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Supercritical antisolvent coprecipitation of PVP/ketoprofen microparticles

Iolanda De Marco*, Paola Franco, Ernesto Reverchon
Department of Industrial Engineering, University of Salerno,
Via Giovanni Paolo II, 132, 84084, Fisciano (SA), Italy
*idemarco@unisa.it
www.supercriticalfluidgroup.unisa.it

ABSTRACT
In order to improve the bioavailability of poorly water-soluble anti-inflammatory drugs, an effective technique is the coprecipitation of the drug with a hydrophilic polymer. In this work, the production of coprecipitated polyvinylpyrrolidone/ketoprofen (PVP/KET) microparticles using Supercritical Antisolvent (SAS) was successfully obtained. The effects of the main process parameters, such as polymer/drug ratio, overall concentration, and operating pressure were systematically investigated to identify successful operating conditions for SAS coprecipitation. Microparticles with a mean diameter ranging between 2.4 and 4.1 μm were successfully precipitated. Powders were characterized using different analytical techniques, such as differential scanning calorimetry to determine the changes in the thermal transition of the drugs and the polymer in the coprecipitates, Fourier transform infrared analysis to identify possible interactions between the polymer and the active principle, and UV–vis spectroscopy to calculate the drug entrapment efficiency and dissolution rate. Precipitation yield was found to be about 98 % with respect to the amount of solute dissolved in the starting solution. Drug release analyses revealed that ketoprofen dissolution rate from PVP/KET microparticles in a 0.1 M HCl buffer was 4 times faster than the dissolution rate of the unprocessed drug. The possible precipitation mechanisms involved in the process were discussed.

INTRODUCTION
Ketoprofen (KET) is a non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic effect [1], which acts inhibiting the body's production of prostaglandin. A major limitation in its usage is due to its reduced solubility in water (< 0.05 mg/mL), which imply the necessary use of large doses to reach the therapeutic level, with consequent adverse side effects including gastrointestinal irritation when administered orally. In order to improve KET dissolution rate, and correspondingly reduce its dose, a possible solution is represented by particle size reduction at micrometric diameters thanks to the larger surface area in contact with the medium [2]. Traditional micronization processes, such as spray-drying, emulsification/solvent evaporation, liquid antisolvent precipitation, freeze drying and jet-milling, suffer from some drawbacks: lack of control over the particle morphology and particle size distribution, large solvent residues and use of high temperatures [3]. 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 successfully applied in different fields: micronization [4-6], liposomes' formation [7], extraction [8], impregnation [9], membranes and scaffolds production [10]. In particular, nanoparticles and microparticles of different kind of materials were successfully obtained by supercritical antisolvent (SAS) precipitation [11-13].
However, when processed using SAS, KET precipitated in form of coalescing sub-microparticles or it is partially extracted by the mixture formed by the organic solvent (dimethylsulfoxide, DMSO) and $scCO_2$, as a result of the partial solubility of this drug in the mixture solvent/antisolvent. In order to overcome this limitation, an effective solution can be the production of drug-polymer composite

microspheres, using a water soluble polymer in which the drug is entrapped. The fast solubilization of the polymer should release the drug in nanometric sub-microparticles obtaining an improvement of their bioavailability. Nevertheless, SAS coprecipitation is difficult to obtain, since the two compounds generally tend to precipitate separately. In SAS literature only few papers are devoted to coprecipitates formation and the results showed in these works are characterized by irregular and coalescing particles, with broad particle size distributions, low drug entrapment efficiency and, in some cases, questionable demonstration of the occurred coprecipitation [14-17]. Recently, Reverchon and co-workers obtained successful results in the production of composite microspheres Polyvinylpyrrolidone (PVP)/folic acid [18], PVP/ β-carotene [19] and PVP/corticosteroids [20], PVP/nimesulide [21], taking advantage of PVP ability to retard crystal growth [22].
In this work, KET coprecipitation with PVP is proposed. The following effects will be tested: polymer/drug ratio, overall concentration, and operating pressure. Samples will be characterized using different techniques in order to verify the occurred coprecipitation and the improvement of KET dissolution rate.

MATERIALS, METHODS AND PROCEDURES

Polyvinylpyrrolidone (PVP, average molecular weight 10 kg/mol), Ketoprofen (KET, purity ≥ 98 %) and Dimethylsulfoxide (DMSO, purity 99.5 %) were supplied by Sigma-Aldrich (Italy). CO_2 (purity 99 %) was purchased from Morlando Group s.r.l. (Italy). All materials were used as received.
The homemade SAS laboratory plant used for the experiments performed in this work consists of two high pressure pumps used to deliver the supercritical CO_2 and the liquid solution, respectively. The precipitator is a cylindrical vessel of 500 cm^3 internal volume (I.V.) (i.d. = 5 cm). The temperature control is assured by a PID controller connected with electrically thin bands and the pressure in the vessel is measured using a test gauge manometer and regulated by a micrometering valve. Carbon dioxide, after a preheating, is co-currently delivered through another port to the chamber. The liquid mixture is delivered to the precipitator through a thin wall 100 μm internal diameter stainless steel nozzle. A stainless steel filter with a pore diameter of 0.1 μm, located at the bottom of the precipitator, is used to collect the produced powder, allowing the CO_2-solvent-solution to pass through. The liquid solvent is then recovered in a second collection vessel located downstream the micrometering valve, whose pressure is regulated by a backpressure valve. At the exit of the second vessel, CO_2 flow rate and the total quantity of antisolvent delivered, are measured by a rotameter and a dry test meter, respectively.
At the beginning of the SAS experiment, CO_2 is delivered to the precipitation vessel to reach the desired pressure; then, pure solvent is sent through the nozzle to obtain steady state composition conditions during the solute precipitation. At this point, the solvent flow is stopped and the liquid solution is delivered through the nozzle, producing the precipitation of the solute. At the end of the injection step, supercritical CO_2 continues to flow, to eliminate residual content of liquid solubilized in the supercritical antisolvent. If the final purge with pure CO_2 is not performed, the organic solvent contained in the fluid phase condenses during the depressurization step and can solubilize or modify the precipitates. At the end of the washing step, CO_2 flow is stopped, the precipitator is depressurized down to atmospheric pressure and the precipitated powder is collected for analysis.
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 (layer thickness 250Å) using a sputter coater (mod. 108 A, Agar Scientific, Stansted, United Kingdom).
Particle size distribution (PSD) of the powders was 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).
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 300 °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 4000–400 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 dissolution studies were performed using an UV/vis spectrophotometer (model Cary 50, Varian, Palo Alto, CA) at a wavelength of 262 nm. Accurately weighted samples containing KET were suspended in a HCl 0.1 M solution (pH 2.5), to simulate the gastric acidity. Each analysis was carried out in triplicate and the proposed curves are the mean profiles.

RESULTS AND DISCUSSION

All SAS experiments were performed using a CO_2 flow rate of 30 g/min and a solution flow rate of 1 mL/min. DMSO was used as the liquid solvent. The effect of PVP/KET w/w ratio, total concentration, and operating pressure was investigated. Each experiment was carried out in duplicate. Ketoprofen alone was first SAS processed at 90 bar, 40 °C and 20 mg/mL; i.e., near above the mixture critical point (MCP) of the system CO_2/DMSO. KET precipitated in form of coalescing sub-microparticles and it is partially extracted by the mixture formed by DMSO and $scCO_2$. PVP precipitated in form of well separated microparticles; therefore, this polymer represents a good candidate as carrier for SAS coprecipitates formation.
First of all, the influence of the operating pressure on particle morphology and mean size was investigated. The experiments were carried out fixing the temperature at 40 °C, the ratio PVP/KET at 20:1 and the total concentration at 20 mg/mL, since these process conditions are characterized by a high drug content in the starting solution. Three values of pressure were investigated: 90, 120 and 150 bar. In correspondence of all the pressures, spherical microparticles were obtained, as shown in Figure 1, where two exemplificative SEM images are reported.

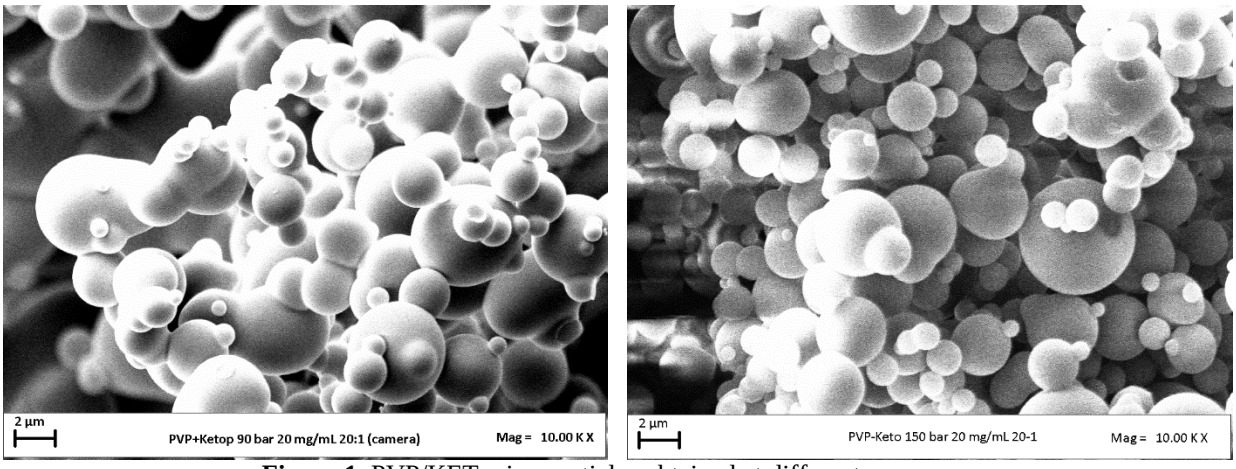

Figure 1. PVP/KET microparticles obtained at different pressures.

The effect of the overall concentration of the solutes in DMSO was studied in SAS experiments performed at 90 bar, 40 °C and fixing PVP/KET at 20:1. The overall concentration was varied in the range 10-100 mg/mL. At 10 mg/mL, coalescing microparticles (with a mean diameter of the single

particle equal to 3.1 µm) were obtained; at 20 and 50 mg/mL, well separated microparticles with a mean diameter in the range 3.6-3.8 µm were obtained; in the end, at 100 mg/mL, irregular crystals were obtained. This effect was attributed to the plasticizer effect of PVP, detectable at high concentrations. The effect of concentration on the mean size and distribution is shown in Figure 2.

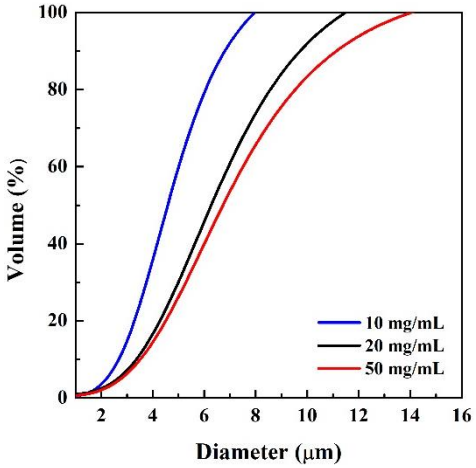

Figure 2. Particle size distributions of PVP/KET particles precipitated from DMSO at 90 bar, 40 °C, 20:1 at different concentrations.

The last set of experiments was carried out varying the polymer/drug ratio from 3:1 to 20:1, at 90 bar, 40 °C, fixing the overall solute concentration at 50 mg/mL. In all the cases, well separated microparticles are obtained; increasing the polymer/drug ratio, the particle mean size increased and the particle size distributions enlarged. These results are detectable in Figure 3, where a SEM image and the particle size distributions are reported.

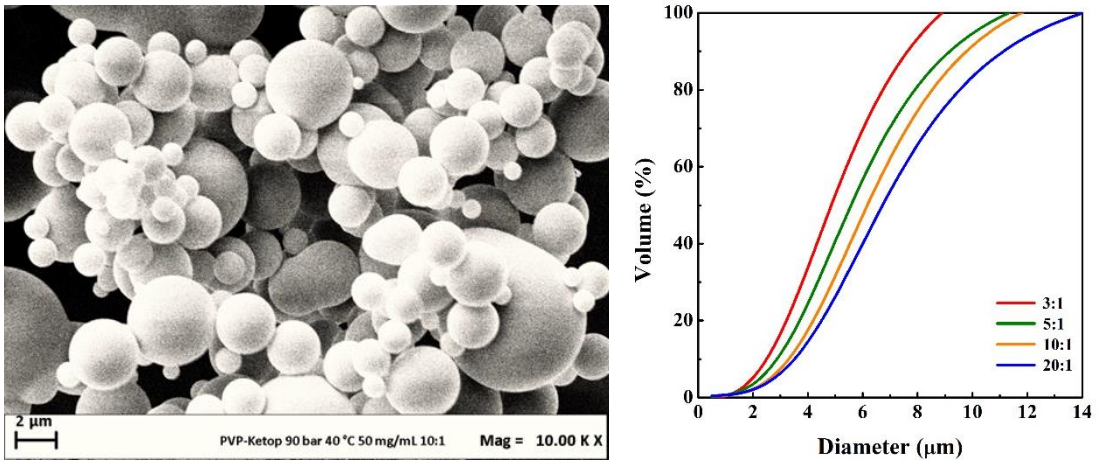

Figure 3. SEM image and particle size distributions of PVP/KET particles precipitated from DMSO at 90 bar, 40 °C, 50 mg/mL at different polymer/drug ratios.

Differential scanning calorimetry (DSC) analyses were performed on the unprocessed drug, the polymer and SAS processed PVP/KET, to determine the changes in the thermal transition of the drug and the polymer in the coprecipitates. DSC thermograms revealed that: the unprocessed drug shows an endothermic peak in correspondence of about 93 °C; unprocessed PVP shows a broad endothermic peak ranging from 80 to 110 °C; SAS processed PVP/KET in correspondence of 3/1 and 10/1 ratios do not show the peak characteristic of the drug. Probably, this behavior can be ascribable to the fact that the polymeric matrix containing the drug is amorphous. 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 the unprocessed drug and PVP, physical mixture PVP/KET 10/1 and

SAS processed PVP/KET 10/1 were determined. The spectrum of the unprocessed drug shows characteristic absorption bands in the range 1600-1700 cm^{-1} related to the stretching vibration of C=O carbonyl groups, two characteristic absorption bands at 1440 and 1370 cm^{-1} related to stretching vibration of the C–H group. 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 mixture PVP/KET and of processed PVP/KET powder show the same characteristic bands 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 KET dissolution rate, drug release tests were performed using UV-vis spectroscopy analyses. The unprocessed KET achieves the 90% release in 6 hours, the physical mixture PVP/KET 10/1 achieves the 90% release in 4 hours, whereas the SAS coprecipitate completes the 90% dissolution in 85 minutes. Summarizing this result, it is possible to observe that the unprocessed KET and its physical mixture with PVP show similar dissolution behavior, whereas PVP/KET coprecipitate shows a very fast dissolution, equal to 4.2 times faster!

CONCLUSIONS

In this work, it was demonstrated that it is possible to enhance KET dissolution rate using SAS in which PVP is used as carrier and selected operating conditions are employed. Indeed, PVP/KET spherical microparticles with improved bioavailability were produced. This result is very interesting: in this way, it is possible to optimize the amount of drug to be administered in patients, avoiding the side effects caused by high dosages.

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