Equation 1 (see Methods section) to perform a more adequate comparison between the particles produced by SAA and commercial jet-milling. The particle density was evaluated by a pycnometer (see Methods section), and the measured mean density is of 1.2 g/cm^3 for SAA micronized particles, while the jet-milled sample shows a density of 1.4 g/cm^3. A shape factor equal to 1 was assumed for the SAA particles and a shape factor of 1.2 was used for the commercial drug sample. This shape factor value was also evaluated by measuring on the SEM images the 2 characteristic dimensions of jet-milled particles: width and length.

According to Equation 1, the calculated MMADs are as follows: 1.6 μm for the particles produced at 50 mg/mL, 2.9 μm for the particles produced at 100 mg/mL, and 3.9 μm for the particles produced at 150 mg/mL by SAA. MMAD of the jet-milled powder is of 6.0 μm. This result confirmed that the SAA particles show more favorable MMADs to address the drug in the deep lung with an aerosol delivery device with respect to the commercial sample. In particular, particles produced at 100 mg/mL are in a range even more restrictive than the standard one requested for drug aerosol delivery.

The difference in PSDs (and MMADs) of the powders produced at different solute concentrations reveal also the possibility of particle size tailoring by SAA, varying the drug concentration in the starting solution. Indeed, at the same pressure, temperature, and R-mass flow ratio operating conditions, the higher the solute concentration in the solution injected, the greater the mean particle size obtained.

To test the effect of the injection device on the PSD of the precipitated drug some SAA experiments were performed using an injector with an 80-μm internal diameter and repeating the experiments previously performed at 150 mg/mL of HMR1031 concentration in methanol. The PSD produced in this case exhibited a D_{50} of 1.9 μm, whereas a D_{50} of 2.6 μm was obtained using the 100-μm injector at the same operating conditions. The calculated MMADs of the powder are 2.1 and 2.9 μm, respectively.

The effects of solute concentration and injector size on the mean particle size and distribution can be explained considering the postulated SAA mechanism.[15] In particular, it was hypothesized that SAA is characterized by the formation of "primary droplets" produced at the exit of the atomization device, from which originate "secondary droplets" owing to the rapid release of CO_2 from inside the primary ones. These secondary droplets are rapidly dried by warm nitrogen, and microparticles are formed. In spray atomization processes, the mean diameter of the droplets is strongly related to the section of the injection device: the smaller the section, the smaller the droplets. In SAA process, the particles are generated by drying the "secondary droplets," therefore the result obtained it is somewhat expected. However, the 80-μm injector has an atomization section that is the 64% of the 100-μm ones, while the decrease observed in the D_{50} size of the particles is only ~25% (ie, is less marked than can be expected from the classical spray atomization theory). Similar results were also obtained in a previous SAA work.[12] Therefore, SAA ability to produce micronic and submicronic particles with sharp PSD mainly relies on the quantity of SC-CO_2 dissolved in the liquid solution and on its release from the primary droplet. This consideration explains the reduced influence of the size of the atomization device on particle dimensions. Moreover, droplet formation during atomization is obviously related to the viscosity and the surface tension of the solution. In particular, viscosity increases with the concentration of solute (drug) in the liquid mixture producing, as a consequence, larger droplets.

As a consequence, SAA process produces an atomization that is strongly enhanced by the presence of SF with respect to the conventional spray drying technique; substantially smaller secondary droplets are produced and, therefore, smaller particles are obtained.

Drug Chemical Characterizations and Solid State

HPLC analyses of SAA-processed samples showed a single chromatographic peak monitored at the same elution time as the standard drug. An example is illustrated in Figure 5, where HPLC traces of the untreated and SAA micronized HMR1031 are reported; both samples show the same retention time of 14.1 minutes. It was calculated that the 98% (± 0.5) of HMR1031 was not degraded after SAA process. This result is particularly relevant because only the 82%

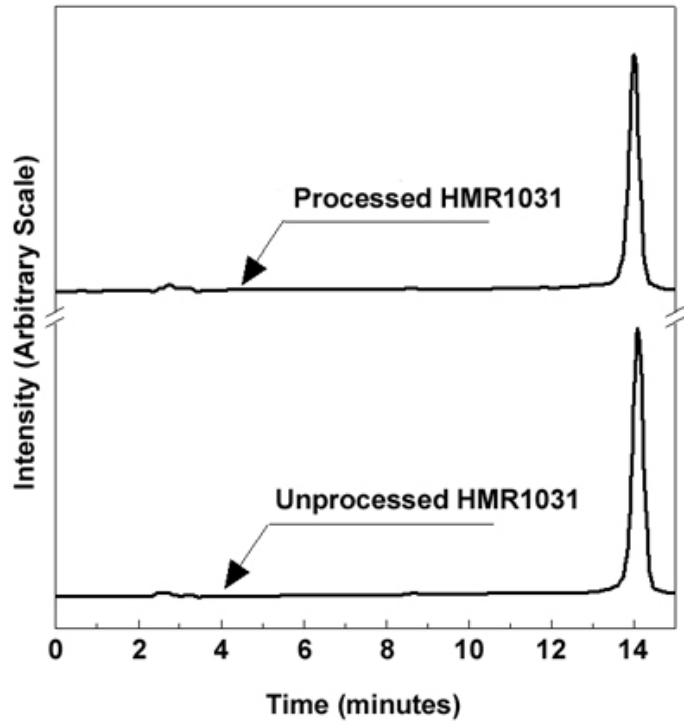

Figure 5. HPLC trace of untreated and SAA treated HMR1031.

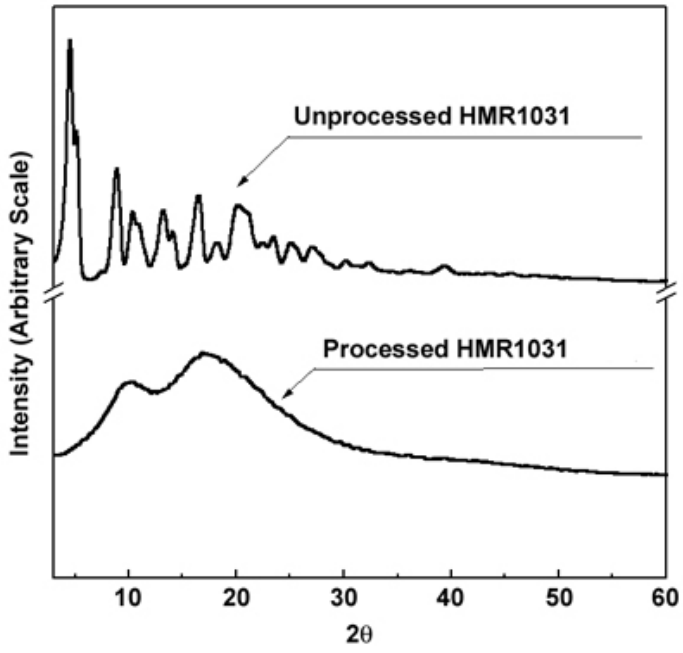

Figure 6. XRD trace of unprocessed and SAA-processed HMR1031.

(± 0.5) of the drug was monitored as not degraded in the jet-milled samples.

A methanol residue ranging from 100 to a maximum value of 500 ppm was measured. These values were obtained in different SAA runs performed at the optimized process condition and varying the solute concentration in the solution injected; however, they are well below the International Conference of Harmonization (ICH) limit that fixed at 3000 ppm the methanol residue because it is a class 2 solvent in pharmaceutical product.[22]

X-ray analyses were performed to evaluate the micronized drug solid state. SAA particles showed a spherical shape when observed by SEM and, in some previous studies, this shape indicated an amorphous solid state.[15,16] The X-ray traces confirmed this general trend; HMR1031 amorphous particles are produced by SAA. An example of X-ray pattern of SAA processes HMR1031 is reported in Figure 6; the X-ray pattern of the untreated drug is also reported for comparison purposes.

A reduction of the crystalline structure of a substance in general improves its bioavailability but can reduce the drug stability. X-ray diffraction (XRD) analyses were repeated after 30 days of drug storage at 40% of relative humidity to monitor if any recrystallization occurred. The micronized drug retained the amorphous habit and, in the same period of time, no relevant variations were observed by SEM in the micronized powder morphology and size. HPLC analyses also confirmed that there was no chemical degradation after 30 days of storage.

CONCLUSION

SAA was successfully applied to a new chemical entity (HMR1031) that was problematic to process using traditional micronization techniques. The study of influence of drug concentration in the liquid solution on SAA performance revealed also the possibility of particle size tailoring depending on the requested target.

ACKNOWLEDGMENTS

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