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Research Article

Formation and Characterization of Beclomethasone Dipropionate Nanoparticles Using Rapid Expansion of Supercritical Solution

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Abstract

Purpose: Particle size of Beclometasone Dipropionate (BDP) was reduced by the rapid expansion of supercritical solution (RESS) process, using CO_2 as supercritical solvent. Also, the effect of RESS parameters such as extraction pressure, pre-expansion temperature, and weight fraction of co-solvent on the size and distribution of BDP particles were investigated.

Methods: The effects of extraction pressure (200-260 bar), pre-expansion temperature (70-110 °C) and weight fraction of menthol as a co-solvent on mean particle size (MPS) of BDP were investigated by design of experiment (DOE). Particles were characterized using Scanning Electron Microscopy (SEM) and Dynamic Light Scattering (DLS).

Results: The average sizes of precipitated BDP were between 64.1 and 294 nm. Analysis of variance (ANOVA) showed that extraction pressure was the most significant parameter and a higher extraction pressure caused production of smaller particles. Also, it was found that higher temperature and weight fraction of co-solvent increased the MPS. The interaction effects of extraction pressure-pre-expansion temperature and pre-expansion temperature-co-solvent ratio were significant through the analysis of variance. It was observed that the MPS of precipitated particles was mostly influenced by pressure.

Conclusion: The smallest MPS of BDP obtained from the RESS process was 65 nm that revealed a significant size reduction from its original MPS of 9 µm. Moreover, a slight change was observed for precipitated particles of BDP into spherical form while the original particles were irregular in shape. RESS process showed as a promising method for production of BDP nanoparticles that may results in improvement of drug's physicochemical properties.

Introduction

Supercritical fluid (SCF) technology has become an important tool of materials processing in several pharmaceutical operations including crystallization, particle size reduction, coating and product sterilization. It has also been shown to be a practical possibility in the formulation of particulate drug delivery systems, such as nanoparticles, microparticles and inclusion complexes which control drug delivery and enhance drug stability. The advantages of SCF technology in the particle design include application of mild conditions, use of environmentally benign nontoxic fluids (such as CO_2), minimization of organic solvents and production of particles with controllable morphology, narrow size distribution and low static charge.[1-4]

Depending on the raw material and the final product, different supercritical processes are employed to produce fine particles. Rapid expansion of supercritical solution (RESS) is a dominant method for production of nano and micro size drugs.[5,6] In this method carbon dioxide acts as a solvent due to its moderate supercritical conditions (31.1°C and 73.8 bar), non-toxic, non-flammable, environmentally friendly and inexpensive characters.[6]

The RESS process consists of pre-extraction, extraction, and precipitation units and it includes the entering of CO_2 into the dissolve chamber (pre-extraction unit), saturation of the supercritical fluid with drug (extraction unit) and then pressure reduction of the solution through a heated nozzle (precipitation unit). As can be expected, the rapid decrease in the pressure reduces the power of solvent which results in nucleation and precipitation of fine particles. The main disadvantage of RESS method is the low solubility of polar substances and high molecular weight compounds (compounds with a molecular weight greater than 500) in supercritical carbon dioxide.

To overcome this problem, the co-solvent can be used due to its molecular interactions (RESS-SC method). Modification in the polarity of supercritical phase depends on the nature and amount of co-solvent.[7] Application of menthol as a co-solvent is recommended due to its high solubility in the supercritical carbon dioxide, non-reacting

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behavior with medicine and supercritical fluid, non-toxic and non-flammable characteristic and high vapor pressure.[8] Beclomethasone Dipropionate (BDP) is an anti-inflammatory corticosteroid which is prescribed in the treatment of asthma and chronic obstructive pulmonary diseases.[9] The solubility of BDP in the supercritical CO_2 (SC-CO_2) over different pressure and temperature ranges was reported previously.[10] In this study, the effect of RESS parameters such as extraction pressure, pre-expansion temperature, and weight fraction of co-solvent on the size and distribution of BDP particles were investigated.

Materials and Methods

Materials

Micronized BDP with MPS of 9 µm was supplied by Sina Daru, Tehran, Iran. Menthol was purchased from Merck, Germany. In addition, the CO_2 (>99.9 % purity) was obtained from Sabalan Company, Iran. The properties of BDP and menthol are listed in Table 1. The particle size distribution of unprocessed BDP is shown in Figure 1(a).

Table 1. Physical properties of Beclometasone Dipropionate (BDP) and menthol

-	BDP	Menthol
Chemical formula	$C_{28}H_{37}CLO_7$	$C_{10}H_{20}O$
Molecular weight	521.05	156.27
Melting point	210 °C	35-38 °C
Water solubility	Slightly soluble	Slightly soluble

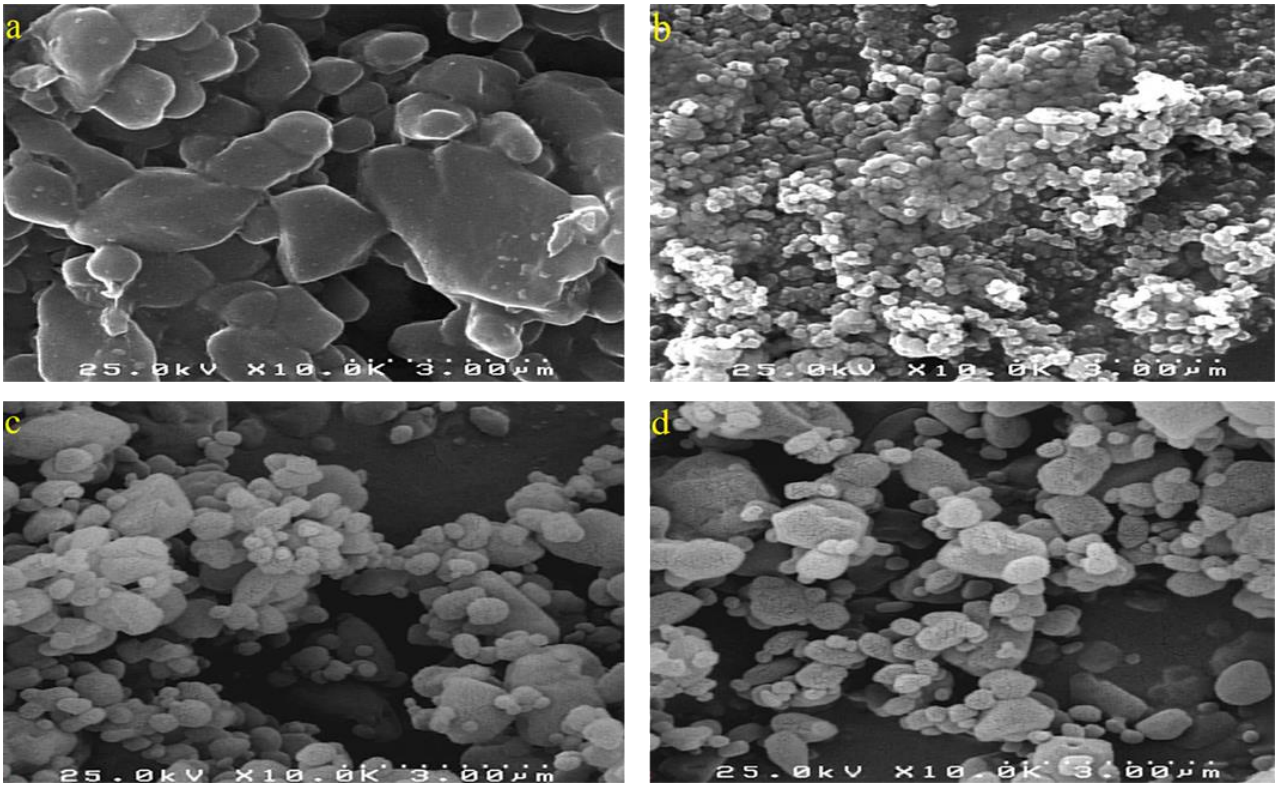

Figure 1. SEM images of: unprocessed BDP (a) and processed by RESS process run 2 (b), run 9 (c) and run 11 (d).

Equipment

RESS Setup

A schematic diagram of the RESS-SC experimental apparatus is shown in Figure 2. The apparatus generally consists of a pre-expansion unit and an expansion unit. Firstly, the gaseous CO_2 was passed through a cooler to make liquid CO_2. Then, it was pressurized to the desired pressure by a high pressure syringe pump prior to entering the extraction vessel which was situated in a controlled temperature oven. High pressure CO_2 was then heated to the supercritical temperature by moving through a coil placed in the oven. Since the process was performed in static mode, the 400 mL extraction vessel was loaded with 200 mg solute and menthol (range of 3 to 7 wt. %) in the presence of 60 mL of 2 mm diameter glass beads to avoid solid solute caking and to increase contact surface area. The SC-CO_2 entered into the extraction vessel while the extraction pressure was set between 200 to 260 bar.

After 75 min as equilibrium time, the supercritical solution was relieved into the expansion unit through a nozzle as the main part of the expansion unit (D=500 µm, L=1 cm). The nozzle was heated by a heater during the expansion process in order to prevent clogging and its temperature was controlled by a PID controller (Cole-Parmer, US). The collision angel was fixed at 90 degree and the distance between the nozzle and collection plate (spray distance) was 7 cm. After precipitation, processed particles were lyophilized for 24 h to evaporate residual menthol.

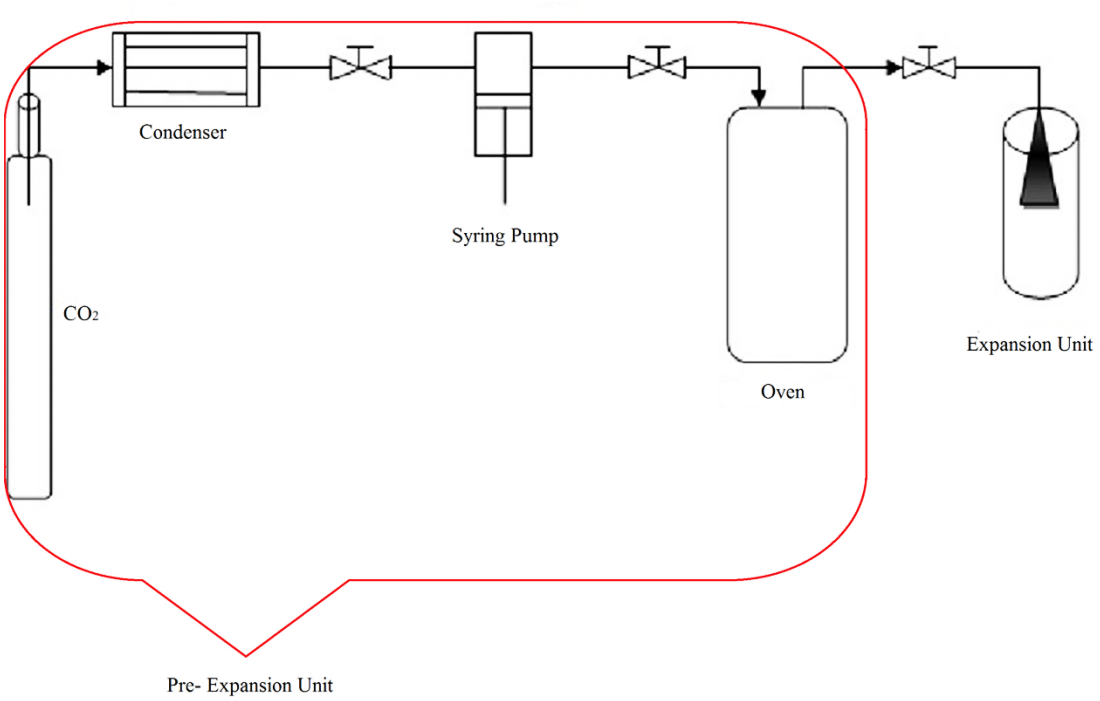

Figure 2. Schematic diagram of the RESS-SC apparatus

Particle Morphology

Morphology of unprocessed and precipitated BDP particles was evaluated by Scanning Electron Microscopy (Hitachi S4160, Japan). Samples were coated by a sputter coater with gold at room temperature for a period of 180s at 24 mA and accelerator voltage for scanning was 25 kV.

Particle Size Analysis

Particle size distribution (PSD) of selected samples were determined by dynamic light scattering (DLS) using a Zetasizer (Nano ZS90, Malvern Instruments, UK). The particles were dispersed in double distilled water containing tween 80 and were sonicated for 3 min before analysis.

Methods

Design of Experiment (DOE) is an effective method to identify the important factors in a process, detect and fix the process problems, and also to evaluate the possibility of estimating interactions. The influences of three parameters (Extraction pressure (A), pre-expansion temperature (B) and wt. % of solid co-solvent (C)) on the MPS were investigated using two-level full factorial design. According to this method, three full factors and two-level factorial design was adopted and 8 experimental conditions were investigated. Furthermore, three additional center point experiments were also performed to find out if any curvature exists in the model. The low and high levels for different parameters are shown in Table 2. As shown in Table 3, the experiments were performed in a random order to minimize the effects of uncontrolled parameters.

Table 2. Independent variables and their levels.

Variable	Symbol	Level		
		Low level (-1)	Center point (0)	High level (+1)
Extraction pressure (bar)	A	200	230	260
Pre-expansion temperature (°C)	B	70	90	110
Wt. % of co-solvent	C	3	5	7

The importance of each experimental parameter and their possible cross-interactions were investigated by analysis of variance (ANOVA) using Design Expert 6.0.6, a DOE software tool from Stat-Ease, Inc. In statistical analysis, a model for the response was obtained by performing a regression on the experimental results. Under the confidence level of 90%, the p value $s<0.1$ were considered as significant terms. The F distribution value of each factor and their cross-interactions were determined based on the experimental results of the MPS listed in Table 3. After eliminating the insignificant terms, the final model was obtained.

Table 3. Experimental design and quantitative results.

Std. order	A	B	C	Mean particle size (nm)	Standard deviation, σ (nm)	Standard error of mean (nm)
1	+1	+1	+1	234.63	70.13	8.44
2	+1	-1	-1	64.09	13.83	1.86
3	0	0	0	216.87	57.70	10.04
4	-1	-1	+1	221.09	92.53	16.11
5	-1	+1	+1	294.05	105.58	20.32
6	+1	+1	-1	117.34	55.48	11.56
7	-1	-1	-1	191.6	63.01	5.93
8	-1	+1	-1	196.67	52.46	6.77
9	0	0	0	195.88	35.79	9.24
10	+1	-1	+1	97.36	39.85	4.6
11	0	0	0	180.32	63.06	9.51

Results and Discussion

As presented in Table 2, the experiments were carried out for studying the effect of extraction pressure (200, 230, and 260 bar), pre-expansion temperature (70, 90, and 110°C), and fraction of solid co-solvent (3, 5, and 7) on MPS of BDP precipitated by RESS-SC process. It should be noted that in all experiments, the extraction temperature was fixed at 55°C.

The SEM image of unprocessed BDP (Figure 1(a)) showed irregular shapes of fine particles and wide size distribution from 5 to about 13 µm. Figure 1(b-d) shows the SEM images of selected precipitated BDP. SEM images show that the processed BDP particles were drastically finer than the original particles.

The quantitative results of experiments are given in Table 3. The average particle size ranged between 64.09 nm and 294.05 nm. The smallest MPS obtained from the RESS process was 64.09 nm (run 2 in Table 3) and its SEM image and DLS analysis are shown in Figure 1(b) and Figure 3, respectively. Figures 1.(c) and (d) that are SEM images of two different runs at center point, show that the particles obtained in these runs (runs 9 and 11) approximately have the same shapes and sizes. According to the SEM images, the formed nanoparticles had irregular shapes but some of the precipitated particles were close to spherical form and some agglomerations between particles were observed, which could be due to the short spraying distance.

Statistical Analysis

After omission of insignificant terms, analysis of variance (ANOVA) is given in Table 4. Extraction pressure had the most significant effect with the highest F-value and coded coefficient. ANOVA shows that there is no curvature in the model. "Prob>F" value of the linear interaction regression model is 0.0041(<0.1), that state the model is significant. Equation 1 is the regression model with 0.969 coefficient of determination (R^2) and 0.9303 adjusted coefficient of determination (R^2_{adj}). It verifies the compatibility of model with experiments for MPS as a function of temperature, pressure, amount of solid co-solvent and two binary interaction of pressure-temperature and temperature-co-solvent in coded units.

$$MPS \quad (nm) = 177.1 - 48.75 \times P + 33.57 \times T + 34.68 \times C + 14.06 \times P \times T + 18.99 \times T \times C \qquad (1)$$

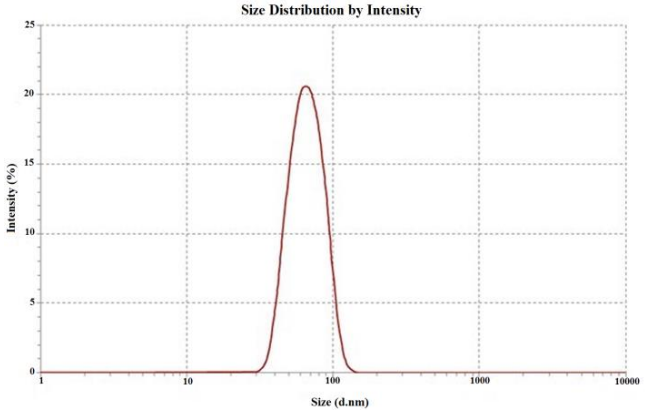

Figure 3. DLS analysis of BDP (extraction pressure: 260 bar, pre-expansion temperature: 70ºC, co-solvent: 3wt. %)

A comparison between the model and experimental MPS is presented in Figure 4. This Figure shows a good agreement between statistical model and experimental results.

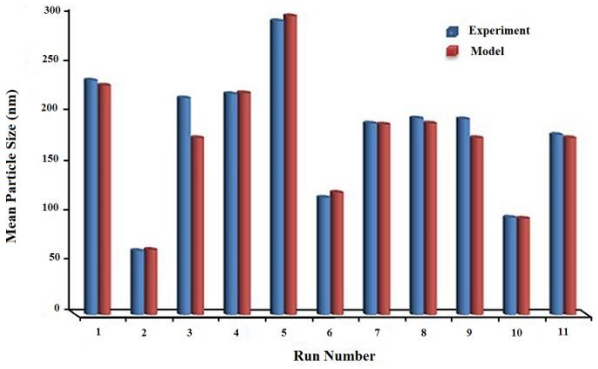

Figure 4. Compression between model and experiment results

Table 4. Analysis of variance for mean particle size

Source	Sum of square	Degree of freedom	Mean square	F value	p-Value Prob.> F	Coefficient (coded)
Model	42113.67	5	8422.73	25.04	0.0041	–
Constant	–	–	–	–	–	177.1
A	19011.53	1	19011.53	56.51	0.0017	-48.75
B	9014.89	1	9014.89	26.8	0.0066	33.57
C	9620.93	1	9620.93	28.6	0.0059	34.68
AB	1581.75	1	1581.75	4.7	0.096	14.06
BC	2884.58	1	2884.58	8.57	0.0429	18.99
Curvature	924.64	1	924.64	2.75	0.1727	–
Residual	1345.73	4	336.43	–	–	–
Lack of fit	672.87	2	336.43	1	0.5	–
Pure error	672.87	2	336.43	–	–	–
Cor. total	44384.04	10	–	–	–	–

Effect of Pre-expansion Temperature

The pre-expansion temperature was studied from 70 to 110°C. The MPS versus pre-expansion temperature at different extraction pressures is presented in Figure 5(a). It shows that higher temperature led to the enhancement of mean average size and this growth was more considerable at higher pressure. An increase in the pre-expansion temperature caused large decrease in the fluid density and the consequent reduction in solid solubility. Increase of temperature led to decrease the supersaturation and nucleation rate of BDP in supercritical solution and the particle size became larger. The existing data[10] indicated that in the pressure range of 213-274 bar, the solubility of BDP in SC-CO_2 decreased with increase of the temperature. Thus, the supersaturation reduced and larger particles were produced. For example, at a pressure of 243 bar, enhancement of the temperature from 75 to 85°C, changed the solubility of BDP in SC-CO_2 from 51.8×10^{-3} (g_{Solute}/L_{CO2}) to 50.8×10^{-3} (g_{Solute}/L_{CO2}).[10]The same results have been reported for Digitoxin,[11] Titanocendicholoride,[12] and benzoic acid.[13] However for salicylic acid[14] and ibuprofen[15] larger particles at lower pre-expansion temperature had been found. For aspirin,[16] the pre-expansion temperature had a little effect on particle size. Therefore the effect of pre-expansion temperature on particle size may be depending on the type of compounds and solute-solvent interactions at process conditions.

Effect of Extraction Pressure

Figure 5(b) shows the effect of pressure on the MPS at three temperature levels. As the extraction pressure was increased from 200 to 260 bar, the MPS decreased. Similar results were obtained for megestrol acetate,[17] benzoic acid[18] and reloxifen;[19] however, the contradicting results for aspirin[16] and taxol[20] were reported. This can be explained by the increase of CO_2 density and consequently, solubility of BDP at higher extraction pressures resulted in at constant temperature. The supersaturation ratio was enhanced in higher extraction pressure due to the increasing of the solubility. It provided a fast nucleation rate and finally caused the production of particles with smaller mean size. At higher extraction pressures the velocity of fluid expansion raises. This results in the increase of fluid turbulence and therefore mass flow rate and consequently shorter particle residence time in the nozzle and expansion chamber. As mass flow rates increase, both particle growth rate and particle size decrease.

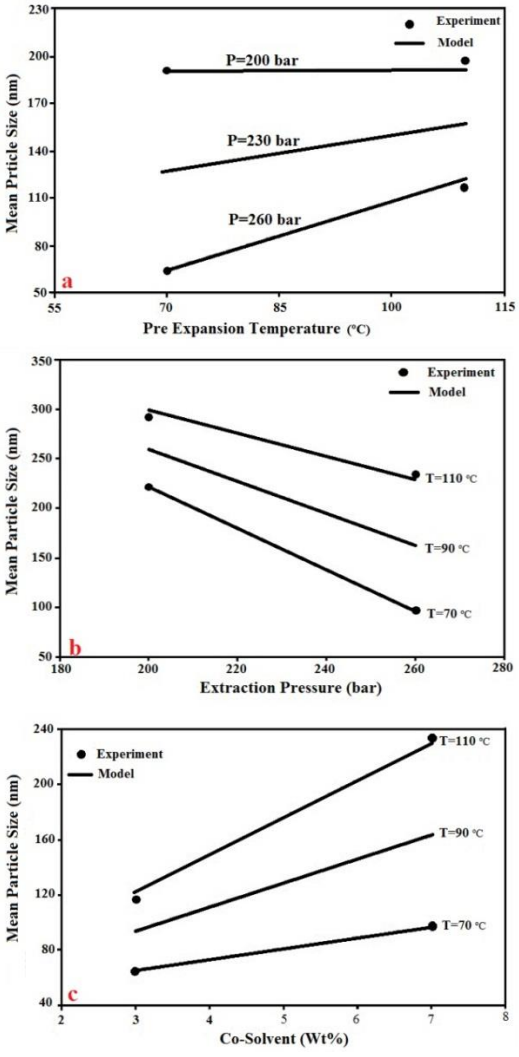

Figure 5. Mean particle size of BDP: (a) vs. pre-expansion temperature (co-solvent=3 wt. %), (b) vs. extraction pressure (co-solvent= 7 wt. %), (c) vs. co solvent (P= 260 bar)

Effect of Solid Co-solvent

The experiments were performed at 3 levels of co-solvent ratios and the effect of this modification on the MPS is presented in Figure 5(c). It shows that larger particles were formed by increasing the amount of co-solvent. The interaction between the solute and menthol is strong, especially through hydrogen bonding. On the other hand, Particle collision rate is directly proportional to the square of particle concentration. Therefore, the increased concentration of menthol may cause coagulation among the particles and result in larger particles.[20]

Effect of Binary Interactions

The 3-dimentional plots of the regression equation (Eq. (1)) are presented in Figure 6(a-b). Figure 6(a) represents the pressure-temperature effect on the MPS at the same time in constant amount of co-solvent (3 wt. %). The simultaneous effect of pressure-temperature reveals that MPS was mostly influenced by pressure than temperature. The model shows that at higher pressure and temperature, the mean particle size was increased tangibly, although at lower pressure and temperature, changes affected the MPS more slightly.

In the Figure 6(b) the effect of temperature and ratios of co-solvent in constant extraction pressure (260 bar) on the MPS can be observed. Increasing the temperature and co-solvent enhanced the MPS. The model indicated that at higher temperature, growth of the MPS with more content of co-solvent was greater than the increase in it at lower temperature. This can be explained as follows: higher temperature caused the density of SCF solvent to fall which resulted in lower drug solubility and adding more co-solvent led to formation of larger particles due to coagulation.

Conclusion

Beclometasone Dipropionate nanoparticles were prepared using the RESS-SC process. A full factorial two-level design was employed as a design of experiment method in order to evaluate the operating parameters including the extraction pressure, pre-expansion temperature, and fraction of co-solvent on the MPS. As extraction pressure was increased, the particle became smaller. While, enhancement of the pre-expansion temperature led to the growth in particle size. By increasing the amount of co-solvent, particle size was increased. The smallest average particle size of BDP obtained from the RESS process was 65 nm that revealed a significant size reduction from its original mean size of 9 µm.

RESS process showed as a promising method for production of BDP nanoparticles that may results in improvement of the drug's physicochemical properties.

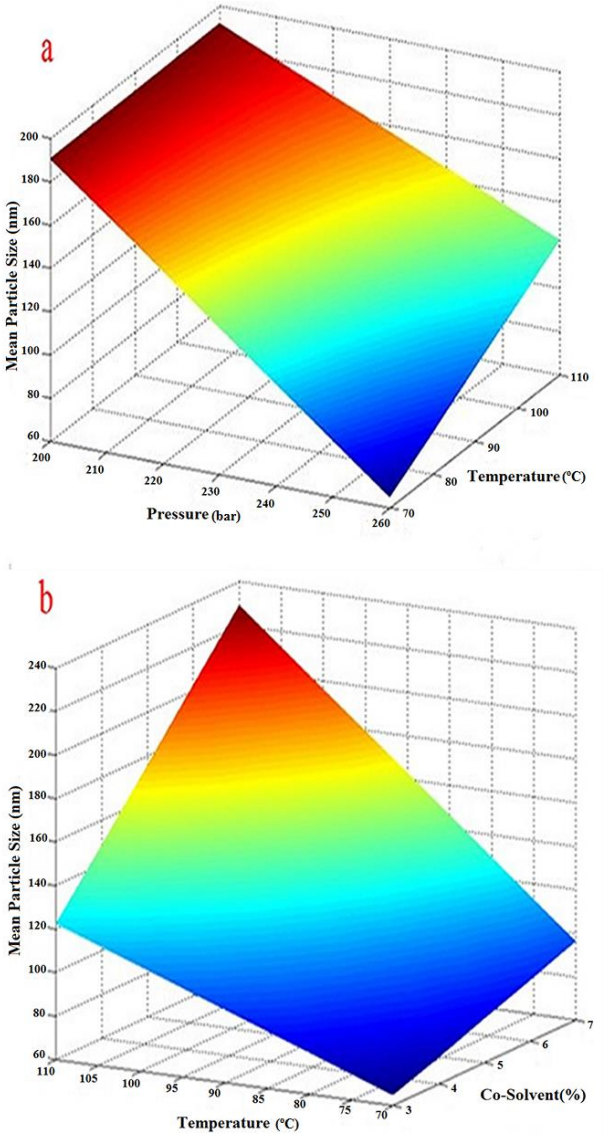

Figure 6. 3D plots of (a) binary effect of pressure–temperature on mean particle size (b) binary effect of temperature – co-solvent on mean particle size

Ethical Issues

Not applicable.

Conflict of Interest

The authors report no conflicts of interest.

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