SUPERCRITICAL ANTISOLVENT PRECIPITATION OF B-LACTAM ANTIBIOTICS: INFLUENCE OF THE LIQUID SOLUTION CONCENTRATION

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Abstract

Ampicillin (AMP) and amoxicillin (AMC), two of the world most widely prescribed antibiotics, have been micronized by Supercritical AntiSolvent (SAS) technique. In our previous works [1, 2], screening designs of experiment (DOE) have been applied to SAS precipitation of both antibiotics independently using carbon dioxide (CO_2) as antisolvent and N – methylpyrrolidone (NMP) as solvent. In this work, the effect of concentration in the liquid solution has been studied to evaluate their influence on particle size (PS) and particle size distributions (PSD) of AMP and AMC and both drugs have been compared. The concentration values used have been 10 and 100 mg/mL and successful micronizations of the antibiotics have been obtained. AMP and AMC size-controlled microparticles have been produced ranging between 100 and 400 nm and, in both cases, an increase in the initial concentration of the solution have led to larger particles sizes with a wider particle size distribution.

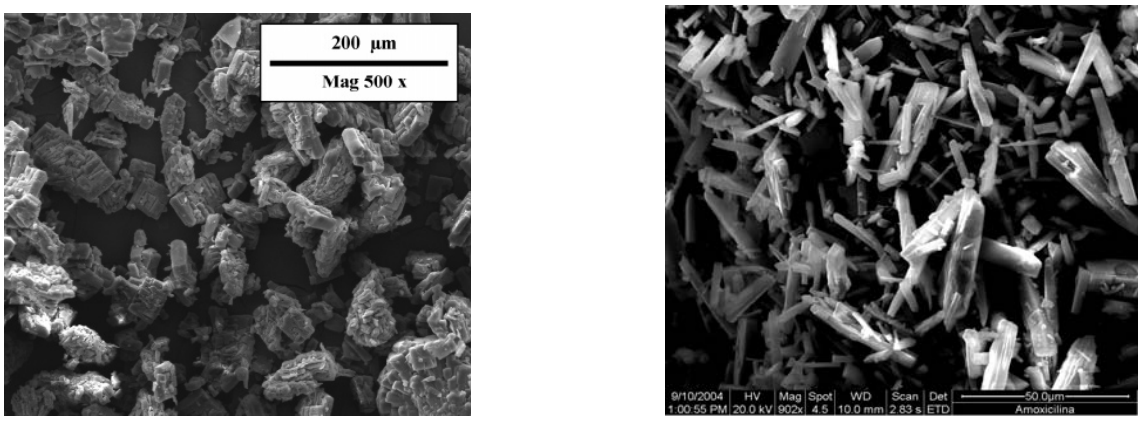
1. Introduction

The SAS process is a promising micronization technique that is based on the particular properties of supercritical fluids (SCFs). The SAS process uses both the high power of supercritical fluids to dissolve the organic solvents and the low solubility of the pharmaceutical compounds in supercritical fluids to bring about the precipitation of these compounds when they are first dissolved in the organic phase and then brought into contact with the supercritical fluid. Therefore, the supercritical fluid has an antisolvent effect with respect of the solute to be micronized. The dissolution of the supercritical fluid into the organic solvent is accompanied by a large volume expansion and, consequently, a reduction of the liquid density, and therefore, of its solvent power, causing a sharp rise in the supersaturation within the liquid mixture. Because of the high and uniform degree of supersaturation, small particles with a narrow particle size distribution are expected [3].

AMP and AMC are two of the world's most widely prescribed antibiotics. Using ampicillin or amoxicillin microparticles of controlled size, it is possible to increase its bioavailability and decrease its therapeutic dosage (by improving efficiency). It is also possible to use various delivery systems for the drug (transdermal, tracheobronchial, and pulmonary delivery systems) [4]. Therefore, the mean PS and PSD must be selected as the specific responses for evaluating process performance.

2. Experimental Section

2.1. Materials and Analytical Methods. Ampicillin sodium salt (91.0% minimum purity) Amoxicillin (97 % minimum purity) and 1-methyl-2-pyrrolidone (NMP) (99.5% purity) were purchased from Sigma-Aldrich Chemical (Madrid, Spain). Carbon dioxide with a minimum purity of 99.8% was supplied by Carburos Metálicos S.A. (Barcelona, Spain). The ampicillin and amoxicillin were soluble in NMP for the fixed experimental concentration at room temperature. SEM images of the as-received ampicillin and amoxicillin are shown in Figure 1.



(a) (b)

Figure 1. SEM images of unprocessed ampicillin (a) and amoxicillin (b)

Samples of the powder precipitated both on the wall and in the frit were observed using a SIRION FEG scanning electron microscope. Previously, the samples had been placed on carbon tape and then covered with a coating of gold using a sputter coater. The SEM images were processed using Scion image analysis software (Scion Corporation) to obtain the particle sizes.

After that, the mean particle size, standard deviation (SD) and coefficient of variation (CV), both as measurements of the distribution width, were calculated using Statgraphics plus 5.1 software. More than 800 particles were counted to perform the analysis in each experiment.

2.2. Design of Experiments (DOE). In previous works [1, 2], screening designs of experiments have been applied to the supercritical antisolvent precipitation of AMP and AMC using carbon dioxide as antisolvent and N-methylpyrrolidone as solvent. The proposed DOE is useful for identifying the key factors involved in the SAS process in just a few runs at an early stage of experimentation. The DOE were applied to the AMP-NMP and AMC-NMP systems. Several factors (concentration of the solution (C), the temperature (T), the pressure (P), the solution flow rate (Q_L), the carbon dioxide flow rate (Q_{CO2}), washing time (t_w) and nozzle diameter ($Ø_n$) and two responses (PS and PSD) were selected and each factor at two different levels was evaluated. Previous works show the details of these designs [1, 2]. Within the range of operating conditions investigated, concentration proved to be the key factors having the greatest effect on both PS and PSD and, thus, the most important factors for controlling the formation of submicrometer particles of ampicillin by the SAS technique. For this reason, in this work, the effect of concentration in the liquid solution has been studied to evaluate their influence on particle size and particle size distributions of AMP and AMC and both drugs have been compared.
The two levels for each factor are shown in Table 1 and were chosen mainly on the basis of previous studies on SAS precipitation. The low level of concentration was set to obtain a sufficient quantity of ampicillin for subsequent analysis, whereas the high level was limited by the saturation of the solution at room temperature.

Table 1. Two-level assessment for each factor

Factor	low level	high level
C (mg/mL)	10	100
T (K)	308.15	328.15
P (bar)	90	180
Q_L (mL/min)	1	5
Q_{CO_2} (g/min)	32	66
t_w (min)	120	180
$Ø_n$ (μm)	100	200

2.3. Experimental Equipment and Procedures. The pilot plant, developed by Thar Technologies (model SAS 200), is described in previous works [1, 2] and was used to carry out all experiments. The SAS 200 system comprises the following components: two high-pressure pumps, one for the CO_2 and the other for the solution, which incorporate a low-dead-volume head and check valves to provide efficient pumping of CO_2 and many solvents; a stainless steel precipitator vessel with a 2 L volume consisting of two parts, the main body and the frit, all surrounded by an electrical heating jacket; an automated back-pressure regulator of high precision, attached to a motor controller with a position indicator; and a jacketed stainless steel cyclone separator with 0.5 L volume, to separate the solvent and CO_2 once the pressure was released by the manual back-pressure regulator. The following auxiliary elements were also necessary: a low pressure heat exchanger, cooling lines, and a cooling bath to keep the CO_2 inlet pump cold and to chill the pump heads; an electric high-pressure heat exchanger to preheat the CO_2 in the precipitator vessel to the required temperature quickly; safety devices (rupture discs and safety valve); pressure gauges for measuring the pump outlet pressure, the precipitator vessel pressure, and the cyclone separator pressure; thermocouples placed inside and outside the precipitator vessel, inside the cyclone separator, and on the electric highpressure heat exchanger to obtain continuous temperature measurements; and a FlexCOR coriolis mass flowmeter to measure the CO_2 mass flow rate and another parameters such as total mass, density, temperature, volumetric flow rate, and total volume. All factors that have an influence on the precipitation process (temperature, flow rate, pressure, etc.) could be controlled either manually or automatically (using ICM software).
All experiments were performed following the same procedure described in previous works [1, 2].

3. Results and Discussion

Results of DOE show that, for both compounds, the concentration is the factor that has the greatest influence on both the PS and the PSD. Therefore, both the PS and the PSD required for the final formulation of ampicillin or amoxicillin could well be adjusted by a change in the initial concentration of the solution.

The effects on the selected responses were calculated for each factor and are reported in Table 2. Moreover, an effect graph was plotted for both the particle size and particle size distribution (coefficient of variation) responses, as shown in Figures 2 and 3.

A change in the concentration from the low level to the high level means an increase of 204.3 nm and 237.7 nm in the particle size response for ampicillin and amoxicillin respectively.

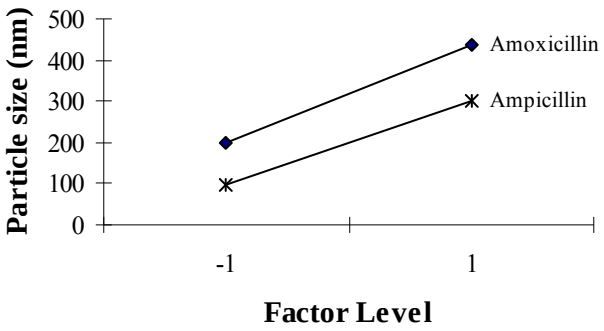


Figure 2. Main effect plots of the factors on PS

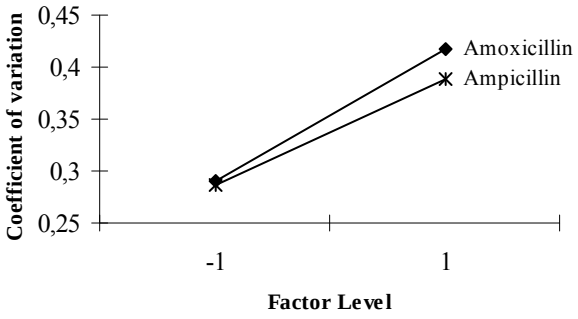


Figure 3. Main effect plots of the factors on PSD.

Table 2. Mean particle size and coefficient of variation

	C	T	P	Q_L	Q_{CO2}	t w	Ø n
AMPICILLIN							
Mean PS (nm)	**204.3**	-181.5	-51.7	10.5	-37.7	-	114.5
C. Variation	**0.102**	-0.049	-0.058	0.07	0.036	-	0.032
AMOXICILLIN							
Mean PS (nm)	**237.7**	-30.2	41	166.5	-56.8	-106.7	14.3
C. Variation	**0.127**	-0.064	-0.013	0.076	0.004	-0.076	0.055

An increase in the initial concentration of the solution has two opposite effects: On one hand, with a higher concentration, it is possible to achieve higher supersaturations, which tend to diminish the particle size. On the other hand, condensation is directly proportional to the concentration of solute, and the increase of the condensation rate at higher concentrations tends to increase the particle size [5].

In our case, an increase in the initial concentration of the solution led to larger particles sizes with a wider distribution. Thus, the second effect (condensation rate) prevailed under the operating conditions used in this work; that is, the higher the initial concentration of the solution, the higher the condensation rate, and thus, the greater the particle sizes produced. This result is consistent with those obtained by Reverchon et al.[6], which were also explained in terms of competition between nucleation and growth processes.

SEM images of ampicillin and amoxicillin samples micronized in these experiments show the formation of spherical nanoparticles with a uniformly distributed mean particle size as reported in Figures 4 and 5 respectively.

(a) (b)

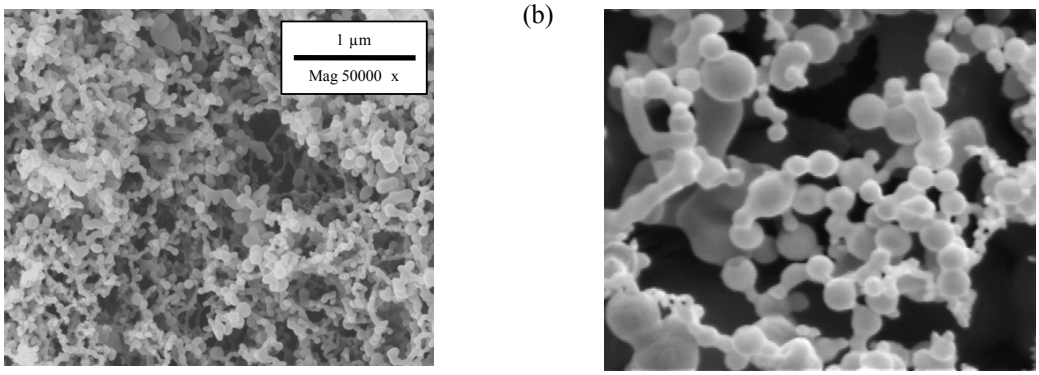


Figure 4. SEM images of ampicillin nanoparticles precipitated from:
(a) 10 mg/mL, 328.15 K, 180 bar, 1 mL/min solution, 32 g/min CO_2, t_W=180 min, noozle 100 µm
(b) 100 mg/mL, 308.15 K, 90 bar, 5 mL/min solution, 32 g/min CO_2, t_W=180 min, noozle 100 µm

(a) (b)

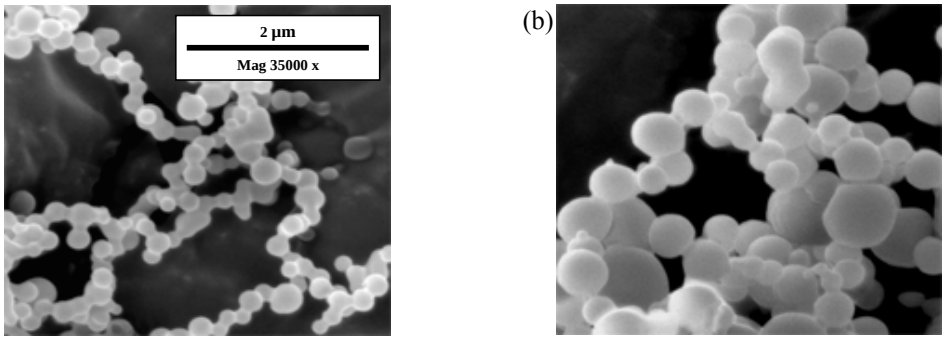


Figure 5. SEM images of amoxicillin nanoparticles precipitated from:
(a) 10 mg/mL, 328.15 K, 180 bar, 1 mL/min solution, 32 g/min CO_2, t_W=180 min, noozle 100 µm
(b) 100 mg/mL, 308.15 K, 90 bar, 5 mL/min solution, 32 g/min CO_2, t_W=180 min, noozle 100 µm

4. Conclusions

The fractional factorial design used in previous works has been used in this work to compare the influence of the liquid solution concentration in the SAS process on the ampicillin and amoxicillin microparticles characteristics. From the results of the calculations of the main effects, in both cases (ampicillin and amoxicillin), the concentration is the factor that has the greatest influence on both the PS and the PSD. An increase of the mean particle size and a broadening of the particle size distribution were observed with the increase of the concentration of the AMC/NMP and AMP/NMP solutions. And this rise is similar in both cases. Therefore, the use of a low initial solution concentration is advised.

5. References

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