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Ampicillin micronization by supercritical assisted atomization

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Abstract

The micronization technique called supercritical assisted atomization (SAA) was used to produce ampicillin microparticles with controlled particle size and particle size distribution suitable for aerosol drug delivery. The process is based on the solubilization of supercritical CO_2 in a liquid solution. The ternary mixture is then sprayed through a nozzle and, as a consequence of enhanced atomization, solid microparticles are formed. Water and organic solvents were tested with ampicillin to determine the influence of the solvent on the process mechanism. SAA process parameters were studied by testing different supercritical/liquid solvent flow ratios, ampicillin concentrations in the liquid solution and nozzle diameters. The effect of these parameters on morphology, particle size and particle size distribution of microparticles was analysed. Ampicillin particles suitable for aerosol delivery in the size range 1–5 μm were obtained using buffered water. Moreover, by varying the solute concentration, ampicillin particles in a narrower range (1–3 μm) than that usually suggested for aerosol deliverable drugs were obtained. This is an example of particle size tailoring by SAA.

Introduction

The delivery of aerosols via the lung provides an attractive alternative to the oral route because of the localized delivery of drug directly to the site of action in the respiratory tract. Moreover, a very large surface area provides a promising portal for the delivery of systemically active drugs. For effective absorption, in order to achieve local or systemic effects, the inhaled compounds must deposit deep in the lung, where the alveolar epithelium thickness is very small (Patton & Platz 1992). It is widely accepted that specific particle properties such as size, shape and density have a major influence on the behaviour of aerosols and these have to be optimized to ensure drug performance. The aerodynamic diameter of the particle is considered to be the most critical. Extensive research has shown that the critical aerodynamic diameter size of particles for aerosol delivery formulations is in the range 1–5 μm. An even more restrictive particle size range (1–3 μm) for effective aerosol delivery has also been suggested (Hickey 1996).

Ampicillin is an aminopenicillin indicated in the treatment of respiratory, gastrointestinal and meningitis infections. It inhibits mucopeptide synthesis in the bacterial cell wall. Ampicillin, like other aminopenicillins, binds to several enzymes in the bacterial cytoplasmic membrane that are involved in cell wall synthesis and cell division, causing lysis and cell death. This β-lactam antibiotic offers clinical efficacy against a broad spectrum of respiratory pathogens, with few serious adverse effects (Nathwani & Wood 1993). The use of the aerosol route for penicillins has been suggested (Hickey 1996). The production of microparticles would allow penicillins to be delivered directly to the lung. Moreover, accurate engineering of particle size would allow selective deposition of the drugs in order to maximize their effectiveness against diseases that do not manifest uniformly within the lung (e.g. bronchitis) and minimize adverse side-effects. Conventional methods involving solvent-based crystallization and precipitation for particle generation do not provide efficient control of size and size distribution, which is necessary for their use in dry-powder inhalation aerosols. High-energy milling and spray-drying techniques are frequently not able to produce very

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narrow and controlled particle size distribution, and can cause thermal degradation of the drug. Some supercritical fluid based micronization processes have been proposed to overcome the limitations of traditional techniques. They take advantage of some specific properties of gases at supercritical conditions, such as the modulation of the solvent power, large diffusivity, solventless or organic solvent-reduced operation and the consequent possibility of controlling powder size, distribution and solvent residue. The techniques proposed are: rapid expansion of supercritical solutions (Matson et al 1987), particle generation from gas saturated solutions (Sencar-Bozic et al 1997; Kerc et al 1999), and supercritical antisolvent precipitation (SAS) (Debenedetti et al 1992, 1993; Bleich et al 1993, 1994; Ticehurst et al 1994; York & Hanna 1994; Johnson 1997; Subramaniam et al 1997; Reverchon 1999; Rehman et al 2000). The last technique has been widely studied and different acronyms have been used by the various authors (e.g. GAS, SEDS, ASES). One of the prerequisites for successful SAS precipitation is the complete miscibility of the liquid in the supercritical CO_2 and the insolubility of the solute in it. For these reasons, SAS is not applicable to the precipitation of water-soluble compounds owing to the very low solubility of water in CO_2 at suitable process conditions.

Supercritical CO_2 assisted micronization processes have been proposed by some authors (Sievers et al 1998, 1999; Sellers et al 2001; Reverchon 2002a, b; Reverchon et al 2002). Sievers and co-workers proposed the supercritical CO_2 assisted nebulization (Sievers et al 1999; Sellers et al 2001). They used a near-zero volume tee and various capillary injectors to produce emulsions of supercritical CO_2 and water or ethanol or water/ethanol solutions, generating small droplets and then microparticles.

Recently, we developed a new process called supercritical assisted atomization (SAA) (Reverchon 2002a, b). In our process configuration, a thermostated packed contactor is used to obtain continuous near-equilibrium solubilization of CO_2 in the liquid solution. The contactor is designed to provide a large contacting surface and an adequate residence time to allow the dissolution of supercritical CO_2 in the liquid solution. The solution formed in the contacting device is then sent to a thin-wall injector and sprayed into the precipitator at atmospheric pressure. We produced micronic and submicronic particles of various compounds with controlled particle size and particle size distribution (Reverchon 2001; Reverchon 2002a, b; Reverchon et al 2002; Reverchon & Della Porta 2003a, b).

The aim of this work was to determine the possibility of controlling particle size by varying some SAA process parameters and to produce particles suitable for aerosol delivery. Ampicillin was selected as a model compound. Some liquid solvents (water, methanol and ethanol), various ratios of supercritical/liquid flow rates, different ampicillin concentrations in the liquid solution and different injector diameters were tested. The effects of these SAA parameters on particle morphology, size and size distribution were monitored. Drug degradation and solvent residue were also monitored to determine the applicability of processed ampicillin to drug formulations.

Materials and Methods

SAA experimental apparatus

The apparatus used for SAA mainly consists of three feed lines used to deliver supercritical CO_2, the liquid solution and warm N_2, and three vessels, the saturator, the precipitator and the condenser. Liquid CO_2 from the high-pressure pump is sent to a heated bath and then to the saturator where it solubilizes into the liquid solution. The liquid solution is pressurized by a high-pressure pump, heated and sent to the saturator. N_2 is heated in an electric heat exchanger and is then sent to the precipitator. The saturator is a high-pressure vessel (50 cm^3) loaded with stainless steel perforated saddles to ensure a large surface for the contact between CO_2 and the liquid solution. The obtained solid/liquid/gas mixture at the exit of the saturator goes to a thin-wall stainless steel injector (i.d. 80 or 100 μm) to produce a spray of liquid droplets in the precipitator. The precipitator is a stainless steel vessel (3 dm^3) operating at near atmospheric pressure; it receives a flow of heated N_2 used to evaporate the liquid droplets and to precipitate the solute in the form of small particles. More detail about the apparatus is given in a previous report (Reverchon 2002b).

Materials

Ampicillin sodium salt (purity 98%) was supplied by ICN Biomedicals Inc. (Milano, Italy). Water (H_2O, HPLC grade), methanol (purity 99.5%) and ethanol (purity 99.8%) were supplied by Carlo Erba Reagenti (Italy). CO_2 (purity 99.9%) was purchased from SON (Naples, Italy). The maximum solubility of ampicillin in water, methanol and ethanol was measured at room pressure and temperature and was 200, 120 and 50 mg mL^{-1}, respectively. Untreated ampicillin consisted of irregular crystals with particle sizes ranging from 6 to 25 μm. All products were used as received.

Product characterization

Scanning electron microscopy (SEM)

Particles precipitated on the metallic frit were sampled onto a carbon tab adhered to an aluminium stub (Agar Scientific, Stansted, UK). The stubs were covered with 250Å of gold/palladium using a sputter coater (Agar model 108A) and observed by SEM (model LEO 420).

Evaluation of drug degradation

Drug degradation was evaluated by HPLC-UV/vis (Hewlett-Packard model G131-132) analysis of the untreated material and SAA processed powder (Xu & Trissel 1999). The elution was obtained using a reverse-phase C_8 column (4.6 × 250 mm; 5 μm particle size; 80Å pore size). The column was equilibrated at a flow rate of 1 mL min^{-1} with a mobile phase consisting of methanol and 0.05 M phosphate buffer at pH 6 (ratio 35:65). The drug was monitored at 210 nm with a retention time of 4.6 min. The HPLC method is reported elsewhere in the

literature and its reproducibility was monitored by using an ampicillin standard with a purity of 99.9% (Lunn & Schmuff 1997).

Solvent residue measurement

Solvent residue was measured using a gas chromatograph interfaced with a flame ionization detector and coupled to a headspace sampler (Hewlett Packard model 50 SCAN). Solvent residue was separated by using a fused silica capillary column (model DB-1; J&W, Folsom, CA, USA) 30 m length, 0.25 mm internal diameter, 0.25 μm film thickness. The gas chromatographic conditions used were: oven temperature of 40 °C for 8 min. The injector was maintained at 180 °C (split mode, ratio 50:50) and helium was used as the carrier gas (7 mL min^{-1}). Head space conditions were: equilibration time 60 min at 100 °C; pressurization time 2 min; loop fill time 1 min. Head space samples were prepared in 10-mL vials filled with 30–60 mg of treated ampicillin.

Statistical analysis

All runs on the SAA apparatus were performed in two replicates. The precipitated powders were sampled in different parts of the precipitation chamber and then observed by SEM. At least 30 SEM images were considered for each sample to verify the uniformity of the produced powder. Particularly, SEM images related to samples taken at different levels in the precipitator and at different positions on each stub were compared.

The particle size and the particle size distribution were measured from the SEM images using Sigma Scan Pro software (version 5.0; Jandel Scientific, San Rafael, CA, USA) and about 1000 particles were considered in each calculation of particle size distribution. Using Microcal Origin software (version 5.0; Microcal Software Inc., Northampton, USA), histograms representing number or volume against particle size were obtained. The histograms were best fitted using log-normal equations, which give a good representation of the non-symmetric distributions obtained.

Results and Discussion

We processed ampicillin and tested different solvents in which the drug was largely soluble. Water was preferred because it is not toxic; ethanol and methanol were also tested because of our experience in using these solvents in the SAA micronization process (Reverchon 2002b).

The process parameters were explored in ranges that were selected on the basis of single-phase formation in the saturator. SAA process performance is strictly related to the solubilization of CO_2 in the liquid solution, which dictates the efficiency of the atomization process. The amount of CO_2 solubilized depends on the liquid solvent and also on temperature, pressure and residence time in the contacting device (saturator). However, if an excess of CO_2 is used, the reverse can also occur (i.e. the solubilization of the liquid in CO_2). In this case, the solute can precipitate in the saturator and the process fails. Moreover, liquid solvent and CO_2 flow rates have to be set in order to obtain an adequate residence time in the contacting device (Reverchon et al 2002; Reverchon & Della Porta 2003a, b).

High-pressure vapour–liquid equilibrium data for methanol, ethanol and water in supercritical CO_2 can be found in the literature (Ohe 1990). The high-pressure vapour–liquid equilibrium of the system solvent/CO_2 define the conditions at which the formation of a homogeneous mixture in the saturator is possible. These conditions are relevant since a homogeneous mixture, saturated by gas, must be obtained before atomization through the nozzle. The starting operating conditions were selected assuming that the presence of ampicillin does not modify the miscibility behaviour of the solvent/CO_2 system.

The temperature selection in the precipitation chamber was driven by the need to minimize temperature stress on the treated drug and to assist droplet evaporation.

Results using methanol

According to the previous considerations, when methanol was used, the saturator operating conditions were set at 9 MPa and 80 °C. These process conditions ensured extensive solubility of CO_2 in the liquid solution. The flow ratio (R) between CO_2 and the liquid solution was regulated at 1.8 w/w (liquid flow rate = 6.5 g min^{-1}; CO_2 flow rate = 11.7 g min^{-1}). This R value should correspond to the operating point A in Figure 1, where a generic system liquid solvent/CO_2 is proposed. The molar fraction of CO_2 in the liquid solution was calculated based on the premise that all CO_2 dissolves in the liquid, and resulted in a value of 0.57. A further increase of the R value will move the operating point in the miscibility hole, where two phases exist in equilibrium. In that case, a gas phase is also formed, containing part of the solvent and solute, and can induce the partial solute precipitation in the saturator.

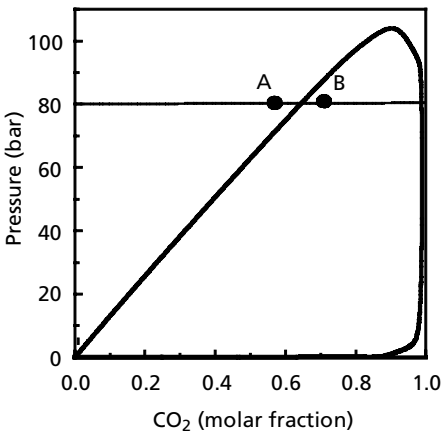

Figure 1 Pressure-composition vapour–liquid diagram for a generic liquid solvent/CO_2 system. The black dots represent the operating points for successful (A) and unsuccessful (B) supercritical assisted atomization.

The precipitation chamber temperature was maintained at 50–60 °C. To minimize thermal stress on the drug, higher temperatures were not tested. Preheated N_2 was added at a flow rate of 60 $Ndm^3 min^{-1}$ to obtain efficient liquid droplet evaporation. A thin-wall injector device with an internal diameter of 80 μm was used in these runs. A 50 $mg mL^{-1}$ solution of ampicillin in methanol was used, which corresponds to a relative concentration of 0.4. The relative concentration is the ratio between the solute concentration and the maximum solubility of ampicillin in the selected solvent. This parameter is useful in order to compare results obtained with different solvents.

Fine white powder was precipitated at the bottom of the chamber in all the experiments. SEM analysis showed that ampicillin was micronized, producing particles with a mean size of 0.5 μm. In all the tests performed with methanol, no particles larger than 1 μm were produced. No chemical modifications were monitored during the SAA process and solvent residue of 500 ppm was measured in the micronized ampicillin powder.

Results using ethanol

Other experiments were performed using ethanol. The saturator operating conditions were set at 8 MPa and 90 °C to ensure extensive solubility of CO_2 in the liquid solution. The precipitation chamber was maintained at 60 °C; the relative concentration of ampicillin used was 0.4 (20 $mg mL^{-1}$) as in the case of the methanol experiments. The R value was again regulated at 1.8 w/w (liquid flow rate = 6.5 $g min^{-1}$; CO_2 flow rate = 3.6 $g min^{-1}$).

At this R value, ampicillin precipitation was observed in the saturator (i.e. the SAA process failed). According to the previous considerations, this behaviour can be owing to the partial solubilization of ethanol in CO_2. Probably, the presence of ampicillin modified the vapour–liquid equilibrium with respect to the binary system ethanol/CO_2. As a consequence, the operating point falls inside the miscibility hole (e.g. see the operating point represented by point B in Figure 1).

Other experiments were performed using R = 1.3, that is, operating at a lower CO_2. molar fraction. In this case, a homogeneous mixture was expected to be generated in the saturator. Indeed, the micronization was successful and spherical particles with a mean size of 0.4 μm were collected in the precipitator. No chemical modifications were monitored by HPLC and a solvent residue of 300 ppm was measured in the micronized ampicillin powder.

Results using water

Ampicillin is also soluble in water. The use of water as a solvent can give some advantages when a pharmaceutical compound is processed, because it does not produce the problem of solvent residues in the precipitate. Therefore, systematic experiments were performed using water, and different saturator pressure and temperature conditions were explored in the ranges of 9–12 MPa and 85–90 °C, respectively. Also in this case, the R value was regulated at 1.8 w/w, which corresponds to an excess of CO_2 with respect to saturation in water. Indeed, the mutual solubility of CO_2 and water is low under our operating conditions. However, solute precipitation in the saturator is not possible owing to the reduced transfer of water in CO_2. In this case, CO_2 excess has the scope of ensuring saturator pressurization at SAA process conditions and thus efficient atomization of the solution.

The optimum precipitation temperature when operating with water solutions was found to be 65 °C. When the chamber temperature was maintained at temperatures less than 60 °C, particle coalescence was observed (see Figure 2). This behaviour can be explained by the fact that low precipitation temperatures induce partial water recondensation on the precipitated particles. Even in small proportions, the presence of water on the surface of particles affects the interparticle forces by the smoothing effect it has on the surface imperfections and its effect of reducing the interparticle distance. These strong boundary forces, resulting from the surface tension of the solvent, draw the particles together, generating the coalescence phenomenon observed (Rhodes 1999).

SAA experiments using water do not require solvent residue monitoring in the drug. However, when HPLC analyses were performed on the SAA treated ampicillin, near complete drug degradation was observed. Figure 3 shows the HPLC trace of the untreated and SAA treated material. This behaviour is probably owing to the fact that when CO_2 is solubilized in water, carbonic acid is formed and acid warm water can induce ampicillin degradation. This hypothesis was confirmed by dissolving ampicillin in acid water (pH 4), warming the solution to 60 °C and then performing an HPLC analysis; ampicillin degradation was also observed after this treatment.

To avoid drug degradation, buffered water containing potassium phosphate at pH 7 was used as the liquid solvent during the SAA process. In this case, no degradation was

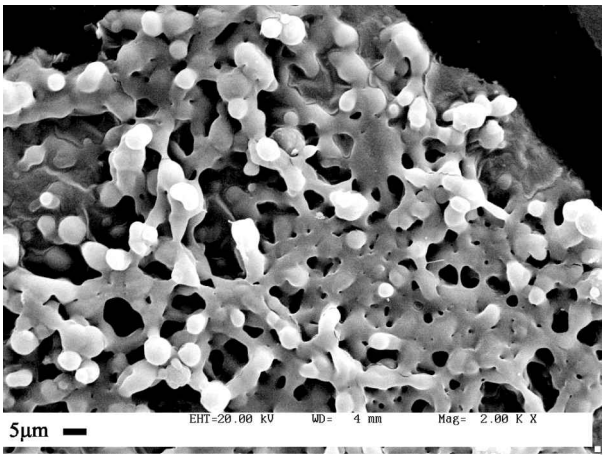

Figure 2 Scanning electron microscopy image of ampicillin particles precipitated by supercritical assisted atomization from buffered water at a precipitation temperature less than 65 °C. Particles are connected by solid bridges.

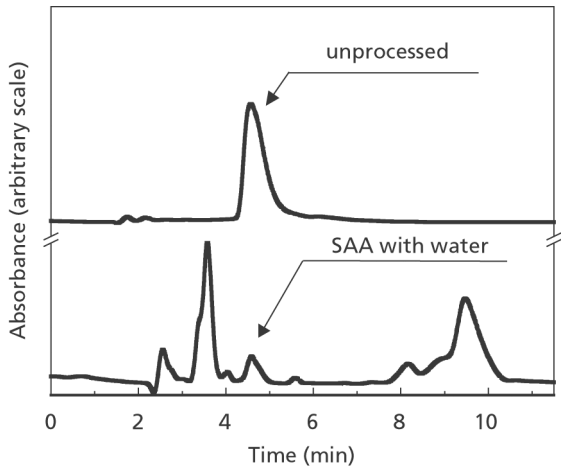

Figure 3 HPLC trace of unprocessed and supercritical assisted atomization (SAA)/water treated ampicillin.

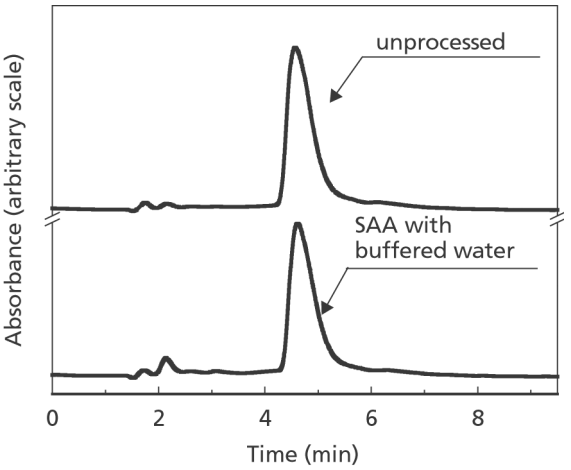

Figure 4 HPLC trace of unprocessed and supercritical assisted atomization (SAA)/buffered water treated ampicillin.

observed. Figure 4 shows the HPLC traces of the untreated and SAA/buffered water treated ampicillin.

Systematic experiments were performed at the optimum operating conditions, that is 12 MPa, 90 °C, using the 80-μm injector and operating at different ampicillin relative concentrations of 0.05, 0.25, 0.4 and 0.75 (10, 50, 80 and 150 mg mL^{-1}, respectively). The morphology of precipitated ampicillin in all these experiments was represented by spherical well-defined micrometric particles. Figure 5 shows sample SEM images of particles obtained in experiments performed at different relative concentrations. The SEM images were obtained at the same magnification and therefore allow a qualitative evaluation of the increase of particle size as the solute concentration increased. Ampicillin particles of larger diameters were produced at higher relative concentrations.

SEM images were then analysed using image analysis software to measure the particle size distribution and the data obtained were treated as described above under statistical analysis. The results are reported in Figure 6. This figure shows particle size distribution calculated on the basis of particle number; the distributions are asymmetric and well described by log-normal curves. The mode (the most frequent particle size) varied from 0.5 to 0.8 μm when the solute relative concentration varied from 0.05 to 0.75. Greater distribution was also observed when the solute concentration was increased. Figure 7 shows the same information but reported in terms of volumetric distribution. These distributions are fairly well fitted by Gaussian curves (i.e. distributions are symmetric) with a mean size that varied from 0.8 to 5.6 μm when solute concentration increased. The volume-based particle size distribution enhances the contribution of the larger particles, since the volume and not the diameter is the relevant parameter. This is important when pharmaceutical compounds are described; 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 ampicillin particles obtained using an ampicillin relative concentration of 0.25 (50 mg mL^{-1}) in water represent an important result in terms of particle size distribution for potential aerosol delivery of ampicillin, since greater than 75% of the particles were between 1 and 3 μm and no particles larger than 3 μm were produced. Another important result was obtained using an ampicillin relative concentration of 0.4 (80 mg mL^{-1}); in this case more than 98% of the distribution was included between 1 and 5 μm and no particles larger than 5 μm were produced. These results confirm that by varying the solute concentration it is possible to tailor the particle size in the exact range required for pharmaceutical applications.

Systematic experiments were also performed at the optimum operating conditions using the 100-μm injector and ampicillin relative concentrations of 0.05 and 0.25. To maintain the fixed optimized pressure in the saturator at 12 MPa, the liquid solution and supercritical fluid flow rates were increased. However, the R value was still fixed at 1.8. When the larger injector was used, no detectable variation in particle size distribution was observed.

Conclusion

The SAA process is characterized by the formation of "primary droplets" produced by the atomization device, from which originate "secondary droplets" as a result of the rapid release of CO_2 from the interior of the primary droplets. The greater the quantity of CO_2 solubilized, the stronger the secondary atomization. Particularly, the experiments performed using alcohols give important information about the mechanism of the SAA process; very small particles were obtained because of the large quantity of CO_2 solubilized in these solvents.

When water was used, larger ampicillin particles were obtained, since the solubility of CO_2 in water is less. For example, using a solute relative concentration of 0.4, particles with a mean diameter of 0.9 μm were obtained from

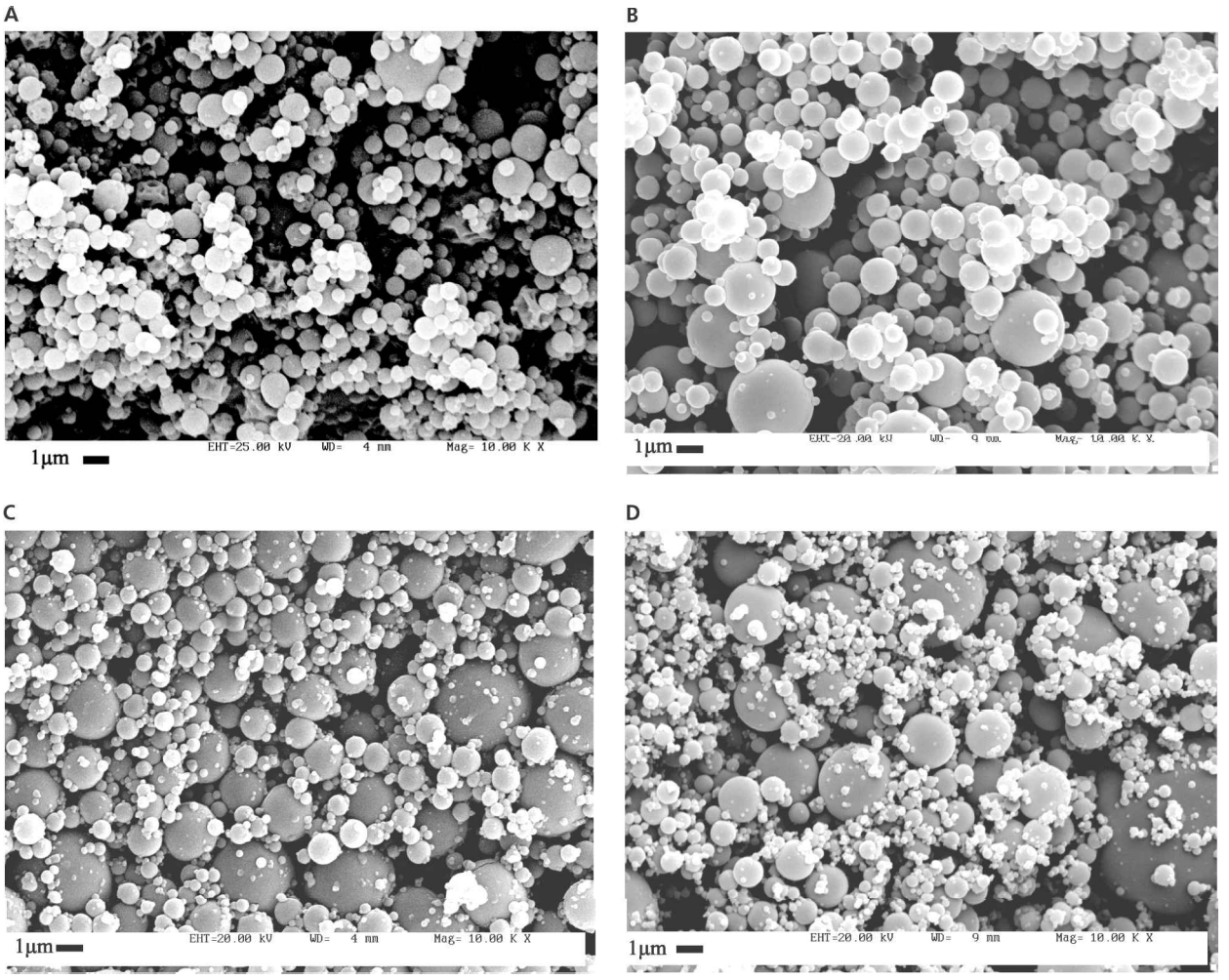

Figure 5 Scanning electron microscopy images of ampicillin precipitated by supercritical assisted atomization from buffered water, varying the solute relative concentration. A. 0.05; B. 0.25; C. 0.4; D. 0.75.

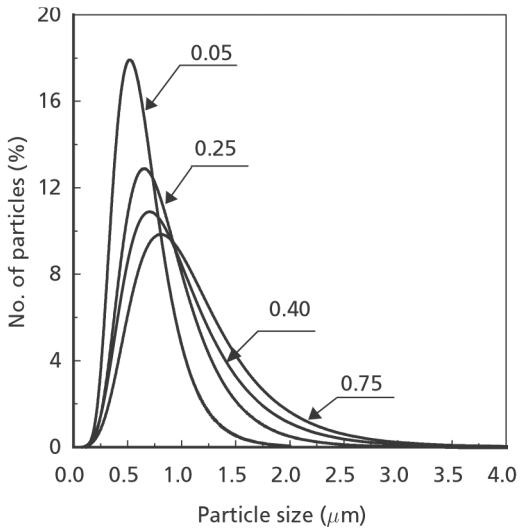

Figure 6 Particle size distribution curves (based on particles number) of ampicillin produced by supercritical assisted atomization from buffered water, varying the solute relative concentration in the solution.

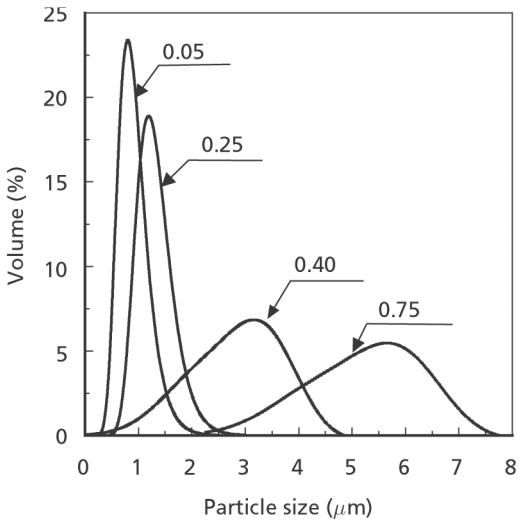

Figure 7 Particle size distribution curves (based on particles volume) of ampicillin produced by supercritical assisted atomization from buffered water, varying the solute relative concentration in the solution.

water, whereas when methanol was used, particles with a mean size of 0.4 μm were produced. Very similar behaviour was previously observed by SAA precipitation of a superconductor precursor from water and methanol (Reverchon 2002b). This difference can also depend on the different strength of the cohesive forces operating on the primary droplets, namely surface tension and viscosity. Although water and methanol (or ethanol) have similar viscosities at SAA operating temperatures, water surface tension is 3 times higher than methanol. Moreover, the dissolution of a gas in a liquid largely reduces its viscosity and its surface tension. Therefore, the greater the amount of gas dissolved, the greater the reduction of the cohesive forces. Therefore, the CO_2/alcohol SAA based process has the further advantage of reducing cohesive forces compared with the CO_2/water SAA process.

SAA processing with buffered water is the most suitable process for the production of microparticles in the range required for aerosol delivery. We have demonstrate that particle size and particle size distribution tailoring is also possible using the SAA process.

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