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Preparation of 5-fluorouracil microparticles and 5-fluorouracil/poly(L-lactide) composites by a supercritical CO_2 antisolvent process

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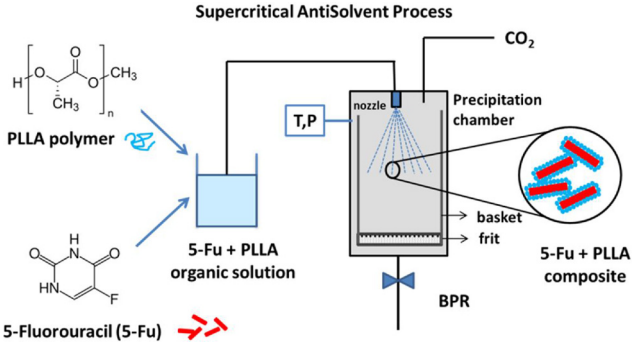
GRAPHICAL ABSTRACT



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ABSTRACT

Microparticles of the anticancer drug 5-fluorouracil (5-Fu) were successfully prepared by supercritical antisolvent (SAS) precipitation using dimethylsulfoxide as solvent. The effect on particle size (PS) of the experiment parameters was studied. The conditions of temperature and pressure were the most important factors. Mean PS values ranged from 220 to 670 nm. Using as solvent a dimethylsulfoxide + dichloromethane mixture, the SAS process was also used to obtain composite particles in which 5-Fu crystals are coated by a layer of poly(L-lactide) spheres. The particle morphology was studied by SEM. Other characterization included powder X-ray diffraction, DSC, TGA, and the measurement of the dissolution profiles. The composite particles were shown to provide a controlled delivery system.

1. Introduction

Particle technology is a key factor in the production of pharmaceuticals. Thus, the micronization of pharmaceuticals facilitates the use of a more appropriate or more convenient administration route, the reduction of the dosage and/or the increase of the drug bioavailability. On the other hand, the pharmaceutical properties may be also improved if microspheres or microcapsules containing the active pharmaceutical ingredient (API) and a carrier are prepared. The carrier may ease the dissolution of poorly water-soluble drugs or may enable a controlled delivery of the API in the targeted media. Conventional micronization techniques such as spray drying, freeze drying or milling very often require operation at temperatures that can denature heat sensitive compounds, lead to broad size distributions or require additional processing to remove the solvents. In the last years, the application of supercritical fluids has resulted in several technologies that overcome

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the limitations of the micronization techniques based on liquid solvents [1–3]. Recently, Esfandiari [4] has reviewed those used to obtain pure API's while Pando et al. [5] have revised those employed to prepare pharmaceutical co-crystals. Carbon dioxide is the most commonly used supercritical fluid because it is nontoxic, non-flammable and has moderate critical temperature and pressure (31 °C and 7.4 MPa). Supercritical fluids densities and solvation power are intermediate between those of gases and liquids and can be easily modified with small changes in temperature and pressure; meanwhile the fluid maintains good transport properties. Thanks to these unique properties, supercritical carbon dioxide ($scCO_2$) may act as a solvent, as an antisolvent or as a molecular mobility enhancer allowing the development of different processes and applications. A pressure reduction after $scCO_2$ treatment allows the preparation of solvent-free pharmaceuticals. Other advantages are operation at moderate temperature in a fairly inert atmosphere thus avoiding the product degradation and the reduction of steps in the production process thus enabling a better control. Micro and nanoparticles with narrow size distributions have been obtained for pure materials and composites [2–5]. Many examples can be found about substantially improving the pharmacological properties of existing compounds using these techniques [6–11]. Nevertheless, the great attention paid to the use of $scCO_2$ is based on its sustainable and environmentally friendly characteristics. Besides being nontoxic, nonflammable and fairly inert, $scCO_2$ is also cheap and readily available either as a byproduct in ammonia synthesis or from atmosphere. This is very appealing for pharmaceutical companies that are urged to develop production processes with low environmental impact, fewer steps and a reduced use of organic solvents.

The aim of this paper is the preparation of two types of pharmaceutical microparticles, those formed by the pure drug 5-fluorouracil (5-fluoro-1*H*-pyrimidine-2,4-dione, 5-Fu, a pyrimidine analog drug widely used in the treatment of several types of cancer) [12], and the composite particles formed by 5-Fu and poly(L-lactide) (PLLA). In both cases a supercritical antisolvent (SAS) process is used [11]. The molecular structures of 5-Fu and PLLA are shown in Fig. 1 together with a scheme of the SAS method. This approach is based on the relatively low solvent power of CO_2 for solutes such as pharmaceuticals and its good miscibility with many organic solvents. Either the drug alone or both the drug and the carrier material are dissolved in the organic solvent. When the solution is mixed with $scCO_2$ in a precipitation chamber, CO_2 acts as an antisolvent and the solute precipitates as solvent–free micro and nanoparticles. The CO_2 + organic solvent mixtures thus formed are led to a separation chamber where the solvent is recovered. Prior to particle collection, the precipitation chamber is washed with pure CO_2 to assure that the residual organic solvent is removed from the precipitate. The particle size and/or morphology are controlled by varying the process parameters such as temperature, pressure, solute concentration, etc.

5-Fu acts as an anticancer drug in several ways, mainly as a thymidylate synthase inhibitor. By interrupting the action of this enzyme, 5-Fu blocks the synthesis of the pyrimidine thymidine, which is a nucleotide required by the cancerous cells for DNA replication [12]. The drug is usually given orally or intravenously (due to its poor water solubility), but can also be applied as an ointment, especially in the case of skin cancer [13] or in dose inhalers or nebulizers and dry powder formulations in the case of lung cancer [14]. The direct delivery of 5-Fu in an aerosol therapy or an ointment is benefited by the drug micronization. On the other hand, intravenous administration of 5-Fu is improved if the drug is embedded or encapsulated in a biodegradable polymeric matrix that provides a controlled delivery system. This allows a better control of dosage and the reduction of side effects [15,16]. To this end, PLLA is an appropriate choice because of its biocompatibility and biodegradability.

5-Fu is a good candidate for SAS micronization because it is soluble in a variety of solvents highly soluble in $scCO_2$ such as methanol, acetone, dichloromethane (DCM) and dimethylsulfoxide (DMSO) while it is scarcely soluble in the supercritical fluid. Several authors [17–19] have measured the solubilities of 5-Fu in $scCO_2$ at pressures ranging from 10 to 30 MPa and temperatures ranging from 35 to 55 °C and found them to vary from 10^{-6} to 10^{-5} in mole fraction. Kalantarian et al. [20] prepared by SAS 5-Fu microparticles using as solvents methanol or binary mixtures formed by methanol and DCM, acetone or ethanol and studied the influence of the solvent in the SAS micronization. As to the polymer, PLLA microparticles can be also successfully obtained by this method [21,22]. Although SAS processed PLLA microparticles have been later impregnated with 5-Fu [22], the SAS coprecipitation of 5-Fu and PLLA has not been attempted so far. In this paper the SAS method is used in the first place to obtain 5-Fu microparticles using dimethylsulfoxide and examining the influence of the SAS process parameters such as temperature and pressure. Next, 5-Fu-

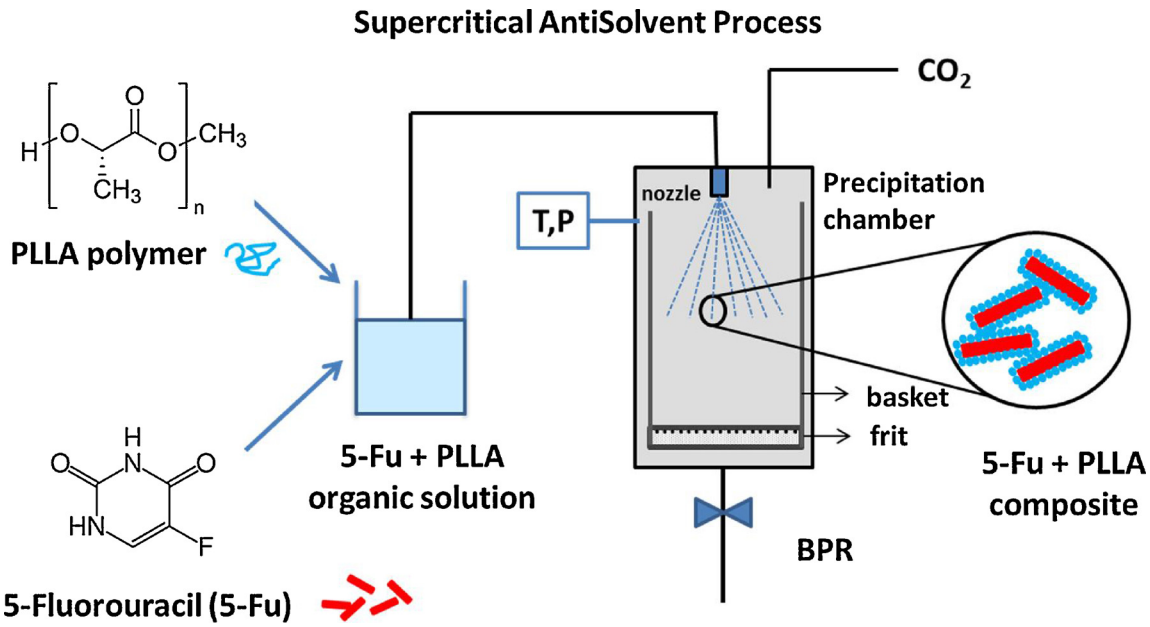

Fig. 1. Molecular structures of 5-Fu and PLLA and schematic representation of the supercritical fluid antisolvent (SAS) micronization technique used to obtain 5-Fu-PLLA composites.

PLLA composites are prepared. In this case, due to the low solubility of PLLA in DMSO a mixture of the organic solvents DCM and DMSO is required.

2. Materials and methods

2.1. Materials

The materials employed were CO_2 (Air Liquide 99.98 mol% pure), 5-Fu (Sigma-Aldrich ≥ 99 mol % pure), poly(L-lactide) (Sigma-Aldrich, T_g 60–65 °C), dimethylsulfoxide, DMSO, (Fluka ≥ 99.9 mol % pure), and dichloromethane, DCM, (Scharlau, ≥ 99.8 mol % pure). All water used was pretreated using the Milli-Q Elix water purification system (Millipore Ibérica, Madrid, Spain).

2.2. Supercritical fluid antisolvent (SAS) micronization and design of experiments (DOE)

The schematic diagram for the laboratory scale SAS apparatus, its validation, and details about this apparatus and the experimental procedure may be found elsewhere [10]. Supercritical carbon dioxide is introduced in the precipitation chamber using a high-pressure pump at constant flow rate. Then the solvent or directly the organic solution containing the drug is sprayed in the precipitation chamber through a small stainless steel nozzle also at a constant flow rate reaching steady state operating conditions and a given supercritical fluid/solvent ratio. The chamber is heated and both temperature and pressure are controlled. When the fluid dissolves in the solution, the mixture becomes supersaturated and precipitation starts. The solute is collected at the bottom and the walls of the precipitation chamber. At the end of the precipitation process, the chamber is washed with the antisolvent to eliminate the liquid solvent. The CO_2 + organic solvent mixture is introduced into a chamber provided with a cyclone separator where solvent is recovered. The factors to be considered in the SAS process are: the concentration of the solution (C), the temperature (T), the pressure (P), the solution flow rate (Q_L), the supercritical CO_2 flow rate (Q_{CO2}), the drying time (t), and the nozzle diameter ($Ø_n$). In this study, the drying time and the nozzle diameter were kept constant at 3 times the time required to fill the precipitation chamber and 100 µm, respectively. SAS experiments described in this paper were carried out at flow rates of 1.0–1.4 mL/min and 15–20 g/min for solution and CO_2, respectively. These values were chosen to obtain CO_2 mole fractions ≥ 0.96 by combining values for the flow rates of the two pumps. In most cases the concentration of solutes in the organic solutions was 1 or 2 g per 100 mL. The temperature and pressure conditions are usually chosen to make sure that precipitation takes place in the supercritical region or near above the mixture critical point where nanoparticles and microparticles are produced, respectively [23]. In the case of the pure 5-Fu SAS micronization this would require the knowledge of the ternary phase equilibria for the CO_2 + DMSO + 5-Fu system. Although DMSO is a solvent frequently used in SAS, ternary data are available only for a few systems. Usually, it is assumed that the solute presence does not modify the phase behaviour and temperature and pressure conditions are chosen taken into account data for the binary CO_2 + organic solvent system [24]. Choosing cefonicid as a model solute, Campardelli et al. [25] have shown that this assumption is valid at low temperatures (40 °C) and low concentrations (up to 9 g per 100 mL) for the ternary system CO_2 + DMSO + cefonicid. However, large modifications are observed at 60 °C. The phase behaviour becomes more complex in the case of the composite materials because of the PLLA interaction with CO_2 and the use of a mixture of solvents. In this case, temperature and pressure conditions are chosen taking into account the critical loci of the two binary systems CO_2 + DMSO and CO_2 + DCM.

In order to minimize the number of experiments in the micronization of 5-Fu, the Design of Experiments (DOE) approach was used. A detailed description of the factorial fractional design of experiments can be found in Refs. [26,27]. The factors that could have effects on particle size were C, T, P, Q_L, and Q_{CO2}. Two levels were used for each factor based on process limits and previous work. From among all the possible designs for five factors at two levels, the factorial fractional design 2^{5-2} was applied to separate the important effects from the unimportant ones. As a result, eight different combinations of signs for the five factors were created. Particle size of the micronized material was chosen as a response to evaluate the process performance.

The main effect (contrast, l_i) of each factor on particle size was calculated as follows:

$$l_i = \frac{1}{N_+}\sum y_+ - \frac{1}{N_-}\sum y_-$$

where N_+ and N_- are the number of runs at high level and low level, respectively, and y_+ and y_- are the response value at high level and low level, respectively.

2.3. Materials characterization

A JEOL-6400 scanning electron microscope (SEM) working at 10 kV was used to characterize the 5-Fu microparticles and the 5-Fu-PLLA composites obtained in the SAS experiments. Prior to analysis, samples were gold coated. The primary particle size distributions were obtained by counting the particles of different sizes in an electron microscope image using the SEMAFORCE program [28]. Values for the standard deviation (SD) were obtained from these distributions. Particle size (PS) was estimated as the equivalent spherical diameter. The mean particle size was obtained considering all the particles that have a definite surface present in the high-resolution SEM image (an average of 100 particles or even more). Energy-dispersive Detection X-ray analysis (EDX) was conducted on the composite samples using the SEM microscope.

Powder X-ray diffraction (XRD) was used to establish the degree of crystallinity and the polymorphic form of the materials. XRD patterns of the solids prior and after micronization were obtained using a Philips X'pert, model MPD powder diffraction system. The X-ray source was nickel-filtered Kα emission of copper (1.541837 Å). Samples were scanned over the range of 10–50 2θ degrees using the Bragg-Brentano geometry.

Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) studies were carried out using a simultaneous DSC and TGA device, a Delta Series TA-SDT Model Q-600 apparatus, in nitrogen atmosphere using open aluminum crucibles.

2.4. Dissolution profiles

The dissolution profile of the 5-Fu samples was followed using a UV/VIS Perkin-Elmer spectrophotometer model Lambda 35 at 265 nm. Approximately, 20 mg of the sample was suspended in 5 mL of a pH 7.4 phosphate buffer medium (0.01 M phosphate buffer) supplied by Sigma-Aldrich and placed into a pretreated dialysis bag (molecular weight cut-off is 12,000). Then the bag was introduced into a flask containing 250 mL of the buffer solution and incubated at 37 °C and 50 rpm speed. Aliquots of 1 mL were taken at time intervals and were analyzed for the drug content by UV/VIS. The volume of the solution was kept constant by adding 1 mL of the phosphate buffer solution each time that samples were withdrawn.

3. Results and discussion

3.1. Micronization and characterization of 5-Fu

Dimethylsulfoxide was chosen as a solvent for the micronization of 5-Fu. Table 1 shows the low and high level values for the five factors used in the DOE: solution concentration, temperature, pressure, solution flow rate and CO_2 flow rate. As explained in the Experimental

Table 1
Level identification of the DOE factors.

Factor	Unit	Symbol	Low level (-)	High level (+)
Concentration	g/100 mL	C	1.0	2.0
Temperature	°C	T	40	50
Pressure	MPa	P	15.0	18.0
Solution flow rate	mL/min	Q_L	1.0	1.4
CO_2 flow rate ($x \geq 0.96$)	g/min	Q_{CO2}	15	20

Table 2
The fractional factorial 2^{5-2} design of experiments with response and contrast.[a].

Experiment	C (1)	T (2)	P(3)	Q_L	Q_{CO2}	Response[b]	
						Mean PS (nm)	SD (nm)
1	–	–	–	+	+	510	170
2	+	–	–	–	–	670	220
3	–	+	–	–	+	–	–
4	+	+	–	+	–	–	–
5	–	–	+	+	–	580	180
6	+	–	+	–	+	580	170
7	–	+	+	–	–	220	65
8	+	+	+	+	+	440	130
Main effect, contrast, l_i							
PS, nm	94.5	−420.5	157	15	13.5		

[a] The values for the low level and high level of solution concentration (C), temperature (T), pressure (P), solution flow rate (Q_L) and supercritical CO_2 flow rate (Q_{CO2}) are given in Table 1.
[b] PS: particle size; SD: standard deviation.

Section, the two levels of temperature and pressure were chosen mainly on the basis of isothermal vapor–liquid equilibrium and critical data for CO_2 + DMSO [24]. Flow rate limits were chosen to obtain CO_2 mole fractions ≥ 0.96, in the carbon dioxide- rich region. The low level of concentration was set to obtain a sufficient amount of precipitate for subsequent analysis; the high level was limited by the saturation of the solutions at room temperature.

The mean particle size and particle morphology obtained from SEM images were used as a response to evaluate the process performance. Table 2 shows the eight combinations of signs resulted from the application of the fractional factorial design 2^{5-2} (experiments 1–8) together with the mean particle size and standard deviation (SD). The main effect (contrast, l_i) is also shown in Table 2. The main effect may adopt positive or negative values. A positive value means it has a positive effect on the process performance while a negative value means it has a negative effect. The alias structure that determines which effects are confounded with each other was generated using the MINITAB 15 SOFTWARE [29] and was simplified by neglecting three order and higher interactions. For screening purposes, the interactions between two factors were also considered negligible [27], so the effect value l_i is caused only by the main factor, by other words, concentration for column 1, temperature for column 2, and so on.

Fig. 2 shows the SEM images of 5-Fu commercial powder. Particles of untreated 5-Fu are greater than 10 μm and exhibit a wide distribution of sizes. As to the SAS micronized 5-Fu, the precipitation was successful in all the experiments and fluffy powders were obtained. Fig. 3 shows SEM images of SAS micronized 5-Fu. Good morphologies (small and nonaggregated particles of similar shape and size) are obtained in experiments 1, 2, 6 and 8. However, at the higher pressure and lower 5-Fu concentration both at 40 and 50 °C (experiments 5 and 7) the elongated particles were somewhat aggregated. On the other hand, much larger particles were obtained in experiments 3 and 4; these particles were not further characterized and for the sake of brevity their SEM images are not shown in Fig. 3. Table 3 lists values for the mean PS and SD. Elongated particles with a mean particle size in the range of

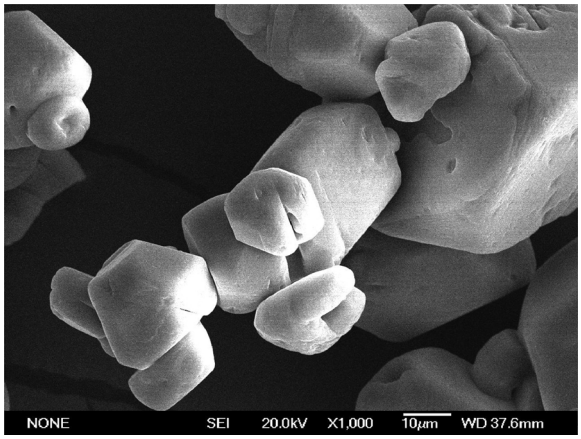

Fig. 2. SEM image of untreated 5-Fu.

220–670 nm and standard deviation within the range of 65–220 nm, respectively, were obtained in experiments 1, 2, 5, 6, 7 and 8.

Fig. 4 shows the effect of the different factors on the particle size. Three key factors with major effect on the particle size and morphology were identified. In order of decreasing importance, these are temperature, pressure and concentration. Taking into account the main effect of the five factors considered their order of importance can be summarized as follows

$$T > P > C > \ > Q_L > Q_{CO2}$$

If the influence of Q_L and Q_{CO2} is neglected, the effect on particles size of the 5-Fu concentration in the liquid solution may be established by directly comparing the experiments in the pairs 1–2, 5–6, and 7-8. Particle size increases when the 5-Fu concentration is increased at a given temperature and pressure. In a similar way the temperature and pressure effects may be discussed by comparing the experiments in the pairs 1–3, 2–4, 5–7 and 6–8 (temperature) and the pairs 1–5, 2–6, 3–7 and 4–8 (pressure). However, these effects cannot be established through the direct comparison of pairs of experiments pointing out a binary P–T interaction. Therefore, we chose to compare simultaneously the eight experiments in terms of particle morphology and the response factor or mean PS: The smallest mean particle size (220 nm) was obtained in experiment 7. However, particles in this case appear aggregated in clusters almost 1 μm long. Therefore, best conditions for the 5-Fu micronization could be those of experiment 8: solution concentration, C = 1%, temperature, T = 50 °C, pressure P = 18 MPa, solution flow rate, Q_L = 1.4 mL/min, supercritical CO_2 flow rate, Q_{CO2} = 20 g/min. In this experiment particles were 440 nm in average.

The crystallinity and polymorphic form of the microparticles obtained by SAS in this paper have been evaluated using X-ray diffraction powder. The XRD patterns of the untreated 5-Fu and 5-Fu obtained by SAS at 50 °C and 18.0 MPa using DMSO as solvent (experiment 8) and using a 1:1 v/v % DMSO + DCM mixture are shown in Fig. 5. This mixture of solvents was used to obtain the 5-Fu-PLLA composite as explained in Section 3.2. The XRD pattern of the untreated 5-Fu exhibits a very intense peak at 28.7 °. The high intensity of this peak suggests a preferred orientation of the untreated drug at this position. On the other hand, the preferred orientation is attenuated in the XRD pattern of the 5-Fu precipitated by SAS using the DMSO + DCM mixture and disappears when DMSO is used as solvent. This could be attributed to the decrease in crystal size due to SAS precipitation with respect to that of the untreated drug. On the other hand, 5-Fu is known to present two well characterized polymorphic forms [30]. The three XRD patterns shown in Fig. 5 correspond to form I; there is no change of the polymorphic form due to SAS treatment. This has been confirmed through differential scanning calorimetry performed using the TA-SDT apparatus model Q-600. The investigation was carried out over the

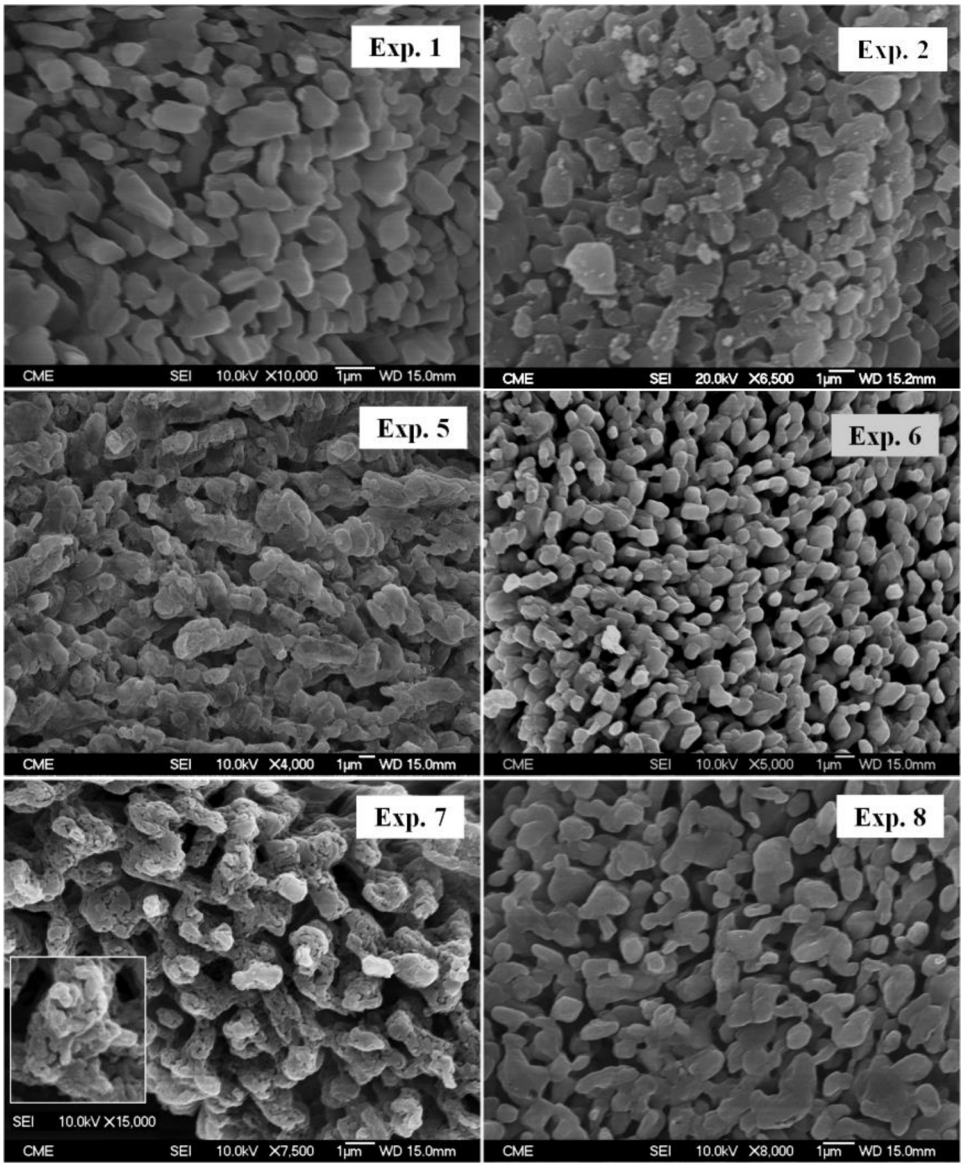

Fig. 3. SEM images of SAS processed 5-Fu. Conditions for SAS experiments are given in Tables 2 and 3.

Table 3
Temperature and pressure conditions for composite preparation using SAS and drug loading obtained.

Experiment	Temperature (°C)	Pressure (MPa)	Drug loading (% w)
9	35	12.0	35
10		18.0	32
11	50	12.0	42
12		18.0	36
13		25.0	18

temperature range 240–360 °C. The melting point was measured reading the onset temperature of the peak and a value of 283 °C corresponding to form I was observed.

Kalantarian et al. [20] micronized 5-Fu by SAS from methanol, methanol + dichloromethane, methanol + acetone, and methanol + ethanol mixtures. The other conditions of the experiments were fixed: a pressure of 10 MPa, a temperature of 40 °C, a solution flow rate of 1 mL/min, and CO_2 flow rate of 20 mL/min. Additionally a pressure of 15 MPa was used for methanol experiments. The particle morphology

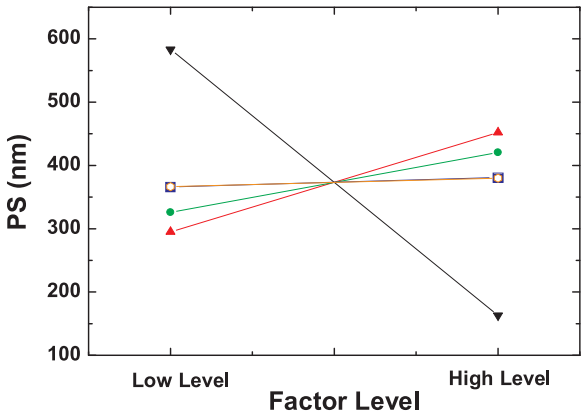

Fig. 4. Effect of temperature (▼, 40 and 50 °C), pressure (▲, 15.0 and 18.0 MPa), solution concentration (●, 1.0 and 2.0 g/100 mL), solution flow rate (■, 1.0 and 1.4 mL/min) and supercritical CO_2 flow rate (◯, 15 and 20 g/min) on particle size for 5-Fu SAS micronization.

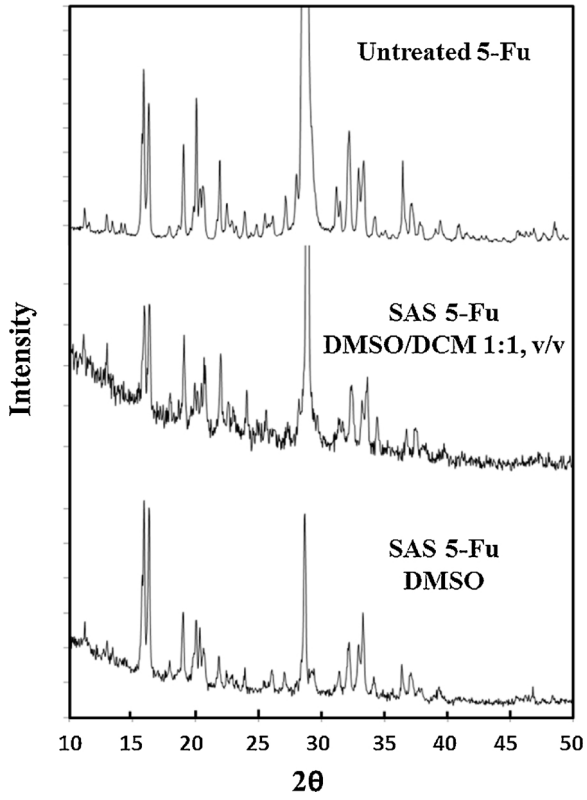

Fig. 5. XRD patterns of untreated 5-Fu and 5-Fu obtained by SAS at 50 °C and 18.0 MPa using DMSO as solvent (experiment 8) and using a 1:1 v/v % DMSO + DCM mixture.

was similar to that found in this study. Mean particle sizes changed significantly with the solvent used. The particles produced from methanol alone and methanol + ethanol mixtures had the biggest sizes (2000–5000 nm) while the particles produced from methanol + acetone had sizes of 730–980 nm and those produced from methanol + dichloromethane mixtures had the smallest sizes (250 to 440 nm). Our study shows that for a given solvent this range of particle sizes may be also attained if temperature, pressure and concentration are adequately chosen. A similar conclusion was reached for the gas antisolvent (GAS) process by Esfandiari and Ghoreishi [31]. On the other hand, 5-Fu microparticles with sizes lower than 500 nm were obtained by Chen et al. [32] through a solution enhanced dispersion by supercritical CO_2 (SEDS method) using a mixture of ethanol and DCM as solvent.

3.2. Micronization and characterization of 5-Fu-PLLA composites

The DOE approach was not used in the SAS micronization of the 5-Fu-PLLA composites because of the high number of parameters involved. On the other hand, due to the very low solubility of PLLA in DMSO, a mixture of a poor PLLA solvent (DMSO) and a good PLLA solvent (DCM), both miscible with supercritical CO_2, was chosen. Gokhale et al. [33] have proposed to use such a mixture in the SAS micronization of pure polymers. The segments of the polymer molecule prefer to be surrounded by the good-solvent molecules (DCM) and the PLLA chain adopts a swollen conformation to maximize contacts between the PLLA segments and DCM. However, the polymer molecule segments avoid contacts with the molecules of the poor solvent (DMSO) and the PLLA chain adopts a compact conformation to minimize contacts between the PLLA segments and acetone. The variation in the solvent ratio provides an effective method to control and manipulate the polymer solubility and the conformation of the polymer molecules in the particles precipitating from the solution injected in the precipitation chamber. Consequently, particle size and morphology may be controlled. At our laboratory we have shown that this is also an

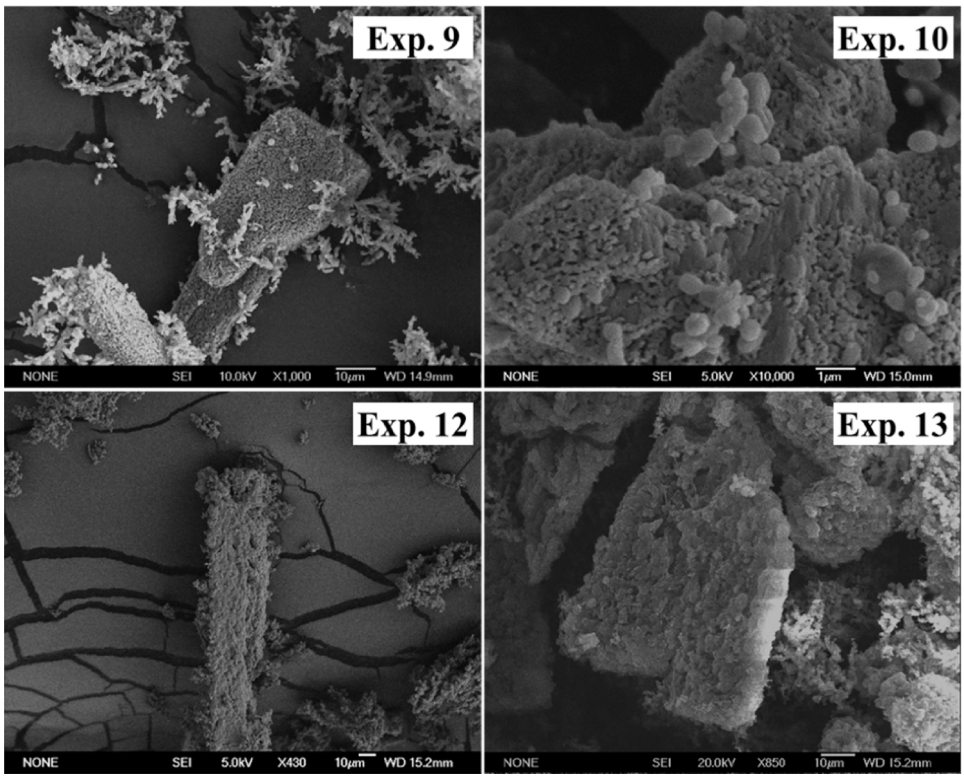

Fig. 6. SEM images of the 5-Fu-PLLA composites.

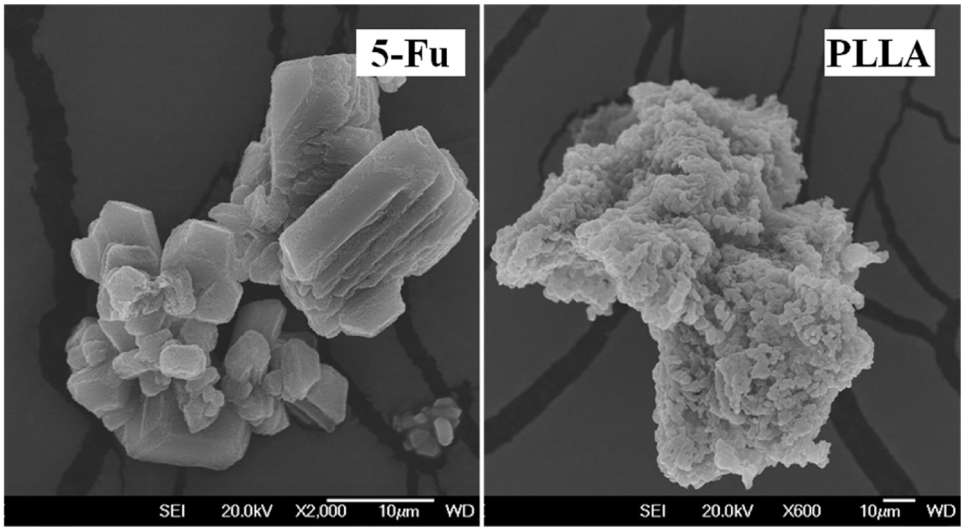

Fig. 7. SEM images of pure 5-Fu and PLLA processed by SAS at 50 °C and 18.0 MPa using as solvent a 1:1 v/v % DMSO + DCM mixture.

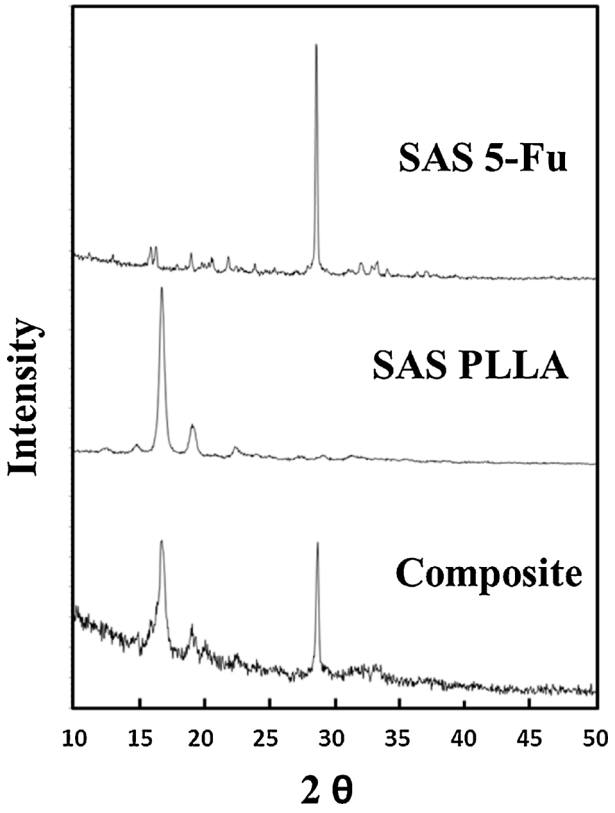

Fig. 8. XRD patterns of 5-Fu, PLLA, and the composite obtained by SAS at 50 °C and 18.0 MPa using as solvent a 1:1 v/v % DMSO + DCM mixture.

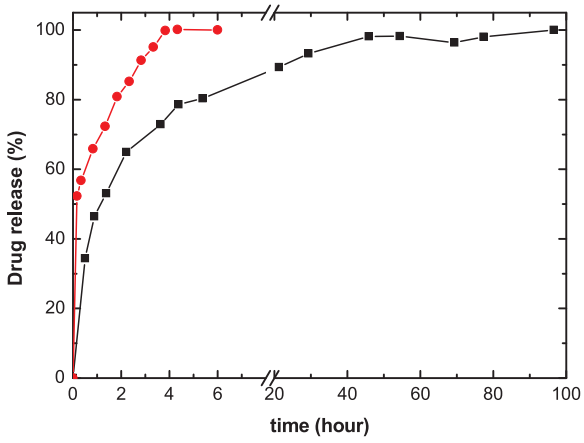

Fig. 9. Dissolution profiles of the pure drug (●) and the 5-Fu-PLLA composite obtained at 50 °C and 18.0 MPa (■).

effective method when the polymer polyvinylpyrrolidone is coprecipitated by SAS together the anti-inflammatory drug diflunisal [10]. After several trials using different DMSO to DCM ratios a 1:1 v/v % DMSO + DCM mixture was chosen to carry out SAS experiments in this study.

As explained in the Experimental Section, conditions of temperature (35 and 50 °C) and pressure (120, 180 and 150 MPa) were chosen taking into account the critical loci of the two binary systems CO_2 + DMSO and CO_2 + DCM [24,34–36]. The other experimental conditions were solution concentrations of 5 mg/mL for both 5-Fu and PLLA, 1 mL/min solution flow rate, and 20 g/min CO_2 flow rate. The liquid and CO_2 flow rates lead to a value of 0.97 for the CO_2 mole fraction in the mixtures formed in the precipitation chamber. Temperature and pressure conditions and drug loadings determined through thermogravimetric analysis are shown in Table 3. 5-Fu loadings were estimated from the mass loss between 100 and 285 °C. Decomposition of PLLA took place from 285 to 370 °C and did not overlap with 5-Fu decomposition. Drug loading for composites obtained at 35 or 50 °C and 12.0 and 18.0 MPa varied from 32 to 42% in mass. The composite obtained at 50 °C and a higher pressure (25.0 MPa) exhibited a much lower loading (18%) Thermogravimetric analysis of our samples was repeated after five months. The samples were kept at ambient conditions and no significant differences were observed in the TGA curves thus showing the composite material stability. SEM images of the composite materials are shown in Fig. 6. Large crystals of 5-Fu (lengths range from 10 to over 100 μm) are coated by a layer of smaller PLLA spheres whose diameters range from 600 nm at 35 °C to 1200 nm at 50 °C (values for the standard deviation are 200 and 300 nm, respectively). EDX analysis confirmed the different chemical composition of the two morphologies and the complete removal of DMSO and DCM. Large crystals of 5-Fu coated by polymer were also obtained by Kalantarian et al. [37] using the GAS and SAS processes, poly(lactide-co-glycolide) (PLGA) as polymer, acetone or methanol + DCM mixtures as solvents, and temperatures and pressures similar to those of this study.

Since the best pressure and temperature conditions for SAS precipitation of 5-Fu using DMSO were 50 °C and 18.0 MPa, for comparison purposes the composite obtained in experiment 12 was chosen for

further characterization and the pure PLLA and the pure 5-Fu were processed by SAS at the conditions of this experiment (50 °C and 18.0 MPa, a 1:1 v/v % DMSO + DCM mixture as solvent, 1 mL/min solution flow rate, and 20 g/min CO_2 flow rate). A solution concentration of 10 mg/mL was used. SEM images of the SAS processed pure PLLA and 5-Fu are shown in Fig. 7. 5-Fu particles are bigger than those obtained in experiments 1, 2, and 5–8 using DMSO. It is confirmed that the solvent plays a key role in the particle size. The polymer particles are much smaller and show aggregation. Fig. 8 shows the XRD patterns for pure 5-Fu, pure PLLA and the composite material obtained at the conditions of experiment 12. As could be expected the composite material pattern is a combination of those of 5-Fu and PLLA.

The dissolution profiles of 5-Fu and the composite material obtained at 50 °C and 18.0 MPa are shown in Fig. 9. Thanks to the PLLA coating, the cumulative release rates of the composite are much slower than those of the pure drug. A burst release is observed for the controlled delivery system in the first hour (37%) followed by a moderate release in the next four hours. A sustained release is reached so that the 100% release requires more than four days. The burst release could decrease if the drug loadings were reduced; nevertheless the dissolution profile is similar to those reported for other 5-Fu controlled delivery systems. The release process is controlled by both polymer erosion and drug diffusion [38,39]. The relationship between drug release and time is linear for systems controlled by polymer erosion whereas a parabolic curve is obtained for systems controlled by drug diffusion. Polymers having erosion controlled properties provide a short release time (less than 8 h) and are not adequate for controlled delivery purposes. Since PLLA degrades very slowly, a parabolic curve is obtained for our composites and for most of the 5-Fu delivery systems previously prepared thus indicating that the goal of controlled delivery is reached.

4. Conclusions

5-Fu microparticles were successfully prepared by SAS micronization using DMSO as solvent. The mean particle size ranged from 220 to 670 nm and could be varied by modifying the experiment parameters. The conditions of temperature and pressure were the most important factors. Using a DMSO + DCM mixture as organic solvent, the SAS process was also used to obtain composite particles in which 5-Fu crystals were coated by a layer of PLLA spheres. These composite particles were shown to provide a controlled delivery system: the release rate was substantially reduced with respect to that of the pure drug.

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