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Research Article

Experimental Design to Predict Process Variables in the Microcrystals of Celecoxib for Dissolution Rate Enhancement Using Response Surface Methodology

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Abstract

Purpose: The purpose of the present investigation was to increase the solubility and dissolution rate of celecoxib (CLX) by preparing microcrystals of drug by solvent change precipitation.

Methods: This procedure was optimized in order to obtain stable and homogeneous particles with a small particle size, high yield and fast dissolution rate. CLX agglomerates were prepared with brij35 (stabilizer agent) using acetone as solvent, water as non-solvent, respectively. The agglomerates were characterized by DSC, XRD, FTIR studies. A full-factorial design was employed to study the effect of independent variables, the amounts of stirring rate (X1), volume of organic solvent (X2), volume of aqueous solvent (X3), time of stirring (X4), concentration of Brij (X5), concentration of Tween 80 (X6), concentration of HPMC (X7) on dependent variables, particle size (PS), drug content (DC), drug released after 15 min (Q15), crystal yield (CY), Gibbs free energy change (ΔG°tr), antalpy change (ΔH) and saturated solubility (Ss).

Results: The DSC and FTIR results indicated the absence of any interactions between drug and stabilizers. These studies showed a decrease in crystalinity in agglomerates. The crystals exhibited significantly improved micromeritic properties compared to pure drug. The drug content and crystal yield were in the range of 32.84-48.22% and 64.55-83.33% with all formulations, respectively. The solubility and drug release rates increased with an increase in concentration of stabilizer.

Conclusion: The results show that microcrystals of the drug in stabilizer considerably enhanced the dissolution rate.

Introduction

Micronization is the common method to increase specific surface area. Various types of mechanical mills are used to reduce the large crystals into smaller. Micronization means transfer of the coarse drug powder to an ultrafine powder with a mean particle size being typically in the range of 2-5 µm, size distributions normally ranges from approximately 0.1 to 25 µm. It is basically applied to class II drugs of the BCS classification system, i.e. drugs having a good permeability but a low bioavailability due to their poor solubility and low dissolution velocity. But, very recently micronization doesn't lead to a very effective increment in the bioavailability.[1]

The bioavailability intrinsically related to drug particle size. By reducing particle size, increased surface area improves the dissolution properties. In this method, typically, low excipient to drug ratios is required. Formulations are generally well tolerated provided that strong surfactants are not required for stabilization. Generally, crystal forms are chemically and physically more stable than amorphous particles.

There are certain disadvantages for the micronization caused by mills. Few of them include extreme inefficiency in reducing size disruption in crystal lattice which may lead thermodynamic imbalance and thus recrystalize when it absorbs atmospheric moisture.[2] Microcrystals can be produced using milling by high pressure homogenizer. The drug crystals with a starting size as small as possible are suspended in an aqueous stabilized surfactant solution.[3,4]

Response surface methodology is a useful tool in the development and optimization of controlled release microcrystals.[5] Different steps involved in response surface methodology include experimental design, regression analysis, constraint optimization and validation. Response surface methodology (RSM) is widely practiced approach in the development and optimization of drug

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delivery devices. Based on the principle of design of experiments (DoEs), the methodology encompasses the use of various types of experimental designs, generation of polymonal equations, and mapping of the response over the experimental domain to determine the optimum formulation(s).[5] The technique requires minimum experimentation and time, thus proving to be far more effective and cost-effective than the conventional methods of formulating dosage forms.

Celecoxib (CLX), a non-steroidal anti-inflammatory drug (NSAID), is the first selective cyclooxygenase-2 inhibitor used in the treatment of osteoarthritis and rheumatoid arthritis in adult patients.[6] CLX exhibits poor flow and compression characteristics and is hence a suitable candidate for *in situ* micronization process to improve the flow properties and compressibility. Also, CLX shows incomplete and poor oral bioavailability due to low aqueous solubility.[7] Hence, the improvement of aqueous solubility in such a case is a valuable goal to improve therapeutic efficacy.[8]

CLX is a highly permeable drug that can be absorbed throughout the GI tract and that dissolution may be a rate-limiting factor for absorption from solid dosage forms. Although these changes would likely result in delayed drug absorption due to delayed gastric emptying, the longer gastric residence time would allow more time for dispersion and dissolution of a poorly soluble, lipophilic drug such as CLX thereby increasing the extent of absorption.

In the present investigation, the effect of factors (RPM, volume of solvent of CLX, volume of non-solvent of CLX, time and rate of stirring, type and concentration stabilizer) that can influence the drug dissolution rate, particle size, crystal yield, and dissolution efficiency of microcrystals CLX was investigated.

Materials and Methods

CLX was obtained from Jaber Ebne Hyane Pharmaceutical Company, Iran, Hydrxypropylmethyl Cellulose (HPMC) low viscosity (Merck, Darmstadt, Germany), Brij 35, Tween 80 and acetone, sodium lauryl sulfate (SLS) (Merck, Darmstadt, Germany) were used.

Micronization

Preparation of microcrystals

In preliminary studies, three different stabilizing agents (HPMC and Brij 35, Tween 80 and HPMC) with different concentrations of CLX (0.5–1g) and stabilizers (0.05–0.1%) were studied by a full factorial design to give the most stable microcrystals with the least particle size.[9,10] Details of the preparation methods of microcrystals are reported previously.[9] Briefly, in solvent-change method, 0.75 g of CLX was dissolved in 15 ml of acetone. One hundred milliliters of water (as the non-solvent of CLX) containing 0.1% of Brij35 (as stabilizer) was poured rapidly into the drug solution under stirring at 26,000 rpm by an ultra-homogenizer (Heidolph Silent Crusher M, Germany) at ice bath temperature. A micron-sized dispersion was formed spontaneously. The mixture was allowed to be mixed for further 15 min, and then aqueous suspension was freeze dried (Christ ALPHA-4, Germany).[9]

Micromeritic Properties

The microcrystals size analysis was performed by laser light scattering particle size analyzer (SALD-2101, Shimadzu, Japan). Samples were suspended in distilled water contained in a 1 cm cuvette and stirred continuously during the particle size analysis. The particle size distribution of the microspheres for all formulations was determined and the results were the mean of three determinations.

Solubility study

The solubility of CLX in various organic solvents was measured by adding excess amount of drug to 10 ml of solvents. The resulting drug suspension was agitated for 24 h at 37°C in glass vials sealed with Teflon-lined closure. These samples were centrifuged and filtered (0.45 µ nylon filter millipore). An aliquot of the filtrate was weighted and diluted appropriately for UV spectrophotometer (Shimatzu-160, Japan) measurement. The Gibbs-free energy of change (ΔG°tr°) of microcrystal occurred during formation of microcrystals from un-treated CLX powder was calculated using equation 1.

$$\Delta G^{\circ}_{tr} = -2.303RT \log \frac{S_0}{S_s} \qquad (1)$$

Where S_0/Ss, is the ratio of the molar solubility of CLX microcrystals in distilled water to that of the untreated CLX powder. R is gas constant (8.31 J $k^{-1}mol^{-1}$) and T is temperature (310.15°K).

Determination of crystal yield

The crystal yield of the microcrystals was determined by calculating accurately the initial weight of the raw materials and the final weight of the polymeric particles obtained. All the experiments were performed in triplicate (Table 1).

Table 1. Effect of drug concentration on the drug content, production yield and particle size and flowability characteristics of CLX microcrystal formulations

Formulation code	Production Yield (%)	Drug Content (%)	Mean particle Size (µm)	Bulk Density (g/cm^3 ± SD)	Tapped Density (g/cm^3 ± SD)	Carr's index (%±SD)	Hausner ratio(±SD)	Angle of repose (°θ ±SD)
F_1	73.33±7.3	2.84±46.10	7.11±0.55	0.069±0.00	0.100 ±0.00	35.00±0.02	11.54 ± 0.0	29.68±0.71
F_2	76.55±5.2	46.55±1.89	7.31±0.52	0.080±0.00	0.099 ±0.00	19.19 ± 0.01	1.14 ± 0.01	30.54 ±0.84
F_3	74.71±6.2	48.22±5.01	7.73±0.56	0.055±0.01	0.070 ±0.01	21.43 ± 0.01	1.27 ± 0.00	30.40 ±0.89

X-ray powder diffractometry (X-RPD)

X-ray diffraction analysis was performed with an apparatus (Siemens D5000, Munich, Germany), using nickel-filtered CuKα radiation (a voltage of 40 KV and a current of 20 mA). The scanning rate was 2° /min over a range of 20-70° and with an interval of 0.02°. Each measurement was repeated three times.

Fourier transforms infrared spectroscopy (FT-IR)

A computerized FT-IR (Bomem, Quebec, Canada) was used to obtain the spectra of various CLX samples. The microcrystal sample (about 10 mg) in potassium bromide discs (0.5% w/w) was placed on the plate of the machine and the handle was placed on the powder sample to generate enough pressure for compression. The spectrum for each sample showed the wavelength of absorbed light, which is a characteristic of the chemical bonds in the sample. The scanning range was 400-4000 cm^{-1} and the resolution was 1 cm^{-1}.

Differential scanning calorimetry (DSC)

A differential scanning calorimeter (DSC 60, Shimadzu, Japan) was used to monitor the thermal events during heating. The DSC was calibrated by the melting point of aluminium oxide (2072°C) standard. Samples weighing 5 mg were placed in open aluminium pans and heated from 30 to 300° C at a rate of 10°C per min. Nitrogen gas was purged at a flux rate of 10 ml/min. The onsets of the melting points and enthalpies of fusion were calculated.

Flow property study

The bulk density and tapped bulk densities were determined by using density apparatus (the apparatus was locally assembled). The Carr's index (%) and the Hausner's ratio were calculated by using CLX. The angle of repose of untreated CLX powder and the microcrystals were assessed by fixed funnel method (Table 1).

In vitro release studies

The *in vitro* release studies of drug-loaded microcrystals were carried out at 37°C in 500 mL of distilled water contain 1%, *m*/*V* sodium lauryl sulfate *(*SLS). Each batch of microcrystals containing 100 mg of drug was individually added to 500 mL of dissolution medium in flask. The dissolution media was stirred at 75 rpm according to USP basket method. Three mL of samples were withdrawn at regular time intervals and the same volume of fresh medium was replaced. After suitable dilution, the drug content of each sample was estimated by using a UV spectrophotometer analysis at 277.6 nm, respectively. Each experiment was repeated three times.

In order to have a better comparison between different formulations dissolution efficiency (DE), t_{50}% (dissolution time for 50% fraction of drug); and difference factor, f_1 (used to compare multipoint dissolution profiles) were calculated and the results are listed in Table 2. DE is defined as the area under the dissolution curve up to a certain time, *t*, expressed as a percentage of the area of the rectangle arising from 100% dissolution in the same time. The areas under the curve (AUC) were calculated for each dissolution profile by the trapezoidal rule. DE can be calculated by the following:

$$\mathrm{DE} = \int y \, \frac{dt}{100t}$$

Where *y* is the drug percent dissolved at time *t*. All dissolution efficiencies were obtained with *t* equal to 1440min.The *in vitro* release profiles of different micrystals formulations were compared with physical mixture formulation using difference factor (f_1), as defined by:[11]

$$f_1 = \frac{\{[\Sigma_t = 1^n |R_t - T_t|]}{[\Sigma_t = 1^n R_t]\}} \times 100$$

Where *n* is the number of time points at which % dissolved was determined, R_t is the % dissolved of one formulation at a given time point and T_t is the % dissolved of the formulation to be compared at the same time point. The difference factor fits the result between 0 and 15 when the test and reference profiles are identical, and approaches above 15 as the dissimilarity increases.

Table 2. Comparison of various release characteristics of CLX from different microcrystal formulations, untreated CLX powder and commercial® capsules

Formulations	Rel $_{15}$[a](%±SD)	Rel $_{30}$[b](%±SD)	Rel $_{60}$[c](%±SD)	DE[d]	t $_{50\%}$[e](min)	f_1[f]
F_1	1.39 ± 35.65	0.48±39.65	59.29±0.79	40.10	19.42	15>
F_2	38.26±1.15	1.15± 45.78	61.99±0.48	44.84	16.60	15>
F_3	0.77±54.21	0.58±61.46	72.68±3.64	56.30	13.53	15>
Cap celecoxib®	6.62±0.67	21.54±1.13	43.01±0.53	22.36	28.81	15>
Untreated celecoxib	1.84±0.34	4.47±0.88	14.62±1.37	5.94	35.61	0

[a] Rel_{15} = amount of drug release after 15 min; [b] Rel_{30} = amount of drug release after 30 min; [c] Rel_{60} = amount of drug release after 60 min ;[d]DE = dissolution efficiency; [e]t 50% = dissolution time for 50% fractions; [f] f_1 = Differential factor ($0<f_1<15$).

Optimization of the CLX microcrystals

The experimental design was a modified Box-Behnken design for seven variables. This design was suitable for exploring quadratic response surfaces and constructing second-order polynomial models. Response surface methodology (RSM) is a very useful statistical technique for optimization of CLX formulations. In this design 7 factors were evaluated, each at 3 levels, and

experimental trials were performed at all 17 possible combinations. The rate of stirring (X_1), volume of organic solvent (X_2), dispersing medium (volume of aqueous solvent) (X_3), time of stirring (X4), concentration of stabilizer (brij) (X_5), concentration of stabilizer (Tween 80) (X_6) and concentration of e stabilizer (HPMC) (X_7) were selected as independent variables. The particle size (PS, Y_1), drug content (DC, Y_2), amount of drug release after 15 minute (Q_{15}, Y_3), crystal yield (CY, Y4), Gibbs free energy change (ΔG°, Y_5), enthalpy change (ΔH, y_6) and saturated solubility (Ss, Y_7) were dependent variables (Table 3).

Table 3. Effect of agitation speed, volume of solvent, volume of non-solvent, time and rate of stirring, type and concentration stabilizer on particle size (PS), drug content (DC), amount of drug release after 15 minute (Q_{15}), crystal yield (CY), Gibbs free energy change (ΔG_{tr}), enthalpy Change (ΔH) and saturated solubility (Ss) of CLX microcrystals

Formulation code	Variable levels in coded form							PS(µm)	DC(%)	Q_{15}(%)	CY(%)	ΔG_{tr} ($Jmol^{-1}$)	ΔH (J/g)	S_s (mg/ml)
	X1	X2	X3	X4	X5	X6	X7							
F_2	26000	15	100	10	0.1	0	0	7.31	46.55	38.26	66.55	-2933.12	-54.52	1.56
F_{2-1}	26000	10	100	10	0.1	0	0	7.33	25.99	30.06	57.65	-2492.96	-41.71	1.29
F_{2-2}	26000	20	100	10	0.1	0	0	10.31	43.70	4.27	82.35	-2911.55	-86.57	1.55
F_{2-3}	26000	15	50	10	0.1	0	0	8.15	13.18	0.60	61.18	-2191.53	-82.72	1.17
F_{2-4}	26000	15	150	10	0.1	0	0	8.34	26.70	8.39	72.94	-2521.94	-29.15	1.33
F_{2-5}	26000	15	100	10	0.05	0	0	7.9	29.76	1.78	51.25	-2463.13	-60.84	1.3
F_{2-6}	26000	15	100	10	0.15	0	0	5.75	42.92	38.26	80	-2916.54	-48.60	1.54
F_{2-7}	26000	15	100	5	0.1	0	0	8.88	26.23	1.55	61.25	-2672.51	0.716	1.41
F_{2-8}	26000	15	100	15	0.1	0	0	13.82	28.62	36.69	66.25	-2866.16	-10.73	1.52
F_{2-9}	20000	15	100	10	0.1	0	0	4.99	26.49	27.37	57.65	-2789.75	-18.52	1.47
F_{2-10}	14000	15	100	10	0.1	0	0	4.30	19.21	4.43	60	-2032.49	-3.87	1.10
F_{2-11}	26000	15	100	10	0	0.05	0	7.53	17.45	6.06	90.59	-2382.57	-80.98	1.26
F_{2-12}	26000	15	100	10	0	0.1	0	7.84	27.33	1.6	67.78	-2443.22	-19.18	1.29
F_{2-13}	26000	15	100	10	0	0.15	0	4.27	18.58	7.3	73.75	-2341.32	-11.25	1.24
F_{2-14}	26000	15	100	10	0	0	0.05	6.89	22.22	2.18	60	-2101.86	-52.63	1.13
F_{2-15}	26000	15	100	10	0	0	0.1	5.08	15.43	19.88	45.56	-2101.85	-45.11	1.13
F_{2-16}	26000	15	100	10	0	0	0.15	7.26	28.08	1.11	98.75	-2008.95	-67.09	1.09
F_{2-17}	26000	15	100	10	0	0	0	9.31	8.03	0.29	76	-512.60	-1.58	0.61

Formulations contained 750 mg CLX and 100 mg Brij 35. 100 ml water, 15 ml acetone, †X_1 is RPM and; X_2 is volume of organic solvent; X_3 is volume of non-solvent; X_4 is time of stirring; X5, X6 and X7 is concentration of stabilizing agents as Brij 35, HPMC and Tween 80. PS particle size; DC drug content; $Q_{15\%}$ indicates drug release after 15 min; CY indicates crystal yield; ΔG° indicates Gibbs free energy change; ΔH indicates enthalpy change; Ss indicates saturated solubility.

X_1 = RPM, X_2 = volume of organic solvent, X_3 = dispersing medium (volume of aqueous solvent), X4 = time of stirring

X5 = concentration of stabilizer (brij), X6= concentration of stabilizer (Tween 80), X7= concentration of e stabilizer (HPMC), PS= particle size, DC = drug content, Q_{15}= amount of drug release after 15 minute, CY= Crystal yield, ΔG_{tr} = Gibbs free energy change, ΔH = enthalpy change, Ss = saturated solubility

Various batches of the selected formulation (F_2) were made, but the stirring rate was the only parameter that was varied between 14000, 20000 and 26000 rpm. Concentration of stabilizers (as brij, HPMC and Tween 80) was shifted between 0.05, 0.075, 0.1 g. Also, while keeping the other parameter constant, time of homogenizer stirring was changed (5, 10 and 15 min). Volume of organic solvent and dispersing medium was changed 5-15 ml and 50-150ml, respectively. After drying, the weighed batch of microcrystals was subjected to particle size, drug content, amount of drug release after 15 minute, crystal yield, Gibbs free energy change, enthalpy change and saturated solubility experiments. The influence of process variables on microcrystals formation, micromeritics and drug release characteristics was investigated.

Regression analysis

The targeted response parameters were statistically analyzed by applying one-way ANOVA at 0.05 levels. Individual response parameters were evaluated using the *F*-test and quadratic models of the form given below

were generated for each response parameter using the multiple linear regression analysis.[12]

Results

CLX microcrystals were prepared by solvent change method using Brij 35 as a hydrophilic stabilizing agent. Solvent ratio (acetone/water) 1:6.7 and 0.1% Brij 35 were optimum parameters for microcrystallization of CLX. Drug was dissolved in organic solvent and then non-solvent was poured rapidly into the drug solution under stirring by an ultrahomogenizer. Microcrystals produced using Brij 35 showed narrow particle size distribution and change in the crystal habit. Higher crystal yield 76.55% was obtained in case of CLX microcrystals prepared using 0.1% Brij 35 (F_1 formulation with 1g CLX) (Table 1).

Measurement of powder flow

According of Table 1, angle of repose for microcrystals was between 29.68° to 30.54°, thus indicating poor flow property for microcrystals. The findings were supported by Carr's (compressibility) index, which was > 20 indicating poor flow characterizes (F_1, F_2 and F_3) (Table 1).

The physical state of the CLX in the Brij 35was studied by classical techniques such as DSC,

XRPD and FTIR. DSC analyses were carried out for CLX, Brij 35, microcrystals (F_1, F_2 and F_3) and physical mixtures (Figure 1). Any abrupt or drastic change in the thermal behavior of rather the drug or polymer may indicate a possible drug-polymer interaction (9). CLX and Brij 35 exhibited crystalline state (Figure 1). In the thermogram of the microcrystals containing Brij 35, endothermic peaks at 155.80°C (F_1), 158.01°C (F_2) and 158.41°C (F_3) corresponds to the melting point of CLX.

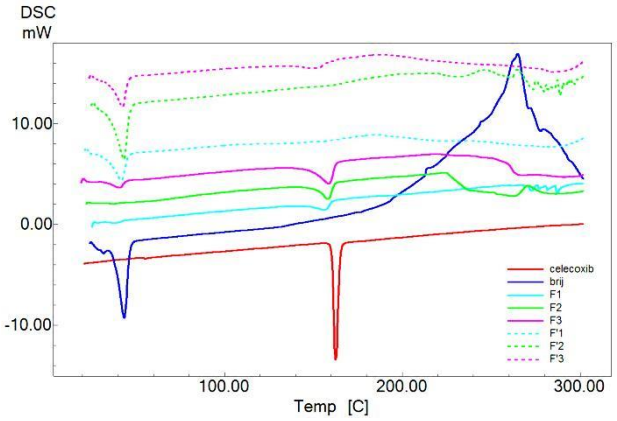

Figure 1. DSC thermogram of CLX powder; Brij35; microcrystals of F_1 , F_2, F_3 and physical mixtures of F'_1, F'_2 and F'_3, respectively.

The X-ray diffraction patterns of pure CLX and Brij 35 showed that the pure drug is crystalline in nature nearly 16 to 20 at 2θ scale (Figure 2). XRPD is a powerful tool to identify any changes in crystallinity of the drug. When the microcrystals were prepared with different drug/stabilizer agent ratios (F_1, F_2 and F_3) it is clear that the samples with lower drug concentration showed similar peaks as the physical mixture.

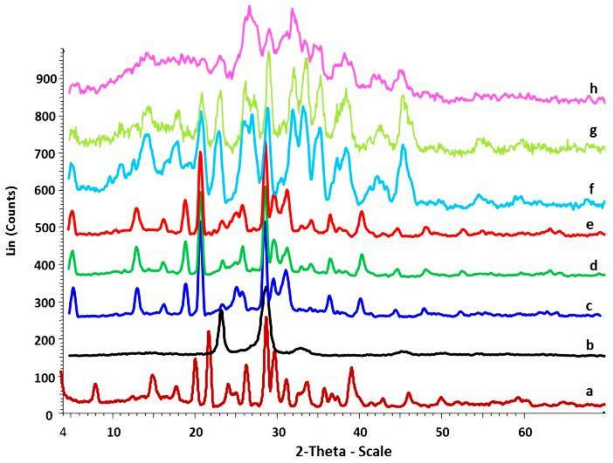

Figure 2. Thermogram of CLX powder (a); brij35 (b); microcrystals of F_1 (c) , F_2 (d), F_3 (e) and physical mixtures of F'_1 (f), F'_2 (g) and F'_3 (h), respectively.

In order to investigate any changes in molecular levels of CLX during the micronization process, FT-IR spectrum was obtained for all formulations and their spectra are shown in Figure 3. FTIR spectra are assigned as follows: (1) CLX: first type amine at 341.51 cm^{-1}, S=O stretching asymmetric band at 1164.58 cm^{-1} and S=O stretching symmetric band 1347.75 cm^{-1}; CF3 band at 1229.99, 1274.96 cm^{-1}, (2) microcrystals F_1, F_2 and F_3: first type amine at 3343.3 cm^{-1}, S=O stretching asymmetric band at 1133.4 cm^{-1} and S=O stretching symmetric band 1345.76 cm^{-1}; CF3 band at 1257.2, 1202.8 cm^{-1} ,respectively (Figure 3). After CLX was micronized and covered with brij, the characteristic peaks for CLX showed by the smaller intensity peaks than pure drug and stabilizer agent. On the other hand, the S=O stretching bands of CLX in microcrystal systems were merged, thus leading to a peak shifting from 1164.58 cm^{-1} to 1133.4 cm^{-1}.

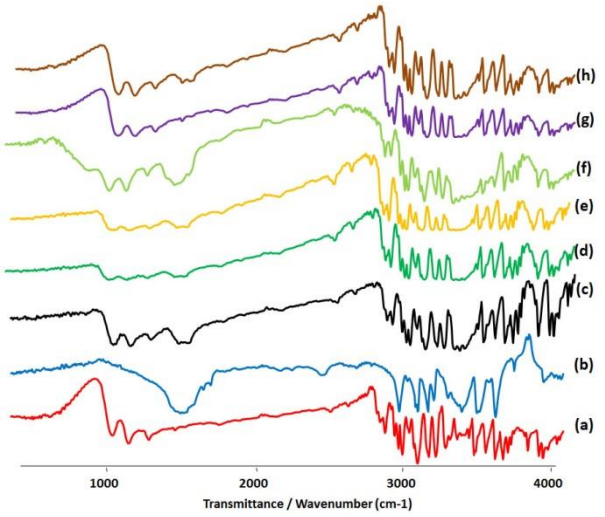

Figure 3. FTIR thermogram of CLX powder (a); brij35 (b); microcrystals of F_1 (c) , F_2 (d), F_3 (e) and physical mixtures of F'_1 (f), F'_2 (g) and F'_3 (h), respectively.

The micronized CLX formulations showed a dramatic enhancement in dissolution rate than CLX powder untreated as illustrated in Figure 4. DE of the studied microcrystals in comparison with the pure CLX powder and commercial capsule CLX was presented in Table 2. Results suggested that dissolution profile of microcrystals was significantly differ ($p<0.05$) from untreated CLX powder. CLX crystals prepared without stabilizing agent did not show significant improvement in drug release when compared with pure CLX drug powder (Figure 4).

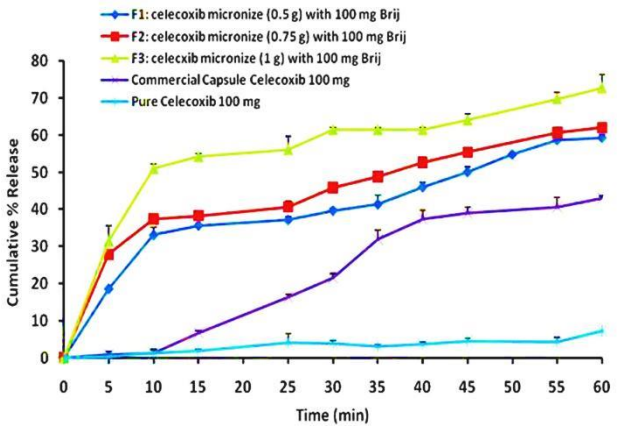

Figure 4. Cumulative percent release of CLX from microcrystals, untreated CLX powder and commercial capsule.

Higher drug release (38.26% for F_2 formulation) after 15 min obtained in case of 0.1% Brij 35 as compared to CLX microcrystals prepared using other concentration of Brij 35 (0.05 and 0.075g). Results of drug release was observed highly significant difference for CLX microcrystals prepared using 0.1 g of Brij 35 with different amounts of drug as compared to untreated CLX powder and commercial capsule CLX (100 mg) ($p<0.05$).

According of Table 2 and 3, CLX microcrystals prepared using 0.1% Brij 35 as stabilizing agent showed small particle size (7.31 µm), higher crystal yield (66.55%), high water solubility (1.56 mg/ml), great reduction in Gibb's free energy (-2933.12 $Jmol^{-1}$), and higher dissolution efficiency (44.84%) as compared to other CLX microcrystals. Therefore, 0.1% concentration was optimum concentration of Brij 35 for microcrystallization of CLX.

The tendency of the solid phase to exhibit solubility is best described by the Gibbs free energy change ($\Delta G^{\circ}tr$). Negative Gibbs free energy values indicate favorable conditions. The $\Delta G^{\circ}tr$ values were all negative at various concentrations, thus indicating that CLX microcrystals had higher aqueous solubility. These values decreased with increasing concentration of Brij 35 up to 0.1 g, thereby demonstrating that drug solubility increased as the concentration of Brij 35 increased up to 0.1 g.

F_1, F_2 and F_3 microcrystals showed higher dissolution efficiency i.e. rapidly dissolution in comparison to respective CLX powder (untreated) and commercial capsule of CLX ($p< 0.05$), (Table 2). According of Table 2 the lowest DE was observed for F_1 (40.10%) and dissolution efficiency of CLX powder (untreated was 5.94% ($p< 0.05$). The value of $t_{50\%}$ varied between 13.53 (F_3 formulation) to 19.42 min (F_1 formulation). The results of difference factor (f_1) showed that the release profile of microcrystals formulations is different from that of CLX powder (untreated) and commercial capsule of CLX (Table 2).

The independent variables and their levels were selected based on the preliminary trials undertaken. The PS, DC, Q_{15}, CY, $\Delta G°$, ΔH and Ss for the formulations (F_1 to F_{17}) showed a wide variation. The data clearly indicated that the PS, DC, CY, $\Delta G°$ and Ss values are strongly dependent on the selected independent variables. The fitted equations (full and reduced) relating the responses PS, DC, Q_{15}, CY, $\Delta G°$, ΔH, Ss and %F to the transformed factors are shown in Table 3. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries (i.e., positive or negative). Table 4 & 5 shows the results of the analysis of variance (ANOVA), which was performed to identify insignificant factors. The high values of correlation coefficient for PS, DC, CY, $\Delta G°$ and Ss indicate a good fit i.e., good agreement between the dependent and independent variables. The equations may be used to obtain estimates of the response as a small error of variance was noticed in the replicates. The significance test regression coefficient was performed by applying the student F test.

The results of statistical analysis are shown in Table 4. The coefficients b_1, b_2, and b_{11} were found to be significant at $p < 0.05$; hence they were retained in the reduced model.

The reduced model was tested in portions to determine whether the coefficients b_{12} and b_{22} contribute significant information for the prediction of PS, DC, CY, $\Delta G°$ and Ss or not. The results for testing the model in portions are. The results of multiple linear regression analysis (response surface regression) reveal that, on increasing the amount of rate of stirring, volume of organic phase, volume of aqueous phase, volume of aqueous phase, time of stirring, type of stabilizer agent (Brij 35, Tween 80 and HPMC) decreased PS is observed; the coefficients b_1 to b_7 are negative sign. The results of statistical analysis are shown in Table 4. The reduced model was tested in portions to determine whether the coefficients b_{11}, b_{12}, and b_{22} contribute significant information for the prediction of %F or not. The results for testing the model in portions are depicted in Table 4.

Hence, conclusions can be drawn considering the magnitude of the coefficient and the mathematical sign (positive or negative) it carries. According to Figure 5, particle size is dependent on major independent factors such as RPM, volume of aqueous solvent and i.e.

Table 4. Model equations obtained from response surface regression analysis

Term	Equation of regression coefficients
PS versus X1, X2, X3, X4, X5, X6, X7	PS = 38.26 - 5.36X1 -1.81X2 – 0.09X3 – 2.93X4 – 17.86X5 – 4.96X6 – 76.78X7 + 0.07X2X2 + 0.00047X3X3 + 0.17X4X4 – 26.53X5X5 – 168.53X6X6 + 421.25X7X7
DC versus X1, X2, X3, X4, X5, X6, X7	DC = 47.98 + 12.42X1 + 8.85X2 + 6.76X3 + 1.19X4 + 17.72X5 + 5.65X6 + 8.83X7 + 5.14X1X1 – 9.21X2X2 – 24.12X3X3 – 16.63X4X4 – 13.64X5X5 – 9.49X6X6 – 1.90X7X7
Q_{15} versus X1, X2, X3, X4, X5, X6, X7	Q_{15}= 45.93 + 12.19X1 – 12.89X2 + 3.89X3 + 17.57X4 + 23.37X5 + 5.32X6 + 4.14X7 – 10.74X1X1 – 11.64X2X2 – 24.31X3X3 – 9.69X4X4 – 2.61X5X5 – 2.61X5X5 – 3.58X6X6 – 13.01X7X7
PY versus X1, X2, X3, X4, X5, X6, X7	PY = 16.24 – 5.83X1 + 12.35X2 + 5.88X3 + 2.5X4 – 1.71X5 – 6.10X6 + 6.39X7 – 3.48X1X1 + 21.67X2X2 + 18.73X3X3 + 15.42X4X4 + 36.24X5X5 – 2.76X6X6 + 41.01X7X7
ΔG°_{tr} versus X1, X2, X3, X4, X5, X6, X7	ΔG_{tr} = -1749.97 – 528.36X1 – 209.29X2 – 165.20X3 – 96.82X4 – 1173.25X5 – 868.46X6 + 1196X7 + 228.90X1X1 + 386.95X2X2 + 732.47X3X3 + 319.87X4X4 + 1154.72X6X6 – 1600.75X7X7
ΔH versus X1, X2, X3, X4, X5, X6, X7	ΔH = -87.18 – 29.28X1 – 22.43X2 + 26.78X3 – 5.73X4 – 14.25X5 + 7.81X6 – 22.92X7 – 14.63X1X1 – 1.70X2X2 + 6.49X3X3 + 57.42X4X4 + 29.22X5X5 + 45.50X6X6 + 9.56X7X7
Ss versus X1, X2, X3, X4, X5, X6, X7	Ss = 2.51 + 0.23X1 + 0.13X2 + .08X3 + 0.55X4 + 0.43X5 + 0.27X6 + 0.20X7 – 0.13X1X1 – 0.15X2X2 – 0.32X3X3 – 0.11X4X4 – 0.37X5X5 – 0.37X6X6 -0.29X7X7

Table 5. Results obtained from the model evaluation

Response/coefficients	DF	SS	MS	F(cal)	R2	p	MPE(%)
PS	14	88.21	6.30	7.27	97.14	0.05	6.29
DC	14	1719.48	122.82	3.13	93.59	0.18	7.48
Q_{15}	14	3246.33	231.881	1.54	87.75	0.40	32.02
PY	14	3173.80	226.70	1.61	88.23	0.38	8.46
ΔG°_{tr}	14	55168743	3440625	22.33	99.05	0.013	8.64
ΔH	14	12099	864.24	1.10	83.75	0.53	28.11
Ss	14	0.88	0.063	7.46	97.21	0.05	5.12

Discussion

According to Noyes–Whitney equation, the dissolution rat is proportional to the surface area exposed to the dissolution medium.[13] In addition to the general solubility enhancement techniques described above; drug particle size reduction has often been used, in regards to the Noyes–Whitney and Ostwald–Freundlich equations, to enhance dissolution of poorly water-soluble compounds.[14]

Disruptions in the crystal lattice can cause physical or chemical instability. Micronized powders with a higher energetic surface show poor flow property and broad size distribution.[15]

The Hausner ratio is correlated to the flowability of a powder or microcrystals. A Hausner ratio greater than 1.25 is considered to be an indication of poor flowability for microcrystals.

XRD, DSC and FTIR studies

The FTIR, DSC, and XRD results showed no chemical interaction between the drug and the stabilizer, and crystalline habit modification has occurred in the microcrystals without any polymorphic changes.

The XRD revealed that crystallinity was reduced significantly in microcrystals. Negative Gibb's free energy change represented higher aqueous solubility of microcrystals. The enhanced dissolution rates attributed to the reduction of the particle size, change in crystal habit, formation of hydrophilic surface and the increased wettability due to adsorption of stabilizer agent and reduction in crystallinity of CLX during microcrystallization.

In vitro studies

Dissolution enhancement effect of CLX microcrystals explained by the reduction in the particle size and as a consequence the increment in the surface area, which is additionally hydrophilized by the adsorbed hydrophilic stabilizing agent. Moreover, the natural crystalline growth creates particles with no electrostatic charge and with better wettability properties.[16]

The *in vitro* release profiles of CLX from microcrystals (Table 2) exhibited initial burst effect, which may be due to the presence of some drug particles on the surface of the microcrystals. In most cases, a biphasic dissolution profile was observed: the initial rapid drug leakage generally ended very early and for the remaining time,

nearly linear behavior was observed. The first portion of the dissolution curves is due to CLX dissolution, which starts immediately after the beginning of the test for the portion of drug on the surface of microcrystals. After such a phase, two phenomena can combine in enhancing in the diffusion of the remaining dispersed drug into the bulk phase as well as the formation of pores within the matrix due to the initial drug dissolution; particle wetting and swelling which enhances the permeability of the microcrystals to the drug (Table 2). Drug release rates were decreased with increasing amounts of drug in the formulation (Table 2). Higher level of CLX corresponding to higher level of the Brij 35 in the formulation resulted in an increase in the drug release rate (F_3). As more drugs are released from the microcrystals, more channels are probably produced, contributing to faster drug release rates. CLX microcrystals of each formulation displayed an immediate and important initial drug release in the first 30 min (22-40%), followed by an 84-87% during 60 min (Table 2).

During the crystal precipitation, surface energy of the system increases. Here, stabilizer agent (Brij 35) adsorbed onto the newly created surface of the precipitated drug in order to lower the interfacial tension. Thereby, the surface energy and consequently the enthalpy of the system are lowered. The formed small particles, which normally would aggregate in order to lower the surface energy, are stabilized sterically against crystal growth by an adsorbed layer of Brij 35. Micron sized particle formed and simultaneously stabilized in the formed dispersion by stabilizer agent.

CLX microcrystals were prepared by solvent change method using Brij 35 as a hydrophilic stabilizing agent. Solvent ratio (acetone/water) 0.1% Brij 35, 15 ml acetone, 100 ml water and 26000 RPM were optimum parameters for microcrystallization of CLX. Microcrystals produced using Brij 35 showed narrow particle size distribution and change in the crystal habit to small crystal type. A volume-based size distribution of drug, stabilizer agent, and drug loaded microcrystals indicated a log–probability distribution.

The results show that linear and interaction components in the proposed model are significant (R^2= 97.14). The optimum condition for CLX microcrystals preparation was 1:7.5 (w/w) drug to stabilizer agent ratio, 0.1 g amount of stabilizer agent, 15 ml (volume of organic phase), 100 ml (volume of aqueous phase), 5 min (time of stirring) and 26000 RPM. RPM, volume of organic phase and aqueous phase and stabilizer agent concentrations were the most effective factors on the particles size, drug content, crystal yield, free energy Gibbs change and solubility (MPE<10%) (Table 5). The results obtained from the predicted model were used to create a contour plot for particle size with rate of stirring and volume of aqueous solvent (Figure 5).

An increase in the rate of stirring, volume of organic phase, volume of dispersing medium, time of stirring and concentration of stabilizer agents leads to a decrease in particle size because the coefficient b_2 bears a negative sign (r^2=97.14, p=0.05 and MPE=6.24%).

An increase in the rate of stirring, volume of organic phase, volume of dispersing medium, time of stirring and concentration of stabilizer agents leads to an increase in drug content because the coefficient b_2 bears a positive sign (r^2=93.95, p=0.18 and MPE=7.48%).

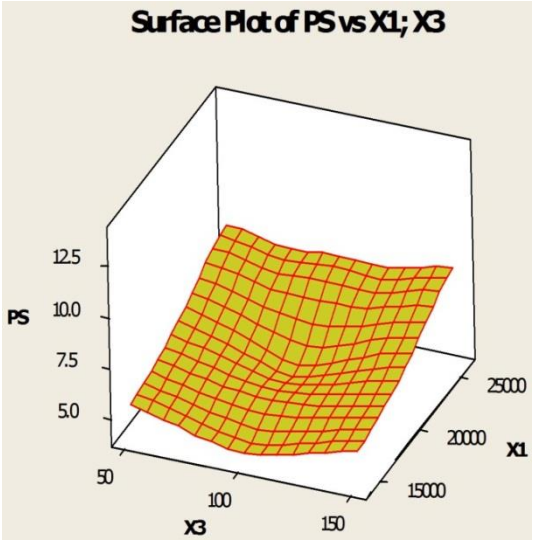

Figure 5. Response surface plot showing the effect of formulation variables (X1=RPM and X3=volume of aqueous phase) on particle size.

An increase in the rate of stirring, volume of dispersing medium, time of stirring and concentration of stabilizer agents (except volume of organic phase) leads to an increase in drug content because the coefficient b_2 bears a positive sign (r^2=87.75, p=0.4 and MPE=32.02%).

An increase in the volume of organic phase, volume of dispersing medium, time of stirring and concentration of stabilizer agent as HPMC (except rate of stirring, concentration of stabilizer agents as Brij 35 and Tween 80) leads to an increase in drug content because the coefficient b_2 bears a positive sign (r^2=88.23, p=0.38 and MPE=8.46%).

An increase in the rate of stirring, volume of organic phase, volume of dispersing medium, time of stirring and concentration of stabilizer agent as Brij 35 and Tween80 (except concentration of stabilizer agents as HPMC) leads to a decrease in drug content because the coefficient b_2 bears a positive sign (r^2=99.05, p=0.013 and MPE=8.64%).

An increase in the rate of stirring, volume of organic phase, volume of dispersing medium, time of stirring and concentration of stabilizer agent as brij35 and HPMC (except concentration of stabilizer agents as Tween80) leads to a decrease in drug content because the coefficient b_2 bears a positive sign (r^2=83.75, p=0.53 and MPE=28.11%).

An increase in the rate of stirring, volume of organic phase, volume of dispersing medium, time of stirring and concentration of stabilizer agents leads to an increase in

drug content because the coefficient b_2 bears a positive sign (r^2=97.21, p=0.05 and MPE=5.12%) (Table 5).

Conclusion

CLX microcrystals were prepared using solvent change method (*insitu* micronization). Stirring speed, time of stirring, volume of solvent, volume of non-solvent and stabilizer agent influenced the characteristics of the microcrystals. The crystal Yield was high for all formulations. It was observed that at higher drug concentration, the mean particle size of the microcrystals is high (p>0.05) but increasing the stirring speed and stabilizer agent resulted in smaller mean particle size of microcrystals. A response surface methodology has been employed to produce CLX microcrystals for oral drug delivery with stabilizer agent by change solvent method. The formulation variables studied exerted a significant influence on PS, DC, Q_{15}, CY, $\Delta G°_{tr}$, ΔH and Ss. The results obtained indicate that response surface methodology can be employed successfully to qualify the effect of several formulation and processing variables thereby minimizing the number of experimental trials and reducing the formulation development cost. In conclusion, the aforementioned technique is a promising tool for effective microcrystal formation during pharmaceutical development in order to increase dissolution rate of poorly water soluble active ingredient.

Ethical Issues

Not applicable.

Conflict of Interest

The authors report no conflicts of interest.

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