Control of particle size, at micrometric and nanometric range, using supercritical antisolvent precipitation from solvent mixtures: application to PVP.

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Abstract

The control of particle size at micrometric and nanometric range is of relevant interest in many industrial applications. In this work, Supercritical AntiSolvent (SAS) precipitation, using solvent mixtures has been used to increase process flexibility in particle size control. The biopolymer polyvinylpyrrolidone (PVP) has been used as a model solute. PVP is slightly soluble in acetone (AC) and largely soluble in dimethylsulfoxide (DMSO), ethyl alcohol (EtOH) and N-methylpyrrolidone (NMP); therefore, solvent mixtures of AC and these other solvents have been used. The experiments using EtOH/AC solvent mixture were performed to study the effect of the solvation power alone, considering that both pure solvents show similar mixing behaviors with $scCO_2$. Experiments using the solvent mixtures NMP/AC and DMSO/AC were performed to study both the influence of the solvation power and the influence of the jet/spray mixing behavior with supercritical carbon dioxide ($scCO_2$) on the characteristics of the PVP precipitates.

Using pure DMSO, NMP or EtOH, it is possible to produce spherical PVP microparticles (with a mean diameter lower than 3.8 μm); whereas, using mixtures of these solvents with acetone, PVP nanoparticles (with a mean diameter down to 0.11 μm) were precipitated.

1. Introduction

Supercritical fluids have been frequently proposed as alternatives to traditional processes in different industrial fields [1,2,3,4,5,6,7]. In particular, the control of microparticles and nanoparticles size and size distribution has been obtained using different supercritical fluids based techniques [8,9,10,11,12,13,14,15,16,17]. Among these, Supercritical AntiSolvent (SAS) precipitation has been proposed for the production of micro and nanoparticles of several kind of compounds [7,18,19,20]. It is based on the rapid mixing of a solution, formed by a solid solute and a solvent, with a supercritical antisolvent. Supercritical carbon dioxide ($scCO_2$) behaves as the antisolvent only with respect to the solute; therefore, during the mixing of the three compounds, the solute supersaturates and precipitates as particles. To assure rapid mixing between the solution and the antisolvent, the solution is usually injected into the antisolvent through a nozzle.

The solute can also be a mixture of solutes; for example, a pharmaceutical compound together with a polymer for the production of coprecipitated microparticles [21]. Also the solvent used in SAS can be a mixture that, in this case, can provide an additional degree of freedom for the manipulation of the characteristics of the products. A variation of the composition of the solvent mixture can influence the SAS process in two distinct ways. Firstly, the **solvation power** of the solvent mixture can be tuned, if, accordingly with the terminology used by Gokhale et al. [22], a "good solvent" with respect to the solute is mixed with a "poor solvent" with respect to the same solute. Secondly, the **mixing behavior** of the injected solution with bulk CO_2 in the precipitation vessel can also change significantly with the composition of the solvent mixture. SAS mechanisms, related to fluid-dynamics, thermodynamics and mass transfer aspects, that lead to the formation of micro and nanoparticles have been the subject of several previous studies [23,24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39]. In some of them, elastic and inelastic *in situ* light scattering techniques have also been used to study the jet/spray mixing behavior of different organic solvents, when injected into $scCO_2$ [35,36,37]. It was demonstrated that:

- Microparticles, generated in the proximity of the mixture critical point (MCP), are frequently produced by solvents, like dimethylsulfoxide (DMSO) and N-methyl-2-pyrrolidone (NMP), showing a large transition pressure range from the two-phase to the one-phase mixing behavior;

- Nanoparticles, precipitated at completely developed supercritical conditions, can be produced both from solvents showing a large transition pressure range from two-phase to one-phase mixing behavior and from solvents, like acetone (AC), characterized by a sharp transition pressure range [37].

The switch between microparticles and nanoparticles formation is controlled by the competition of three characteristic time scales of the process [40]: the time of jet break-up (τ_{jb}), the time of interfacial tension vanishing (τ_i) and the time of particle precipitation (τ_p).

Solvent mixtures, formed at different proportions of DMSO and AC [37], show a jet behavior that is intermediate between the one of the two pure solvents [41,42].

In this paper, we want to show that, choosing polyvinylpyrrolidone (PVP) as a model compound and using solvent mixtures, it is possible to modulate the product morphological characteristics, separating the influence of "solvation power" and "mixing behavior". This result is achieved, firstly, by characterizing single solvent behavior with respect to PVP, then, identifying couples having different solvation power and mixing behaviors. It was, therefore, analyzed:

- the influence of the solvation power on the particle characteristics alone, when the solvents couple did NOT change its mixing behavior with compressed CO_2;
- the effect of the solvation power and of the mixing behavior, when the solvents couple mixing behavior with compressed CO_2 is significantly a function of the solvents mixture's composition.

These results could add some further general knowledge on SAS and its applications when specific product diameters in the micrometric and nanometric range are required.

2. Materials and procedures

2.1. Materials

Dimethylsulfoxide (DMSO, purity 99.5%), N-methyl-2-pyrrolidone (NMP, purity 99.0%), ethanol (EtOH, purity 99.8%), acetone (AC, purity 99.8%), and Polyvinylpyrrolidone (PVP, average molecular weight equal to 10 kg/mol) were supplied by Sigma–Aldrich (Italy). Carbon dioxide (CO_2, purity 99%) was purchased from SON (Italy). All the products were used as received.

2.2. SAS experimental setup

In the SAS apparatus, a HPLC pump (Gilson, mod. 305) was used to deliver the liquid mixture. A diaphragm high-pressure pump (Milton Roy, mod. Milroyal B) equipped with a cooling system for the pumping head, was used to deliver liquid carbon dioxide. A cylindrical vessel (NWA, Germany) with an inner volume of 375 cm^3 (i.d. = 4.4 cm), equipped with two quartz windows along the longitudinal section, was used as the SAS vessel. The liquid mixture was delivered to the precipitator through a stainless steel nozzle with a diameter of 80 μm. A second collection chamber located downstream of the expansion valve was used to recover the liquid solvent. The pressure in the precipitation vessel was measured using a digital gauge manometer (Parker, USA), while the temperature was measured by a K-type thermocouple and controlled by a PID controller (Watlow, USA). Temperature uniformity inside the vessel was guaranteed by the presence of 16 cartridge heaters distributed in the upper and lower part of the walls of the precipitation chamber.

2.3. SAS procedure

SAS experiments start by delivering supercritical CO_2 at a constant flow rate to the precipitation chamber until the desired pressure is reached. Then, the mixture of organic solvents (without PVP dissolved in it) is sent through the nozzle to the chamber with the aim of obtaining steady state composition conditions of the fluid phase during the solute precipitation. At this point, the flow of the pure solvent is stopped and the liquid solution is delivered through the nozzle at the same flow rate, resulting in the precipitation of the solute. At the end of the solution delivery, supercritical CO_2 continues to flow and to wash the chamber from the residual solvent content. The continuous flow of supercritical carbon dioxide guarantees the continuous removal of the organic solvent from the particles. At the end of the washing step, CO_2 flow is stopped and the vessel is gradually depressurized to atmospheric conditions.

2.4. Analyses

Samples of the precipitated powder were collected at different points inside the precipitation chamber and examined using a Field Emission Scanning Electron Microscope (FESEM, mod. LEO 1525, Carl Zeiss SMT AG, Oberkochen, Germany). FESEM samples were covered with 250 Å of gold using a sputter coater (Agar, mod. 108A). Particle size (PS) and particle size distribution (PSD) were measured using an image processing software (Sigma Scan Pro, Jandel Scientific) that counts, measures, and analyzes digital images. About 1000 particles, coming from different images, were considered for each PSD calculation. The number of particles was chosen in agreement with the criteria generally used in the image analyses [43]: 500 to 1500 measured particles represent a good compromise between the time spent for the analysis and the accuracy of results.

PVP thermograms were obtained using a Differential Scanning Calorimeter (DSC, mod. TC11, Mettler-Toledo, Inc., Columbus, USA) using Mettler STARe system. Fusion temperature and enthalpy were previously calibrated with indium standard (melting point 156.6 °C, enthalpy of fusion 28.52 J/g). PVP powder samples (5 ± 0.5 mg), prepared in duplicates, were accurately weighed, crimped into an aluminium pan and heated from 40 to 170 °C at 5 °C/min under a nitrogen purge (50 mL/min).

X-ray diffractograms were recorded using an X-ray powder diffractometer (model D8 Discover, Bruker, USA) with a Cu sealed tube source. The measuring conditions were: Ni-filtered CuKα radiation, λ = 1.54 A˚, 2θ angle ranging from 5° to 50° with a scan rate of 0.3 s/step and a step size of 0.028°.

Solvent residues were measured by a headspace sampler (model 7694E, Hewlett Packard, USA) coupled to a gas chromatograph equipped with a flame ionization detector (GC-FID, model 6890 GC-SYSTEM, Hewlett Packard, Agilent Technologies Mfg. GmbH & Co. KG, USA). The solvents were separated using two fused silica capillary columns connected in series by press-fit: the first column (model Carbowax EASYSEP, Stepbios, Italy) connected to the detector, 30 m length, 0.53 mm i.d., 1 μm film thickness and the second (model Cp Sil 5CB CHROMPACK, Stepbios, Italy) connected to the injector; 25 m length, 0.53 mm i.d., 5 μm film thickness. GC conditions were: oven temperature at 160 °C for a total time equal to 8.8 min. The injector was maintained at 250 °C (split mode, ratio 5:1), and helium was used as the carrier gas (2 mL/min). Headspace conditions were: equilibration time 9 min at 170 °C; pressurization time 0.3 min; loop fill time 0.4 min. Head space samples were prepared in 20 mL vials filled with 50 mg of drug dissolved in water. Analyses were performed on each batch of processed drug, in triplicates.

2.5. Elastic Light scattering setup

A home-made elastic light scattering setup was used to analyze the mixing behavior between the injected solvents (without PVP dissolved in the solvents) and the scCO$_2$; that setup was coupled with the SAS apparatus, as sketched in Figure 1. The general setup is based on illuminating the spray/jet mixing zone diagonal from the bottom while detecting the light scattered elastically in horizontal backward direction. This type of setup was already used in previous studies and was introduced in detail in previous papers [35,37]. The benefit of this configuration is that light scattered from refractive-index-gradients and light scattered from real interfaces can be detected at the same time with one detector. This enables us to simultaneously visualize the mixing regimes in which there are still real interfaces between the injected solvent and the CO$_2$ (two-phase-mixing regime) and in which the interfaces have already disappeared and mixing between the solvent and the CO$_2$ takes place in a single phase. If we did not use this configuration, it was not possible to visualize two-phase and single-phase

mixing regimes simultaneously with one detector, as the intensities of light scattered elastically from two-phase and single-phase mixing regimes are different several orders of magnitudeThe angle between the direction of illumination and detection is crucial for the simultaneous detection of light scattered from real phase boundaries (two phase mixing regime) and from gradients of the refractive index (single phase mixing regime). For the identification of a suitable angle, we tilted the mirror during online measurements of the mixing process at the respective operation condition until the acquired photos simultaneously showed the two phase and the single mixing regimes in the transcritical range. Therefore, the angle was optimized slightly for each operation condition. At fully developed supercritical conditions, we were not able to identify any angle for which elastic light scattering signals from phase boundaries could be detected.

The setup we used in this work is different to the ones used in previous studies [35,37]. Here we used a charge coupled device (CCD) camera with a CCD chip of 1376 times 1056 pixels. To freeze the turbulent motion of the mixing process in "snap shots", short camera exposure times (shorter than 50 µs) had to be realized. Aiming to compensate the weak signal levels accompanied with the short exposure times, we binned two times two pixels on the camera chip, which increased the signal levels by a factor of four (two times two). To resolve the jet/spray mixing regime as the best as possible, the camera was equipped with a long distance microscope positioned approximately 25 cm away from the jet/spray mixing zone. Finally, an area from the jet/spray mixing zone of 2.3 times 1.7 cm^2 (vertical times horizontal) was imaged on the CCD. The elastic light scattering setup was calibrated with respect to its spatial resolution and its magnification using a 1951 USAF resolution test chart. The intensity values of the pixels were not calibrated, as the interpretation of the acquired photos solely relies onto the structures identifiable in the photos but not on their intensities.

3. Results and discussion

3.1 Comparison of the jet behavior of solvent mixtures AC/EtOH and AC/NMP

The evolution of the interface between the injected solvent and $scCO_2$ was analyzed for some solvent mixtures AC/EtOH and AC/NMP of different compositions, varying the operating pressure from subcritical to supercritical conditions. All the experiments were carried out at 40 °C, using an 80 µm diameter nozzle, with a liquid flow rate of 2.5 mL/min and with a CO_2 flow rate equal to 7 kg/h (to obtain a CO_2 mole fraction in the precipitator equal to 0.99). These process conditions assure that, independently of the pressure, the operating point is located outside the miscibility gap of the system CO_2-mixture of solvents. Figure 2 and Figure 3 show jet images of solvent mixtures of AC/EtOH and AC/NMP, respectively, in $scCO_2$ at the previously indicated process conditions. The left column shows jet images at subcritical conditions (pressures lower than the mixture critical point) at which a two-phase mixing between liquid phase and bulk CO_2 takes place. At subcritical conditions, when the jet break-up occurs, the liquid jet is atomized into droplets; the interfacial tension vanishing time τ_i is higher than the jet break-up time τ_{jb}, therefore, mixing occurs in a two-phase flow (liquid droplets mixed with bulk carbon dioxide). The right columns of Figures 2 and 3 show jet images where mixing occurs in a single-phase flow, typical of completely developed supercritical conditions. Indeed, in this case, the interfacial tension between solvent and bulk CO_2 has reached the zero value before the jet break-up. Therefore, liquid droplets do not form and a completely developed single-phase mixing can be observed. The minimum pressure at which no phase boundaries (droplets) are detectable is the one indicated in the images represented in the right columns

of Figures 2 and 3. The jet images reported in the middle columns are related to the transition from a two-phase to a single phase mixing that, in terms of pressure range, depends on the kind of solvent. In the region of transition, the two-phase mixing can be observed in the vertical centre of the jet (high elastic light scattering intensities can be detected from interfaces); whereas, a single-phase mixing can be observed in the outer jet mixing regions (low elastic light scattering intensities can be detected from refractive-index-gradients).

Summarizing, adding a narrow transition solvent (AC) to another solvent (like EtOH or NMP), the pressure at which the transition from a two-phase to a single-phase mixing flow takes place decreases, if compared to the pressure at which this transition occurs when these solvents are used alone.

In Figure 2, it can be observed that all the mixtures formed by AC and EtOH at 8.0 MPa are in the two-phase mixing region. Increasing EtOH content, the transition region from two-phase to one-phase behavior widens from 8.6 MPa for pure AC to 9.0 MPa for pure EtOH and the mixture containing 70 % EtOH.

In Figure 3, the jets of the mixtures formed by acetone and NMP, when in contact with $scCO_2$, are shown. Also in the case of the mixture formed by these two solvents, at 8.0 MPa, a two-phase mixing can be detected for all the mixture compositions. Increasing NMP content (whose transition region from two-phase to one-phase mixing behavior is wider with respect to pure EtOH), it can be noted that the span of the transition region from two-phase to one-phase behavior increases from 8.6 MPa (pure AC) to 9.7 MPa (for 70 % NMP). For pressures larger than 9.2 MPa (in the case of AC-rich mixture) and 9.7 MPa (in the case of the NMP-rich mixture) no signals originating from two-phase mixing zones are detectable; therefore, one-phase mixing occurred.

3.2. SAS precipitation experiments

Solubility tests performed at room temperature showed that PVP solubility in AC, identified as a "poor solvent", is about 10 mg/mL; whereas, its solubility in the so-called "good solvents" is about 200 mg/mL in EtOH, 250 mg/mL in DMSO and 350 mg/mL in NMP. SAS experiments were, then, performed, precipitating PVP from mixtures, at different compositions, of AC/EtOH, AC/NMP and AC/DMSO; the latter mixing behavior was the subject of a previous study [37]. Preliminary tests showed that, when processed by SAS using EtOH, DMSO or NMP (solvents in which PVP is very soluble), PVP always precipitated in the form of microparticles, even at pressures well above the mixture critical point (MCP) of the systems organic solvent/carbon dioxide, corresponding to well-developed supercritical conditions. We may hypothesize that the addition of PVP strongly modifies the mixing behaviour and enlarges the transition region from two-phase to one-phase mixing regime. To control PVP particle size and eventually produce nanoparticles, we added acetone that features two characteristics as previously mentioned:

    (1) AC is a "poor solvent" with respect to PVP;

    (2) AC shows a sharp transition region between two-phase and one-phase mixing behavior [37].

The experiments were carried out at 10 MPa, 40 °C, PVP concentration in the liquid solution (50 mL injected for each experiment) equal to 1 % wt, liquid flow rate equal to 2.5 mL/min, and a CO_2 molar fraction of 0.99. A list of the experiments performed indicating the solvent-mixtures used (and the related compositions), the morphology obtained, the mean diameter (m.d.) and the standard deviation (s.d.), is reported in Table 1. All the experiments were performed in triplicates.

The first set of experiments was performed using pure ethanol and the mixtures AC/EtOH at different percentages of the two solvents (# 1-4 in Table 1), to evaluate the effect of the content of AC in the mixture on PVP morphology and on the mean size of the particles. It is possible to observe that using pure ethanol (Figure 4a), well separated spherical microparticles were obtained; whereas, using EtOH/AC mixtures (the images reported in Figures 4b-5d are taken at the same magnification), sub-microparticles and nanoparticles were produced; in particular, increasing the content of AC, the mean particle sizes decreases and the particle size distributions shrinks.

The contribution of Acetone in the reduction of particles mean size is also evident from the particle size distributions (PSDs) reported in Figure 5.

When DMSO and AC/DMSO mixtures were used (# 5-8 in Table 1), we also observed that acetone acted as a morphology modifier, leading to the production of nanoparticles; again, increasing acetone content in the mixture, the mean size of PVP particles decreased.

In the case of PVP precipitated from NMP and from AC/NMP mixtures, the same proportions of the two solvents were used (# 9-12 in Table 1), as in the previous set of experiments. The obtained results followed the same trend of the particles obtained from the mixtures AC/EtOH and AC/DMSO. Particle mean diameter decreased and PSDs sharpened, increasing the percentage of AC in the solvent mixture. A comparison of FESEM images and PSDs of particles obtained from pure NMP and from the mixture AC/NMP 50/50 is reported in Figures 6a-6c.

For all the performed experiments, the yield of the process was about 90 %.

Summarizing the results, these experiments showed that the use of proper solvent mixtures is effective in the production of nanoparticles of PVP that have never before been obtained by SAS using pure solvents.

In particular, ethanol and acetone show a similar behavior from the point of view of mixing regimes. Therefore, the decreasing of PVP mean particle size and the shrinkage of the particle size distribution is in agreement with the "poor solvent"-"good solvent" interpretation [22]. Adding acetone, that is a "poor solvent" with respect to PVP, the polymer tends to reject acetone molecules, precipitating in a more compact conformation; therefore, particles with a lower mean particle size were obtained.

In the case of the AC/DMSO and AC/NMP mixtures, the addition of acetone has a double-effect: it is a "poor solvent" with respect to PVP and it is a "narrow transition solvent" from two-phase to one-phase mixing, if compared to DMSO and NMP. Therefore, adding acetone to DMSO and NMP produced a more distinct effect. As indicated in Table 1, in these cases, smaller nanoparticles, compared to the ones obtained for PVP precipitated from the AC/EtOH mixture, were produced. This more distinct effect can be explained also considering that PVP solubility in DMSO and NMP (and, consequently, in DMSO/AC and NMP/AC mixtures) is higher with respect to PVP solubility in EtOH and EtOH/AC mixtures. Therefore, when precipitated from EtOH/AC mixtures, PVP nucleation early occurs, because the supersaturation is earlier reached with respect to DMSO/AC and NMP/AC mixtures; therefore, growth phenomenon is favored and higher mean diameters are obtained.

3.3 Analyses

Differential scanning calorimetry (DSC) thermograms of untreated and SAS processed PVP samples are shown in Figure 7a. DSC diagrams show a broad endothermic peak ranging from 50 to 130 °C for all the samples that

indicate the loss of water due to the hygroscopic nature of PVP. This analysis shows that the SAS process did not alter the product structure. X-ray diffraction (XRD) analyses of untreated and SAS processed PVP samples were carried out to evaluate the presence of possible crystalline modification, as a consequence of the processing. XRD patterns are reported in Figure 7b; they show similar and very broad peaks, suggesting that no changes occurred in the amorphous structure of the polymer.

Solvent residue was analyzed to verify that it was lower than the Food and Drug Administration (FDA) limit, that, according to the USP 467 for the residual solvents, is equal to 530 for NMP (belonging to class 2), and to 5000 ppm for AC, DMSO and EtOH (belonging to class 3). These analyses revealed that the solvent residue was around 1300 ppm in the case of AC, 2000 ppm in the case of EtOH, 3000 ppm in the case of DMSO and 100 in the case of NMP, therefore, it is, in any case, below the allowed limits.

4 Conclusions and perspectives

The results obtained in this work showed that, precipitating PVP from a mixture formed by a "poor solvent" (like AC) and a "good solvent" (like EtOH, DMSO or NMP) allows the production of nanoparticles, that cannot be obtained using single solvents alone. Moreover, considering that AC and EtOH are sharp two-phase/one-phase mixing transition solvents, whereas DMSO and NMP are broad two-phase/one-phase mixing transition solvents, the experiments performed using the mixture EtOH/AC took into account the solvation power alone and the experiments performed using the mixtures DMSO/AC and NMP/AC allowed the study of the influence of the solvation power and the influence of the spray mixing behavior. This kind of results could be readily extended to other solutes; therefore, it represents a significant advancement in SAS knowledge and potential industrial use.

5. Acknowledgments

Figures and tables captions

Figure 1: Schematic representation of SAS apparatus. V: CO_2 supply; S: organic solvent supply; RB: refrigerating bath; P1, P2: pumps; PV: precipitation vessel; MV: micrometering valve; LS: liquid separator; BPV: back pressure valve; R: rotameter; CCD: charged-coupled device camera; LDM: long distance microscope; LS: light source; M: mirror.

Figure 2: Jet images of solvent mixtures of AC and EtOH injected at 40 °C with a flow rate of 2.5 mL/min through a d= 80 μm nozzle into $scCO_2$.

Figure 3: Jet images of solvent mixtures of AC and NMP injected at 40 °C with a flow rate of 2.5 mL/min through a d= 80 μm nozzle into $scCO_2$.

Figure 4: FESEM images of PVP precipitated at 40 °C, 10 MPa, 1% wt from different AC/EtOH mixtures percentages: (a) 0/100 (w/w); (b) 30/70 (w/w); (c) 50/50 (w/w); (d) 70/30 (w/w).

Figure 5: Volumetric particle size distributions of PVP precipitated at 40 °C, 10 MPa, 1% wt from pure EtOH and from different AC/EtOH mixtures percentages.

Figure 6: PVP precipitated at 40 °C, 10 MPa, 1% wt. (a-b) FESEM images from pure NMP (a) and AC/NMP 50/50 (b); (c) volumetric particle size distributions.

Figure 7: Unprocessed and SAS processed PVP using the different mixtures; (a) Differential scanning calorimetry thermograms; (b) XRD analysis**.**

Table 1: SAS experiments performed using PVP at 40 °C, 10 MPa and 1% wt (NP: nanoparticles; MP: microparticles; SMP: sub-microparticles; m.d.: mean diameter; s.d.: standard deviation).

6. References

[1]   K. Byrappa, S. Ohara, T. Adschiri, Nanoparticles synthesis using supercritical fluid technology – towards biomedical applications, Advanced Drug Delivery Reviews 60 (2008) 299–327.

[2]   E. Reverchon, S. Cardea, Supercritical fluids in 3-D tissue engineering, J. of Supercritical Fluids 69 (2012) 97– 107.

[3]   A.I. Cooper, Porous materials and supercritical fluids, Advanced Materials 15(13) (2003) 1049–1059.

[4]   G. Caputo, M. Scognamiglio, I. De Marco, Nimesulide adsorbed on silica aerogel using supercritical carbon dioxide, Chemical Engineering Research and Design 90(8) (2012) 1082–1089.

[5]   I. Smirnova, S. Suttiruengwong, W. Arlt, Feasibility study of hydrophilic and hydrophobic silica aerogels as drug delivery systems, Journal of Non-Crystalline Solids 350 (2004) 54–60.

[6]   Y. Zhang, C. Erkey, Preparation of supported metallic nanoparticles using supercritical fluids: A review, J. of Supercritical Fluids 38 (2006) 252–267.

[7]   E. Reverchon, Supercritical antisolvent precipitation of micro- and nano-particles, J. of Supercritical Fluids 15 (1) (1999) 1–21.

[8]   S.-D. Yeo, E. Kiran, Formation of polymer particles with supercritical fluids: A review, J. of Supercritical Fluids 34 (2005) 287–308.

[9]   E. Reverchon, A. Antonacci, Polymer microparticles production by supercritical assisted atomization, J. of Supercritical Fluids 39 (2007) 444–452.

[10]   N. Ventosa, S. Sala, J. Veciana, DELOS process: a crystallization technique using compressed fluids 1. Comparison to the GAS crystallization method, J. of Supercritical Fluids 26 (2003) 33–45.

[11]   Z. Huang, G.-B. Sun, Y.C. Chiew, S. Kawi, Formation of ultrafine aspirin particles through rapid expansion of supercritical solutions (RESS), Powder Technology 160 (2005) 127–134.

[12]   I. De Marco, E. Reverchon, Supercritical carbon dioxide + ethanol mixtures for the antisolvent micronization of hydrosoluble materials, Chemical Engineering Journal 187 (2012) 401–409.

[13]   J. Fages, H. Lochard, J.-J. Letourneau, M. Sauceau, E. Rodier, Particle generation for pharmaceutical applications using supercritical fluid technology, Powder Technology 141 (2004) 219–226.

[14]   G. Della Porta, R. Campardelli, E. Reverchon, Monodisperse biopolymer nanoparticles by Continuous Supercritical Emulsion Extraction, J. of Supercritical Fluids 76 (2013) 67– 73.

[15]   K. Mishima, Biodegradable particle formation for drug and gene delivery using supercritical fluid and dense gas, Advanced Drug Delivery Reviews 60 (2008) 411–432.

[16]   R. Campardelli, E. Oleandro, R. Adami, E. Reverchon, Polymethylmethacrylate (PMMA) sub-microparticles produced by Supercritical Assisted Injection in a Liquid Antisolvent, J. of Supercritical Fluids 92 (2014) 93-99.

[17]   J. Li, M. Rodrigues, A. Paiva, H.A. Matos, E. Gomes de Azevedo, Modeling of the PGSS Process by Crystallization and Atomization, AIChE Journal 51(8) (2005) 2343–2357.

[18]   A. Shariati, C.J. Peters, Recent developments in particle design using supercritical fluids, Current Opinion in Solid State & Materials Science 7 (4–5) (2003) 371–383.

[19]   Y. Hakuta, H. Hayashi, K. Arai, Fine particle formation using supercritical fluids, Current Opinion in Solid State & Materials Science 7 (4–5) (2003) 341–351.

[20]   E. Reverchon, I. De Marco, Mechanisms controlling supercritical antisolvent precipitate morphology, Chemical Engineering Journal 169 (2011) 358–370.

[21]   I.N. Uzun, O. Sipahigil, S. Dinçer, Coprecipitation of Cefuroxime Axetil–PVP composite microparticles by batch supercritical antisolvent process, J. of Supercritical Fluids 55 (2011) 1059–1069.

[22]   A. Gokhale, B. Khusid, R.N. Dave, R. Pfeffer, Effect of solvent strength and operating pressure on the formation of submicrometer polymer particles in supercritical microjets, J. of Supercritical Fluids 43 (2007) 341–356.

[23]   C. S. Lengsfeld, J. P. Delplanque, V. H. Barocas, T. W. Randolph, Mechanism governing microparticle morphology during precipitation by a compressed antisolvent: atomization vs. nucleation and growth, J. Physical Chemistry B 104 (2000) 2725–2735.

[24]   J.O. Werling, P.G. Debenedetti, Numerical modeling of mass transfer in the supercritical antisolvent process, J. Supercrit. Fluids 16 (2) (1999) 167–181.

[25]   J.O. Werling, P.G. Debenedetti, Numerical modeling of mass transfer in the supercritical antisolvent process: miscible conditions, J. Supercrit. Fluids 18 (1) (2000) 11–24.

[26]   F. Chavez, P.G. Debenedetti, J.J. Luo, R.N. Dave, R. Pfeffer, Estimation of the characteristic time scales in the supercritical antisolvent process, Ind. Eng. Chem. Res. 42 (13) (2003) 3156–3162.

[27]   J. Bałdyga, D. Kubicki, B.Y. Shekunov, K.B. Smith, Mixing effects on particle formation in supercritical fluids, Chem. Eng. Res. Des. 88 (9) (2010) 1131–1141.

[28]   M. Sarkari, I. Darrat, B.L. Knutson, Generation of microparticles using CO2 and CO2-philic antisolvents, AIChE J. 46 (9) (2000) 1850–1859.

[29]   J. Sierra-Pallares, D.L. Marchisio, M.T. Parra-Santos, Juan García-Serna, F. Castro, M.J. Cocero, A Computational Fluid Dynamics Study of Supercritical Antisolvent Precipitation: Mixing Effects on Particle Size, AIChE J. 58(2) (2012) 385-398.

[30] Y. Pérez de Diego, H.C. Pellikaan, F.E. Wubbolts, G.J. Witkamp, P.J. Jansens, Operating regimes and mechanism of particle formation during the precipitation of polymers using the PCA process, J. of Supercritical Fluids 35 (2005) 147–156.

[31] S. S. Dukhin, Y. Shen, R. Dave, R. Pfeffer, Droplet mass transfer, intradroplet nucleation and submicron particle production in two-phase flow of solvent–supercritical antisolvent emulsion, Colloids and Surfaces A: Physicochemical and Engineering Aspects 261 (2005) 163–176.

[32] A. Martin, M. J. Cocero, Numerical modeling of jet hydrodynamics, mass transfer, and crystallization kinetics in the supercritical antisolvent (SAS) process, J. of Supercritical Fluids 32 (2004) 203–219.

[33] T. Petit-Gas, O. Boutin, I. Raspo, E. Badens, Role of hydrodynamics in supercritical antisolvent processes, J. of Supercritical Fluids 51(2) (2009) 248–255.

[34] I. De Marco, E. Reverchon, Influence of pressure, temperature and concentration on the mechanisms of particle precipitation in supercritical antisolvent micronization, J. of Supercritical Fluids 58 (2011) 295– 302.

[35] E. Reverchon, E. Torino, S. Dowy, A. Braeuer, A. Leipertz, Interactions of phase equilibria, jet fluid dynamics and mass transfer during supercritical antisolvent micronization, Chemical Engineering Journal 156 (2) (2010) 446–458.

[36] A. Braeuer, S. Dowy, E. Torino, M. Rossmann, S.K. Luther, E. Schluecker, A. Leipertz, E. Reverchon, Analysis of the supercritical antisolvent mechanisms governing particles precipitation and morphology by in situ laser scattering techniques, Chemical Engineering Journal 173 (2011) 258–266.

[37] I. De Marco, O. Knauer, F. Cice, A. Braeuer, E. Reverchon, Interactions of phase equilibria, jet fluid dynamics and mass transfer during supercritical antisolvent micronization: the influence of solvents, Chemical Engineering Journal 203 (2012) 71–80.

[38] A. Erriguible, S. Vincent, P. Subra-Paternault, Numerical investigations of liquid jet breakup in pressurized carbon dioxide: Conditions of two-phase flow in Supercritical Antisolvent Process, J. of Supercritical Fluids 63 (2012) 16–24.

[39] E. Torino, I. De Marco, E. Reverchon, Organic nanoparticles recovery in supercritical antisolvent precipitation, J. of Supercritical Fluids 55 (2010) 300–306.

[40] F. Marra, I. De Marco, E. Reverchon, Numerical analysis of the characteristic times controlling supercritical antisolvent micronization, Chemical Engineering Science 71 (2012) 39–45.

[41] I. De Marco, V. Prosapio, F. Cice, E. Reverchon, Use of solvent mixtures in supercritical antisolvent process to modify precipitates morphology: Cellulose acetate microparticles, J. of Supercritical Fluids 83 (2013) 153–160.

[42] M. Rossmann, A. Braeuer, E. Schluecker, Supercritical antisolvent micronization of PVP and ibuprofen sodium towards tailored solid dispersions, J. of Supercritical Fluids 89 (2014) 16–27.

[43] M.-N. Pons, H. Vivier, V. Delcour, J.-R. Authelin, L. Pailleres-Hubert, Morphological analysis of pharmaceutical powders, Powder Technology 128 (2–3) (2002) 276–286.