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Study on optimization of ternary complex of piroxicam-β-cyclodextrin-lysine inclusion in supercritical CO_2

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Abstract:

Piroxicam is a bioactive compound classified as a non-steroidal anti-inflammatory drug (NSAID). However, its low solubility in water imposes a serious limitation for its application in the pharmaceutical industry. Using cyclodextrins to form complexes can enhance the dissolution rate, solubility, and bioavailability of piroxicam. In this study, piroxicam/β-cyclodextrin complexes are prepared in supercritical carbon dioxide (SC-CO_2) in the solid state and the process was optimized using response surface methodology (RSM). UV-Vis spectroscopy, differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FTIR), and dissolution profile in water were used to characterized the complex under optimized temperature, residence time, moisture, and ternary agent. Finally, the maximum reaction yield of the inclusion complex was predicted to be 95% at the optimal conditions of 266 bar, 136°C, 1.84:1 ratio of cyclodextrin:piroxicam, and 1.5:1 ratio of lysine:piroxicam. Large scale production of inclusion complexes can be developed from the results of this work on optimizing conditions.

Keywords: **β-cyclodextrin, CO_2 supercritical fluid, piroxicam, piroxicam solubility**.

Classification number: **2.2**

Introduction

A variety of pharmaceutical agents including piroxicam (PC), a non-steroidal anti-inflammatory drug, have inaccessible bioavailability and dissolution. Molecular encapsulation is one approach available to produce a higher solubility compound, such as with the use of cyclodextrins (CDs), through the formation of inclusion complexes [1, 2]. CDs, also known as cycloamyloses and cycloglucans, are cyclic oligosaccharides composed of α (1, 4)-linked glucose units that have the shape of a truncated cone or torus as illustrated in Fig. 1, left [3, 4]. The cavity of the CD molecule is relatively non-polar compared to water, while the outer edge is polar due to the presence of hydroxyl groups on the faces of the structure. The chemical structure of the three native CDs α-, β-, and γ-cyclodextrin, are composed of 6, 7, and 8 glucose units, respectively. Due to their unique chemical structure, CDs are interesting host molecules for hydrophobic guest molecules in aqueous solutions [5]. In an inclusion compound or complex, a hydrophobic guest molecule can move into the cavity of the CD molecule (see Fig. 1, left). The hydrophilic character of the CD molecule's surface, when replaced by the complex, can lead to improved aqueous solubility. When the inclusion complex is placed in an aqueous solution, the guest molecules are released from the CD's internal cavity through equilibrium association/dissociation, which drives the continuous release of the guest molecule [1, 6].

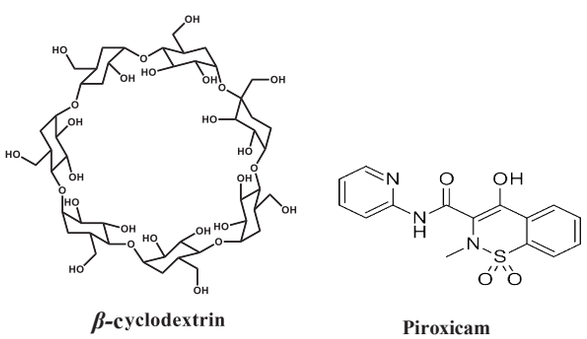

Fig. 1. Chemical structure of β-cyclodextrin (left) and piroxicam (right).

The formation procedures of CD-drug inclusion complexes use a diversity of techniques including methods that require considerable quantities of water

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rather than toxic organic solvents [3]. To be specific, kneading, coprecipitation, and methods that freeze dry or spray dry can provide high inclusion efficiencies but come along with a substantial amount of toxic solvents [4]. However, applying SC-CO_2 to form an inclusion complex can significantly reduce the amount of organic solvents used in the process. Another advantage of using CO_2 is it's nontoxicity at ambient conditions and mild toxicity at critical conditions, which simplifies the problem of solvent residues. Supercritical CO_2 is known to be non-flammable, relatively non-toxic, and inert [7-9], which is ideal for a "green solvent" media that is suitable for the preparation and modification of a drug-carrier. Processes that generate particles using supercritical fluids (SCF), rapid expansion of a supercritical solution (RESS), supercritical anti-solvent (SAS), and particles from a gas-saturated solution (PGSS), may show higher rates of powder dissolution by increasing the specific surface area. However, techniques using SC-CO_2 to form inclusion complexes are reported to be limited by solubility of the drug in CO_2. Ternary agents, such as L-lysine, have been investigated as additions in the solid state to promote complex formation [10, 11].

In the present study, SC-CO_2 was used as a synthesis solvent to prepare a piroxicam/lysine/β-cyclodextrin complex. The reaction was conducted in batch conditions using a high pressure induced autoclave. The effects of process and reaction parameters such as reaction ratio, reaction temperature, and pressure were investigated and analysed by RSM. The addition of L-lysine and water showed a significant influence on reaction yield. A UV-Vis spectrometer, FTIR, and DSC instruments were used to measure the reaction yield, product solubility, and related properties.

Materials and methods

Materials

Piroxicam (PC, 99,5%), β-cyclodextrin (β-CD, 98%), L-lysine (98,5%) from Sigma-Aldrich, methanol (HPLC grade, Merck), acetonitrile (HPLC grade, Merck), double distilled de-ionised water (DI), and CO_2 (99.99%, air liquid) were purchased and used as received. All other reagents and solvents were of analytical grade.

Synthesis of ternary complex in CO_2 media

In each experiment, the amounts of all related solvents were calculated by the supercritical density as well as the volume of reaction. Supercritical density was determined by the NIST REFPROP database from Aspen Plus. The PC: β-CD: lysine ternary complex was synthesized from a mixture composed of piroxicam, lysine, and β-cyclodextrin in a molar ratio of reaction materials that varied depending on the experimental design conditions. The equipment included a volumetric pump, a 100-ml stainless steel autoclave, and a depressurisation system at the designed pressure and temperature. In a typical experiment, piroxicam (0.33 g), β-cyclodextrin (2.27 g), and lysine (0.146 g) were physically mixed and ground by a mortar and pestle and the molar ratio of reaction was 1:2:1, respectively. The white powder mixture was transferred to the autoclave. Then, 5 ml of deionized water (DDW) was added and stirred by glass rod until it became a homogeneous suspension. The autoclave was carefully closed and connected to the experimental system. The reactor was filled with SC-CO_2. The heat was slowly increased to 110°C, the pressure was fixed, and a magnetic stirrer ensured continuous stirring. After 3 h of reaction time, the reaction was slowly cooled down and the pressure was vented before collecting the product. Then, it was freeze dried.

Determination of the reaction yields and solubility

The reaction yield was calculated based on the relative amount of insoluble piroxicam in product and in the solid material mixture. Specifically, 15 mg of powder product was dissolved in 10 ml of DDW with sonication for 30 s. The solution was then centrifuged at 9,000 rpm for 5 min and then the supernatant was decanted. This was repeated two more times until there was no more product to be dissolved. The remaining solid in the centrifuge tube was dissolved by acetonitrile acidified with 0.05 M HCl (2.08 ml of 12 M HCl in 500 ml of acetonitrile). Solution is transferred to the volumetric flask and solvent was added to 25 ml. UV absorption at 334 nm is measured in the solution and the amount of PC was calculated based on a premade calibration of PC.

The reaction yield is then calculated based on the equation:

$$H(\%) = \frac{PC_{tol} - PC_{fr}}{PC_{tol}} \times 100$$

where PC_{tol} and PC_{fr} are the amount of piroxicam in the solid material before the reaction and amount of piroxicam insoluble in the product, respectively.

The water solubility of piroxicam in a complex product was measured by dissolving 25 mg of powder product in 10 ml of DDW with sonication for 30 s. The solution was then centrifuged at 9,000 rpm for 5 min and the saturation solution was decanted. All the clear supernatant solution was collected and DDW was added to 25 ml in a volumetric flask. The measurement was conducted on an HPLC equipped with a Zorbax Eclipse

Plus C18 (4.6x250, 5 µm) column using a DAD detector and the amount of piroxicam was calculated based on a premade calibration of PC.

Characterization of complex product

Thermal analysis: approximately 3 mg of sample was measured by Differential Scanning Calorimetry (DSC, Model 204 F1 Phoenix, NETZSCH, Germany) from 40 to 220°C at a heating rate of 5°C/min under an N_2 gas stream.

FTIR spectroscopy: study of the piroxicam and product were conducted with an FTIR Tensor 27, Brucker, Germany spectrophotometer. The powder (10 mg) was kneaded with KBr (150 mg) and analysed using 16 scans, resolution of 4 cm^{-1}, and scan from 400 to 4,000 cm^{-1}.

Optimization of process parameters

RSM was chosen as the system to start the development, improvement, and optimization of complex production stages. According to the results from single-factor experiments, the effects of three factors, namely, temperature, pressure, and ratio of *β*-CD/PC, were chosen for further optimization. The inclusion product was evaluated by employing RSM based on the Box-Behnken design. The factor levels were coded as -1 to +1 as presented in Table 1. The input ratio of 1.5:1 LL:PC was selected based on preliminary experiments carried out in the laboratory [10, 11]. The optimum combination and the effect of process parameters on reaction yields were the target of the experiment and was performed through CCD. The relations between the response and process variables were observed after fitting the model, which was expressed as a second-order polynomial. The following equation was fit to the model:

$$y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=1+1}^{3} b_{ij} X_i X_j \qquad (1)$$

where y is the expected response; X_i X_j denoted the independent variables; b_0 is the model constant; b_i is the coefficient of the linear parameter; b_{ii} is the coefficient of the quadratic parameter; b_{ij} is the coefficient of the crossed parameter, and $\sum$ is the residual associated with the experiments.

Table 1. Experimental ranges and levels of the independent variables for RSM study.

Factors	Units	-1	0	+1
Temperature	°C	110	120	130
Pressure	Bar	200	250	300
Ratio *β*-CD/PC	-	1	1.5	2

Finally, the validity of the model was evaluated by the coefficient of determination (R^2) and its adjusted value. In addition, the significance of the obtained model and its terms were verified by using the analysis of variance (ANOVA).

Results and discussion

Characterization of ternary complex

Solubility: the solubility of the piroxicam complexation product in water is presented in Table 2. Under different reaction conditions, the solubility of piroxicam is 0.7 mg/ml in DI water corresponding to 2.1×10^{-3} mol/l, while trace amounts of free piroxicam was obtained in DI water. Based on the results, the water solubility of piroxicam complex is approximately 30.4 times more than free piroxicam.

Table. 2. Box-Behnken design for inclusion PC complex and the observed yield for each experiment.

Experiment no.	Temperature (°C) X_1	Pressure (MPa) X_2	*β*-CD/PC X_3	% Yield actual	% Yield predict
1	140	250	1	52.9	55.03
2	130	250	1.5	82.3	84.00
3	140	300	1.5	79.8	81.24
4	130	250	1.5	80.1	84.00
5	120	200	1.5	35.5	34.06
6	120	300	1.5	40.8	42.12
7	130	200	1	22.3	21.49
8	130	250	1.5	88.1	84.00
9	130	200	2	38.7	42.28
10	140	200	1.5	45.5	44.18
11	130	250	1.5	85.5	84.00
12	120	250	1	36.8	39.05
13	130	300	1	41.9	38.29
14	130	300	2	69.8	70.62
15	140	250	2	92.5	90.23
16	130	250	1.5	84	84.00
17	120	250	2	59.1	56.97

UV-Vis absorption spectrum: the complexation obviously changed the solubility as well as the spectrum of the product with respect to that of free piroxicam. In contrast, the complexation product in DDW was quickly dissolved and formed a clear-to-slightly green coloured solution. The UV-Vis absorption spectrum of the product in Fig. 2 clearly showed the characteristic absorption peak of piroxicam at 358 nm and the shoulder at 291 nm assigned as the interaction of the piroxicam molecule with a protic solvent. In the case of free piroxicam and cyclodextrin, the spectra did not show absorption in the visible region [12, 13].

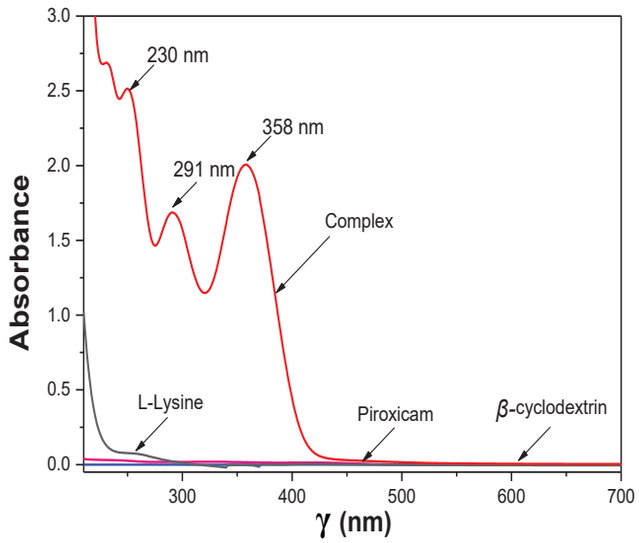

Fig. 2. The UV-Vis absorption spectra of piroxicam/β-cyclodextrin complex, free piroxicam, β-cyclodextrin, L-lysine in H_2O solvent as indicated.

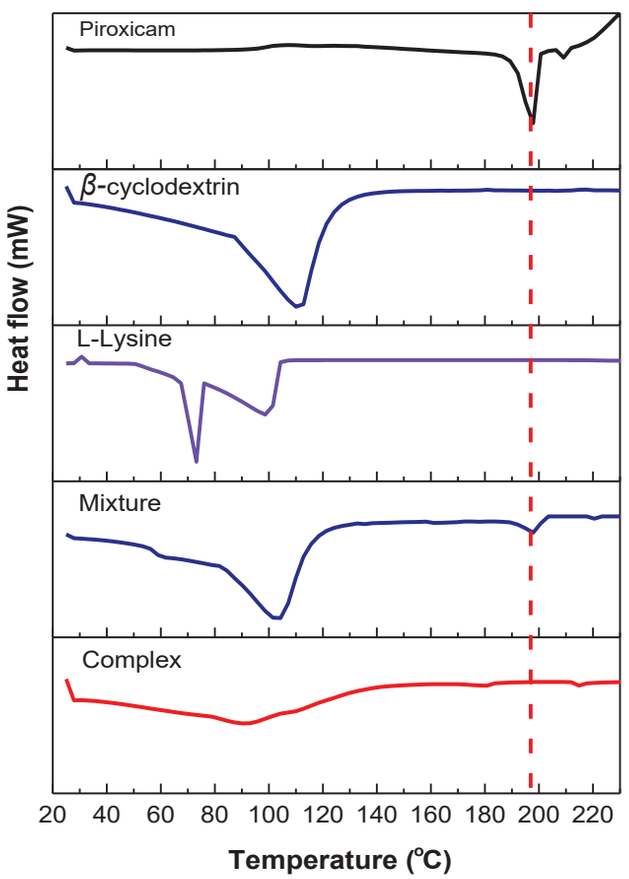

Fig. 3. The DSC curve of L-lysine, piroxicam, β-cyclodextrin, physical mixing and complex.

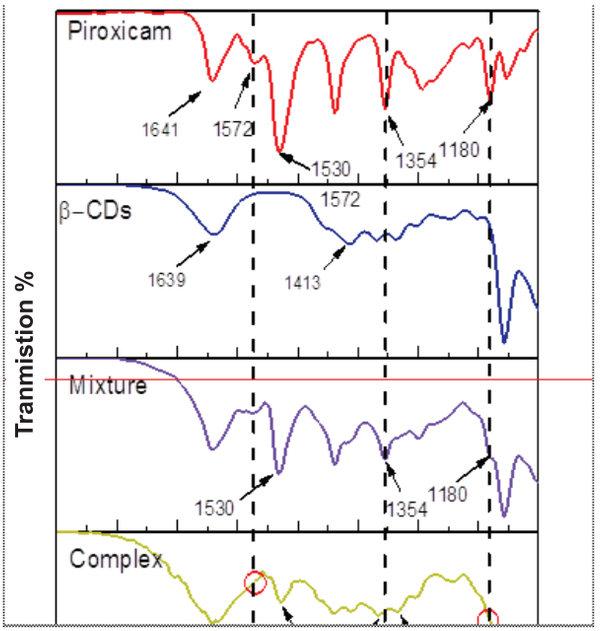

Fig. 4. The FTIR absorption spectra of piroxicam, β-cyclodextrin, their physical mix, and the complex.

Differential Scanning Calorimetry: DSC curves were analysed for pure piroxicam, a physically mixed sample, and a complexation product with a 1:2 ratio of piroxicam/*β*-CD, which are shown in Fig. 3. The thermogram of L-lysine showed an endothermal peak starting from 70 to 100°C corresponding to water loss. Fig. 3 clearly showed an endothermal peak in free piroxicam that appeared as a sharp peak at 200-205°C, which is the melting point of the high crystallinity solid. The DSC profile of piroxicam may indicate a cubic crystal polymorph that can be confirmed by FT-IR analysis [14]. Similar to piroxicam, *β*-CD is a slightly hydrophobic compound. The results did not show the peak as water loss in both cases. However, the thermogram of *β*-CD showed two thermal phase transfer peaks at 132 and 166°C. In case of the physically mixed sample, a broad peak appeared in the region of 70-100°C and another peak at same position as the free piroxicam case.

With the complexation product, this thermogram is almost flat and the endothermal peak due to molten piroxicam has disappeared. This result reveals that the initial crystalline structure of piroxicam had collapsed. On the molecular level, the piroxicam molecules in the product are in a disordered arrangement. This is consistent with the above discussion in regard to the inclusion of a piroxicam molecule into the cavity of *β*-CD [7, 14, 15].

Fourier transform infrared spectrometry: Fig. 4 represents a graph of the FTIR analysis of piroxicam, *β*-cyclodextrin, their physical mix, and the complex.

Characteristic peaks of the amide group is suspected in the crystal form of piroxicam exhibited by the FT-IR spectrum at 3,338.94 cm^{-1} and 1,641 cm^{-1} due to N-H and C=O stretching, respectively. This result confirms the cubic crystal polymorph that was supposed above according to the DSC curve [10]. However, those peaks are broader and lower in intensity in both the case of PC/β-CD complex. The vibration peak of C=C of the aromatic ring and methyl group in free piroxicam and the physical mix occur at 1,572 cm^{-1} and 1,354 cm^{-1}, which are unobserved in the case of the complex. Moreover, the peak at 1,180 cm^{-1} due to the vibration mode of $S(=O)_2$ stretching is clearly seen in the spectra of piroxicam and the physical mix, however, in the complex, the vibration signal of $S(=O)_2$ clearly decreases suggesting an interaction of PC with β-CD during complex formation. Based on the characterization of the spectra, it is highly possible that piroxicam molecules are encompassed in the cyclodextrin cavity [14, 16]. This inclusion then reduces the flexibility and hinders the vibration of the aromatic ring.

Effect process parameter

The influence of temperature on the reaction was investigated and is shown in Figs. 5A, 5B. The reaction yield increased with increasing the temperature. However, when the reaction temperature was further increased, a dark colour residue formed beside the bright suspension. The dark-coloured product was insoluble in water. For other later investigations, the reaction temperature was chosen from 110-130°C. Pressure was built up inside the reactor due to the occupancy of SC-CO_2 over all the reactor's volume. The pressure rapidly increased with increase in temperature. The influence of pressure on the reaction yield is shown in Figs. 5A, 5C. When the reaction without using SC-CO_2 was carried out and the reaction temperature was 120°C, the observations did not indicate increased pressure on the pressure gauge. At the same time, the yield of the reaction was rather low. However, in the presence of SC-CO_2, the reaction yields dramatically increased. Further, when the pressure was controlled in the range of 200 to 300 bar, reaction yields slightly increased. This result was quite consistent with the reported work in [7]. The influence of the β-cyclodextrin-to-piroxicam molar ratio in the starting materials on the reaction yield is shown in Figs. 5B, 5C. When increasing the amount of cyclodextrin from a ratio of 1.0 to 2.0, the yield almost increases further. This result reveals that there is a high possibility that PC complexation with piroxicam was successful, where piroxicam is contained within a cyclodextrin cavity.

Table 3. Box-Behnken design for inclusion PC complex and the observed yield for each experiment.

Source	Sum of squares	df	Mean square	F value	p-Value
Model	8,327.88	9	925.32	71.24	<0.0001
A-Temperature	1,212.29	1	1,212.29	93.34	<0.0001
B-Pressure	1,018.58	1	1,018.58	78.42	<0.0001
BCD:PC	1,410.60	1	1,410.60	108.60	<0.0001
AB	210.25	1	210.25	16.19	0.0050
AC	74.65	1	74.65	5.75	0.0477
BC	33.35	1	33.35	2.57	0.1531
A^2	284.76	1	284.76	21.92	0.0023
B^2	2,711.39	1	2,711.39	208.75	<0.0001
C^2	1,005.88	1	1,005.88	77.44	<0.0001
Residual	90.92	7	12.99		
Lack of fit	53.76	3	17.92	1.93	0.2666
Pure error	37.16	4	9.29		
Cor total	**8,418.80**	**16**			

Optimization of inclusion complex

RSM and Box-Behnken experimental design were used in this work to analyse the data when applying process parameters to an inclusion complex using SC-CO_2. The yield of the complex formation obtained in each experiment is presented in Table 2. Temperature (X_1), pressure (X_2) and piroxicam/β-cyclodextrin ratio (X_3) are presented as independent variables in the second-order equation of Eq. (2):

$$\% \text{ Yield} = 84 + 12.31X_1 + 11.2838\ X_2 + 13.2788\ X_3 + 7.25X_1X_2 + 4.32X_1X_3 + 2.8875X_2X_3 - 8.22375X_1^2 - 25.3762X_2^2 - 15.4562X_3^2 \quad (2)$$

The analysis of variance (ANOVA) was used to confirm the model's reliability. Table 3 illustrates the results of supercritical inclusion on the quadratic model. Because $p<0.001$, the model is statistically significant. The lack-of-fit value of the model was 0.2666 and the coefficient of determination (R^2) and its adjusted value (R^2 adjusted) were 0.9753 and 0.8909, respectively, which point out the similarity between the experimental data and the model. Obviously, this model was proven to be a proper means of estimating the yield of the product. Moreover, the significance of the different terms belonging to the model can be identified using an ANOVA table. As shown in Table 3, the linear terms of temperature, pressure, and ratio of β-CD/PC are highly significant ($p<0.001$). The effects of process parameters are factors that have been optimized for further study.

Figure 5 shows 3D and contour plots that illustrate the interactive influence of different variables on the yield of complex formation. Fig. 5A illustrates that both negative

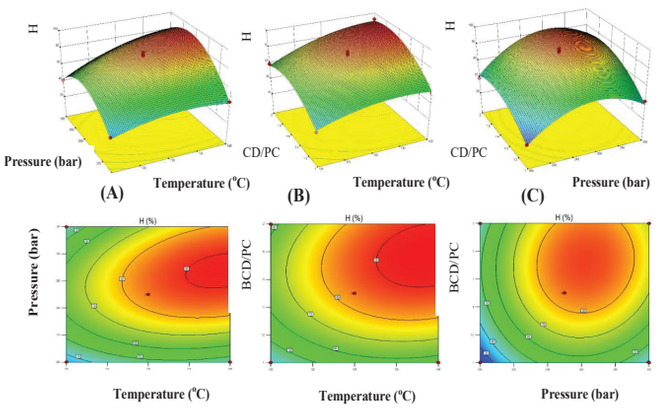

Fig. 5. 3D (top) and contour (bottom) representation of response surface method for supercritical inclusion yield demonstrates the interactive effects of (A) temperature and pressure; (B) temperature and *β*-CD/PC ratio; (C) pressure and *β*-CD/PC.

and positive effects of pressure and temperature are retained on the yield throughout the studied range. Figs. 5B, 5C demonstrated the interactive effects of temperature and pressure with *β*-CD/PC ratio. In these figures, despite the similarity of the impacts of temperature, pressure, and *β*-CD/PC ratio, the *β*-CD/PC ratio is not a significant factor and its effect on yield is relatively lower than the two other variables. The crossed effect of pressure and *β*-CD/PC ratio was the only interaction term that was significant. The rising trend of inclusion yield related to supercritical temperature is shown in Fig. 5B to be outstanding at higher levels of *β*-CD.

Surveying the results of RSM and Design Expert optimization, the ideal condition was a temperature of 136.5°C, pressure of 266 bar, and *β*-CD/PC ratio at or below 1.84 where the reaction yield was proved to reach to 95.5%. By repeating the process at these optimum conditions, an inclusion yield of about 95% was achieved, which suggests the model's validity and precision.

Conclusions

The liquid dissolution of hydrophobic drugs has been shown to improve by making complexes with *β*-cyclodextrin. Specifically, SC-CO_2 was used in this experiment to prepare an inclusion complex of PC with *β*-CD. SC-CO_2 was pressurized to submit the powder through a fixed procedure. L-lysine was used as a ternary agent in cooperation with a considerable amount of water and a temperature above 130°C, which were combined to achieve a high inclusion yield. Finally, a complete inclusion was achieved after 3h at 266 bar and 136°C with a 1.84 and 1.5 molar ratio of *β*-CD and L-lysine, respectively. The characterization of the complexation product was studied by UV-Vis, FTIR, and DSC. The aqueous dissolution rate of the ternary-complex made in SC-CO_2 was determined to be significantly higher than that of the physical mixture of the components by more than 30.4 times, which has a high potential to bring this complexation product to practical application.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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