PVP/corticosteroid microspheres produced by supercritical antisolvent coprecipitation

Valentina Prosapio, Iolanda De Marco*, Ernesto Reverchon

Department of Industrial Engineering, University of Salerno,

Via Giovanni Paolo II, 132, 84084, Fisciano (SA), ITALY

*idemarco@unisa.it Fax: +39-89-964057

ABSTRACT

Coprecipitation of poorly water soluble drugs with an hydrophilic polymer can enhance the drug dissolution rate and, therefore, its bioavailability. In this work, Supercritical Antisolvent (SAS) precipitation is proposed for the coprecipitation of corticosteroids. This class of compounds cannot be directly processed by SAS, since it precipitates in form of large crystals. Dexamethasone (DMS), prednisolone (PDN) and budesonide (BDS) were tested. To control microspheres size and morphology, the effect of polymer/drug ratio, precipitation pressure and concentration were investigated.

For all the processed systems polyvinylpyrrolidone (PVP)/corticosteroids, spherical microparticles were obtained at selected operating conditions and at proper PVP/corticosteroid ratios. Particles mean diameter ranged between about 1.8 and 2.5 μm for PVP/DMS, 2.0-3.1 μm for PVP/PDN and 3.0-3.6 μm for PVP/BDS. All precipitates were characterized, to determine the effective drug

entrapment efficiency and drug release rate. The performed analyses showed that the drug entrapment efficiency was about 90-95 % with respect to the initial concentration in all samples. The drug dissolution rate in phosphate buffered saline solution (PBS) was largely improved: it was more than 4 times faster than the one of unprocessed drug in the case of DMS and about 5 times faster in the case of PDN and BDS.

KEYWORDS: Microspheres, Corticosteroids, Polyvinylpyrrolidone, Supercritical Antisolvent process, Precipitation mechanisms.

INTRODUCTION

Corticosteroids are a class of steroid hormones, largely used as anti-inflammatory drugs for the treatment of ocular [1] and pulmonary [2] diseases, hepatitis [3] and ulcers [4]. Some of the most widely used drugs in this category are dexamethasone (DMS), prednisolone (PDN) and budesonide (BDS). They show a low water solubility (< 1 mg/100 mL) and consequently low bioavailability [5, 6]; therefore, large doses are required to reach the therapeutic level, that can produce some undesired effects, like high sugar concentration in blood, hypertension, stomach and intestinal ulcers, fluids retention [7]. To improve their dissolution rate, and correspondingly reduce their dose, a possible solution is represented by their particle size reduction at micrometric diameters. Traditional micronization techniques such as spray drying, jet milling and solvent evaporation show several drawbacks: lack of control over the particle morphology and particle size distribution, difficulty in elimination of the solvent and use of high temperatures [8]. Tajber et al. [9] proposed the micronization of budesonide using a spray drying technique: they obtained amorphous microparticles in the range 1-7 μm; but, they did not demonstrate the improvement of the dissolution rate. Rasenack et al. [10] used a controlled crystallization process to micronize several anti-inflammatory drugs, among which budesonide and prednisolone, obtaining large and irregular crystals; also in this case, dissolution tests were not performed.

Another approach to increase drug dissolution rate is to produce composite microspheres drug-polymer, using a water soluble polymer in which the drug is entrapped. The fast solubilization of the polymer should release the drug in nanometric subparticles obtaining an improvement of their bioavailability. Rodrìguez et al. [11] prepared cellulose acetate butyrate-budesonide microspheres by emulsion-solvent evaporation using acetone and methanol as solvents; they obtained particles larger than 200 μm with an encapsulation efficiency lower than 75 %, and did not report neither the

residual solvent analysis, nor the comparison of the dissolution profiles between unprocessed drug and coprecipitated powder.

An alternative to conventional techniques is represented by supercritical carbon dioxide ($scCO_2$) based processes, characterized by fast mass transfer, high solvent power, high density, near zero surface tension, low viscosity and high diffusivity, that can be tuned varying pressure and temperature. They have been proposed for several applications, such as extraction of natural matters [12], supercritical adsorption of drugs in aerogels [13], membranes and scaffolds production [14, 15] and micronization [16-19]. It has been demonstrated that it is possible to accurately control particles size and particle size distribution. In particular, nanoparticles, microparticles and expanded microparticles of different kind of materials have been successfully obtained by supercritical antisolvent (SAS) precipitation [20-30]. However, when processed using SAS, corticosteroids precipitate in form of large crystals [31-33], without improvement of the dissolution rate with respect to the unprocessed drugs. SAS is governed by complex high-pressure equilibria, fluid dynamics interactions and mass transfer [34-36]. In the case of corticosteroids, large crystals are produced as a result of the partial solubility of these drugs in the mixture solvent/antisolvent, that can modify the corresponding vapor-liquid equilibria (VLE). As previously discussed, to improve the dissolution rate, it is possible to coprecipitate the drug with a biodegradable polymeric carrier in form of microspheres. However, examples of SAS successful coprecipitation are rather limited, because of the difficulty to produce composite microspheres using this process, since the two compounds tend to precipitate separately. The results are generally unsatisfactory since irregular and coalescing particles [37] are produced, with broad particle size distributions [38, 39], low drug loadings [8, 40], and, in some cases, the occurrence of coprecipitation is questionable [41-44]. In two recent papers, Prosapio et al. [45, 46] successfully obtained coprecipitates using SAS; these authors produced polyvinylpyrrolidone (PVP)/folic acid microparticles in the range 0.8–3.8 μm and

PVP/β-carotene microparticles in the range 1.0–2.4 μm. The microparticles showed a dissolution rate of the active compound significantly larger than the one of the unprocessed compound.

Therefore, in this work, to overcome SAS limitation in producing corticosteroids microparticles and to extend the application of SAS coprecipitation, three corticosteroids: dexamethasone (DMS), prednisolone (PDN) and budesonide (BDS) are processed, trying to take advantage of PVP ability to retard crystal growth [47-49]. The effect of polymer/drug ratio, operating pressure and overall solute concentration is investigated to understand their effect on the success of coprecipitation and on particle morphology, mean size and particle size distribution. Precipitation mechanisms are discussed and loading efficiency and dissolution tests of coprecipitates are performed.

MATERIALS, METHODS AND PROCEDURES

Materials

Polyvinylpyrrolidone (PVP, average molecular weight 10000 g/mol), dexamethasone (DMS, purity ≥ 98 %), prednisolone (PDN, purity ≥ 99 %), budesonide (BDS, purity ≥ 99 %) and ethanol (EtOH, purity 99.5 %) were supplied by Sigma−Aldrich (Italy). CO2 (purity 99 %) was purchased from SON (Italy). All materials were used as received. Solubility tests performed at room temperature showed that the solubilities of the materials in EtOH are about: 200 mg/mL in the case of PVP, 17 mg/mL in the case of DMS, 25 mg/mL in the case of PDN and 12 mg/mL in the case of BDS.

SAS experimental setup

The SAS laboratory plant used for the experiments (Figure 1) consists of two high pressure pumps to feed the liquid solution (Milton Roy, mod. Milroyal D) and carbon dioxide (Milton Roy, mod. Milroyal B). The precipitation vessel consists of a cylindrical vessel with an internal volume of 500 cm^3. The liquid mixture is sprayed in the precipitator through a thin wall, 100 μm internal diameter stainless steel nozzle. Supercritical CO_2, after preheating, is delivered to the precipitator through

another port located on the top of the vessel. Considering that, at the process conditions, the carbon dioxide and the liquid solvent are perfectly miscible, their co-current or countercurrent feed positioning is indifferent; in this conformation of the plant, they are co-currently delivered to the precipitator. The collection of the produced powders is realized using a stainless steel filter (pore diameter of 0.1 μm) located at the bottom of the precipitator, that also allows the CO_2–organic solvent solution to pass through. A second collection vessel located downstream the precipitator at a lower pressure (18–20 bar) is used to recover the liquid solvent. Further details were published elsewhere [45].

SAS procedure

At the beginning of a SAS experiment, the precipitation vessel is pressurized delivering CO_2 until the desired pressure is reached; then, pure solvent is sent through the nozzle to the vessel for at least 15 min. Once quasi-steady state composition of solvent and antisolvent is reached inside the chamber, the solvent flow is stopped and the liquid solution is delivered through the nozzle, producing the precipitation of the solute.

When the solution delivery is completed, the precipitator is washed, flushing only supercritical CO_2, to eliminate the solution formed by the liquid solubilized in the supercritical antisolvent. At the end of the washing step, CO_2 flow is stopped, the precipitator is gradually depressurized down to atmospheric pressure and the precipitated powder is collected for analysis.

Analytical methods

Samples of the precipitated material were observed by a Field Emission Scanning Electron Microscope (FE-SEM, mod. LEO 1525, Carl Zeiss SMT AG, Oberkochen, Germany). Powder was dispersed on a carbon tab previously stuck to an aluminum stub (Agar Scientific, United Kingdom);

then, was coated with gold-palladium (layer thickness 250 Å) using a sputter coater (mod. 108 A, Agar Scientific, Stansted, United Kingdom).

Particle size distributions (PSDs) of the powders were measured from FE-SEM photomicrographs using the Sigma Scan Pro image analysis software (release 5.0, Aspire Software International Ashburn, VA). Approximately 1000 particles, taken at high enlargements and in various locations inside the precipitator, were analyzed in the elaboration of each particle size distribution. Histograms representing the particle size distributions were fitted using Microcal Origin Software (release 8.0, Microcal Software, Inc., Northampton, MA). We were not able to calculate PSDs using the dynamic laser scattering (DLS) technique, since we did not find an effective dispersant in which both PVP and corticosteroids are not soluble.

The thermal behavior of samples was measured by 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). Powder samples (5 ± 0.5 mg), prepared in duplicates, were accurately weighed, crimped into an aluminium pan and heated from 40 to 280 °C at 10 °C/min under a nitrogen purge (50 mL/min).

Fourier transform infrared (FT-IR) spectra were obtained via M2000 FTIR (MIDAC Co, Costa Mesa, CA), at a resolution of 0.5 cm^{-1}. The scan wavenumber range was 4000–450 cm^{-1}, and 16 scan signals were averaged to reduce the noise. The powder samples were ground and mixed thoroughly with potassium bromide (KBr) as infrared transparent matrix. KBr discs were prepared by compressing the powders in a hydraulic press.

Drug entrapment efficiency and powder dissolution studies were performed using an UV/vis spectrophotometer (model Cary 50, Varian, Palo Alto, CA). Accurately weighted samples containing an equivalent amount of drug (1 mg) were suspended in 1.5 mL of phosphate buffered saline

solution (PBS) and placed into a dialysis sack; it was then incubated in 300 mL of PBS at pH 7.4, continuously stirred at 200 rpm and 37 °C. Each analysis was carried out in triplicate and the proposed curves are the mean profiles. Drug entrapment efficiencies were measured by UV–vis analysis, measuring the absorbance obtained in the release medium at the end of the drug release; i.e., when the entire drug was released from the microspheres to the outer water phase. The absorbance, then, was converted into drug concentration, using a calibration curve.

Ethanol residues were measured using 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 solvent was 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 8.80 min. The injector was maintained at 250 °C (split mode, ratio 5:1), and helium was used as the carrier gas (2 mL/min). Head-space 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.

Considering that particles could be not stable during the time, DSC, FE-SEM and dissolution rate analyses were replicated after 30 and 90 days.

EXPERIMENTAL RESULTS

SAS experiments were performed using a CO_2 flow rate of 10 NL/min, a solution flow rate of 1 mL/min and EtOH as the liquid solvent. The effect of polymer/drug w/w ratio and operating pressure was investigated for the three corticosteroids. In the case of PDN, additional experiments were performed to evaluate also the effect of total concentration. Table 1 reports a list of the experiments, with the indication of the obtained morphology, mean diameter (m.d.) and standard deviation (s.d.).

A first set of experiments was performed processing by SAS the corticosteroids alone, to confirm the results found in the literature [31-33]. These experiments were performed operating at 90 bar, 40 °C and a solute concentration of 20 mg/mL (#4,11,21 in Table 1). At these process conditions, all the corticosteroids (DMS, PDN and BDS) precipitated in form of large crystals. Then, to verify if these results are limited to lower SAS operating pressures, the three compounds were processed at 150 bar and 40 °C (#3,10,20 in Table 1). A pressure of 150 bar was chosen because, for the binary system EtOH-CO_2, it is well above the mixture critical point, assuring the precipitation at well-developed supercritical conditions. Also at 150 bar, the same morphology was observed, as shown, for example, in the FE-SEM images reported in Figure 2. Therefore, it is possible to confirm that during SAS, corticosteroids precipitate in form of crystals, irrespective of the process conditions selected.

To complete the analysis of the single compounds, PVP was processed at the same two operating conditions used for corticosteroids (#1,2 in Table 1). Rossmann et al. [50] and De Marco et al. [35] observed that, when PVP is SAS processed from ethanol, it precipitates in form of microparticles, independently on the operating pressure, in the range 90-300 bar. Our results confirmed their observation: at 90 and 150 bar, well-separated spherical microparticles were produced. This experimental result is ascribable to the remarkable shift towards higher pressures of the mixture critical point (MCP), caused by the modification of binary EtOH/CO_2 VLE due to the presence of PVP. In Figure 3, an example of FE-SEM image of PVP particles obtained at 150 bar is reported. The mean

sizes and the standard deviations of the particles obtained in these two experiments were compared: increasing the operating pressure, the mean diameter of the particles decreased and the particle size distribution shrank (Table 1).

The comparison of the FE-SEM images for corticosteroids and PVP (Figures 2 and 3) clearly shows that the polymer and the drugs precipitate with a completely different morphology, even when processed at the same SAS operating conditions. In previous works [45, 46], it was observed that PVP is a good candidate for coprecipitates formation, since it can interfere with the crystallization kinetics of some compounds like folic acid and β-carotene, producing composite microspheres. In order to verify if PVP can work in the same manner for corticosteroids, SAS experiments using PVP as the carrier and DMS, PDN or BDS as the active compounds were performed. To this purpose, the effect of: polymer/drug ratio, operating pressure and total concentration of the two solutes in EtOH was studied.

Effect of PVP/drug ratio

For each corticosteroid, the effect of polymer/drug ratio on particles morphology, mean size and particle size distribution was investigated at 90 bar and 40 °C. When PVP/drug 2:1 ratio (#5,12,23 in Table 1) was processed, crystals and coalescing sub-microparticles were observed in the case of DMS and PDN, as shown in the FE-SEM images in Figures 4a and 4b; therefore, the samples could not be characterized in terms of particle diameter, since coprecipitation was substantially unsuccessful: the two compounds precipitated as separated particles/crystals. In the case of BDS at 2:1 ratio, instead, microparticles with a mean diameter equal to 3.06 μm were produced, as shown in Figure 4c. Since this last result differs from the ones obtained for DMS and PDN, a further experiment was performed fixing PVP/BDS at 1:1, to identify a possible transition from crystals to microparticles; indeed, in this test, crystals and coalescing sub-microparticles were obtained.

In the subsequent experiments, the ratio PVP/drug was increased at 3:1 (#6,13,24 in Table 1) for all systems. Operating at these conditions, microparticles were obtained in all cases. Also when the polymer/drug ratio was increased at 5:1 (#7,14,25), as showed in the exemplificative FE-SEM images reported in Figure 5, and at 10:1 (#8,15,26) and 20:1 (#9,19,27), well separated spherical microparticles were obtained.

The volumetric PSDs obtained at different polymer/drug ratios were compared. In the case of PVP/DMS and PVP/PDN particles, as shown in Figure 6, increasing the percentage of polymer in the injected solution, the mean size of the particles increases and the PSD becomes wider; in the case of PVP/BDS particles (see Table 1), not significant variations in the mean diameter were observed.

Effect of the operating pressure

In order to study the effect of the SAS operating pressure, an experiment was performed at 150 bar for the system PVP/PDN 10:1 (#16 in Table 1), keeping constant all the other operating parameters at the values previously proposed. The FE-SEM image and the PSD of the powders produced confirmed that they were formed by microparticles, as shown in Figure 7.

This result is interesting, since at these process conditions the SAS operating point is located far above the MCP of the binary system CO_2/EtOH and, as a consequence, nanoparticles should be produced, as largely discussed in the literature [28, 36]. The system PVP/PDN follows, instead, the same behavior of PVP alone that, as mentioned above, precipitates from ethanol in form of microparticles at pressures up to 300 bar [50]; i.e., the quantity of polymer that is present in the system definitely modified VLE and, therefore, controls coprecipitates overall morphology even at the highest pressure tested. This experimental evidence for PVP is still under investigation.

Effect of concentration

The effect of the total concentration PVP/corticosteroid in EtOH was investigated for the system PVP/PDN 10:1 at 90 bar and 40 °C, varying the overall concentration from 10 mg/mL to 30 mg/mL (#17-18 in Table 1); data at 20 mg/mL was already available (#15). Using an overall concentration of 10 mg/mL, microparticles were produced, as shown in Figure 8a, with a mean diameter of about 2 µm; increasing the concentration at 30 mg/mL, as shown in Figure 8c, microparticles were still obtained, but with a mean size of 3.5 µm.

Comparing the volumetric PSD of the particles obtained at different concentrations, it is possible to observe that, increasing the concentration, the particle mean size increases and the PSD enlarges, as reported in Figure 9; this result is in agreement with several other experimental observations on SAS precipitates, reported in the literature [51].

In Table 2, the properties of the PVP/EtOH solution at different concentrations are reported [52, 53]. Usually, dimensionless numbers employed to describe jet fluid dynamics are the Reynolds (Re) and the Ohnesorge (Oh) number. Reynolds number ($Re = Dv\rho/\mu$) gives a measure of the ratio of inertial forces to viscous forces; whereas, Ohnesorge number ($Oh = \mu/\sqrt{D\rho\sigma}$) relates the viscous and the surface tension forces [28]. D is the nozzle diameter, ρ is the density of the fluid, v its velocity, µ its viscosity and σ is the surface tension. The dimensionless numbers evaluated at different concentrations are also reported in Table 2.

Characterization of precipitates

Differential scanning calorimetry (DSC) analyses were performed on unprocessed drugs and polymer, physical mixtures PVP/corticosteroids and SAS processed PVP/corticosteroids 5:1, to determine the changes in the thermal transition of the drugs and the polymer in the coprecipitates.DSC thermograms are reported in Figure 10 and revealed that: the unprocessed drugs show all a narrow endothermic peak in correspondence of about 250 °C; unprocessed PVP shows a broad endothermic peak ranging from 50 to 130 °C; the physical mixtures show both the

polymer and drug peaks; SAS processed PVP/corticosteroids show both the endothermic peaks but, in all the cases, the peak of the drug is remarkably reduced in its intensity (results not shown). This last result could be ascribable to the large presence of the polymer with respect to the drug; it indicates the presence of both the compounds in the powder.

Fourier transform infrared (FT-IR) analyses were performed to identify possible interactions between the drug and the carrier in the coprecipitates. FT-IR spectra of unprocessed drugs and PVP, physical mixtures PVP/corticosteroids 5:1 and SAS processed PVP/corticosteroids 5:1 are reported in Figure 11. The spectra of unprocessed corticosteroids show characteristic absorption bands in the range 1600-1700 cm^{-1} related to the stretching vibration of C=O carbonyl groups, a characteristic absorption band in the range 2750-3100 cm^{-1} related to the stretching vibration of the C–H group and a characteristic absorption band in the range 3100-3700 cm^{-1} related to the stretching vibration of the –OH groups. The spectrum of PVP shows a characteristic absorption band at 1653 cm^{-1} which belongs to the stretching vibration of C=O groups, a C–H stretching vibration at 2873 cm^{-1} and a –OH stretching vibration at 3469 cm^{-1}. The spectra of the physical mixtures PVP/corticosteroids and of processed PVP/corticosteroids show the same characteristic bands related to the carbonyl stretching, –OH stretching and C–H stretching; this result suggests the presence of both the compounds in the samples, but does not indicate the existence of a well-defined interaction between them.

To demonstrate the successful coprecipitation of the drug and the polymer and the improvement of corticosteroids dissolution rate, drug entrapment efficiencies and drug release tests were performed using UV-vis spectroscopy analyses. Entrapment efficiency analyses revealed that the samples of PVP/DMS, PVP/PRN and PVP/BDS microparticles show a drug content ranging between 90 and 95 % with respect to the initial value. In the dissolution tests, the samples taken into account were: unprocessed drugs, SAS processed drugs (#4,11,21), physical mixtures and SAS processed

coprecipitates PVP/drug 5:1 (#7,14,25). During the analyses, the dissolution rate of each sample in PBS was monitored plotting the percentage of dissolved drug as a function of time. In Figure 12a, the dissolution profiles of dexamethasone are reported; it is possible to observe that unprocessed DMS, physical mixture PVP/DMS and SAS processed DMS achieve the complete dissolution in 25 hours; whereas, the sample PVP/DMS 5:1 has a faster dissolution rate and arrives at 100 % in about 6 hours. In Figure 12b, the dissolution profiles of prednisolone are shown: unprocessed PDN achieves the complete dissolution in about 70 hours; SAS processed PDN achieves the 100 % release also in 70 hours, but, the curve shows a higher slope in the first part of the release; the physical mixture PVP/PDN shows an intermediate behavior; the coprecipitate PVP/PDN 5:1, instead, concludes its dissolution in only 14 hours. At the end, in Figure 12c, the dissolution profiles of budesonide are reported; unprocessed BDS reaches the complete dissolution in 90 hours; SAS processed BDS dissolves slightly faster, but achieves the 100 % release also in 90 hours; the physical mixture PVP/BDS shows an intermediate behavior; the coprecipitate PVP/BDS 5:1, instead, completes its dissolution in 20 hours. Summarizing, in all cases, the unprocessed and SAS processed corticosteroids and their physical mixtures with PVP show very similar dissolution behavior, whereas PVP/drug coprecipitates show a very fast dissolution, from about 4.2 to 5 times faster!

DSC, FE-SEM and dissolution rate analyses were replicated after 30 and 90 days to demonstrate the stability of the powders in time: no difference among these analyses and the ones previously performed was detected.

According to Food and Drug Administration (FDA) guidelines, ethanol belongs to class 3 solvents; therefore, its maximum acceptable concentration in the final product is 5000 ppm. A headspace sampler, coupled to a gas chromatograph, was used to verify the solvent residue content in SAS produced microspheres. The analysis revealed that the solvent residue was around 100 ppm in all cases!

DISCUSSION

The major SAS processing difficulties resolved in this work concern the following aspects:

- difficulty in producing coprecipitates;
- crystals formation for corticosteroids.

To explain the results obtained, it is possible to recall the SAS precipitation mechanisms [28] and how they worked, in this case, in controlling particles formation. Two characteristic times of the process are involved: the time of jet break-up (τ_{jb}) and the surface tension vanishing time (τ_{stv}). In particular:

- when $\tau_{stv}<\tau_{jb}$, the surface tension disappearance phenomenon prevails and nanoparticles are produced by nucleation and growth mechanism; coprecipitation fails because the two species tend to precipitate separately, due to two different nucleation and growth times, forming at best a sort of physical mixture;
- when $\tau_{jb}<\tau_{stv}$, the jet break-up phenomenon prevails and microparticles are obtained by jet break-up and drying mechanism; in this case, coprecipitation is successful since each droplet works as a confined drying system.

Another important key factor that favors the success of coprecipitation is the right choice of the carrier. Indeed, when the active compound tends to assume a crystalline structure, as in the case of corticosteroids, it is necessary to use a polymer that inhibits crystal growth, such as PVP.

Dissolution tests in PBS confirmed the coprecipitation, since the samples PVP/DMS, PVP/PDN and PVP/BDS showed a dramatic reduction of the time needed to obtain the complete dissolution, with respect to unprocessed and SAS processed corticosteroids. It is simple to hypothesize that this improved dissolution rate is due to the presence of the corticosteroid in form of nanoparticles inside

the polymeric matrix [46, 54]. In Figure 12, it is also possible to observe that there is no “burst effect”: the drug is substantially located inside the microparticles and not on their surface. This result definitively confirms the successful coprecipitation.

In addition to coprecipitates production, the further goal reached in this work was the evaluation of the influence of the process parameters on precipitates. Indeed, it is possible to control the morphology tuning the polymer/drug ratio, starting from a PVP/drug ratio of 2/1 to 20/1. Particle size can be controlled varying pressure and concentration; in particular:

- increasing the pressure, the mean diameter reduces and the PSD becomes sharper due to an increase of atomization efficiency in the case of higher pressures;
- increasing the overall concentration, the particle mean size increases and the PSD becomes wider because the solution viscosity increases, leading a decrease of atomization efficiency.

These results can also be explained using dimensionless parameters characteristic of atomization processes. Jet atomization depends on Reynolds and Ohnesorge dimensionless numbers. An increase of concentration produces an increase of the viscosity of the solution (as evident from data reported in Table 2); therefore, the cohesive forces operating on the liquid jet are stronger: Re number decreases and Oh number increases, leading the precipitation of larger particles. An increase of pressure, instead, produces an increase of the density of the solution, thus, an increase of the dispersive forces, with consequent formation of smaller particles.

CONCLUSIONS

In this work, it was demonstrated that, at specific operating conditions and using PVP as carrier, it is possible to obtain for the first time a successful SAS coprecipitation of three corticosteroids, with a yield of the process higher than 90%. It is expected that this result could be readily extended to other compounds of the same family.

Dissolution tests confirmed the improvement of the dissolution rate of all the coprecipitated microparticles that is between 4 and 5 times faster with respect to unprocessed corticosteroids. This result is very relevant from a pharmaceutical point of view, since it can open the way to more efficient and focused administration routes for corticosteroids.

TABLES AND FIGURES CAPTIONS

Table 1: Summary of SAS coprecipitation experiments (MP: microparticles, SMP: sub-microparticles; C: crystals; m.d.: mean diameter; s.d.: standard deviation).

Table 2: Physical properties and dimensionless numbers of PVP/EtOH solutions at different concentrations. Densities and viscosities were obtained from [52], surface tension was obtained from [53].

Figure 1: Schematic representation of SAS apparatus. S1: CO_2 supply; S2: liquid solution supply; RB: refrigerating bath; P1, P2: pumps; PV: precipitation vessel; MV: micrometering valve; LS: liquid separator; BPV: back-pressure valve; R: rotameter and DM: dry test meter.Figure 2: FE-SEM images of corticosteroids precipitated from EtOH at 150 bar, 40 °C and a concentration of 20 mg/mL: (a) DMS; (b) PDN; (c) BDS. Large crystals were obtained in all cases.

Figure 3: FE-SEM image of PVP microparticles precipitated from EtOH at 150 bar, 40 °C and 20 mg/mL.

Figure 4: FE-SEM images of PVP/corticosteroids 2:1 ratio particles precipitated from EtOH at 90 bar, 40 °C and 20 mg/mL: (a) PVP/DMS; (b) PVP/PDN; (c) PVP/BDS.

Figure 5: FE-SEM images of PVP/corticosteroids 5:1 particles precipitated from EtOH at 90 bar, 40 °C and 20 mg/mL: (a) PVP/DMS; (b) PVP/PDN; (c) PVP/BDS.

Figure 6: Volumetric cumulative PSDs of microparticles precipitated from EtOH at 90 bar and 40 °C at different polymer/drug ratios: (a) PVP/DMS; (b) PVP/PDN.

Figure 7: PVP/PDN 10:1 particles precipitated from EtOH at 150 bar, 40 °C and 20 mg/mL: (a) FE-SEM image; (b) particle size distribution.

Figure 8: FE-SEM images of PVP/PDN 10:1 particles precipitated from EtOH at 90 bar, 40 °C and different concentrations: (a) 10 mg/mL; (b) 20 mg/mL; (c) 30 mg/mL.

Figure 9: Volumetric cumulative PSDs of PVP/PDN 10:1 particles precipitated from EtOH at 90 bar and 40 °C at different concentrations.

Figure 10: DSC thermograms of unprocessed, physical mixtures and SAS processed PVP/corticosteroids: (a) dexamethasone; (b) prednisolone; (c) budesonide.

Figure 11: FT-IR spectra of unprocessed, physical mixtures and SAS processed PVP/corticosteroids: (a) dexamethasone; (b) prednisolone; (c) budesonide.

Figure 12: Dissolution profiles in PBS at 37 °C and pH 7.4: (a) DMS; (b) PDN; (c) BDS.

REFERENCES

[1] S. Muñoz-Fernández, E. Martín-Mola, Uveitis, Best Practice & Research Clinical Rheumatology 20 (2006) 487-505.

[2] G.B. Diette, A.A. Dalal, A.O. D'Souza, O.E. Lunacsek, S.P. Nagar, Treatment patterns of chronic obstructive pulmonary disease in employed adults in the United States, International journal of chronic obstructive pulmonary disease 10 (2015) 415.

[3] A.D. Yeoman, R.H. Westbrook, Y. Zen, W. Bernal, T. Al-Chalabi, J.A. Wendon, J.G. O'Grady, M.A. Heneghan, Prognosis of acute severe autoimmune hepatitis (AS-AIH): The role of corticosteroids in modifying outcome, Journal of hepatology 61 (2014) 876-882.

[4] T. Zhou, P. Zhou, H. Hua, X. Liu, Beneficial Effects and Safety of Corticosteroids Combined with Traditional Chinese Medicine for Pemphigus: A Systematic Review, Evidence-Based Complementary and Alternative Medicine 2015 (2015).

[5] X.-Y. Chen, Y.-L. Shang, Y.-H. Li, J.-X. Wang, A.G. Maimouna, Y.-X. Li, D. Zou, N.R. Foster, J. Yun, Y. Pu, Green preparation of uniform prednisolone nanoparticles using subcritical water, Chemical Engineering Journal 263 (2015) 20-26.

[6] C. Gómez-Gaete, N. Tsapis, M. Besnard, A. Bochot, E. Fattal, Encapsulation of dexamethasone into biodegradable polymeric nanoparticles, International journal of pharmaceutics 331 (2007) 153-159.

[7] J. Urbańska, A. Karewicz, M. Nowakowska, Polymeric delivery systems for dexamethasone, Life sciences 96 (2014) 1-6.

[8] W. Wang, G. Liu, J. Wu, Y. Jiang, Co-precipitation of 10-hydroxycamptothecin and poly (l-lactic acid) by supercritical CO 2 anti-solvent process using dichloromethane/ethanol co-solvent, The Journal of Supercritical Fluids 74 (2013) 137-144.

[9] L. Tajber, D. Corrigan, O. Corrigan, A. Healy, Spray drying of budesonide, formoterol fumarate and their composites—I. Physicochemical characterisation, International journal of Pharmaceutics 367 (2009) 79-85.

[10] N. Rasenack, H. Steckel, B.W. Müller, Micronization of anti-inflammatory drugs for pulmonary delivery by a controlled crystallization process, Journal of pharmaceutical sciences 92 (2003) 35-44.

[11] M. Rodríguez, J.L. Vila-Jato, D. Torres, Design of a new multiparticulate system for potential site-specific and controlled drug delivery to the colonic region, Journal of controlled release 55 (1998) 67-77.

[12] M. Herrero, J.A. Mendiola, A. Cifuentes, E. Ibáñez, Supercritical fluid extraction: Recent advances and applications, Journal of Chromatography A 1217 (2010) 2495-2511.

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

[14] L. Baldino, S. Cardea, E. Reverchon, Supercritical assisted enzymatic membranes preparation, for active packaging applications, Journal of Membrane Science 453 (2014) 409-418.

[15] P. Pisanti, A.B. Yeatts, S. Cardea, J.P. Fisher, E. Reverchon, Tubular perfusion system culture of human mesenchymal stem cells on poly-L-lactic acid scaffolds produced using a supercritical carbon dioxide-assisted process, Journal of Biomedical Materials Research Part A 100 (2012) 2563-2572.

[16] R. Campardelli, L. Baldino, E. Reverchon, Supercritical fluids applications in nanomedicine, The Journal of Supercritical Fluids 101 (2015) 193-214.

[17] R. Campardelli, I. Espirito Santo, E.C. Albuquerque, S. Vieira de Melo, G. Della Porta, E. Reverchon, Efficient encapsulation of proteins in submicro liposomes using a supercritical fluid assisted continuous process, The Journal of Supercritical Fluids 107 (2016) 163-169.

[18] S. Liparoti, R. Adami, E. Reverchon, Supercritical Assisted Atomization: effect of operative conditions on PVP microparticle size and morphology, The Journal of Supercritical Fluids 97 (2015) 31-35.

[19] E. Reverchon, R. Adami, M. Scognamiglio, G. Fortunato, G. Della Porta, Beclomethasone microparticles for wet inhalation, produced by supercritical assisted atomization, Industrial & Engineering Chemistry Research 49 (2010) 12747-12755.
[20] F. Cardoso, R. Rezende, R. Almeida, V. Cabral, E. Zanoelo, D. Noriler, H. Meier, L. Cardozo-Filho, A model for precipitation of sub-micrometric particles of PHBV poly (3-hydroxybutyrate-co-3-hydroxyvalerate) by supercritical assisted-atomization, The Journal of Supercritical Fluids 97 (2015) 88-99.
[21] I. De Marco, V. Prosapio, F. Cice, E. Reverchon, Use of solvent mixtures in supercritical antisolvent process to modify precipitates morphology: Cellulose acetate microparticles, The Journal of Supercritical Fluids 83 (2013) 153-160.
[22] I. De Marco, E. Reverchon, Supercritical carbon dioxide+ ethanol mixtures for the antisolvent micronization of hydrosoluble materials, Chemical Engineering Journal 187 (2012) 401-409.
[23] M. Henczka, J. Bałdyga, B.Y. Shekunov, Particle formation by turbulent mixing with supercritical antisolvent, Chemical Engineering Science 60 (2005) 2193-2201.
[24] F. Marra, I. De Marco, E. Reverchon, Numerical analysis of the characteristic times controlling supercritical antisolvent micronization, Chemical Engineering Science 71 (2012) 39-45.
[25] D.L. Obrzut, P.W. Bell, C.B. Roberts, S.R. Duke, Effect of process conditions on the spray characteristics of a PLA+ methylene chloride solution in the supercritical antisolvent precipitation process, The Journal of supercritical fluids 42 (2007) 299-309.
[26] V. Prosapio, E. Reverchon, I. De Marco, Antisolvent micronization of BSA using supercritical mixtures carbon dioxide+ organic solvent, The Journal of Supercritical Fluids 94 (2014) 189-197.
[27] V. Prosapio, E. Reverchon, I. De Marco, Production of lysozyme microparticles to be used in functional foods, using an expanded liquid antisolvent process, The Journal of Supercritical Fluids 107 (2016) 106-113.
[28] E. Reverchon, I. De Marco, Mechanisms controlling supercritical antisolvent precipitate morphology, Chemical Engineering Journal 169 (2011) 358-370.
[29] M. Rossmann, A. Braeuer, S. Dowy, T.G. Gallinger, A. Leipertz, E. Schluecker, Solute solubility as criterion for the appearance of amorphous particle precipitation or crystallization in the supercritical antisolvent (SAS) process, The Journal of Supercritical Fluids 66 (2012) 350-358.
[30] Y. Wang, Y. Wang, J. Yang, R. Pfeffer, R. Dave, B. Michniak, The application of a supercritical antisolvent process for sustained drug delivery, Powder technology 164 (2006) 94-102.
[31] H. Steckel, J. Thies, B. Müller, Micronizing of steroids for pulmonary delivery by supercritical carbon dioxide, International journal of pharmaceutics 152 (1997) 99-110.
[32] S.P. Velaga, R. Berger, J. Carlfors, Supercritical fluids crystallization of budesonide and flunisolide, Pharmaceutical research 19 (2002) 1564-1571.
[33] S.P. Velaga, S. Bergh, J. Carlfors, Stability and aerodynamic behaviour of glucocorticoid particles prepared by a supercritical fluids process, European journal of pharmaceutical sciences 21 (2004) 501-509.
[34] F. Chávez, P.G. Debenedetti, J. Luo, R.N. Dave, R. Pfeffer, Estimation of the characteristic time scales in the supercritical antisolvent process, Industrial & engineering chemistry research 42 (2003) 3156-3162.
[35] I. De Marco, M. Rossmann, V. Prosapio, E. Reverchon, A. Braeuer, Control of particle size, at micrometric and nanometric range, using supercritical antisolvent precipitation from solvent mixtures: Application to PVP, Chemical Engineering Journal 273 (2015) 344-352.
[36] 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 (2010) 446-458.
[37] A. Montes, M. Gordillo, C. Pereyra, E.M. de la Ossa, Co-precipitation of amoxicillin and ethyl cellulose microparticles by supercritical antisolvent process, The Journal of Supercritical Fluids 60 (2011) 75-80.
[38] V. Patomchaiviwat, O. Paeratakul, P. Kulvanich, Formation of inhalable rifampicin–poly (L-lactide) microparticles by supercritical anti-solvent process, Aaps Pharmscitech 9 (2008) 1119-1129.

[39] İ.N. Uzun, O. Sipahigil, S. Dinçer, Coprecipitation of Cefuroxime Axetil–PVP composite microparticles by batch supercritical antisolvent process, The Journal of Supercritical Fluids 55 (2011) 1059-1069.
[40] A.Z. Chen, X.M. Pu, Y.Q. Kang, L. Liao, Y.D. Yao, G.F. Yin, Preparation of 5-Fluorouracil-Poly (L-lactide) Microparticles Using Solution-Enhanced Dispersion by Supercritical CO2, Macromolecular Rapid Communications 27 (2006) 1254-1259.
[41] N. Elvassore, A. Bertucco, P. Caliceti, Production of protein-loaded polymeric microcapsules by compressed CO2 in a mixed solvent, Industrial & engineering chemistry research 40 (2001) 795-800.
[42] C.G. Kalogiannis, C.M. Michailof, C.G. Panayiotou, Microencapsulation of amoxicillin in poly (l-lactic acid) by supercritical antisolvent precipitation, Industrial & engineering chemistry research 45 (2006) 8738-8743.
[43] H. Nerome, S. Machmudah, R. Fukuzato, T. Higashiura, Y.-S. Youn, Y.-W. Lee, M. Goto, Nanoparticle formation of lycopene/β-cyclodextrin inclusion complex using supercritical antisolvent precipitation, The Journal of Supercritical Fluids 83 (2013) 97-103.
[44] L. Wenfeng, L. Guijin, L. Lixian, W. Juan, L. Yangxiao, Y. JIANG, Effect of process parameters on co-precipitation of paclitaxel and poly (l-lactic acid) by supercritical antisolvent process, Chinese Journal of Chemical Engineering 20 (2012) 803-813.
[45] V. Prosapio, I. De Marco, M. Scognamiglio, E. Reverchon, Folic acid–PVP nanostructured composite microparticles by supercritical antisolvent precipitation, Chemical Engineering Journal 277 (2015) 286-294.
[46] V. Prosapio, E. Reverchon, I. De Marco, Coprecipitation of PVP/β-carotene by supercritical antisolvent processing, Industrial & Engineering Chemistry Research (2015).
[47] G.A. Ledet, R.A. Graves, E.Y. Glotser, T.K. Mandal, L.A. Bostanian, Preparation and in vitro evaluation of hydrophilic fenretinide nanoparticles, International journal of pharmaceutics 479 (2015) 329-337.
[48] Y. Sun, L. Zhu, T. Wu, T. Cai, E.M. Gunn, L. Yu, Stability of amorphous pharmaceutical solids: crystal growth mechanisms and effect of polymer additives, The AAPS journal 14 (2012) 380-388.
[49] G. Van den Mooter, M. Wuyts, N. Blaton, R. Busson, P. Grobet, P. Augustijns, R. Kinget, Physical stabilisation of amorphous ketoconazole in solid dispersions with polyvinylpyrrolidone K25, European journal of pharmaceutical sciences 12 (2001) 261-269.
[50] M. Rossmann, A. Braeuer, E. Schluecker, Supercritical antisolvent micronization of PVP and ibuprofen sodium towards tailored solid dispersions, The Journal of Supercritical Fluids 89 (2014) 16-27.
[51] I. De Marco, E. Reverchon, Influence of pressure, temperature and concentration on the mechanisms of particle precipitation in supercritical antisolvent micronization, The Journal of Supercritical Fluids 58 (2011) 295-302.
[52] M.T. Zafarani-Moattar, Z. Khoshsima, Measurement and correlation of density and viscosity of polyvinylpyrrolidone solutions in alcohols at different temperatures, The Journal of Chemical Thermodynamics 40 (2008) 1569-1574.
[53] J.I. Wells, C.V. Walker, The influence of granulating fluids upon granule and tablet properties: the role of secondary binding, International Journal of Pharmaceutics 15 (1983) 97-111.
[54] N. Shah, H. Sandhu, D.S. Choi, H. Chokshi, A.W. Malick, Amorphous Solid Dispersions: Theory and Practice, Springer2014.