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Screening design of experiment applied to supercritical antisolvent precipitation of amoxicillin: Exploring new miscible conditions

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ABSTRACT

Microparticles of amoxicillin (AMC) have been precipitated by supercritical antisolvent process (SAS) using carbon dioxide and N-methylpyrrolidone (NMP) as antisolvent and solvent, respectively. A fractional factorial design of experiment (DOE) with 2^{7-4} experiments has been used. Mean particle size (PS) and particle size distribution (PSD) of the processed amoxicillin have been chosen as responses to evaluate the process performance. In a previous work, a DOE was applied too, but now, the range of operating conditions investigated has been changed to let the process take place in a single supercritical phase. Within this range, concentration is again the key factor having most effect on both PS and PSD and thus, the most important factor for controlling the formation of sub-microparticles of amoxicillin by the SAS technique. Moreover, all the experiments included in the new design matrix led to a successful precipitation of amoxicillin.

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1. Introduction

In a previous work, the importance of pharmaceutical preparations was described [1]. These are the final product of a technological process that gives the drugs the characteristics appropriate for easy administration, proper dosage and enhancement of the therapeutic efficacy. Among several kinds of development of modified release preparation, the design of pharmaceutical preparations in nanoparticulate form has emerged as a new strategy for drug delivery [2]. Particle size and particle size distribution are critical parameters that determine the rate of dissolution of the drug in the biological fluids and, hence, have a significant effect on the bioavailability of those drugs [3,4].

Supercritical antisolvent (SAS) processes have been widely used for the last ten years to precipitate Active Pharmaceutical Ingredients (APIs) [5–10]. The features and advantages of these processes compared to conventional processes were described in the previous work [1]. The SAS process exploits the fast diffusion of the supercritical fluid, normally CO_2, into the organic solvent, in which the solute is dissolved; this generates a high volumetric expansion, thus decreasing its solvent power. As a consequence, a high degree of uniform supersaturation is produced promoting the precipitation of the solute as particles in the micrometer and nanometer size ranges. Moreover, this process enables particle size distribution and morphology to be controlled. The mixing between the solvent and antisolvent is achieved, in this semicontinuous processing mode, basically by spraying the organic solution of API via a nozzle into the flowing stream of supercritical carbon dioxide.

In the previous work amoxicillin nanoparticles were precipitated from NMP making a screening design of experiment selecting other ranges of operating conditions. The particles obtained depended on these values. Spherical amoxicillin nanoparticles with a mean particle size in the range of 182–450 nm, uniformly distributed, were obtained [1]. The pressure condition limits (90 and 180 bar) were set with the aim of ensuring a high solubility of supercritical carbon dioxide in NMP at two levels of temperature (308 and 328 K). We noted that a pressure of 180 bar brought the CO_2–NMP system to miscible conditions [11]. These conditions result in faster mass transfer, causing a higher degree of supersaturation that result in higher nucleation rates, thus producing a narrow particle size distribution. This fact can also be explained on the basis of the numerical modeling of mass transfer proposed by Werling and Debenedetti [12].

We noted, in a previous work [1], that in the experiments situated below the critical point of the mixture CO_2–NMP at the operating temperature, which took place in the presence of two phases, two types of precipitates were observed: nanoparticles of amoxicillin that were formed in the phase rich in CO_2 located in the upper part of the precipitator, and solid aggregates that precipitated in the phase rich in solvent located in the lower part of the precipitation chamber. For that, we concluded that a complementary set of experiments should be designed using the same design generators but selecting some operating conditions (P, T) where all experiments should be situated above the critical point

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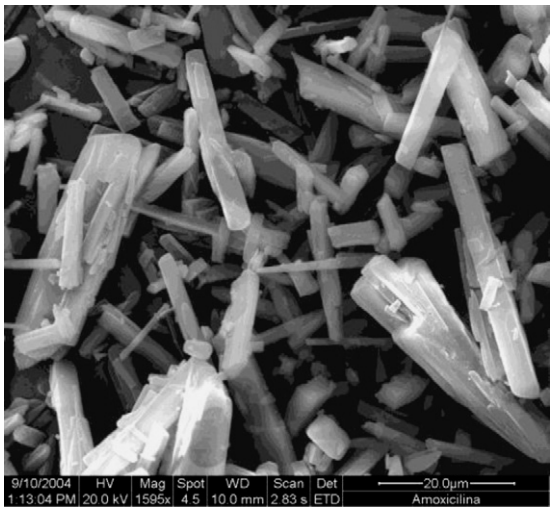


Fig. 1. SEM image of unprocessed amoxicillin.

of the mixture amoxicillin–NMP–CO_2 for all possible combinations of the design. In accordance with usual practice in these ternary systems it has been considered that the presence of amoxicillin does not affect the NMP–CO_2 equilibrium, therefore a NMP–CO_2 pseudo-binary equilibrium will be considered [13]. Now, a DOE has been built to let the process take place in a single phase and therefore the mixing can take place without interface limitations and consequently a homogeneous precipitate constituted by spherical nanoparticles of amoxicillin can be obtained.

Therefore, the objective of this work is to investigate the effect of processing parameters (pressure, temperature, flow rate of solution, CO_2 flow rate, initial concentration of the solution, nozzle diameter and washing time) on the precipitation of amoxicillin micro- and nanoparticles using SAS technique, focusing on particle size and particle size distribution, in a new range of the operating conditions.

2. Experimental

2.1. Materials and analytical methods

Amoxicillin (purity ≥97%) and 1-methyl-2-pyrrolidone (NMP) (purity 99.5%) were bought from Sigma–Aldrich Chemical (Madrid, Spain). Carbon dioxide with a minimum purity of 99.8% was supplied by Carburos Metálicos S.A. (Barcelona, Spain). Amoxicillin was soluble in NMP for the fixed experimental concentration at room temperature. Scanning electron microscope (SEM) pictures of the as-received amoxicillin are shown in Fig. 1.

Table 1
Two-level assessment for each factor.

Factors	Low level	High level
C (mg/mL)	10	60
T (K)	328.15	338.15
P (bar)	180	275
Q_L (mL/min)	2	5
Q_{CO_2} (g/min)	32	66
t_w (min)	120	180
$Ø_n$ (µm)	100	200

2.2. Design of experiment (DOE)

As Subra and Jestin [14] suggested and we published in the previous work [1], we considered all the factors that could have effects on the performance of the SAS process: P, pressure of the precipitator vessel (bar); T, temperature of the precipitator vessel (K); C, initial concentration of the solution (mg/mL); Q_L, liquid flow rate (mL/min); Q_{CO_2}, supercritical CO_2 flow rate (g/min); t_w, washing time (min) and $Ø_n$, nozzle inner diameter (µm) [1]. The coding scheme used to describe the factor levels is based on the "+" and "−" signs, where "+" and "−"denote the high and low level of a factor, respectively. In this work we have changed the choice of the allied factors because of the conclusions of our previous work [1]. In this previous work the liquid flow rate was allied with the 123 interactions because it was considered a priori would be the factor that had the major influence on the particle response, modifying the degree of mixing of CO_2 and the sample solution. However, in another previous work [15] and by other authors [16–19], the influence of the liquid flow rate on the degree of mixing, and thus on the particle size, was not noticeable. So, from the four factors: liquid flow rate, CO_2 flow rate, washing time, and nozzle diameter, this last one was considered a priori as one of the factor that has the major influence, because the system can change from an atomization process to a mixing process if the nozzle diameter is varied; hence, this factor was allied with the interaction of concentration/temperature/pressure.

The new two levels for each factor are shown in Table 1 and they were chosen mainly on the basis of previous studies on SAS amoxicillin precipitation [1]. The low and high levels of concentration were 10 and 100 mg/mL in our previous work, and now, 10 mg/mL is chosen to obtain a sufficient quantity of amoxicillin for its subsequent analysis, and 60 mg/mL to avoid the saturation of the amoxicillin solution at room temperature, and the consequent partial precipitation of the amoxicillin. If the solute is not completely soluble in the liquid the set solution concentration is different to the actual solution concentration that will be fed to the system. In order to avoid such difference, the concentration of the solution was set below its saturation point. The low and high temperature levels were 308 and 328 K in the previous work, and the low and high pressure levels were 90 and 180 bar. Now, the low

Table 2
Experimental design, response and contrasts.

Runs	C(1)	T(2)	P(3)	Q_L (4 = 12)	Q_{CO_2} (5 = 13)	t_w (6 = 23)	$Ø_n$ (7 = 123)	I	Responses	
									Means PS (nm)	C.V.
12	+	+	−	+	−	−	−	+	355	0.346
22	+	−	+	−	+	−	−	+	505	0.440
39	−	+	+	−	−	+	−	+	251	0.268
57	−	−	−	+	+	+	−	+	255	0.248
77	−	−	+	+	−	−	+	+	216	0.273
83	−	+	−	−	+	−	+	+	348	0.304
98	+	−	−	−	−	+	+	+	392	0.382
128	+	+	+	+	+	+	+	+	352	0.301
Means PS (nm)	133.5	−15.5	−6.5	−79.5	61.5	−43.5	−14.5	334.2		
C.V.	0.094	−0.032	0.0005	−0.056	−0.005	−0.042	0.010	0.320		

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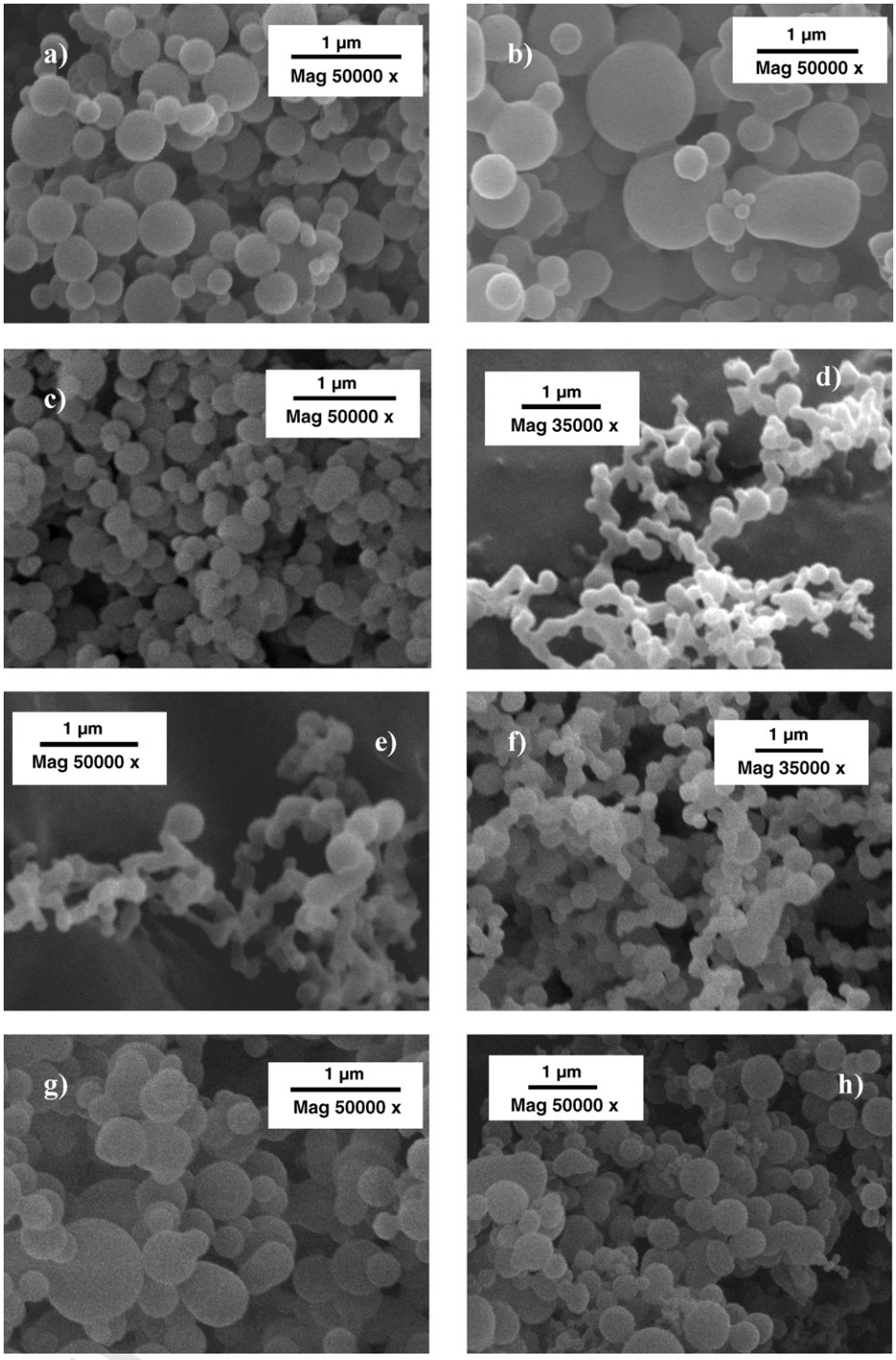


Fig. 2. SEM images of amoxicillin precipitated from (a) run 12, (b) run 22, (c) run 39, (d) run 57, (e) run 77, (f) run 83, (g) run 98 and (h) run 128.

and high temperature levels are increased till 328 and 338 K and the low and high pressure levels till 180 and 275 bar.

In the new range of pressure and temperature, we can affirm that all experiments are located above the critical point of the mixture [11].

The low level liquid flow rate is increased from 1 to 2 mL/min because we noted that when the 200 μm diameter nozzle was used with a liquid flow rate of 1 mL/min, the solution was not atomized, and we did not obtain any precipitation. We thought that a higher flow rate would be needed to atomize the solution. The fact of getting nanoparticles at such conditions in the present work agreed with our hypothesis. After that, an empirical hydrodynamic model was applied to correlate the result of such experiment with the predicted disintegration regimen, Rayleight, sinuous break-up and atomization [20]. The results proved that a change of the disintegration regimen by increasing flow rate from 1 to 2 mL/min was required to get nanoparticles at optimum thermodynamic conditions (pressure and temperature) and utilizing a 200 μm nozzle. The CO_2 flow rate, nozzle diameter and the washing time are the same as the previous work [1].

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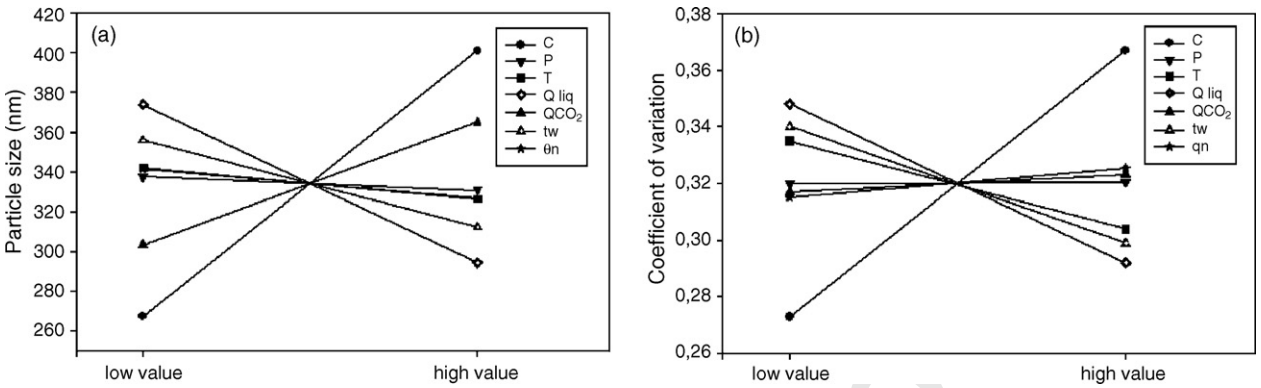


Fig. 3. Main effect plot of the factors on (a) particles size and (b) particle size distribution.

As a result, eight different combinations of signs for the seven factors were generated; that is, eight different trials (12, 22, 39, 57, 77, 83, 98 and 128) out of the full factorial design at two levels 2^7. Thus, the design of Table 2 is a 1/16 fraction of the full 2^7 design.

Among the most important solid-state properties defined by the crystallization process are the dimensional properties of the final product (PS, PSD and particle morphology) since these define some of its key specific characteristics such as processing behaviour, particle permeability (API adsorption) and bioavailability (API absorption) [21]. Consequently the mean particle size of the processed amoxicillin was chosen as response to evaluate the process performance.

2.3. Experimental equipment and procedures

A pilot plant, developed by Thar Technologies® (model SAS 200) was used to carry out the experiments shown in Table 2. This plant was explained in details in the previous work [1].

Samples of the powder precipitated were observed using a SIRION FEG scanning electron microscope. Previously, the samples had been placed on the carbon tape and were 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, mean particle size was calculated using Statgraphics Plus 5.1 software. More than 500 particles were counted to perform the analysis in each experiment.

3. Results and discussion

All experiments of experimental design showed in Table 2 were carried out following the same experimental procedure, at operating conditions indicated by the coding scheme (high and low levels of each factor).

All experiments included in the experimental design led to a successful precipitation of amoxicillin. The SEM images of amoxicillin samples micronized in these experiments show the formation of spherical nanoparticles with a mean particle size in the range of 216–505 nm, uniformly distributed, and coefficients of variation (C.V.) within the range 0.248–0.440, as reported in Fig. 2 and Table 2. The precipitation of homogeneous spherical nanoparticles of amoxicillin that are obtained from a fine precipitate with foamy texture is verified. They are very small and have a very narrow particle size distribution. All the experiments were precipitated on the wall of the precipitator vessel, in the upper part. For this reason, particles of amoxicillin forming aggregates, compact films, and rods were not obtained. These are obtained when the precipitation happens in the lower part of the precipitation chamber. The effects on the selected responses for each column in Table 2 were calculated and can be observed in Fig. 3.

As can be observed in this figure, the lowest slopes are for the temperature, the pressure and the nozzle diameter. The concentration was identified as a key factor. It could be mentioned, at this point, that an increase in the initial concentration of the solution has two opposing effects: on the one hand, higher supersaturations from higher concentration tend to decrease the particle size; and on the other hand, ~~once the condensation is directly proportional to the concentration of solute and increase in condensation rate, with higher concentrations, tends to increase the particle size~~ [22]. In our case, an increase in the initial concentration of the solution leads to larger particles sizes with a wider distribution. Thus, the second effect (condensation rate) prevails under the operating conditions used in this work; that is, the higher the initial concentration of the solution, the faster the condensation rate; thus bigger particle sizes are produced. This result is consistent with those obtained by Reverchon et al., which were also explained in terms of competition between nucleation and growth processes [17].

An increase in the liquid flow rate leads to smaller particle sizes. This finding can be explained considering that the degree of mixing may be improved at higher liquid flow rates under miscible conditions (two phases are fully miscible) resulting in a higher supersaturation and thus, smaller particles are expected [22]. Temperature, pressure and nozzle diameter have not had a significant influence on the particle size.

In the SAS process the disperse phase is formed by the solute to be micronized dissolved in an organic solvent; the continuous phase is formed by supercritical CO_2, in such a way that the organic solvent and the supercritical CO_2 have to be partial (below the critical point of the mixture, MCP) or completely miscible (above the MCP) at the operating conditions of the process. The solution is dispersed in the fluid at high pressure by nebulisers or capillaries with orifice diameters of the order of hundreds of micrometer, generating sufficient contact area between the phases to increase the transfer of matter in the two directions; that is, the solvent diffuses into the supercritical fluid (evaporation) and CO_2 diffuses into the liquid phase, generating an antisolvent effect (supersaturation) that causes the precipitation of the solute [20]. It is possible to select the optimum thermodynamic conditions for this process by studying the volumetric expansion in the solvent caused by CO_2, so, a key role in the precipitation process is played by the volumetric expansion of the liquid solvent [23]. Volumetric expansion of NMP with CO_2 at various temperatures is presented by Rajasingam et al. [24]. Reverchon noted that for pressures larger than the asymptotic volume there were no large effect of pressure on particle size and an increase of temperature produced a non-negligible increase of particle size [5] as were tested in this work.

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4. Conclusions

Homogeneous spherical amoxicillin nanoparticles with a very narrow particle size were obtained by SAS process being situated above the critical point of the mixture amoxicillin–CO_2–NMP, where all experiments were in miscible conditions, to let the process to take place in a single supercritical phase. A successful precipitation of amoxicillin with foamy texture was obtained under all conditions studied. The concentration was the factor that had the greatest influence on the PS and PSD. An increase in the initial concentration of the solution led to larger particles sizes with a wider distribution. An increase in the liquid flow rate led to smaller particle sizes. Temperature, pressure and nozzle diameter did not have a significant influence on the particle size.

References

[1] A. Tenorio, M.D. Gordillo, C.M. Pereyra, E.J. Martínez de la Ossa, Screening design of experiment applied to supercritical antisolvent precipitation of amoxicillin, J. Supercrit. Fluids 44 (2008) 230–237.
[2] M.I. Pasquali, R. Bettini, F. Giordano, Solid-state chemistry and particle engineering with supercritical fluids in pharmaceutics, Eur. J. Pharm. Sci. 27 (2006) 299–310.
[3] M. Perrut, J. Jung, F. Leboeuf, Enhancement of dissolution rate of poorly-soluble active ingredients by supercritical fluid processes. Part I. Micronization of neat particles, Int. J. Pharm. 288 (2005) 3–10.
[4] T. Van Nijlen, G. Van Den Mooter, R. Kinget, P. Augustijns, N. Blaton, K. Brennan, Improvement of the dissolution rate of artemisinin by means of supercritical fluid technology and solid dispersions, Int. J. Pharm. 254 (2003) 173–181.
[5] E. Reverchon, Supercritical antisolvent precipitation of micro- and nanoparticles, J. Supercrit. Fluids 15 (1999) 1–21.
[6] C.G. Kalogiannis, E. Pavlidou, C.G. Panayiotou, Production of amoxicillin microparticles by supercritical antisolvent precipitation, Ind. Eng. Chem. Res. 44 (2005) 9339–9346.
[7] P. Chattopadhyay, R.B. Gupta, Production of antibiotic nanoparticles using supercritical CO_2 as antisolvent with enhanced mass transfer, Ind. Eng. Chem. Res. 40 (2001) 3530–3539.
[8] M. Rehman, B.Y. Shekunov, P. York, P. Colthorpe, Solubility and precipitation of nicotinic acid in supercritical carbon dioxide, J. Pharm. Sci. 90 (2001) 1570–1582.
[9] S.P. Velaga, R. Ghaderi, J. Carlfors, Preparation and characterisation of hydrocortisone particles using a supercritical fluids extraction process, Int. J. Pharm. 231 (2002) 155–166.
[10] S.D. Yeo, J.C. Lee, Crystallization of sulfamethizole using the supercritical and liquid antisolvent processes, J. Supercrit. Fluids 30 (2004) 315–323.
[11] C.W. Lee, C.Y. Jung, H.S. Byun, High pressure phase behaviour of carbon dioxide + 1-methyl-2-pyrrolidinone and carbon dioxide + 1-ethyl-2-pyrrolidinone systems, J. Chem. Eng. Data 49 (2004) 53–57.
[12] J.O. Werling, P.G. Debenedetti, Numerical modeling of mass transfer in the supercritical antisolvent process: miscible conditions, J. Supercrit. Fluids 18 (2000) 11–24.
[13] E. Reverchon, G. Caputo, I. De Marco, Role of phase behaviour and atomization in the supercritical antisolvent precipitation, Ind. Eng. Chem. Res. 42 (2003) 6406–6414.
[14] P. Subra, P. Jestin, Screening design of experiment (DOE) applied to supercritical antisolvent process, Ind. Eng. Chem. Res. 39 (2000) 4178–4184.
[15] A. Tenorio, M.D. Gordillo, C.M. Pereyra, E.J. Martínez de la Ossa, Relative importance of the operating conditions involved in the formation of nanoparticles of ampicillin by supercritical antisolvent precipitation, Ind. Eng. Chem. Res. 46 (2007) 114–123.
[16] E. Reverchon, G. Della Porta, Production of antibiotic micro- and nano-particles by supercritical antisolvent precipitation, Powder Technol. 106 (1999) 23–29.
[17] E. Reverchon, G. Della Porta, M.G. Falivene, Process parameters and morphology in amoxicillin micro and submicro particles generation by supercritical antisolvent precipitation, J. Supercrit. Fluids 17 (2000) 239–248.
[18] W.K. Snavely, B. Subramaniam, R.A. Rajewski, M.R. Defelippis, Micronization of insulin from halogenated alcohol solution using supercritical carbon dioxide as an antisolvent, J. Pharm. Sci. 91 (2002) 2026–2039.
[19] P. Subra, A. Vega-González, C.-G. Laudani, E. Reverchon, Precipitation and phase behavior of theophylline in solvent-supercritical CO_2 mixtures, J. Supercrit. Fluids 35 (2005) 95–105.
[20] A. Tenorio, P. Jaeger, M.D. Gordillo, C.M. Pereyra, E.J. Martínez de la Ossa, On the selection of limiting hydrodynamic conditions for the SAS process, Ind. Eng. Chem. Res. (2009) (available on web).
[21] B.Y. Shekunov, P. York, Crystallization processes in pharmaceutical technology and drug delivery design, J. Cryst. Growth 211 (2000) 122–136.
[22] A. Martin, M.J. Cocero, Numerical modeling of jet hydrodynamics, mass transfer, and crystallization kinetics in the supercritical antisolvent (SAS) process, J. Supercrit. Fluids 32 (2004) 203–219.
[23] A. Martín, M.J. Cocero, Micronization processes with supercritical fluids: fundamentals and mechanism, Adv. Drug Deliv. Rev. 60 (2008) 339–350.
[24] R. Rajasingam, L. Lioe, Q.T. Pham, F.P. Lucien, Solubility of carbon dioxide in dimethylsulfoxide and N-methyl-2-pyrrolidone at elevated pressure, J. Supercrit. Fluids 31 (2004) 227–234.

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