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Production of Eudragit/Ampicillin Microparticles by Supercritical Antisolvent Coprecipitation

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In this paper, the supercritical antisolvent (SAS) technique was proposed to coprecipitate ampicillin sodium (AMPI), an antibiotic chosen as the model drug, by using Eudragit L100-55 as the polymeric carrier. In the last years, supercritical carbon dioxide ($scCO_2$) based techniques were frequently used to produce active principle/biopolymer composites with fast or controlled drug release. In this work, the Eudragit L100-55 micronization was studied and, as desired, the attainment of spherical microparticles of polymer with mean size in the range 1.64 - 1.99 μm was achieved. Then, SAS coprecipitation Eudragit/AMPI was investigated to verify the potential of Eudragit as the carrier for drug controlled delivery. Working at the best operating conditions, in terms of pressure (100 bar) and of overall concentration in the liquid solution (50 mg/mL$_{DMSO}$), microspheres Eudragit/AMPI 20/1 and 10/1 w/w were obtained, with mean diameters of 2.52 μm and 1.53 μm, respectively. Release studies showed that the dissolution rate of ampicillin was prolonged 4 and 3 times, respectively in the case of SAS coprecipitated powders at 20/1 and 10/1 ratios. This outcome allowed to reduce the frequency of administration up to once a day, with fewer side effects due to antibiotic overdosing.

1. Introduction

Antibiotic therapies are commonly prescribed to prevent or treat bacterial infections; ampicillin was the first semi-synthetic active principle able to inhibit both Gram-positive and some Gram-negative bacteria. Short (Shah et al., 2018) or long-term (Roy and Grove, 2000) antibiotic therapy could be required based on the type of infections, including endocarditis, skin, respiratory and urinary tract diseases, but also during/after surgical procedures to avoid nosocomial infections. However, ampicillin has a very short half-life, as well as most antibiotics, and, as a consequence, high and repeated dosages are needed. Furthermore, antibiotic overuse is nowadays very common, often due to wrong ways/times of administration. All these reasons lead to complications and side effects, especially the antibiotic-resistance and the alteration of the beneficial human intestinal microbiota. Controlled-release systems allow to overcome the antibiotics short-half life and to reduce the frequency of administrations, resulting in fewer adverse effects caused by high dosages (Gürsel et al., 2000). In this context, the choice of a polymeric carrier suitable for achieving a controlled or prolonged release is very important. Among various carriers, Eudragit polymers have gained considerable interest in the pharmaceutical field due to their wide versatility, due to the different solubilities at different pH values (Singh et al., 2015). Moreover, these polymers offer moisture protection and odor/taste masking, in addition to their capability to modify drug release. For example, Eudragit L100-55 protects the drug against the aggressive gastric fluid, promoting a controlled drug release at pH higher than 5.5, whose value is associated with the first intestinal tract (i.e., duodenum) (Moustafine et al., 2006). The development of composite polymer/drug particles can be obtained by many conventional techniques, such as spray-drying, freeze-drying, centrifugal extrusion, jet -milling, but irregular particles with a wide particle size distribution (PSD) are often produced (Khadka et al., 2014). Moreover, the use of these processes can involve organic solvents residues, low encapsulation efficiencies and/or the thermal degradation of the compounds. On the contrary, supercritical carbon dioxide ($scCO_2$) based techniques allow obtaining regular particles with narrow PSDs and very low solvent residues, operating at bland process conditions because of low $scCO_2$ critical parameters (T_c = 31.1 °C, P_c = 73.8 bar) (Reverchon et al., 2008). CO_2 has been frequently used in the supercritical state in

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extraction (Tirado et al., 2019), fractionation (Fernández-Ponce et al., 2019), and micronization (Ghaderi et al., 1999). Among the micronization processes, the supercritical antisolvent (SAS) precipitation has been widely used for the attainment of nanoparticles (Kim et al., 2008), microparticles (De Marco et al., 2013), crystals (Clercq et al., 2018), and nanostructured filaments (De Marco and Reverchon, 2011) of different materials. According to the literature already published on SAS, to achieve a massive coprecipitation polymer/drug by the SAS process, microdroplets have to be formed and, after the subsequent solvent extraction, composite microspheres are produced. However, the coprecipitation implies the formation of a quaternary system solvent+scCO_2+polymer+drug with complex phase behaviors that can lead to the failure of coprecipitation (Franco et al., 2018). Up to now, few polymers allow reaching a successful coprecipitation in the form of microspheres by SAS process, namely polyvinylpyrrolidone (PVP) (Prosapio et al., 2017), L-PLA (Song et al., 2002), and, only recently, zein (Franco et al., 2019). Montes et al. tried to coprecipitate Eudragit L100 with ibuprofen (Montes et al., 2014a) and naproxen (Montes et al., 2014b), producing particles with mean diameters in the range 0.08 - 0.51 μm and 0.08 – 0.31 μm respectively for Eudragit L100/ibuprofen and Eudragit L100/naproxen systems. Low loading efficiencies between 0.94-7.88 % for ibuprofen and 4.45-25.55 % for naproxen were reached because the nanometric and sub-micrometric particle size obtained did not permit a massive coprecipitation polymer/drug. Similarly, in another paper (Montes et al., 2016), the same authors studied the coprecipitation of ellagic acid using Eudragit L100 as carrier varying the polymer/drug ratio only. FESEM images revealed the presence of crystals at Eudragit L100/ellagic acid ratios equal to 1/1 and 2/1, whereas slightly irregular and coalescent sub-microparticles precipitated using 4/1 polymer/drug ratio. From the previous discussion, it is evident that, up to now, SAS coprecipitation studies using Eudragit as the carrier is not yet fully satisfactory. Therefore, the purpose of this paper is to investigate the possible application of Eudragit L100-55 as a new polymeric carrier for SAS coprecipitation to develop drug controlled delivery systems. First of all, the micronization of Eudragit L100-55 only was optimized by studying the effect of some process conditions, to obtain spherical microparticles. Then, some experiments were focused on SAS coprecipitation using ampicillin as a model drug, to evaluate the effectiveness of Eudragit L100-55 as the polymeric carrier.

2. Materials and methods

2.1 Materials

Eudragit L100-55 (Eudragit) was supplied by Degussa (Darmstadt, Germany). Ampicillin sodium salt (AMPI, average molecular weight 371.39 g/mol) and Dimethylsulfoxide (DMSO, purity 99.5 %) were purchased from Sigma-Aldrich (Italy). CO_2 (purity 99 %) was brought from Morlando Group s.r.l. (Italy). All materials were used as received. The solubilities at room temperature of Eudragit L100-55 and AMPI in DMSO are about 55 mg/mL and 25 mg/mL, respectively.

2.2 Apparatus and procedure

The SAS bench-scale plant used for the micronization and coprecipitation experiments consists of two high-pressure pumps to feed carbon dioxide and the liquid solution, respectively. The precipitator is a cylindrical vessel with an internal volume of 500 cm^3. The temperature control is assured by a proportional integral derivative (PID) controller connected with electrically thin bands, and the pressure in the vessel is measured using a test gauge manometer and regulated by a micrometric valve. The liquid solution is injected in the precipitator through a thin wall, 100 μm internal diameter stainless steel nozzle. Supercritical CO_2, after preheating, is co-currently delivered through another port to the chamber. 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 and allows the CO_2–solvent solution to pass through. The liquid solvent is then recovered in a second collection vessel located downstream of the precipitator at a lower pressure (18–20 bar), regulated by a backpressure valve. At the exit of the second vessel, the CO_2 flow rate and the total quantity of antisolvent delivered are respectively measured by a rotameter and a dry test meter.

A SAS experiment starts pressurizing the precipitation vessel with CO_2 until the desired pressure is reached; then, the pure solvent is sent through the nozzle to obtain a steady-state composition of solvent and antisolvent inside the chamber. Afterward, the solvent flow is stopped, and the liquid-solution is delivered to the precipitator, producing the precipitation of the solute. At the end of solution injection, scCO_2 continues to flow, to eliminate residual content of liquid solubilized in the supercritical antisolvent. When the washing step is completed, CO_2 flow is stopped, the precipitator is depressurized down to atmospheric pressure and the precipitated powder can be collected and characterized.

2.3 Analytical Methods

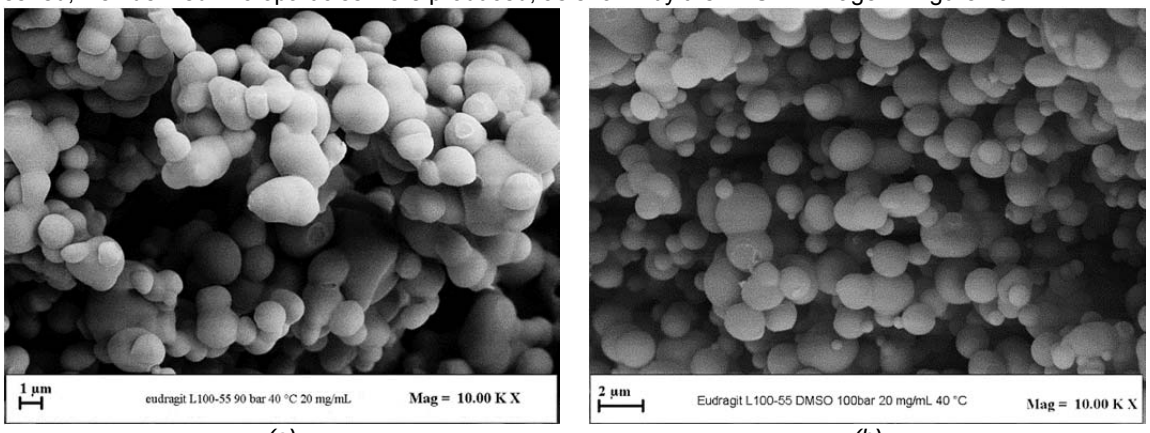
The morphology of the samples was evaluated by a Field Emission Scanning Electron Microscope (FESEM, mod. LEO 1525, Carl Zeiss SMT AG, Oberkochen, Germany). The powder was dispersed on a carbon tab previously stuck to an aluminum stub (Agar Scientific, United Kingdom) and coated with gold-palladium (layer thickness 250 Å) using a sputter coater (mod. 108 A, Agar Scientific, Stansted, United Kingdom). Mean dimensions and standard deviation of particles were measured from FE-SEM photomicrographs by using the Sigma Scan Pro image analysis software (release 5.0, Aspire Software International Ashburn, VA), considering about 1000 particles for each sample. Particle size distributions (PSDs) were determined by Microcal Origin Software (release 8.0, Microcal Software, Inc., Northampton, MA).
AMPI loadings and drug dissolution studies were performed using a UV/vis spectrophotometer (model Cary 50, Varian, Palo Alto, CA) at a wavelength of 220 nm. An equivalent amount of drug of 5 mg was considered to compare the dissolution rate of unprocessed drug and that of coprecipitates rightly. The powders were suspended in 3 mL of phosphate-buffered saline solution (PBS) at pH 7.4 and placed into a dialysis sack; then, the system was incubated in 300 mL of PBS, continuously stirred at 200 rpm and heated at 37 °C. Each analysis was performed in triplicate, so the mean release profiles were reported in this paper. AMPI loadings were obtained by measuring the absorbance in the release medium at the end of the drug release, namely when all the antibiotic was released from the particles to the outer PBS phase. Then, the absorbance was converted into AMPI concentration using a calibration curve; thus, it was possible to calculate the antibiotic entrapment efficiency. The calibration curve was determined through very diluted standards at different concentrations of AMPI in PBS at pH 7.4.

3. Results and discussion

All SAS tests were carried out in duplicates using an operating temperature of 40 °C and DMSO as the solvent. A CO_2 flow rate equal to 30 g/min and a solution flow rate of 1 mL/min were fixed, because, at the chosen temperature, these values permit to work at molar fractions approximately equal to 0.98; i.e., on the right of the Mixture Critical Point (MCP) of the binary system solvent/antisolvent that ensures the supercritical mixture conditions.

3.1 Micronization of Eudragit L100-55

The first part of the experimentation was dedicated to the study of Eudragit L100-55 micronization to investigate its possible applicability as the polymeric carrier for SAS coprecipitation. Aiming at obtaining particles of micrometric dimensions, the effect of the solvent used (EtOH or DMSO), of the operating pressure (P), and the overall concentration in the liquid solution (C_{tot}) was studied on the morphology and dimensions of precipitated particles.
First of all, two tests were conducted using ethanol as solvent. Fixing a Eudragit concentration in the liquid solution equal to 20 mg/mL, the operating pressure was varied from 90 to 120 bar: very coalescing sub-microparticles were obtained from both the experiments. Due to the formation of non-separated and non-micrometric particles by using ethanol, we decided to use DMSO as the organic solvent. In particular, fixing the same concentration of polymer (20 mg/mL), a SAS experiment was performed working at 90 bar. As desired, well-defined microparticles were produced, as shown by the FESEM image in Figure 1a.



(a) *(b)*

Figure 1: FESEM images of Eudragit particles precipitated from DMSO at 40 °C and 20 mg/mL. Effect of the operating pressure: (a) 90 bar; (b) 100 bar.

Since DMSO allowed to improve the micronization of Eudragit, all the following experiments were carried out using it as the solvent. The effect of the pressure variation was studied fixing the concentration of Eudragit in DMSO at 20 mg/mL and gradually increasing the operating pressure from 90 to 120 bar. Coalescing microparticles were precipitated at 120 bar, whereas well-separated spherical microparticles were obtained working at 90 and 100 bar (Figure 1a and 1b, respectively). In particular, the microparticles obtained at 100 bar seemed to be well-defined.

3.2 Eudragit/AMPI coprecipitation

After optimizing the micronization of Eudragit alone, SAS coprecipitation was attempted using ampicillin sodium as the model drug. Based on the previous results, 90 and 100 bar were selected as operating pressures to perform the subsequent coprecipitation tests. A preliminary test was carried out by micronizing ampicillin alone at 40 °C, 90 bar, and a drug concentration in DMSO equal to 20 mg/mL: sub-microparticles characterized by a mean size of around 0.23 µm were obtained. The effect of pressure, total concentration in DMSO, and polymer/drug ratio w/w were investigated on the morphology and the mean size of coprecipitated particles. Fixing the overall concentration in DMSO at 40 mg/mL and the polymer/drug ratio at 20/1 w/w, the effect of pressure on the Eudragit/AMPI system was investigated in the range from 90 to 100 bar. A higher degree of coalescence of the particles was observed operating at 90 bar instead of 100 bar. In particular, well-separated Eudragit/AMPI microparticles with a mean diameter of about 2 µm were produced working at 100 bar. Since the pressure of 100 bar led to obtaining more defined microspheres, this pressure was set to study the influence of the overall concentration in DMSO on Eudragit/AMPI composites at a polymer/drug ratio of 20/1. Very coalescing particles precipitated at 20 mg/mL, whereas well-separated microparticles were produced by increasing the total concentration at 40 and 50 mg/mL. Moreover, a slight increase in mean particle size occurred increasing the total concentration in DMSO.
Once found the optimal pressure and overall concentration to produce microspheres, i.e., 100 bar and 50 mg/mL, the effect of the polymer/drug ratio w/w was investigated. Decreasing the Eudragit/AMPI ratio at 10/1 w/w, well-separated microparticles were produced, and a reduction of particle mean size was noted with respect to the 20/1 ratio, as highlighted through the comparison of volumetric cumulative PSDs in Figure 2.

3.3 Characterization of samples

The comparison of the dissolution rate of pure AMPI and SAS coprecipitates Eudragit/AMPI 20/1, and 10/1 w/w was performed by UV/vis spectroscopy. Drug dissolution curves were reported in Figure 3, plotting the percentage of AMPI dissolved in PBS at pH 7.4 as a function of time. Unprocessed AMPI completely dissolved in about 6.5 h, whereas SAS processed Eudragit/AMPI 20/1 and 10/1 took about 26 and 22 h, respectively. Therefore, their dissolution rates are 4 and 3 times slower than the one of the pure drug.
Regarding the AMPI entrapment efficiency, its value was in the range of 99.4-99.7 % for all the composite samples.

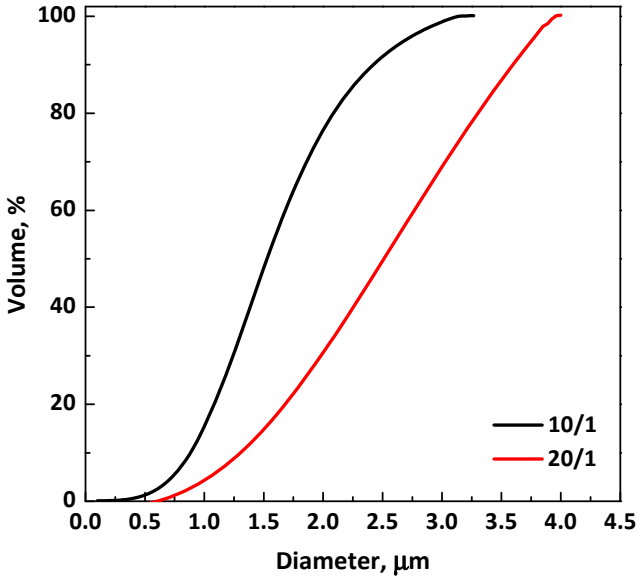


Figure 2: Volumetric cumulative PSDs of Eudragit/AMPI particles precipitated from DMSO at 40 °C, 100 bar, and 50 mg/mL; effect of the polymer/drug ratio.

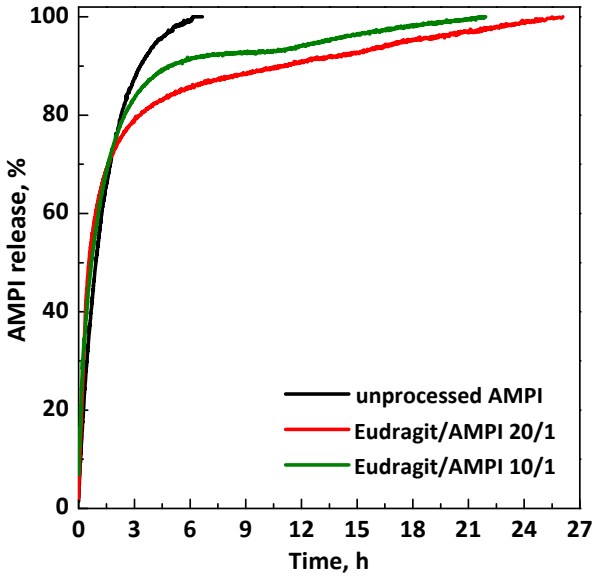


Figure 3: Dissolution profiles in PBS at 37 °C and pH 7.4.

4. Conclusions

In this paper, the application of Eudragit L100-55 as a new polymeric carrier for SAS coprecipitation was investigated using ampicillin sodium as the model drug.
First of all, Eudragit L100-55 micronization was optimized studying the effect of the pressure and the polymer concentration in DMSO and selecting respectively 100 bar and a concentration higher than 20 mg/mL as the best conditions. Well-separated microparticles of Eudragit L100-55 with mean size ranging from 1.7 to 2 μm were obtained. Then, SAS coprecipitation of Eudragit L100-55/AMPI was studied by varying the pressure and the total concentration of solutes in DMSO and finding 100 bar and concentrations higher than 20 mg/mL as optimal conditions again. Polymer/drug ratio was also varied from 20/1 to 10/1 w/w, and coprecipitated microparticles with a mean size of 2.52 and 1.53 μm were respectively produced. Dissolution tests confirmed the effectiveness of coprecipitation using Eudragit L100-55 as a polymeric carrier. Indeed, SAS composite particles allowed to prolong the release of ampicillin sodium up to 4 times with respect to the pure drug. These results are of great interest since controlled-release formulations can be developed by SAS coprecipitation Eudragit L100-55/AMPI, reducing the frequency of administration up to once a day with reduced side effects.

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