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Terbutaline microparticles suitable for aerosol delivery produced by supercritical assisted atomization

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Abstract

A new micronization technique called supercritical assisted atomization has been used to produce terbutaline microparticles with controlled particle size distribution in the range of drug particles deliverable by aerosol. The process is based on the solubilization of a fixed amount of supercritical carbon dioxide in a liquid solution; then, the ternary mixture is sprayed through a nozzle and atomized in order to produce microparticles. Water has been used as the liquid solvent; heated nitrogen has also been delivered into the precipitator to evaporate the liquid droplets. The process has been first optimized with respect to pressure and temperature (mixing temperature and pressure, precipitation temperature) and very mild operation conditions have been selected; then, the influence of the solute concentration in the liquid solution on particle size has been studied. The terbutaline produced powders were characterized with respect to morphologies and particle size. Spherical particles with very narrow volumetric particle size distributions were produced. Particularly, operating at 30 and 50 mg of terbutaline per ml of water, more than 90% of the two distributions ranged between 1 and 3 μm; at 80 mg/ml more than 99% of the distribution ranged between 1 and 4 μm. HPLC analysis confirmed that no chemical degradation occurred in the drug as a consequence of the supercritical processing.

Keywords: Terbutaline; Supercritical fluids; Micronization

1. Introduction

Terbutaline is a beta-adrenergic receptor antagonist that acts as a bronchodilator in the treatment of asthma and chronic bronchitis (Barnes, 1989; Popa, 1986). It is mainly delivered as solid powder by aerosol.

Extensive research has shown that the critical size and, even more important, critical particle size distribution for aerosol delivery formulations lays in the range from 1 to 5 μm. Some authors report that a more reduced range between 1 and 3 μm is even more effective. Indeed, particles larger than about 5 μm collide with the walls in the upper airways; then, they are carried by ciliary flow to the mouth and reach the system primarily by ingestion. Particles smaller than 1 μm can remain suspended in the inspired and expired air and do not reach the lung. Only particles ranging approximately between 1 and 3 μm are effectively delivered in the deep lung, where they can perform their therapeutic action (Hickey, 1996).

Conventional techniques like jet milling and spray drying are usually not able to produce powders with very narrow and controlled particle size distributions; moreover, they can induce thermal degradation of the drug. Therefore, in this last decade, several supercritical fluids based techniques have been proposed for the production of micronic and nanometric particles of pharmaceuticals compounds. Supercritical fluid

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micronization processes can take advantage of some specific properties of gases at supercritical conditions, like large diffusivities, possibility of solvent power modulation and, as a consequence, the possibility to produce powders with controlled size and distribution. The solventless or organic solvent reduced operation is another advantage of these techniques. Supercritical fluids based micronization techniques proposed are: the rapid expansion of supercritical solutions (RESS) (Debenedetti et al., 1993; Reverchon et al., 1995; Kim et al., 1996), the particles generation from gas saturated solutions (PGSS) (Weidner et al., 1994; Sencar-Bozic et al., 1997; Kerc et al., 1999) and the supercritical antisolvent precipitation (SAS) (Yeo et al., 1993; Hanna et al., 1995; Reverchon, 1999; Reverchon et al., 2000, 2001, 2002). RESS concept can be implemented in relatively simple equipments although particle collection from the gaseous stream is not easy; the applications are limited since many drugs are not soluble enough into the supercritical fluid. Limitations for PGSS application are the drug thermal stability up to melting conditions. The prerequisite for successful SAS is the complete miscibility of the liquid in the supercritical CO_2 and the insolubility of the solute in it. All these techniques cannot be used to process water soluble compounds.

Recently, supercritical carbon dioxide assisted atomization process has been proposed. The process can be considered a modification of the classical spray drying. This technique allows drug micronization using water as liquid solvent; but, can work using organic solvents too (Sievers et al., 1999; Sellers et al., 2000, 2001; Reverchon, 2002; Reverchon and Della Porta, in press). Sievers and co-workers proposed the use of 1 ml tee with the scope of minimize the time of contact between the two phases. Various capillary injectors were also proposed to obtain a supercritical CO_2 assisted nebulization (Sievers et al., 1999; Sellers et al., 2000, 2001). Sievers and co-workers used solutions of water or ethanol or water/ethanol mixtures, to produce aerosols with very small droplets that rapidly expanded from an emulsion formed by supercritical CO_2 and successfully processed several compounds. We have developed an evolution of the supercritical assisted atomization (SAA) (Reverchon, 2002). In our arrangement, a thermostated packed contactor is added to the process and it is used to obtain continuous equilibrium solubilization of CO_2 in the liquid solution. The contactor has the opposite function of the near-zero volume tee; indeed, it is designed to provide large contacting surface and an adequate residence time in order to allow the efficient dissolution of supercritical CO_2 in the liquid solution. The solution formed in the contacting device is then send to a thin wall injector and sprayed into the precipitator. Moreover, thin wall injectors are used, instead of capillary devices. The SAA process has been tested on various drugs like: carbamazepine, dexametasone, triclabenzadol (Reverchon, 2002) rifampicin and tetracycline (Reverchon and Della Porta, in press) producing controlled micronic particle size distributions of compounds precipitated from water, methanol or acetone.

Terbutaline is water soluble and, therefore, it represents a good candidate for SAA micronization. Thus, the aim of this work is to test the possibility of terbutaline microparticles production using SAA. SAA-produced powders are characterized with respect to their morphology, particle size and particle size distribution in order to optimise the operating parameters for the application selected. We study the influence of some process parameters on terbutaline particle size distribution to evaluate the possibility of particle size tailoring. HPLC analyses are performed on the untreated and on the SAA-processed powders to ascertain if chemical degradation occurred in the drug as a consequence of SAA processing. The comparison with a commercial micronized terbutaline sample is also proposed.

2. Experimental apparatus, materials and methods

2.1. Experimental apparatus

The apparatus used for SAA is reported schematically in Fig. 1. It consists of three fed lines used to deliver supercritical CO_2, the water solution and warm N_2 and of three vessels: saturator, precipitator and condensator. Liquid CO_2 is taken from a cylinder and sent to the high pressure pump (Gilson model 305); then, it is sent to a heated bath (Forlab, Carlo Erba model TR12) and to the saturator where it solubilizes into the liquid solution. The water solution is taken from a graduate glass vessel; it is pressurised by a high pressure pump (Gilson model 305), heated and

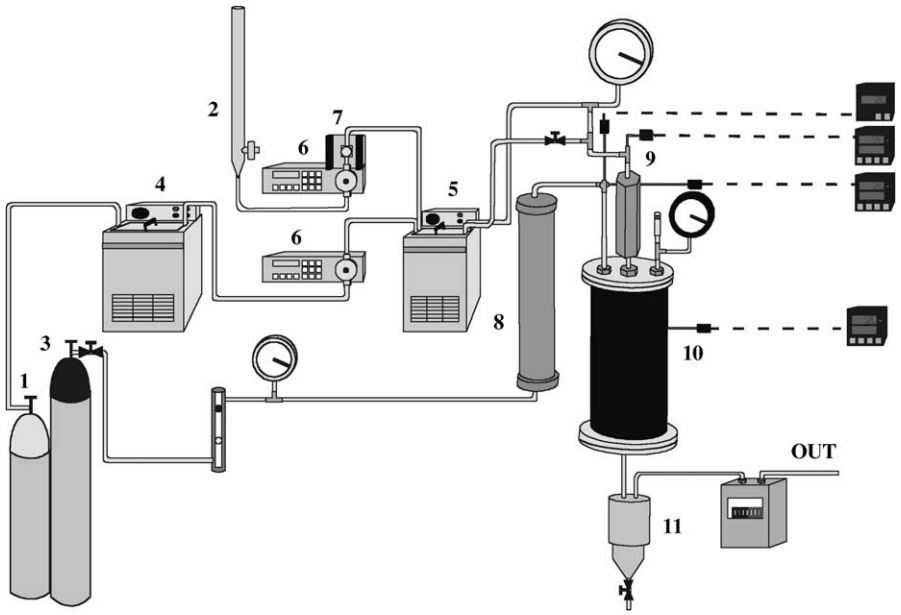


Fig. 1. Schematic representation of the SAA apparatus: (1) CO_2 cylinder; (2) liquid solution; (3) N_2 cylinder; (4) cooling bath; (5) heating bath; (6) high pressure pumps. (7) dampener; (8) heat exchanger; (9) saturator; (10) precipitator; (11) condensator.

sent to the saturator. N_2 is taken from a cylinder, it is heated in an electric heat exchanger (Watlow model CBEN 24G6) and then, is sent to the precipitator. The saturator is a high pressure vessel (i.v. 50 cm^3) loaded with stainless steel perforated saddles to assure a large surface for the contact between CO_2 and the water solution obtaining the dissolution of the gaseous stream in the water solution. The solid–liquid–gas mixture at the exit of the saturator is sent to a thin wall stainless steel injector (i.d. 80 μm) to produce a spray of liquid droplets in the precipitator. The precipitator is a stainless steel vessel (i.v. 3 dm^3) operating at near atmospheric pressure. It also receives the flow of heated N_2 to evaporate water from the droplets. The powder and the gas mixture (CO_2/N_2/water) generated in the precipitator are forced to assume an ordinate motion by a flux conveyor. It consists of a helicoidal stainless steel device that occupies all the horizontal precipitator section. The powder is collected at the bottom of the precipitator on a stainless steel sintered frit (mean pore diameter 0.1 μm), whereas, the gaseous stream passes through the frit and reaches a cooled separator where water is condensed. The resulting gas mixture (CO_2 and N_2) is sent to a dry test meter (Schlumberger, model 2000AP LPG G2.5) to measure the overall flow rate. Calibrate thermocouples, manometers, check valves, high pressure piping and connections complete the apparatus. More details on this apparatus have been given in a previous paper (Reverchon, 2002).

2.2. Materials

Terbutaline sulphate with a purity of 99.9% was supplied by ICN Biomedicals (Milano, IT). Distilled water (residue after maximum evaporation at 2 ppm) was supplied by Carlo Erba Reagenti (Italy). CO_2 (purity 99.9%) was purchased from SON (Naples, Italy). All products were used as received. The solubility of terbutaline in water is of 90 mg/ml at room pressure and temperature. Terbutaline melting temperature is 121 °C. A commercial sample of micronized terbutaline sulphate was kindly supplied by Micromacinazione SA (Switzerland).

2.3. Analytical methods

Samples of the powder precipitated on the metallic frit were observed by scanning electron microscopy

(SEM, model LEO 420). SEM samples were covered with 250 Å of gold–palladium using a sputter coater (Agar model 108A). The particle size (PS) and the particle size distribution (PSD) were measured using the Sigma Scan Pro software (Jandel Scientific); about 1000 particles were considered in each PSD calculation performed. The untreated materials consisted of irregular crystal with particle sizes ranging between 5 and 20 μm.

Drug stability was evaluated by performing HPLC-UV-Vis (Hewlett-Packard model G131–132) analysis on the untreated material and on the SAA-treated powder. The elution was obtained using a reverse phase C18 column (4.6 mm × 250 mm; 5 μm particle size). The column was equilibrated at a flow rate of 1 ml/min with a mobile phase consisting of: phosphate buffer at pH 7 (77%) and methanol (23%). Terbutaline was monitored at 220 nm with a retention time of 4.8 min. All chromatographic analyses were carried out at room temperature. The average column back pressure was of about 2 bar.

3. Results and discussion

As discussed in the introduction, the solubilization of CO_2 in the liquid solution is one of the key parameters controlling the efficiency of SAA. The maximum quantity of CO_2 that can be solubilized in water (solubility) depends on the temperature and pressure in the contacting device. Another condition that has to be obtained in the saturator, is an adequate residence time of the water solution and of the compressed gas to assure gas saturation in the liquid solution. This last condition can be pre-determined by setting adequate flow rates for the gas and the liquid that allow contacting times of several seconds. To set pressure and temperature conditions in the saturator, precise values of CO_2 solubility in the liquid solvent should be known. In the case of water, some data is reported in the literature (Takenouchi and Kennedy, 1964); but, the knowledge of the ternary system behaviour (CO_2/water/terbutaline) at high pressures is not available. Solubility of water in SC-CO_2 is very low at every operating condition; therefore, this aspect of the process does not represent a problem for SAA operation. As a consequence, CO_2 in excess with respect to the expected solubilization concentration was used. This last condition also assures a readily obtainment of the process pressure in the saturator. According to these considerations, some preliminary tests were performed by setting the saturator operating conditions in a pressure range from 100 to 150 bar and in a temperature range between 70 and 80 °C; these conditions should assure good solubility of CO_2 in water and very small solubility of water in CO_2.

In our previous general work on SAA (Reverchon, 2002) we used precipitation temperatures up to 80–100 °C. In the case of terbutaline, since the drug has a low melting temperature (121 °C), we started the experiments at lower precipitation temperatures to avoid problems of particles sintering or of drug degradation. Particularly, the precipitatior temperature was initially set at 50 °C. The precipitator was set at 1.2 bar and warm N_2 with a flow rate of 60 N dm^3/min was delivered. The flow rate ratio between CO_2 and the water solution was alwasys regulated at 1.8 w/w.

With the precipitator temperature set at 50 °C, terbutaline particles formed large agglomerates like the ones reported in Fig. 2, were a SEM image of the precipitate is reported. When the precipitator temperature was increased to 60 °C, partly connected particles were produced (see SEM image in Fig. 3). The explanation of this phenomenon is that: at the temperatures used in the precipitator, water condensates on the internal surfaces of the vessel causing the partial resolubilization of the terbutaline particles that coalesced together. Fig. 3 is particularly significant from this point of view, because the solid bridges that connect the spherical particles are evidenced. Only when the precipitator temperature was increased up to 70 °C, well separated spherical particles were obtained (see Fig. 4a–c).

After these preliminary tests, we selected, as the best process parameters, a pressure of 95 bar and a temperature of 82 °C in the saturator; a temperature of 70 °C was maintained in the precipitation chamber. Then, systematic experiments were performed in duplicate operating at different terbutaline concentrations in water to explore the effect of this process parameter on the precipitated powder. Indeed, we know from previous works (Reverchon, 2002; Reverchon and Della Porta, in press), that this parameter is the most relevant in the control of particle size and particle size distribution. The morphology of terbutaline particles obtained in all these experiments was

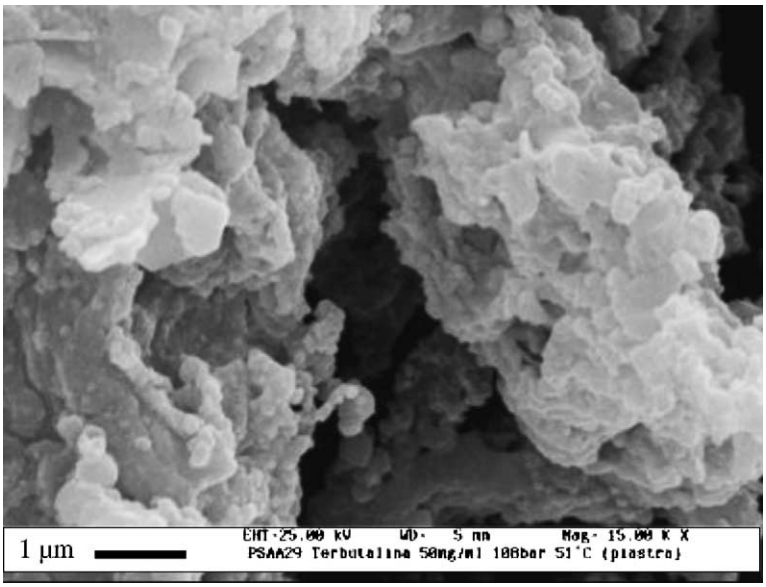


Fig. 2. SEM image of terbutaline precipitated by SAA from water operating at 100 bar, 80 °C in the saturator and at a temperature of 50 °C in the precipitator.

always spherical with well-defined and non-coalescing nano and micrometric particles. Some examples of the particles collected are shown in the SEM images reported Fig. 4a–c that are referred to some of the experiments performed at solute concentrations in water between 10 and 80 mg/ml. These SEM images have been obtained with the same enlargement (10 K); therefore, they allow a qualitative evaluation of the

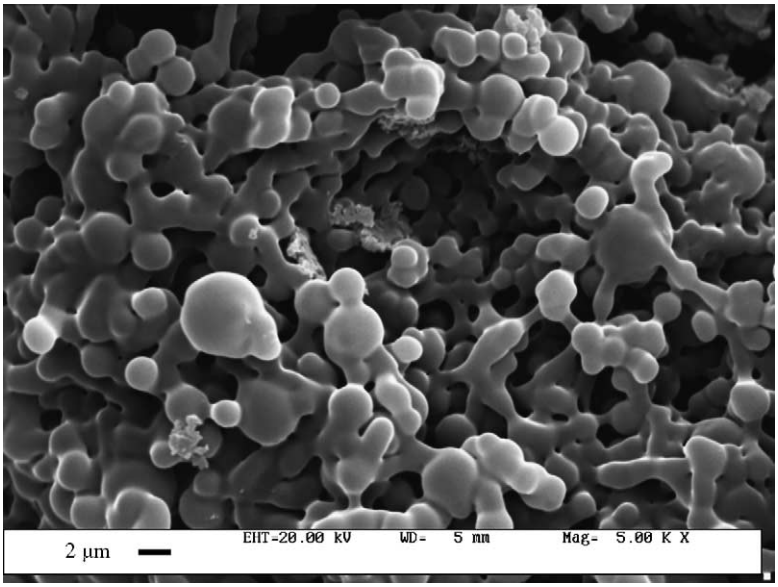
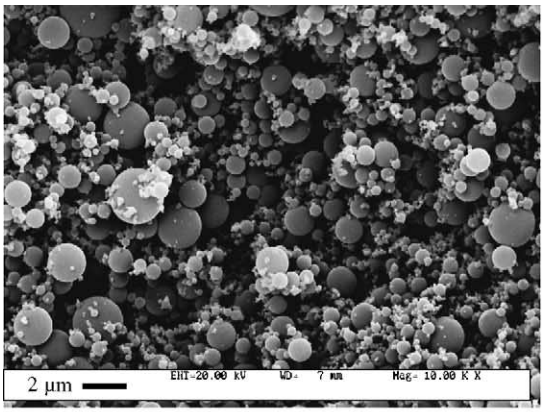
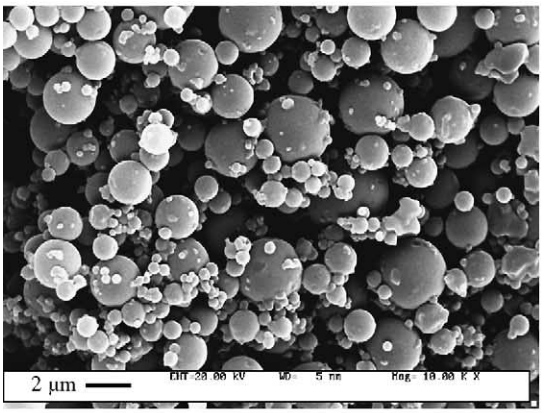


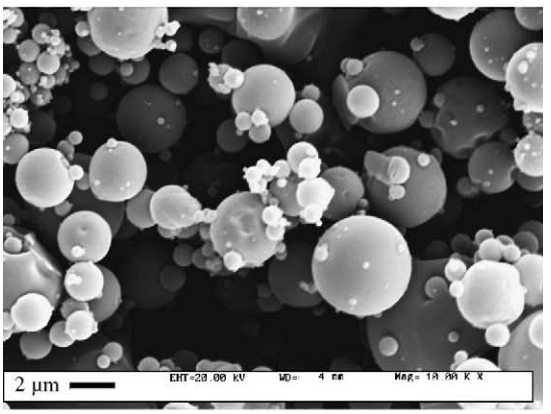
Fig. 3. SEM image of terbutaline precipitated by SAA from water operating at 100 bar, 80 °C in the saturator and at a temperature of 60 °C in the precipitator.



(a)



(b)



(c)

Fig. 4. (a–c) SEM images of terbutaline precipitated by SAA from water operating at 95 bar, 82 °C in the saturator and at a temperature of 70 °C in the precipitator; the concentration of terbutaline in the water solution was of 10, 30 and 80 mg/ml, respectively.

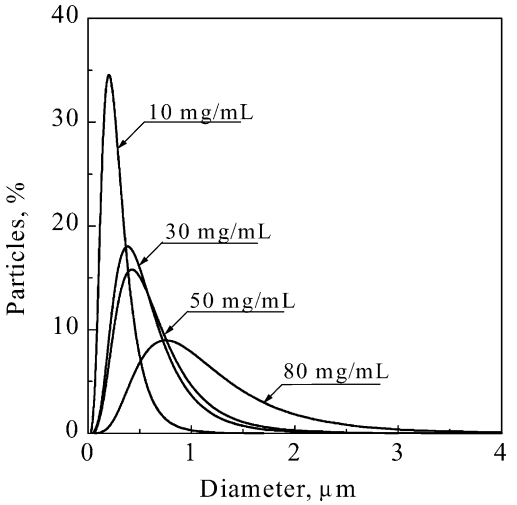


Fig. 5. Particle size distribution curves (based on the number of particles) of terbutaline microparticles produced by SAA from water operating at 95 bar, 82 °C in the saturator and at a temperature of 70 °C in the precipitator. Terbutaline concentrations in water from 10 to 80 mg/ml were reported.

increase of particle size and of polydispersity when the terbutaline concentration in water is increased.

SEM images have been studied using an image analysis software (as described in Section 2) to obtain

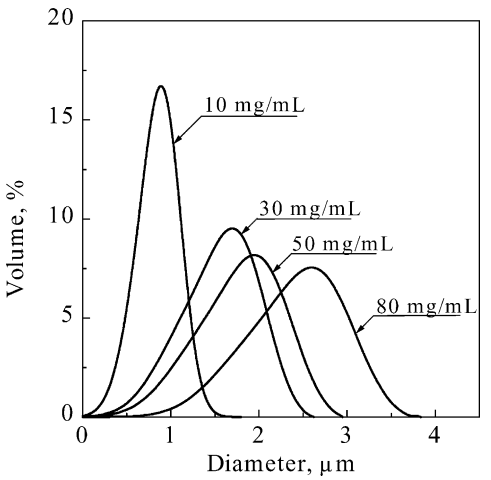


Fig. 6. Particle size distribution curves (based on particles volume) of terbutaline microparticles produced by SAA from water operating at 95 bar, 82 °C in the saturator and at a temperature of 70 °C in the precipitator. Terbutaline concentrations in water from 10 to 80 mg/ml were reported.

a measure of the particle size distributions. The results have been illustrated in the diagram in Fig. 5, where distributions based on the number of particles are reported. They are asymmetric and have been described using a log-norm distribution. The mode (the most frequent particle size) varies from 0.3 to 0.7 μm, when the solute concentration varies from 10 to 80 mg/ml. An enlargement of the distributions is also observed when the solute concentration is increased.

Fig. 6 shows the same information of Fig. 5, but reported in terms of volumetric distributions. These distributions are fairly well fitted by GCAS curves with modes ranging between 0.7 and 2.6 μm, when solute concentration was increased again from 10 to 80 mg/ml. The volume based particle size distributions enhance the contribution of the larger particles since the volume and not the diameter is the relevant parameter. These distributions are very important when a pharmaceutical compound is described. Indeed, in this case, the number of particles having a fixed diameter is not particularly relevant, since the weight of the drug with a given particle size is the key parameter with respect to therapeutic performance. The microparticles produced operating with a terbutaline solution in water of 10 mg/ml, strictly ranged from 0.1 to 1.7 μm; more than 70% of the particles is smaller than 1 μm; therefore, as discussed in the introduction, they are too small for aerosol delivery system formulations. The microparticles obtained using the terbutaline solutions in water of 30 and 50 mg/ml represent, instead, a good result in terms of the required particle size distributions. In these cases, particles ranging from 0.1 to 1 μm represent only 4 and 2.5% of the two distributions, respectively. A maximum particle size of 2.6 and 2.9 μm is also obtained, respectively. In both cases, more than 90% of the particle size distribution is included between 1 and 3 μm. No particles larger than 3 μm have been produced; therefore, these particles can be used to address the drug to particular targets in the lung, as discussed by Hickey (1996). Moreover, if a particle size distribution between 1 and 5 μm is preferred, the powders produced operating at 80 mg/ml are the best in terms of particle size distribution, because particles ranging between 1 and 4 μm represent about 99% of the whole distribution.

In all cases very regular and homogenous particles have been obtained by SAA with respect to the particles that can be obtained with the traditional techniques that are irregular and characterized by larger particle size distributions. An example of commercial micronized terbutaline, produced by jet milling, is reported in Fig. 7. A comparison between the particle size distribution curves (based on particles volume) of

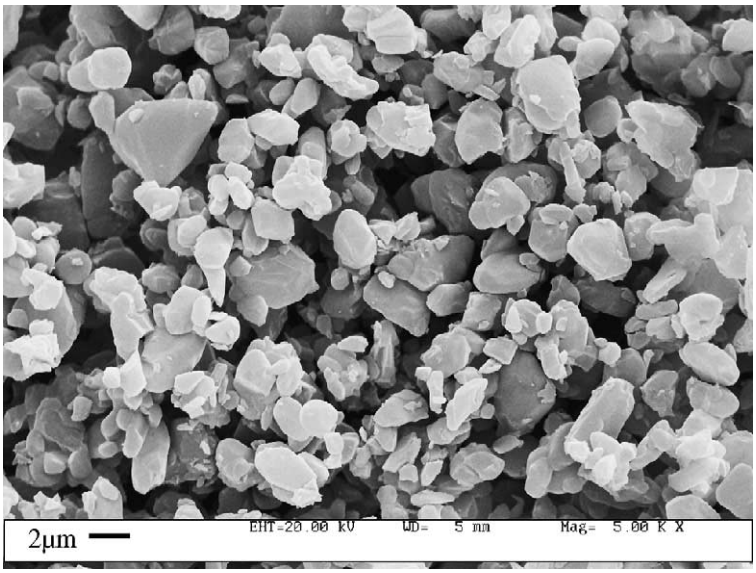


Fig. 7. SEM image of a commercial sample of micronized terbutaline.

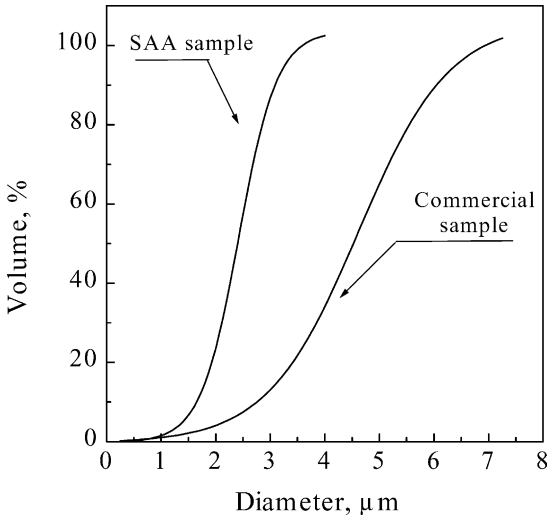


Fig. 8. Comparison between the particle size distribution curves (based on particles volume) of terbutaline produced by SAA at 80 mg/ml and of the micronized commercial sample.

terbutaline produced by SAA from water at 80 mg/ml and of the micronized commercial sample is reported in Fig. 8. From this diagram, it is evident that nearly 30% of the commercial sample is formed by particles larger that 5 μm, whereas, the SAA sample ranges between 1 and 4 μm.

The efficient particle size control obtained by SAA can be explained considering the probable process mechanism (Reverchon, 2002). We think that SAA is characterized by the formation of "primary droplets" produced at the atomization device, from which originate "secondary droplets" due to the rapid release of CO_2 from the internal of the primary ones. These secondary droplets are rapidly dried by warm N_2 and particles are formed on the basis of a "*one droplet one particle*" mechanism. This mechanism seems the only one able to explain the exceptional efficiency of SAA in producing very small micronic and submicronic particles.

HPLC analysis performed on the SAA-treated and raw terbutaline showed that no degradation occurred during the process. This result is illustrated in Fig. 9, where HPLC traces of the untreated and of SAA-treated terbutaline are reported. Therefore, thermal and mechanical stress due to SAA process had no effect on drug stability.

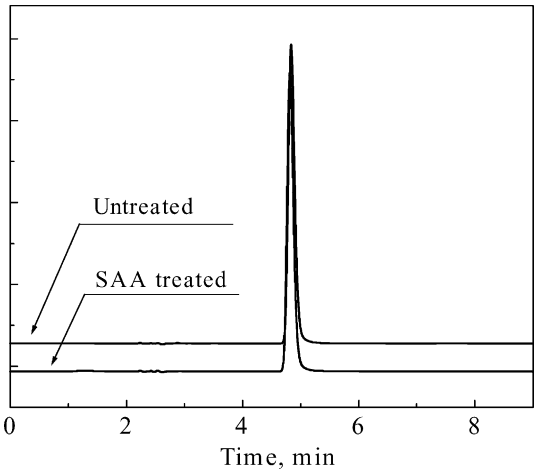


Fig. 9. HPLC trace of untreated and SAA-treated terbutaline.

4. Conclusions

SAA has confirmed to be a very promising process to produce micronic particles of controlled particle size and particle size distribution also in the case of terbutaline. Using water as liquid solvent we produced powders with very narrow particle size distributions centered in the particle size range of aerosilizable drugs. From the various particle size distributions obtained, it is evident that it would also be possible to obtain even more specialized particles tailored for different targets inside the lung.

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