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"CENTRAL COMPOSITE DESIGN TO ENHANCE THE SOLUBILITY OF CELECOXIB"

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ABSTRACT

One of the major challenges in pharmaceutical development is the poor dissolution performance of drugs. Celecoxib (CLX) is a poorly water soluble drug with its bioavailability being limited by its poor dissolution. In this study solvent evaporation method by rota evaporator was employed to prepare solid dispersion drug and polymers. Central composite design employed statistical experimental design tool and investigated the combined effect of experimental factors, i.e., % of PVP K30, PVP K90 and % of β-cyclodextrin (BCD) on responses like saturation solubility (SS), dissolution efficiency (DE) and mean dissolution time (MDT). Dissolution profile of physical mixture of drug and polymers was performed, and results so obtained were compare with the results of solid dispersion prepared by solvent evaporation technique. Design of experiment was used in the context of quality by design, which requires a multivariate approach for understanding the multifactorial relationships among experimental factors. Desirability function was used to attain optimization of responses. The theoretical desired goals were not achieved for SS, DE and MDT using drug or physical mixtures, and therefore there was a need of highly efficient technique like solvent evaporation. Predicted values were very close to the experimental values obtained from the optimized formulation, thus it confirmed that the generated mathematical model was valid. Results of dissolution study showed that PVP K30 increases more solubility compare to PVP K90. The results demonstrated the effect of the proposed combined use of BCD, PVP K90 and PVP K30. Validated optimized solid dispersion was characterized by DSC, XRD, SEM and particle size analysis. Characterization results confirmed the formation of partial amorphous drug complex with average particle size 1703.1±996.3 nm.

KEYWORDS: Celecoxib, PVP K30, β-cyclodextrin, solid dispersion, PVP K90.

INTRODUCTION

Celecoxib (CLX), 4-[5-(4-methylphenyl)-3-trifluromethyl-1H-pyrazol-1-yl} benzenesulfon- amide is a cycloxygenase-2 (COX-2) inhibitor and widely used as analgesic and anti-inflammatory (NSAID) agent in the treatment of osteoarthritis, rheumatoid arthritis, familial adenomatous polyposis (FAP), primary dysmenorrhea and dental problems.[1,2,3]

As CLX is having poor water solubility and high permeability, it belongs to Class -2 drugs of biopharmaceutical classification system (BCS). Poor water soluble drugs are difficult to develop into therapeutically effective pharmaceutical formulations, as they usually exhibit poor and variable bioavailability because of poor aqueous solubility.[4] As per study in dogs the oral bioavailability of CLX was only 30% and the absorption was dissolution rate limited.[5] Therefore, increase in solubility and dissolution rate of CLX may increase its bioavailability. Moreover CLX possess poor pre-formulation properties such as poor flowability and low compatibility because of its long needle-shaped crystalline form. Thus, CLX is good drug candidate for enhancement of solubility and improvement of pre-formulation properties.

Increase in solubility and dissolution rate has several promising approaches such as particle size reduction which works by increase in surface area.[6] formation of amorphous form which is highly soluble than crystalline form. This is because an amorphous form of the drug carriers high free energy and weaker bonding strength between the molecules compared to its crystalline form.[7] Solvent evaporation is unique and single step operation that transforms solution or suspension into solid amorphous nano / micro particles. Thus, Literature has supported solvent evaporation as the convenient technique for enhancement of solubility of poorly water soluble drugs.

Recently, a systematic Design of Experiment (DoE), is being extensively practiced in the development of the drug delivery devices. Such systematic approaches are far more advantageous, because they require fewer

experiments to achieve an optimum formulation. Optimization using DoE represent effective and cost-effective analytical tools to yield the "best solution" to a particular "problem." Through quantification of Polymer, these approaches provide an ability to explore and defend ranges for formulation factors.[8,9]

Therefore, the aim of present study was to develop and optimize solid dispersion (CLX-Polyvinylpyrrolidone K30 - β Cyclodextrin) complex by systematic DoE using central composite design (CCD) with the help of Design expert software.

MATERIALS AND METHODS

Materials

Celecoxib (CLX) (Batch No: 225/KL/CLX/2014/062), Polyvinylpyrrolidone K30(PVP K30), Polyvinylpyrrolidone K90(PVP K90) and β Cyclodextrin (BCD) were received kind gift from Lupin Research Park, Pune, India, All other solvents and chemical used were of analytical grade.

Methods

Design of Experiments (DoE)

Design Expert V10 software (DES) used for design of experiments. A central composite design with 5 central points and 8 no central points was employed as per standard protocol.[10,11] The amount of PVP (A) and BCD (B) were selected as experimental factors. All other formulation and processing variable were kept invariant throughout the study. Table 1 summarizes an account of the 13 experimental runs. Saturation Solubility (SS), percent dissolution efficiency (%DE) and mean dissolution time (MDT) were taken as response variables.

TABLE 1: FACTOR COMBINATIONS AS PER CENTRAL COMPOSITE DESIGN

Run	PVP K30	BCD
1	0.58579	2
2	3	1
3	2	2
4	3.41421	2
5	3	3
6	2	2
7	2	2
8	2	2
9	1	1
10	2	3.41421
11	1	3
12	2	0.58579
13	2	2

Preparation of solid dispersion (SD)

The Solvent evaporation operation was performed using a Rota evaporator (Evator rotary evaporator). Appropriate weights of CLX, PVP K30 and BCD as per DoE in all runs were added to 99.8% Methanol. The solvent evaporation was performed with the following conditions: temperature was set at 50^0 C, and RPM was set at 120/min. The evaporated solid dispersion were stored in a desiccator until used for further studies.

Solubility Studies

Solubility studies were performed on raw CLX, solvent evaporated CLX, physical mixtures of CLX-PVP K30, CLX-PVP K90, CLX-β CD, CLX-BCD-PVP K30 and CLX-BCD-PVP K90 ternary complexes of all 13 runs. An excess amount of sample was added to a 15 ml screw-capped glass vials containing 10ml double distilled water which was shaken at 100 rpm in an orbital shaker (Biomedica BM-262-D) at 25^0C for 48 hr. The resulting solution was filtered through whatman filter 42. The concentration of CLX was determined spectrophotometrically at 252 nm (Shimadzu Corporation, Japan-UV 1700). The saturation solubility, Dissolution efficiency and mean dissolution time of each sample was determined using PCP disso software and the values are the mean and standard deviation of three observations.

Dissolution Parameters

Dissolution efficiency (DE) is defined as the area under dissolution curve (y) up to certain time t, express as a percentage of area of rectangle describe by 100% dissolution in the same time.[12]

$$DE = \frac{\int_{t_1}^{t_2} y \cdot dt}{y_{100} \times (t_2 - t_1)} \times 100$$

Another parameter that describes the rate of dissolution is the mean dissolution time (MDT);

MDT is the statistical analysis that reflects to the mean time of dissolution. MDT is calculated by the following equations.

$$MDT = \frac{\int_0^{\infty} t \, dM(t)}{\int_0^{\infty} dM(t)}$$

Where t is the midpoint of the time period during which the fraction ….of the drug has been released from sample.[13]

STATISTICAL ANALYSIS

Saturation solubility, dissolution efficiency, and mean dissolution time were calculated using PCP Disso V3 software. All data of SS, DE and MDT were inserted in to design expert software as response variables to be optimized. Multivariate linear regression was used to generate the models. Response surface and desirability function were used to define the design space and find the optimum conditions. Analysis of variance (ANOVA) was applied for testing the significance and validity of the models.

X-ray Diffraction

X-Ray diffraction patterns of selected sample, were obtained using a Bruker, D8 Advance, Germany diffractometer, using Ni filtered Cu K (α) radiations, a

voltage of 35 kv, current of 30 mA and receiving slit of 0.2 In. Samples were analyzed over 2θ range of 5-70^0 and 5-50^0 for stability interpretation.

Differential Scanning Calorimetry (DSC)

DSC thermograms of samples were recorded using a DSC 1 Mettler-Toledo (Mettler-Toledo, Switzerland) equipped with a refrigerated cooling system and calibrated using indium standard. Sample (4-5mg) of pure CLX and CLZ-PVP-BCD ternary system were placed in aluminum pans hermetically sealed with aluminum lids. The program temperature was set from 30-300˚C and increased at a rate of 10˚C/min. The nitrogen gas flow rate was adjusted to 50 ml/min. Onset temperature and melting points of the samples were automatically calculated using the software provided (STARe Ver. 12.1 Mettler Toledo, Switzerland).

Morphological Analysis

Scanning electron microscopy has been used to study the surface topography, texture and to examine the morphology of nanoparticles. SEM studies were carried out by using scanning electron microscope (JEOL, JSM-6360A Japan).The samples were coated with gold ion sputtering using auto fine coater JFC-1600 (JEOL, Japan) and coating was done for 5-6 minutes. The sample was kept on the sample holder and the scanning electron micrographs were taken.

Particle Size Measurement

Particle size measurement of CLX-PVP-BCD optimize ternary system was done using particle sizer NICOMP 380 (PSS-NICOMP USA). Gaussian distribution plot was obtained using particle sizing system Santa Barbara California USA.

RESULT AND DISCUSSION

Solubility studies

Table 2 shows the saturation solubility of raw CLX, solvent evaporated CLX, and various physical mixture at different ratios of CLX:PVP K30, CLX:PVP K90, CLX-β CD, CLX-BCD-PVP K30 and CLX-BCD-PVP K90. The solubility study of raw CLX indicated a very low solubility in water (2.23±0.14 µg/ml) which is slightly lower than literature data (~3 µg/ml) [14]. As shown in Table 2 in comparison with raw CLX, the evaporated CLX did not show any improvement in the saturation solubility ($p > 0.05$), however the presence of PVP K30, PVP K90, BCD, BCD- PVP K90, and BCD- PVP K30 in physical mixture samples increased the solubility of CLX (ANOVA test, $p < 0.05$). ANOVA test showed that there was no significant difference between the solubility of untreated CLX, physical mixtures containing 1:1 weight ratio of CLX and PVP K30, PVP K90, BCD, and BCD ($p > 0.05$). When the concentration of PVP K30, PVP K90, BCD and various ratios of BCD with PVPK30 and PVPK90, were further increased to 1:2 and 1:3 weight ratio, the solubility of the drug was significantly increased ($p < 0.05$). This could be due to the solubilizing effect of PVP K30, PVPK90, and complex formation of CLX with BCD which was discussed earlier These data were used in design of experiments in for development of solid dispersion. Solvent evaporation technique was adopted for development of solid dispersion. PVP K90 was rejected for solvent evaporation technique as PVP K90 produced very sticky dispersion and showed poor result compare to PVPK30. However PVP K30 and BCD produced free flowing non sticky fine powder. Thirteen runs were prepared as per central composite design for solvent evaporation. These runs were evaluated and optimized on the basis of results of saturation solubility (SS), % dissolution efficiency (DE) and mean dissolution time (MDT).

TABLE 2: SATURATION SOLUBILITY IN DISTILLED WATER OF RAW CLX, EVAPORATED CLX AND PHYSICAL MIXTURE (PM) OF CLX AND CARRIERS.

CLX-Carrier PM	CLX: Carrier Ratio	Saturation Solubility µg/ml
Raw CLX	--	2.23±0.14
SD CLX	- -	2.45±0.27
	1:1	5.96±0.43
PVP K30	1:3	12.58±0.21
	1:1	7.34±0.53
PVP K90	1:3	13.93±0.62
	1:1	6.76±0.27
β - CD	1:3	14.21±0.31
	1:1:3	13.37±0.65
BCD:PVPK30	1:3:3	19.84±0.35
	1:3:1	17.43±0.85
	1:1:3	12.51±0.75
BCD:PVPK90	1:3:3	17.24±0.88
	1:3:1	16.78±0.64

The results in Table 3 shows that the evaporated CLX obtained in the presence of different ratios of PVP K30 and BCD combinations presented greater solubility than when CLX was physically mixed with the same polymers. This was confirmed by ANOVA ($p < 0.05$). The presence of PVP K30 and BCD in evaporated samples increased the solubility of CLX significantly. Run 4 shows maximum saturation solubility 77±0.17 but dissolution efficiency and mean dissolution time was not to the mark 84% and 13 min respectively. It may be because of high concentration of PVP K30. As concentration of PVP K30 increases saturation solubility was increased; whereas it retarded drug release. Run 10 shows 72±0.36 saturation solubility, 91% dissolution efficiency and 6 min mean dissolution time. It is because run 10 contains highest concentration of BCD. It reveals that BCD formed complex with CLX and formation solid dispersion. DSC and XRD results also implies the same.

Optimization was done by response surface methodology. Optimized solid dispersion (SD) was evaluated for solubility. It was found that optimized SD shows maximum apparent equilibrium solubility of 74.68±0.67 µg/ml, dissolution efficiency 89.37% and

MDT was minimum i.e. 5.88 min. It is because of particle size is reduced, CLX converts in partial amorphous form, PVP K30 forms hydrophilic layer over all particles and BCD form inclusion complex with CLX.

TABLE 3: SATURATION SOLUBILITY (SS), MEAN DISSOLUTION TIME (MDT) AND DISSOLUTION EFFICCINECY (DE)

Run	SS µg/ml	MDT in Minutes	DE in %
1	48	23	51
2	59	17	61
3	61	12	69
4	77	13	84
5	72	6	85
6	61	14	70
7	62	15	69
8	61	14	70
9	47	18	58
10	72	6	91
11	68	12	81
12	54	20	66
13	61	14	59

RSM OPTIMIZATION RESULTS

Mathematical Modeling

Mathematical relationship generated using MLRA for the studied response variables are expressed as following coded equations.

$SS= +61.20+7.13*A+7.43*B-2.00* AB+0.34* A^2+0.59* B^2$….(1)

$DE=+67.40+6.71* A+10.29* B+0.25* AB-0.39* A^2+5.11* B^2$…(2)

$MDT=+13.80-2.64* A-4.60* B-1.25* AB+1.54* A^2-0.96* B^2$….(3)

A: PVP K30, B: BCD. All the polynomial equations were found to be statistically significant ($p<.01$), as determined using ANOVA, as per the provision of Design Expert software. The polynomial equations comprise the coefficient for intercept, first order main effects. The sign and magnitude of main effects signify the relative influence of each factor on the response. The values obtained for main effects of each factor in equation 1 to 3 revels that BCD has more effect on all three responses (SS, DE and MDT) compared to PVP.

Response Surface Analysis

Figures 1A to 3A portray the 3-dimentional response surface plots, while 1B to 3B are the corresponding counter plots for studied response properties viz SS, DE, and MDT . Figure 1A and 1B nonlinear trends of saturation solubility in an ascending order, with augmentation of PVP and BCD levels. This may be explained on the basis of mathematical models generated for the response variable saturation solubility (Equation 1). It can be deduced from the model that at high level of PVP, BCD and their combinations shows positive effect on saturation solubility.

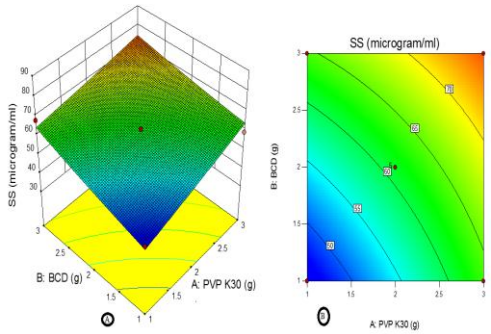

FIG 1: EFFECT OF PVP AND BCD ON SS A. 3D GRAPH B. CONTOUR GRAPH

Figures 2A and B also exhibited that dissolution efficiency vary in a non linear manner, but in an ascending pattern with an increase in amount of each polymer and its combinations.

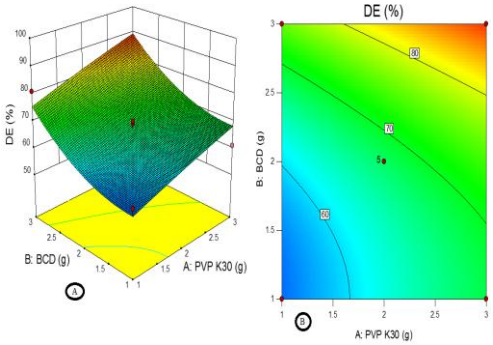

FIG 2: EFFECT OF PVP AND BCD ON DE A. 3D GRAPH B. CONTOUR GRAPH

Except high level of PVP K30 this decline trend was observed at highest level of PVP K30. The counter plot Figure 2B shows that BCD has comparatively greater influence on dissolution efficiency than PVP K30.

Figures 3A and B shows an effect on PVP K30 and BCD on third response mean dissolution time PVP K30 and BCD shows inverse relation with the mean dissolution time. As per the equation 3, BCD is more effective than PVP K30. Figure 3B Counter plot shows minimum MDT (3- 4 min) at high concentration of BCD whereas at high level of PVP K30 MDT was slightly increased.

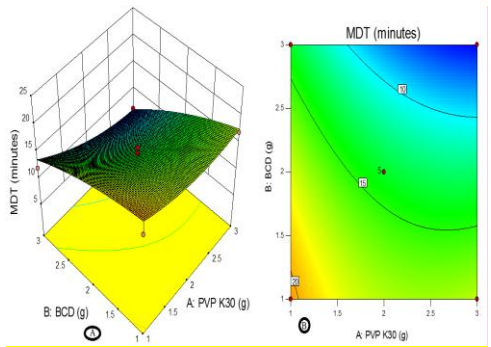

FIG 3: EFFECT OF PVP AND BCD ON MDT A. 3D GRAPH B.CONTOUR GRAPH

CHARACTERIZATION OF OPTIMUM CLX-PVP-BCD SOLID DISPERSION COMPLEX

The solid CLX-PVP-BCD ternary system obtained by solvent evaporation was characterized by DSC and X-ray powder diffractometry, to confirm the formation of the solid complex.

Differential Scanning Calorimetry (DSC)

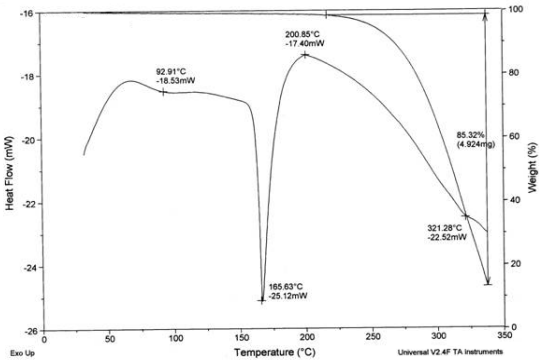

FIG 4: DSC THERMOGRAM OF RAW CLX

The thermal curve of CLX was typical of a pure anhydrous crystalline substance, exhibiting a sharp fusion peak at 165.63^{0}C indicating melting point of drug as shown in figure 4. The endothermic peak of CLX which appeared at 165.63^{0}C in case of the SD was absent shown in figure 5. This result confirmed the formation of inclusion complex.

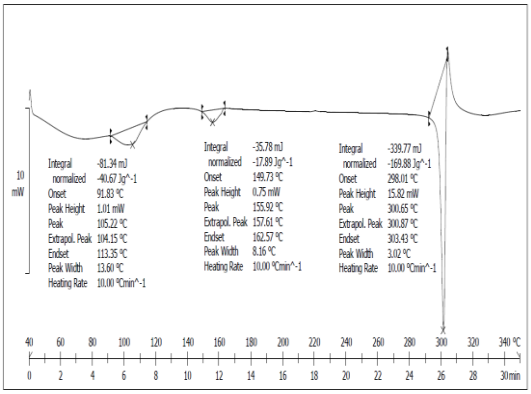

FIG 5: DSC THERMOGRAM OF OPTIMUM SD

X-Ray diffraction

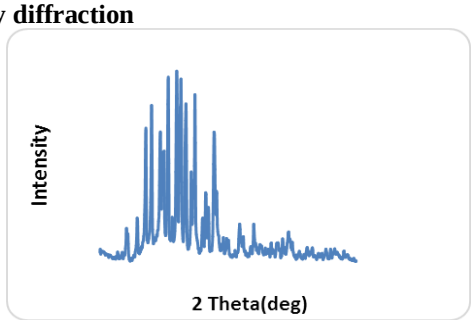

FIG 6: X-RAY DIFFRACTION OF CLX

The X-ray diffraction pattern of the drug was characterized by the presence of several intense and sharp peaks as shown figure 6, confirming its crystalline nature, while they partialy disappeared in the corresponding CLX-PVP –BCD complex shown in figure 7, indicating its partial amorphization in agreement with DSC results. Dissolution rate studies demonstrated very better dissolution properties of the ternary evaporated product.

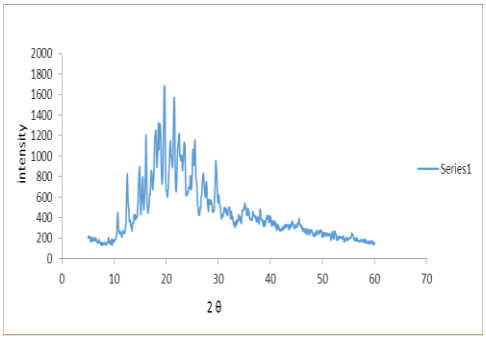

FIG 7: X-RAY DIFFRACTION OF SD

Morphological analysis

The scanning electron microphotograph of CLX-PVP-BCD amorphous ternary complex was obtained as depicted in figure 8. Pure CLX exhibited typical crystalline pattern as per DSC and XRD study. The SEM of solid dispersion complex was observed. Free drug crystals were also not observed. The original morphology of CLX, PVP K30 and BCD disappeared and it was not possible to differentiate the three components. Rota evaporated solid dispersion showed partial amorphous and homogenous aggregates of flake like structure. The drastic change in the surface morphology of the evaporated complexes was indicative of the presence of a new solid phase, which may be due to the molecular encapsulation of drug in the BCD. XRD patterns of SD also indicated formation of amorphous complex. This indicated the presence of strong interaction between the CLX, PVP K30 and BCD.

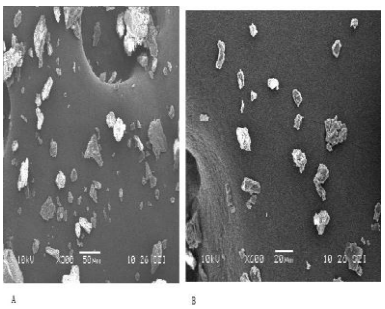

FIG 8: SEM OF SD

Particle size measurement

Figure 9 shows the optimum SD of evaporated product had a mean particle diameter between 1703.1±996.3 nm. Gaussian distribution plot of optimum SD shows that particle size of the evaporated product was ranged between 200-5000 nm and average particle size was found to be 1703.1±996.3nm. Particle size range of 500 to 1000 nm contained maximum number of particles.

This indicates that particle size of CLX was significantly reduced.

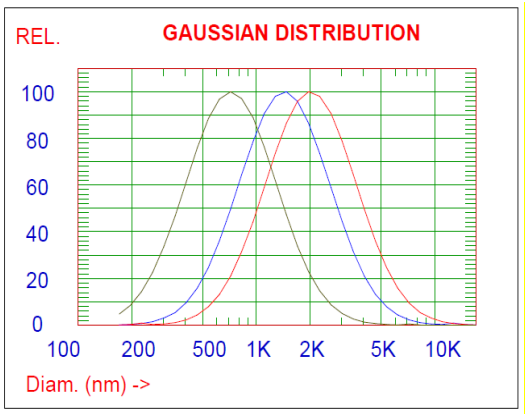

FIG 9: PARTICLE SIZE ANALYSIS OF SD

CONCLUSION

Present study showed that DoE approach can be successfully used in the development of solid dispersion of CLX with predictable dissolution properties. In particular, DoE allowed the simultaneous evaluation, by a response surface study. The effect of the selected factors or variables, i.e., PVP K30 and BCD on the saturation solubility, dissolution efficiency and mean dissolution time were optimized. The use of desirability function was use to find out the best compromise which allowed simultaneous optimization of all considered response variables. It can be expect that this application of the DoE tools in QbD approach could be useful for further formulation studies. The results conclude that PVP-K30 and BCD are potential carrier in developing solid dispersion of CLX for the dissolution improvement. As particle size is not reduced significantly, CLX does not completely covert into amorphous nature, another effective method like spray drying can be adapted using same experimental design for better results.

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