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Micronization of Clozapine Particles Using Rapid Expansion of Supercritical Solution with Solid Cosolvent (RESS-SC) Process

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Abstract
The rapid expansion of a supercritical solution (RESS) process has limited commercial applicability due to the extremely low solubility of polar drugs in supercritical CO_2 (sc CO_2). To overcome this major limitation, a modified process of rapid expansion of supercritical solution with solid cosolvent (RESS-SC) is proposed. Here, the RESS-SC is examined for clozapine using menthol solid as a cosolvent to reduce particle size and to increase drug solubility. In addition, the effect of extraction pressure and extraction temperature was investigated on the size and morphology of precipitated particles of clozapine. The properties of the micronized clozapine were assessed using scanning electron microscopy (SEM) and powder x-ray diffraction (XRD) techniques. In this study, the average particle size of clozapine was reduced from 29.15 (before RESS-SC) to 3.15 µm (after RESS-SC).

Keywords
Clozapine; Rapid expansion of supercritical solution; solid cosolvent; Increase the drug solubility.

1. INTRODUCTION

About two-thirds of the products utilized in the pharmaceutical industry are in the form of particulate solids [1]. Particle characteristics such as the size, shape, surface, crystal structure, and morphology are also important factors for physical and chemical stability, uniformity, flowability, tabletability, crystallographic quality, dissolution rate and bioavailability of the drugs [2-6]. The traditional manufacturing processes of drugs, such as mechanical milling, are not sufficient to control their particle characteristics. For example, the routine mechanical milling for particle size reduction resulted in a broad size distribution and degradation of heat-sensitive materials. The supercritical fluid (SCF) and dense gas technology are expected to afford an interesting and effective technique of particle production. Using this technology, we will be able to avoid most of the drawbacks of the traditional methods. These can be divided into two major processes: the rapid expansion of supercritical solution (RESS) for CO_2-soluble drugs and the supercritical antisolvent (SAS) process for CO_2-insoluble drugs.

In RESS process, the desired solute is solubilized in SCF and then resulting solution is expanded through a nozzle to cause a sudden decrease in the solubility and hence, particle formation [7, 8]. Homogenous nucleation in RESS is caused by supersaturation and several mathematical models have been presented to explain this process theoretically [9-11]. Appreciable solubility of the material in the SCF is the major prerequisite for the use of RESS depending on the density of the SCF, the solute chemical structure and the SCF-solute contact time. The characteristics of the RESS precipitated material (morphology and particle size distribution) are a function of the pre-expansion concentration and expansion conditions [12]. The pre-expansion concentration depends on the supercritical fluid, nature of the solute (crystalline or amorphous, composite or pure), addition of a cosolvent, and operating pressure and temperature. The higher the pre-expansion concentration, the narrower the particle size range will be. The expansion conditions depend on the nozzle temperature, geometry and size, distance and angle of impact against the surface of the jet stream. The nozzle can be maintained at a suitable pre-

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expansion temperature to prevent the premature precipitation of the solute. The main limitation of the use of RESS technique is the poor solubility of pharmaceutical products in supercritical CO_2. The low solubility can, in some instances, be overcome with appropriate tuning of the fluid density and the use of an adequate cosolvent. Other limitations are poor predictive control of particle size, morphology along with the difficulty to scaling-up the process because of particle aggregation and nozzle blockage caused by cooling effects due to the rapid expansion of the supercritical solution [2]. Some of the advantages of these techniques include mild operating temperatures and the absence of residual solvent. Nucleation and growth are two important phenomena that govern particle size and morphology in the RESS process. As the pressure reduces in the nozzle, supersaturation causes nucleation of the solute particles at the nozzle tip. On the Mach disk, (where the speed of the fluid changes from supersonic to subsonic) a significant amount of the solute starts coagulating. This coagulation leads to growth of the particles in the expansion chamber [11, 13]. Smaller particles can be maintained if the coagulation is reduced in the expansion chamber. For this purpose, a method of rapid expansion of supercritical solution with solid cosolvent (RESS-SC) has been proposed which overcomes this particle growth in expansion zone resulting in smaller particles. Furthermore, this method not only inhibits the agglomeration of particles during the process, but also improves the solubility of the materials in SCF-CO_2 [14-16]. In RESS, all the nuclei or small particles of solute are surrounded by the same kind of particles. In contrast, in RESS-SC process, nuclei or small particles of the solute are surrounded by excess solid cosolvent particles. This reduces the probability of solute-particle growth by coagulation. Menthol (Fig. 1) is a solid compound that satisfies the necessary requirements as a solid cosolvent [14].

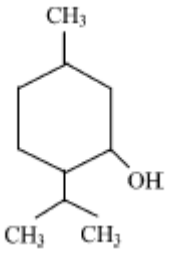

Fig. 1. Molecular structure of menthol (solid cosolvent, mp 32-34 °C).

In this study, clozapine was chosen as a model drug compound. The RESS-SC technique was used to reduce the particle size to <5 μm using menthol as the solid cosolvent. Figure 2 depicts the chemical structure of Clozapine. As is shown, Clozapine is a highly polar compound including both H-donor/acceptor groups which are mainly responsible for its solubility in alcohols and ethers. Clozapine is described as a typical antipsychotic agent due to its effectiveness in the treatment of both positive and negative symptoms of schizophrenia and has low extrapyramidal side effects. The absolute solubility of clozapine in the binary system (clozapine/SCF CO_2) is typically very low [17] also, solubility of clozapine and menthol in SCF CO_2 in mixed solid system (clozapine/ menthol/ SCF CO_2) was previously determined [18].

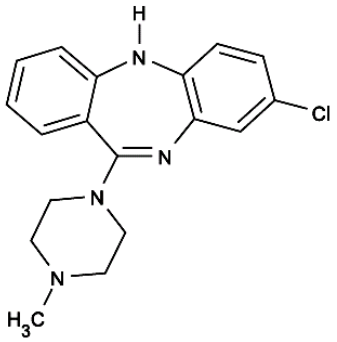

Fig. 2. Molecular structure of clozapine (mp 183 °C).

Menthol is a solid compound that has appreciable solubility in CO_2 [19]. Menthol naturally occurs in mint-flavored plants and is widely used in antipruritic agents, mouthwashes, nasal sprays, food, etc. without any harmful effects. The solubility of clozapine in the binary system is about 4.2×10^{-5} [17], which can be enhanced to as high as 4.48×10^{-4} mol fraction by adding menthol as a cosolvent in the mixed solid system [18]. The size and morphology of the particles obtained in RESS-SC process are characterized by SEM and XRD methods. Also, the effect of extraction pressure and temperature was investigated on the size and morphology of the precipitated particles of clozapine.

2. EXPERIMENTAL

2.1. Materials

Carbon dioxide (99.99%) was purchased from Arish gas (Tehran, Iran). All the drugs (purities more than 99.5%) were obtained from the Food and Drug Quality Control Lab (Tehran, Iran), and used without any further purification except for the vacuum-drying.

2.2. Apparatus and Procedures

2.2.1. Preparation of micronized clozapine

Figure 3 shows a schematic diagram of a laboratory apparatus configured in the RESS-SC mode. The CO_2 is drawn from a dip tube cylinder (A) and precooled in a pump head before being delivered to the vessel by a piston pump (C) (P200, Thar Design Inc., Pittsburgh, PA, USA). Pump heads are cooled to 2 °C by a cryostat (B)

(minichiller, Huber, Offenburg, Germany). The pure solvent is continuously pumped to the desired pressure and pre-heated to extraction temperature by electrical heating jacket (D). RESS-SC process was accomplished with a 25- mL extraction vessel (G) and the equilibrium time was reached after 60 min. The oven temperature and pressure were held constant during running the experiments. The vessel was placed in the oven of the system (E) to provide precise temperature control (±0.2 K) during the RESS-SC processes. When the thermal equilibrium was reached, a piston pump was used in the constant pressure mode to provide enough pressurized CO_2 into the extraction vessel. The extraction vessel consisted of a mixture of 200 mg of Clozapine, 2 g of Menthol and glass beads in a packed form. This procedure prevents channeling, increase the contact surface between the sample and the supercritical fluid and consequently, reduced the equilibration time. For RESS-SC, the expansion chamber (J) having 3-liter capacity was used for expansion of the menthol/clozapine/CO_2 mixture from a desired pressure to atmospheric pressure. When the equilibrium is reached, the saturated supercritical CO_2 was loaded into expansion vessel through a 10- port, 2- position valve (H) and an orifice nozzle (OD: 20 µm) (L). An electrical heating jacket (80 °C) was used in order to prevent the blocking of the nozzle. Particles were collected in the expansion chamber (at a distance of 10 cm from the nozzle) and subjected to a vacuum of 10 mbar for about 40 h to remove any excess of menthol before they were analyzed for their size and morphology. Also, the lyophilized powder did not give any mint smell. These were the sufficient test for checking menthol presence in lyophilized clozapine particles. The obtained Clozapine particles were analyzed by SEM, and XRD.

2.2.2. SEM analysis

SEM images were obtained on an XL30 scanning electron microscope (Philips, Netherland) at 20 KV. Samples were deposited on carbon sticky tabs and were coated using a sputter- coater with gold in a Balzer SDC- 050 apparatus (Bal- Tec AG, Liechtenstein) in the presence of argon (99.9 %< purity) at room temperature for two runs of 1 min with 40 mA current each. In mixed solid system, where in menthol was the cosolvent, processed powder was first kept in a high vacuum of 10 mbar to remove all the menthol from the particle mixture. All the SEM analyses were done after 5 days.

2.2.3. X-ray powder diffraction

The XRD pattern of the unprocessed solutes and the micronized substances were evaluated using an X-ray powder diffractometer (STOE MP, Germany), with Cu target tube, and exposed to all lines. A monochromator was used to select the $K\alpha_1$ line ($\lambda = 1.54056$). The scanning angle ranged from 0° to 90° and the counting time was 0.6 s/step in steps of $2\theta = 0.05$. The scanning rate used was 5°/min. The excitation current used was 30 mA and the excitation voltage used was 40 KV.

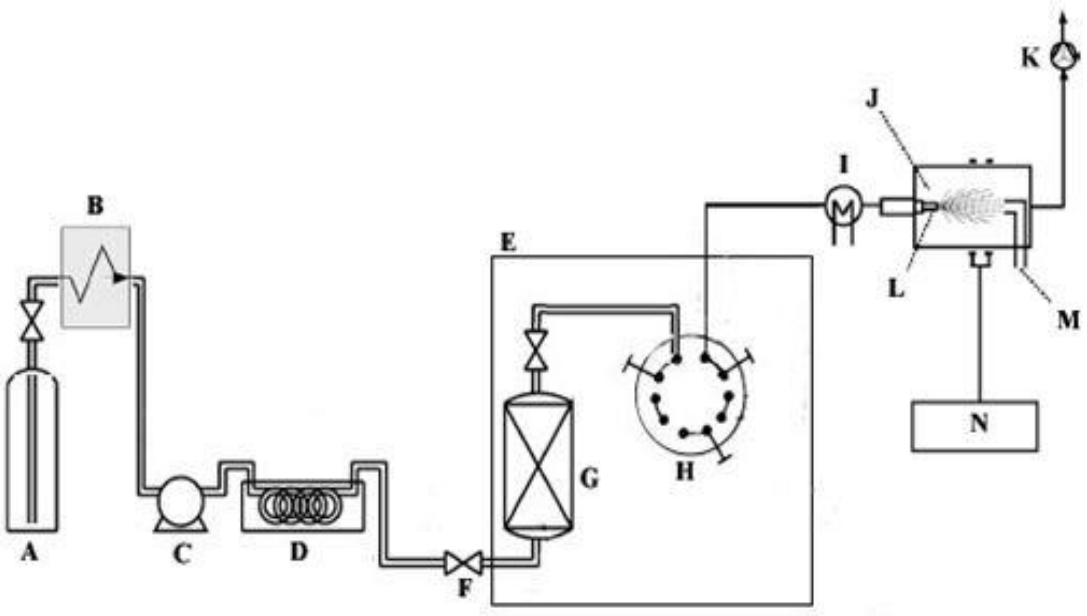

Fig. 3. Schematic diagram of experimental apparatus used for RESS-SC processes: (A) CO_2 gas cylinder; (B) minichiller; (C) piston pump; (D) electrical heating jacket; (E) oven; (F) on/ off valve; (G) 25- mL equilibrium cell; (H) 10- port, 2- position valve; (I) electrical heating jacket; (J)expansion chamber; (K) vent; (L) nozzle; (M) sample for SEM; (N) retainer.

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3. RESULTS AND DISCUSSION

3.1.Effect of the pressure and temperature on the clozapine micronized particles size

In this study, 8 experiments were conducted to study the influence of the pressure and temperature of the extraction vessel on clozapine particles (Table 1).

Table 1. The quantitative results for the effect of the extraction temperature at the two different extraction pressures on the micronized clozapine particles

Pressure (bar)	Extraction vessel temperature (K)	Standard deviation (μm)	Mean particle size (μm)
123	313.15	0.59	7.09
	323.15	0.20	8.99
215	313.15	0.59	3.94
	323.15	0.03	3.15

The precipitated particles were analyzed off- line and its mean particle size was calculated by counting at least for 100 particles. The mean particle size of the primary formed Clozapine was 29.15 μm. The particle size distributions of Clozapine and SEM images are given in Figure 4. The other RESS- SC parameters such as spraying distance (10 cm), nozzle diameter (20 μm) and pre-expansion temperature (80 ˚C) were held constant. The results of experiments relevant to the morphology of the precipitated particles of clozapine and the particle size distribution in different pressures and temperatures (Figs. 5- 7) and the cumulative particle size distribution (Fig. 8), reveal that the precipitated particles of the clozapine are decreased while the extraction pressure is increased from 123 bar to 215 bar.

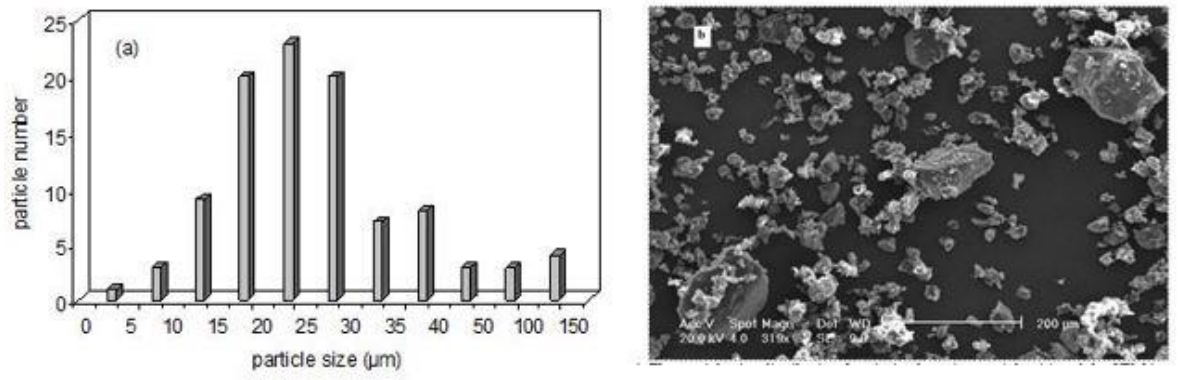

Fig. 4. The particle size distribution for virgin clozapine particles (a) and the SEM image (b).

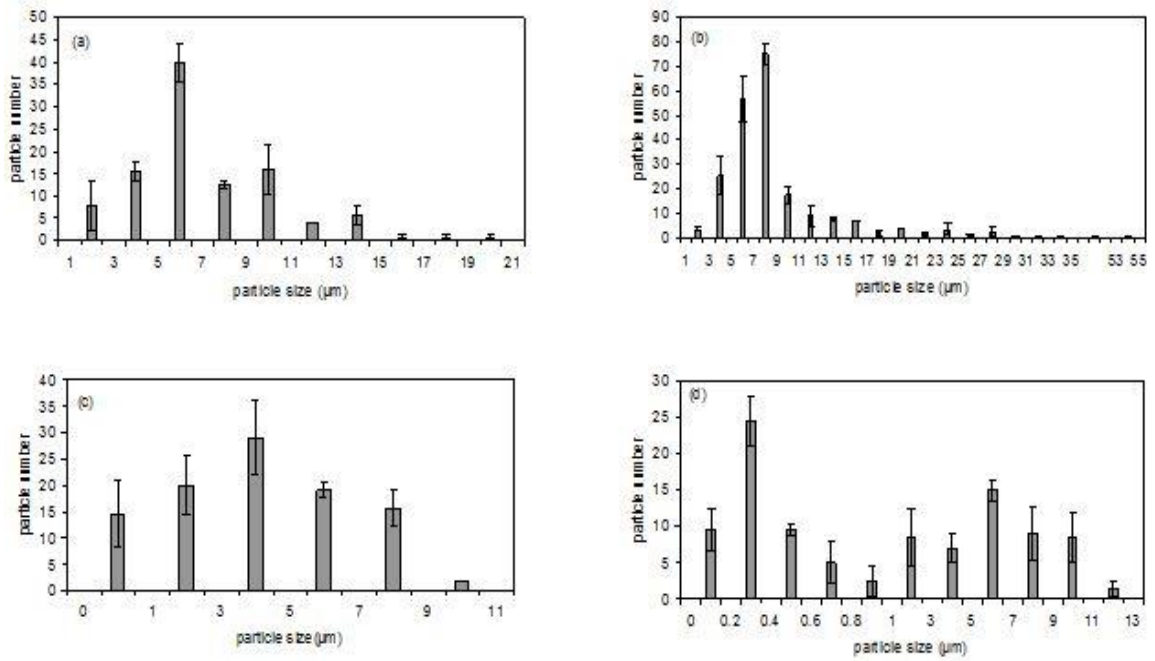

Fig. 5. The particle size distribution for different pressures and temperatures of extraction vessel; (a): P=123 bar, T= 313.15 K; (b): P=123 bar, T= 323.15 K; (c): P= 215 bar, T= 313.15 K; (d): P=215 bar, T= 323.15 K.

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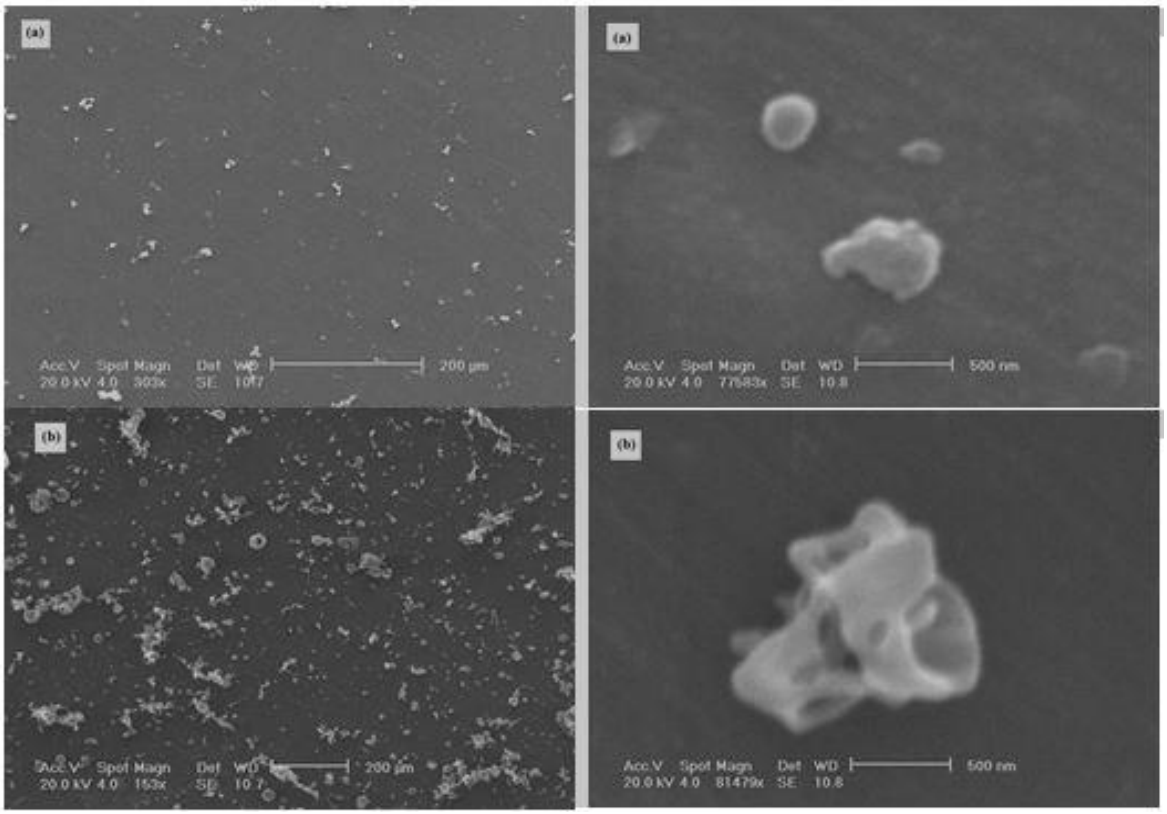

Fig. 6. The SEM images for clozapine particle with 123 bar extraction vessel pressure: (a) 313.15 K and (b) 323.15 K.

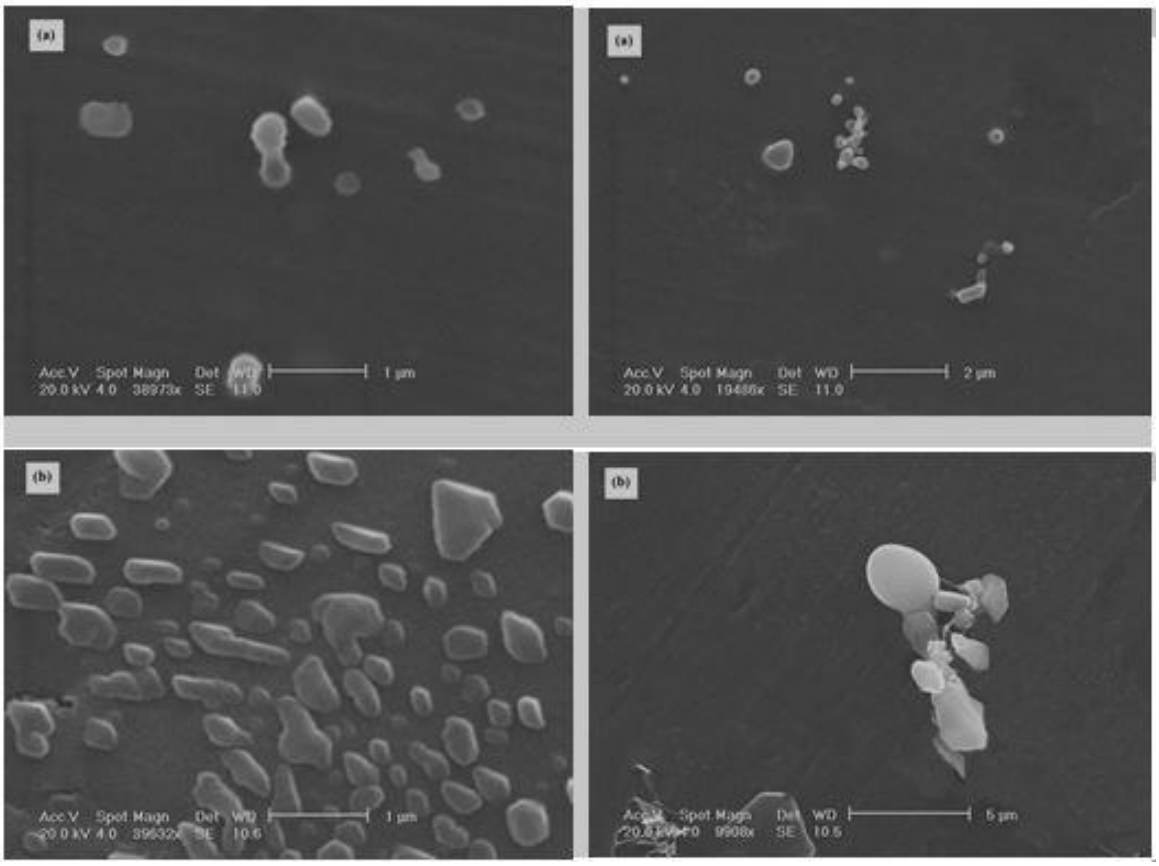

Fig. 7. The SEM images for clozapine particle with 215 bar extraction vessel pressure: (a) 313.15 K and (b) 323.15 K.

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As can be seen from the SEM images, the lower extraction pressure not only is the cause of bigger particle formation, but also affects the morphology of the precipitated particles. On the other hand, the SEM images suggest that the morphology of the precipitated particles is changed from rectangular to almost spherical by increasing the extraction pressure.

Additionally, it seems that a reduction in particle size can be obtained by increasing the pressure of extraction. When the extraction pressure is increased, the density of the supercritical fluid would be increased. This increase in density enhances the solvating power of the supercritical fluid and solute concentration in the SCF. When the concentration increases, the super-saturation and the nucleation rate would increase during the expansion period and finally cause a smaller particle size generation [14, 16, 20]. The obtained results show that the increase of extraction temperature under extraction pressure (123 bar) increases the particle size of precipitated clozapine particles.

Also, the obtained results exhibit that at a higher extraction pressure, the effect of the extraction temperature will reduce and the variation of the extraction temperature cause little change in the mean particle size of the precipitated particles. An increase in the extraction temperature leads to a decrease in the density of CO_2 and a concurrent increase in the solute's vapor pressure. The decrease of the solvent density causes a decrease in the solvent strength. On the other hand, a concurrent increase in the solute's vapor pressure leads to an increase in the drug solubility [17]. The total effect of the two competing phenomena will be equal. This point is defined as the crossover point. At pressures below the crossover region the concentration of the solute in the supercritical phase decreases as temperature is increased. Beyond the crossover region, the solubilities increase with increasing both the temperature and pressure. Results in previously work [18] show that at a pressure of 123 bar, increasing the extraction temperature leads to a decrease in the solubility of Clozapine in SCF, and the decrease of the supersaturation and nucleation rate occurs as a result. The decrease of nucleation rate leads to an increase in the particles grow time, which consequently favors bigger particle. At a pressure of 215 bar [18], the increase of the extraction temperature leads to an increase in the solubility of Clozapine in SCF.

The crystallinity of a pharmaceutical substance has a significant effect on its bioavailability, and its physical and chemical stability. Most of the pharmaceutical products contain drug in the crystalline form. Exposure to changes in temperature, pressure, and relative humidity is encountered during most of the pharmaceutical processes such as drying, granulation, milling and compression. The stresses applied to crystals during these processes can cause defects in their crystal lattice and cause a decrease in the degree of the crystallinity. Figure 9 illustrates typical XRD pattern of the unprocessed and the processed Clozapine at 215 bar and 323.15 K. The intensity of the XRD-peaks of the micronized Clozapine was slightly lower compared to the unprocessed Clozapine. This indicates a reduction in the degree of crystallinity of clozapine after being processed by RESS-SC. The decrease in crystallinity can be helpful for better dissolution of Clozapine particles [21]. Also, the reduced particle size increases in the surface area of microparticles which accounts for the decreasing in peak intensity. Figure 10 shows the XRD pattern of pure menthol particles. The two major peaks for menthol are at 8° and 18° angles, whereas there are no peaks at these angles for clozapine. This further suggests that RESS-SC processed clozapine after lyophilization was menthol free.

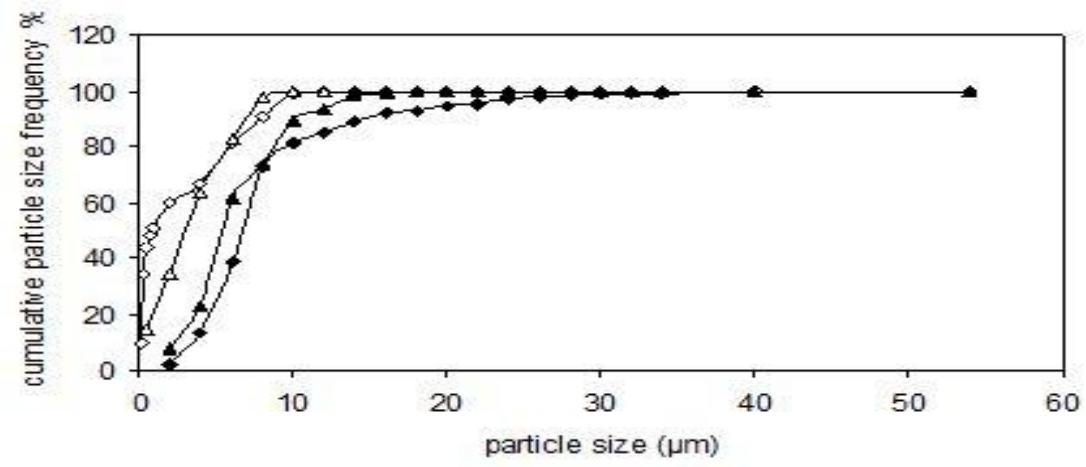

Fig. 8. The cumulative particle size frequency for different pressure and temperature; (▲): P= 123 bar, T= 313.15 K; (♦): P= 123, T= 323.15 K; (Δ): P= 215 bar, T= 313.15 K; (◊): P= 215 bar, T= 323.15 K.

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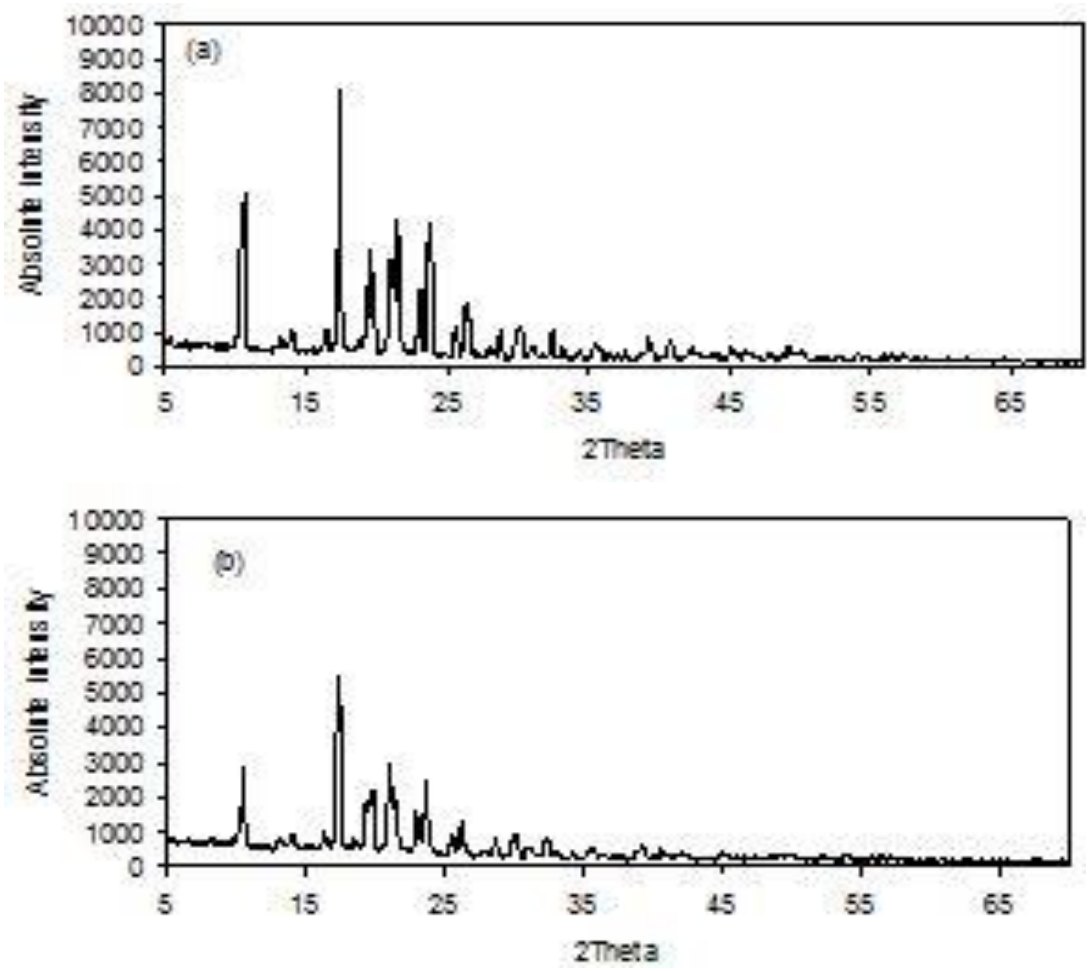

Fig. 9. XRD analysis of: (a) unprocessed and (b) RESS-SC processed clozapine particles.

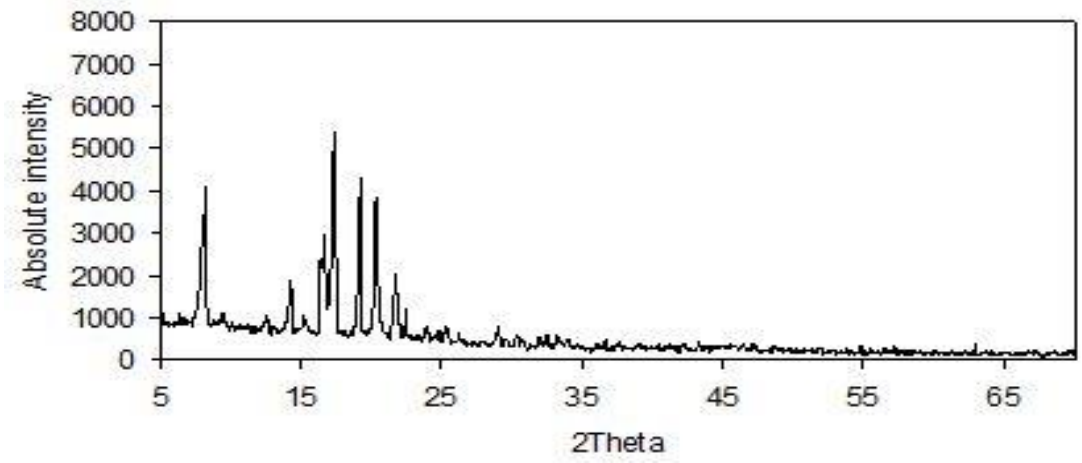

Fig. 10. XRD of pure menthol particles.

4. CONCLUSION

The rapid expansion of supercritical solution with solid cosolvent was successfully used to produce the clozapine microparticles. The limited solubility of clozapine in CO_2 is the major challenges of RESS process that was addressed with using menthol as solid cosolvent. The solubility of clozapine in CO_2 can be increased by a factor of 56 in the presence of menthol as a solid cosolvent. The influences of the pressure and the temperature in the extraction vessel, on the morphology, size and size distribution were investigated. The results show that the pressure is most effective operation parameter while the temperature has little effect on the particles.

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میکرونی کردن ذرات کلوزاپین با استفاده از روش پخش سریع محلول سیال فوق بحرانی با کمک حلال جامد

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چکیده

روش پخش سریع محلول سیال فوق بحرانی (RESS) به دلیل حلالیت بسیار کم داروهای قطبی در CO_2 فوق بحرانی ($SC\text{-}CO_2$) کاربرد تجاری محدودی دارد. برای غلبه بر این محدودیت، یک فرآیند اصلاح شده از پحش سریع محلول سیال فوق بحرانی کمک شده با حلال جامد (RESS-SC) پیشنهاد شده است. در اینجا، روش RESS-SC با استفاده از جامد منتول به عنوان یک کمک حلال جامد برای کاهش اندازه ذرات و افزایش حلالیت داروی کلوزاپین مورد بررسی قرار می‌گیرد .علاوه بر این، اثر فشارو دمای مرحله استخراج بر اندازه و مورفولوژی ذرات تولیدی کلوزاپین مورد بررسی قرار گرفت. خواص ذرات میکرونی کلوزاپین با استفاده از میکروسکوپ الکترونی روبشی (SEM) و تکنیک پراش اشعه ایکس پودری (XRD) ارزیابی شد. در این مطالعه، میانگین اندازه ذرات کلوزاپین از ۲۹.۱۵ قبل از روش (RESS-SC)به ۳.۱۵ میکرومتر بعد از روش (RESS-SC)کاهش یافت.

کلید واژه ها

کلوزاپین؛ پخش سریع محلول سیال فوق بحرانی؛ کمک حلال جامد؛ افزایش حلالیت دارویی.

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