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Research articles

Prediction of Process Variables in Indomethacin Ternary System Using Experimental Design Solid State Characterization and *In-vivo* Studies

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ABSTRACT

The aim of the present investigation is (i) to apply and analyze response surface methodology to optimize the preparation method of indomethacin solid dispersion which involves three significant independent variables: amounts of each carrier β-Cyclodextrin (βCD), Polyvinyl pyrrolidone (PVP K30), and solvent mixture (ethanol-water), (ii) to characterize by solid state analysis, and evaluate by *in vivo* study. A 3-factor, 3-level 'Central Composite Face Centered design' was used to explore the quadratic and linear response surfaces and to construct a second order polynomial models which can predict responses 'aqueous solubility', and '% release' with minimum % error. In addition, the desirability function approach was applied to obtain the best compromise among the multiple responses. It was found that both the independent variables played a significant role on the solubility and percentage drug release. The solid state studies enable the phase (crystalline) transform of drug which influences its solubility and drug release. The enhancement of C_{max} and $AUC_{0\text{-}24}$ of indomethacin (in solid dispersion) with that of pure drug imply the increase in bioavailability of drug.

Keywords: Solubility, Dissolution rate, Response surface methodology, Desirability function.

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INTRODUCTION

The delivery of poorly water-soluble drugs using solid dispersion is currently an area of great concern in pharmaceutical research [1]. The preparation of binary solid dispersion was simple, but the effect of solubility and dissolution rate of poorly water soluble drugs such as indomethacin (IDM) were quite limited[2-5]. Incorporation of water soluble carrier in a binary mixture is limited to a variety of factors which includes cost, production capability and the amount of carrier such as cyclodextrins [6]. So, a logical design method should be established, in order to achieve the maximum response with a minimum number of trials. A number of well-designed experiments were carried out in the preparation of solid dispersion by following proper methodology with the parameters associated with the response factors to achieve the desired goal [7-8].

Response surface methodology (RSM) is an empirical statistical technique employed for multiple regression analysis by using quantitative data obtained from systematically designed experiments[9]. The graphical representation of this equation is called response surface, which is used to describe the individual and cumulative effect of the test variables and their subsequent effect on the response. To determine quantitative analysis between the process variables and the response function, a central composite face centered design (CCD) is adopted in which the experiments are randomized in order to minimize the effects of unexplained validity on the observed response due to extraneous factors [10]. The function is approximately assumed as a second-degree polynomial equation (Eq. (1))

$$Y = \beta_{k0} + \sum_{i=1}^{n} \beta_{ki} X_i + \sum_{i=1}^{n} \beta_{kii} X_i^2 + \sum_{i \neq j=1}^{n} \beta_{kij} X_i X_j + \in \quad \ldots.(1)$$

where Y is the predicted response, X_i is the variables in the coded form of the input variables, β_{k0} is the value of fitted response at the center point of design, i.e., point (0, 0, 0), and β_{ki} , β_{kii} and β_{kij} are the linear, quadratic and cross-product regression terms, respectively and ε is the residual term associated with the experiment.

Therefore, an attempt has been made to employ response surface methodology to optimize the solid dispersion technique. The parameters investigated include, the amount of carrier's (β-Cyclodextrin (βCD), polyvinyl pyrrolidone (PVP K30)) and solvent

mixture. In addition, the desirability function approach was used simultaneously to optimize the responses. The ternary solid dispersion was characterized by solid state analysis and *in vivo* test was performed to assess the bioavailability

MATERIAL AND METHOD

Indomethacin (Mol. wt. 357.79) was gifted by Micro Labs, Hosur, India. β-Cyclodextrin (Mol.wt. 1135) and PVP K30 (Av. Mol. wt. 45,000), were from Roquette (Lestrem, France) and Dr. Sains Laboratories (Kolkata India), respectively. Chemicals used for the buffer preparation and other solvents were of analytical grade.

Experimental design and optimization by using response surface methodology (RSM)

In this study, second order polynomial model was constructed using RSM, which helps in optimizing several independent variables with minimum experiments. Besides, the central composite face centered design (CCD) is well suited for fitting a quadratic surface which usually works well for the process optimization [10]. A set of 20 experiments were designed to optimize three independent variables such as the amount of βCD in mg (A), amount of PVP K30 in mg (B) and composite solvent (ethanol- water) in mL (C) to achieve desired responses (aqueous solubility (Sol_{aq}, mg/mL) and release in 5min ($Rel_{5\ min}$, %) of Indomethacin) by solid dispersion technique. Six replications at the design center point were utilized to provide information on response variation about the average and the residual variance. The level of independent variables studied was determined through a series of preliminary trials (Table 1).

Table 1: Process Control parameters and their limits

Parameters	Units	Notations	Limits		
			-1	0	1
βCD	mg	A	300	500	700
PVP K30	mg	B	100	300	500
Composite Solvent*	mL	C	15/35	25/25	35/15

* Composite solvent consists of ethanol/water system (v/v)

Preparation of physical mixtures

The physical mixtures of IDM and respective carriers were prepared by homogeneous blending in a mortar for 5min and sieved through a mesh (120μm).

Preparation of ternary solid systems

The ternary solid dispersion was prepared as per the design matrix (Table 2). The specified amount of drug (IDM) and carrier (βCD) were dispersed in 50mL of composite solvent (ethanol-water) with constant stirring (60 ± 0.5°C using magnetic stirrer (REMI-2MLH)). Then, PVPK30 was added to the above mixture and stirring was continued for 30min. The solvent was subsequently evaporated at room temperature for 2h and at 50 ± 0.5°C in a hot air oven for 48h. The resulting blend was pulverized and sieved through a 120μm mesh.

Drug content and yield

The drug content of solid dispersion was determined by dissolving 50mg of solid dispersion in 5mL of methanol and was diluted up to 50mL with phosphate buffer (pH 7.2). Then the drug content was assayed spectrophotometrically (UV-1240, ANALAB, Mumbai, India) at 320nm.

Table 2 – Design matrix and measured responses

Process Variables(ML)			% yield	Drug Content (mg)	Response Variables	
A	B	C			Sol_{aq} (mg/mL)	rel 5min (%)
700	500	-1	95.33±1.288	89.91±1.215	20.74±0.235	64.82±1.3
700	100	-1	96.67±2.099	87.42±1.899	19.01±0.185	48.26±2.052
300	100	-1	96.34±1.89	85.95±1.686	10.12±0.362	45.67±2.72
500	300	0	94.65±1.548	93.95±1.536	29.37±0.398	82.32±2.525
500	300	0	95.97±1.293	93.99±1.266	30.56±0.843	81.15±1.775
500	100	0	93.59±1.591	90.99±1.357	27.16±0.404	65.35±1.251
500	300	-1	96.23±1.52	89.58±1.415	19.22±0.596	70.17±1.872
500	300	0	97.01±1.237	95.01±1.211	27.45±0.281	79.44±1.811
300	500	-1	93.23±1.837	81.85±1.613	17.97±0.306	78.02±1.165
500	300	1	95.18±1.588	97.84±1.632	30.88±0.404	75.76±1.752
300	500	1	94.38±2.08	87.44±1.927	25.03±0.495	94.55±0.539
500	500	0	95.27±2.887	89.9±2.724	29.8±0.723	95.2±0.352
700	300	0	95.72±2.814	90.84±2.671	27.9±0.442	55.35±2.169
300	300	0	93.68±1.98	92.9±1.964	18.6±0.404	73.29±1.456
500	300	0	97.02±1.251	91.6±1.181	28.69±0.358	83.14±2.369
300	100	1	95.92±2.642	94.74±2.609	18.44±0.33	62.26±1.5
700	500	1	96.33±1.654	95.76±1.644	25.08±0.477	61.52±1.117
500	300	0	94.5±1.508	91.3±1.457	27.34±0.417	84.79±1.634
500	300	0	94.56±2.603	96.78±2.664	28.32±0.355	81.76±0.943
700	100	1	97.46±1.788	97.18±1.782	31.05±0.37	48.64±1.412

Aqueous solubility

An excess quantity of solid dispersion was added to the test tubes containing 5mL of phosphate buffer (pH 7.2), sealed with parafilm and kept in a thermostated water bath at 37 ± 0.5°C until it attained equilibrium (~12 h) with vortex-mixing and sonicated for 2min at every 1h interval. Then the samples were filtered by Whatman filter paper (pore size: 11 μm), diluted and the drug concentration was determined spectrophotometrically at 320nm.

Dissolution studies

The dissolution studies were conducted in a USP dissolution apparatus II (TDT 06P, Electro Lab, New Mumbai, India) by adding 50mg of drug equivalent solid dispersion to 900mL of simulated intestinal fluid without pancreatin (pH 7.2) at a stirring speed of 25rpm, maintained at 37 ± 0.5°C[11]. At predetermined time intervals (5, 10, 15, 20 and 30min), samples were withdrawn and the same amount of fresh medium was replaced to maintain the sink condition throughout the test. The samples were filtered and drug concentration was determined at 320 nm by UV - spectrophotometer. Each test was repeated three times.

Solid state characterization

Fourier transform infrared spectrophotometric analysis

FTIR spectra were obtained using a Shimadzu FTIR-8300, Kyoto, Japan, equipped with Quick Snap sampling modules. The samples were scanned in solid state by the KBr disc method over the

wave number range of 4000–400 cm^{-1} at 4 cm^{-1} resolution.

Powder X-ray diffraction analysis

The powder X-ray diffraction patterns were recorded using Miniflex diffractometer; Rigaku Co. Ltd., Japan. The small sample in a rotating holder was exposed to a Kb filter, Cu radiation with continuously spun and scanned at a rate of 1°/min over a 2θ range of 5 –50°, at voltage of 30 kV and a 15 mA current.

Differential scanning calorimetric analysis (DSC)

The DSC analysis was performed using differential scanning calorimeter (Pyris diamond TG/DTA; P, Perkins Elmer Instruments). Approximately 3-5mg of samples were placed in a sealed aluminum pan, and was heated at a scanning rate of 10°C/min over 30° to 250°C, with alpha alumina in the reference pan under nitrogen flow of 150mL/min.

Surface morphology

The morphological features of pure components and solid dispersion were investigated using scanning electron microscopy (SEM) (JEOL, JSM5200, and Tokyo, Japan) after palladium-gold coating of the sample on a brass stub using a gold sputter module.

Desirability function

An empirical statistical technique searches for the better combination of process variables to achieve the requirements placed (i.e. optimization criteria) on each of the responses and process factors. In order to achieve the desired goal, the multicriteria problem can be treated as a single criterion problem by using the desirability function approach. It reflects the desirable ranges for each response ranging from 0 to 1, corresponding to the least and the most desire respectively. The simultaneous objective function is a geometric mean of all transformed responses

$$D = \left(\prod_{i=1}^{k} d_i \right)^{1/k} \quad (2)$$

where, *k* is the number of responses and D is the overall desirability. The optimum formulation of Indomethacin ternary solid dispersion systems was selected based on the criteria of attaining the maximum value of aqueous solubility (mg/mL) and release at 5min (%) and by applying constraints on factors.

***In-vivo* study (Pharmacokinetic parameters)**

The *in-vivo* studies on animals were conducted to assess the bioavailability of Indomethacin solid dispersion (optimized formulation) which is compared to that of pure Indomethacin. All the experimental procedures and protocols used in this study were reviewed by the Institutional animal ethics committee (367001/C/CPCSEA), Jadavpur University**,** Kolkata, India, and were in accordance with the guidelines of the IAEC. Animal care was given as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The *in-vivo* studies were conducted on 12 Albino Wister rats of either sex weighing 150- 200gm, which was obtained from institutional animal house of Jadavpur University, Kolkata. The animals were acclimatized in animal house for 1 week and were fed with a fixed standard diet. Twelve rats were divided into two groups of 6 in each and were kept fasting for 24h prior to drug administration. The pure drug (5mg IDM equivalent/kg) was suspended in 1mL of methyl cellulose (0.5 % w/v) and administered orally with the help of canulla to control group animals. Similarly, solid dispersion was administered to test group animals. The animal was anesthetized with ether and blood samples (0.25mL) were withdrawn carefully via the retro-orbital route at various intervals of 0(as blank), 0.5, 1, 2, 4, 8, 12 and 24h and collected in a heparinized Eppendorf tubes to prevent blood clotting. Then, the samples were centrifuged at 13,000 rpm for 5min and the plasma obtained was stored at −20°C for further analysis[12]. A total 100µl of plasma sample was mixed with 0.5mL of acetonitrile and centrifuged. The supernatant was evaporated under nitrogen stream, and the residue was dissolved in 500µl of the mobile phase and the sample was subjected to HPLC analysis using CN-RP column (Princeton SPHER 300 CN-RP, 5µm). The mobile phase which comprises a mixture of acetonitrile - water (60:40, v/v) with 0.1% Trifluoroacetic acid in both phases runs at an isocratic flow rate of 1mL/min. The column effluent was monitored by Waters 2489 (UV-VIS) dual lambda absorbance detector set at 320 nm.

Pharmacokinetic and statistical analysis

From the observed values, various pharmacokinetic parameters such as AUC, C_{max}, t_{max}, K_{el}, and $t_{1/2}$ were calculated using Microsoft Excel-PK software tool version 2.0 individually for each group and the values were expressed as mean ± SD. The pharmacokinetic data of test formulations and control (pure drug) were compared for statistical significance by the non-paired, two-tailed Student's t-test using Graph Pad Instate software (trial version).

RESULT AND DISCUSSION

The design matrix for the tested factors and the observed responses for all the experiments done including replicates, are shown in Table 2. The assayed drug content in the ternary solid dispersion ranges between 81.85 ± 1.613 and 97.84 ± 1.632 of initial drug load. The percentage yield of the solid dispersion was found to be 93.23±1.837 to 97.46±1.788. The loss in yield of solid dispersion was accountable for the loss of some material on the wall of container and might be due to retention of some insoluble particles on the filter paper. The solubility of Indomethacin in pH 7.2 phosphate buffer solutions at 37°C was found to be 0.990mg/mL. A remarkable increase in solubility (vide Table 2) and dissolution rate (Fig. 1) of indomethacin was observed from the ternary solid dispersion which can be attributed to the reduction of drug's crystallinity upon complexation with cyclodextrin and anti plasticizing activity of PVP K30, retards the formation of crystal lattice[13].

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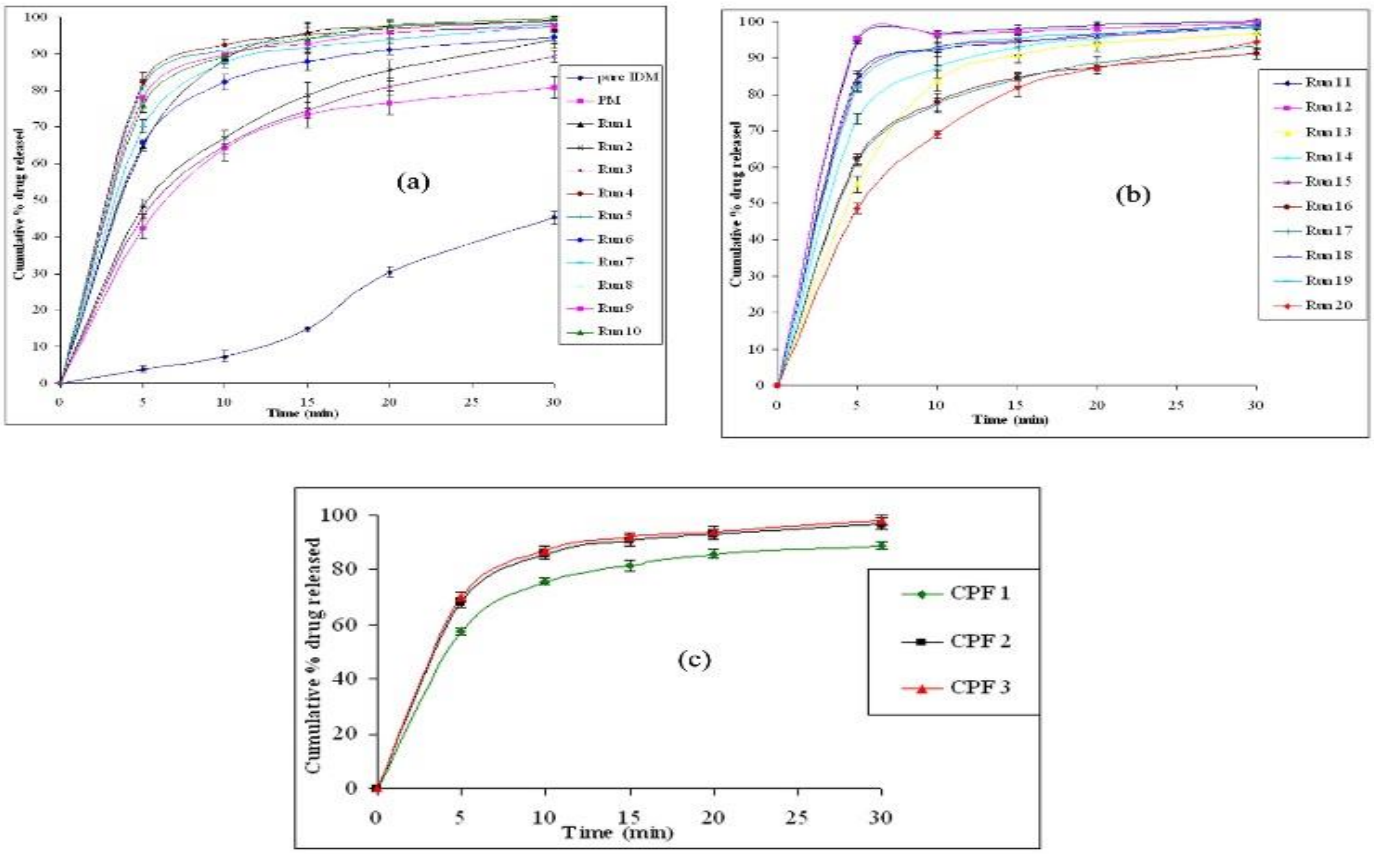
Figure 1 Dissolution profiles of Indomethacin ternary complex prepared as per the Experimental Design (a & b) and check point formulation ©



Statistical analysis and validation of the developed model

The analysis of variance (ANOVA) results calculated using the Design Expert software was summarized in Table 3.

Table 3 – Regression coefficients and their p –values for the regression models for predicting optimized responses (mg/mL), release at 5min (%)

Sol_{aq} (mg/mL)				**rel 5min (%)**		
Factor	**b-Coefficient**	**p -value**		**Factor**	**b-Coefficient**	**p-value**
Intercept	28.46	< 0.0001		Intercept	80.91	< 0.0001
A	3.36	< 0.0001		A	-7.52	< 0.0001
B	1.28	0.0135		B	12.39	< 0.0001
C	4.34	< 0.0001		C	3.57	0.00142
AB	-2.33	0.000506		AB	-4.4	0.000691
BC	-1.12	0.0433		AC	-4.5	0.000575
A^2	-4.72	< 0.0001		A^2	-13.66	< 0.0001
C^2	-2.92	0.00285		C^2	-5.01	0.00679
Other Statistics				Other Statistics		
R^2 = 0.9627				R^2 = 0.9779		
Adjusted R^2 = 0.9409				Adjusted R^2 = 0.965		
Predicted R^2 = 0.8448				Predicted R^2 = 0.9338		
Adequate Precision = 25.15				Adequate Precision = 28.62		
	Sum of Squares	df	p-value	Sum of Squares	Df	p-value
Model	609.42	7		4016.6	7	
Residual	23.61	12		90.64	12	
Lack of Fit	16.19	7	0.323	74.15	7	0.109
Pure Error	7.41	5		16.48	5	
Corr Total	633.03	19		4107.25	19	
F-value of model = 44.2				F-value of model = 76.0		

The developed models were validated by performing three confirmative experiments chosen within the range. The low percentage error (vide Table 4) observed from the confirmation experiments indicates the better model adequacy.

Table 4 – Validation Test Results

Code	**Experimental Composition**			**Response Variable**	**Experimental Value**	**Predicted Value**	**Percentage Error**
	βCD (mg)	**K30 (mg)**	**Composite Solvent (mL)**				
CPF 1	300	300	15/35	Sol_{aq} mg/mL	14	13.11	-6.78
				rel % 5min	57.47	61.63	6.74
CPF 2	500	200	35/15	Sol_{aq} mg/mL	28.97	29.72	2.52
				rel % 5min	68.35	67.07	-1.9
CPF 3	700	500	25/25	Sol_{aq} mg/mL	24.23	26.04	6.95
				rel % 5min	70.2	67.68	-3.72

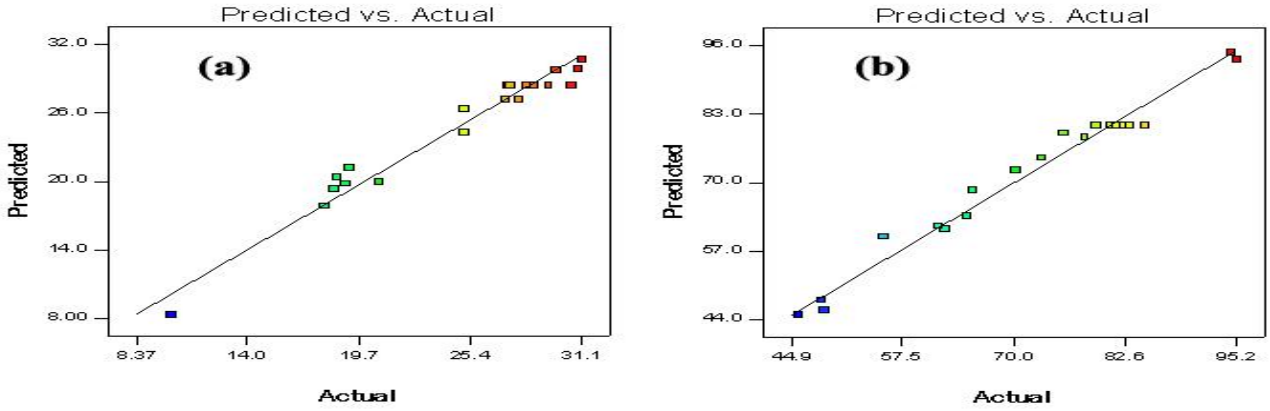
The best correlation was observed in predicted vs. actual plot for the two responses are evident from Fig. 2. The significant terms ($p<0.05$) with low probability values (<0.0001) are remained in the equation after elimination. The higher p values (0.323 for Sol_{aq} mg/mL and 0.109 for $rel_{5min,}$ %) attained from the F-test (Lack-of-fit) showed the better model adequacy. The predicted R^2 and adjusted R^2 values 0.9627 and 0.9409, 0.9779 and 0.965 were in reasonable agreement for Sol_{aq} and (rel_{5min}) respectively. Therefore, this model can be used for

the prediction of aqueous solubility (Sol_{aq}) (Eq. 3) and dissolution (rel_{5min}) (Eq. 4) of Indomethacin within the design space,

Sol_{aq} =+28.46+3.36*A+1.28* B+4.33* C-2.34* AB-1.12* BC-4.72* A^2-2.92* C^2.....(3)

rel_{5min} (%) =+80.91-7.52* A+12.39* B+3.57* C-4.40*AB-4.50*AC-13.66*A^2-5.01*C^2…….(4)

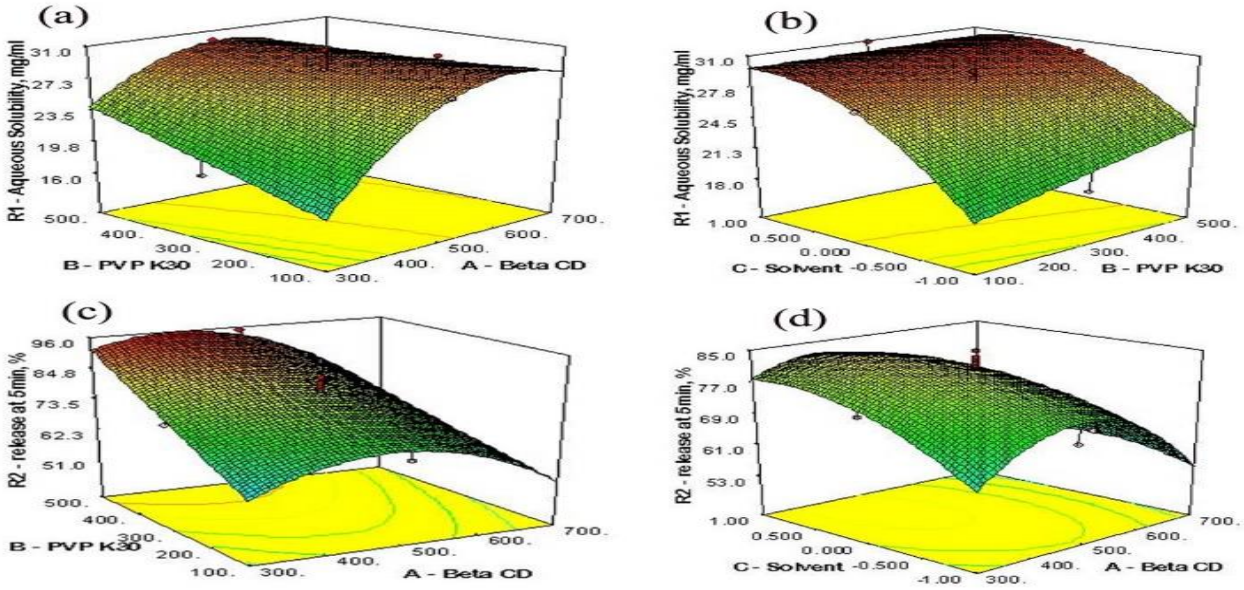
Figure 2. Plot of predicted versus actual response of (i) aqueous solubility and (ii) rel_{5min} (%) results



Response surface analysis

The effect of independent variables on the responses was interpreted using three-dimensional response plot is presented in Figure 3. It is observed that the solubility increases with increasing concentration of βCD upto intermediate concentration (500mg) and not showed much variance upon further addition of βCD as showed in Fig. 3a, owing to the formation of electrostatic bonds in the aqueous solution and heat–reversible gels retard the further solubility enhancement of IDM at higher concentration of βCD. From Table 2, dominant effect of solvent mixture (factor C) on Sol_{aq} was observed in Run 7, 10, 18. The maximum aqueous solubility was observed in Run 20 &16 and Run 10 &11 containing higher amount of βCD. There was not much difference when βCD increased from 500 to 700mg as evidenced in Run 20 & 10. This is because of the formation of macromolecular cluster by the addition of excess polymer which may hinder solubilization effect of drug/CD complex alone[14]. By keeping βCD concentration at constant level, the effect of PVP K30 and composite solvent were observed as depicted in Fig. 3b. A linear increment was observed in the enhancement of solubility upon increasing the concentration of PVP K30 and composite solvent (up to certain ethanol fraction). However, the solubility remained unchanged upon further addition of composite solvent fraction due to the higher dilution of drug in ethanol and insufficient amount of water to dissolve βCD completely. As we have accounted earlier, solvent in which the drug molecules readily solubilized, favored complex formation[15]. The above results show that addition of ethanol to the aqueous solutions of IDM ternary complexes leads to their gradual dissociation into the components[16] and no complex was observed over 75% ethanol. The solubility was not enhanced appreciably with the increase of PVP as we observed in Run 4, 6 & 12 when βCD (500mg) and composite solvent (35/15) were kept constant.

Figure 3. Response surface plot showing effect of (a) βCD (A) and PVP K30 (B), (b) PVP K30 (B) and composite solvent (C) on aqueous solubility, mg/mL and (c) βCD (A) and PVP K30 (B), and (d) βCD (A) and composite solvent (C) on release at 5min %.



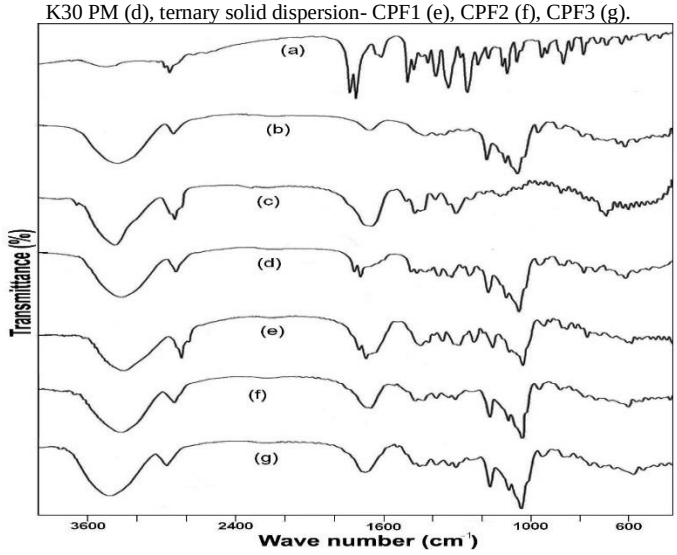
The response surface plots for the dissolution rate (rel at 5min, %) of ternary solid dispersion were depicted in Fig.3c & d. The % drug release increased with increasing the concentration of PVP K30 and βCD (upto 500mg) and then decreased above midpoint with increasing amount of βCD as showed in Fig. 3c. This can be attributed to the structural disorder of carriers (βCD and PVP K30) when combined together, favored an enhancement of dissolution rate[17]. The effect of PVP K30 on drug release is high in comparison to that of βCD which may due to the increase in drug wettability and anti plasticizing activity of PVP K30. The Semi-spherical response surface (Fig. 3d) showed the increase in % release of IDM with increasing solvent composition upto certain composite solvent fraction (30mL ethanol/20mL water).

Solid state characterization

Fourier transform infrared spectrophotometric analysis

FTIR spectrum of Indomethacin displays a peak characteristic of benzyl carbonyl (1691 cm^{-1}, 1716 cm^{-1}) and band at 1606 cm^{-1} and 3471cm^{-1} indicative of the carbonyl and hydroxyl group respectively (Fig. 4a). The bands corresponding to IDM C=O stretching vibration showed reduced intensities in the physical mixture (Fig. 4d). The broad peak corresponds to C=O stretching at 1689 cm^{-1}, 1716 cm^{-1}, and 2856 cm^{-1} with lower intensity was observed in 'Check point formulation ,CPF1' (Fig. 4e) which was possibly due to complexation of drug by hydrogen bonding with the carriers. In CPF1 contribution of polymers is lesser than that of CPF2 and CPF3, so characteristics peaks of indomethacin owing to C=O stretch were predominant features in CPF1. Whereas, the single broad peaks at 1650 cm^{-1} and 1688 cm^{-1} were observed in the CPF2 (Fig. 4f) and CPF3 (Fig. 4g) respectively, resembles to the carrier peak. There is a possible explanation that drug's characteristic peaks are less visible/prominent where, binding between drug and carriers is strong enough in presence of sufficient number of carriers which is substantiated by the enhanced solubility and dissolution of drug.

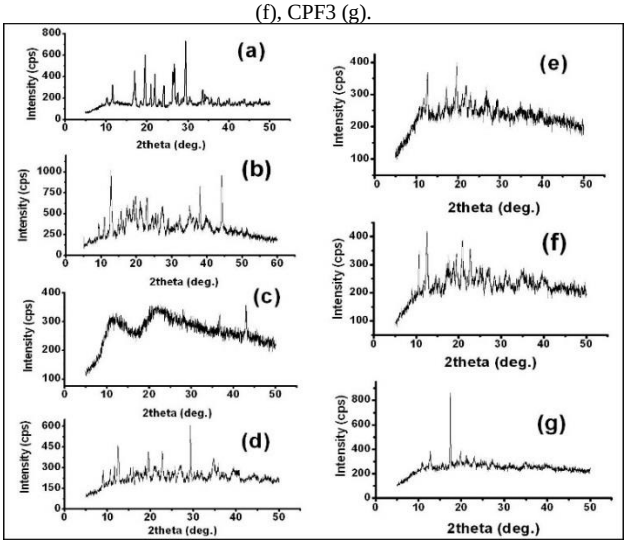
Figure 4. FTIR of Indomethacin (a), βCD (b), PVP K30 (c), IDM: βCD: PVP K30 PM (d), ternary solid dispersion- CPF1 (e), CPF2 (f), CPF3 (g).



Powder X-ray diffraction analysis

The diffractogram patterns of IDM (Fig. 5a) appeared at different angles (2θ – 11.56, 17, 19.56, 21.8, 26.2, 26.6 and 29.36°) have indicated the crystalline nature of Indomethacin. The diffractogram of βCD (Fig. 5b) showed characteristics peaks at 9.32, 10.97, 12.8, 15.7, and 19.8° (2θ) owing to its crystalline nature, while a halo-pattern was recorded for PVP K30 (Fig. 5c) be the evidence for amorphous nature. The reduced intensity of peaks at 2θ equal to 26.51° and 29.3° corresponding to the pure drug (IDM) were observed in physical mixture (Fig. 5d) and in CPF1 and CPF2 (Fig. 5e & 5f) imply the occurrence of partial amorphization of drug. Whereas, most of the sharp peaks disappeared, and a single peak at 2θ equal to 17.42° was observed in the CPF 3 (Fig. 5g). This indicates predominant effect of amorphous PVP[18] and strong binding of drug with sufficient quantity of carriers. The high proportion of carrier causes dilution of drug resulting in disappearance of its characteristic peaks.

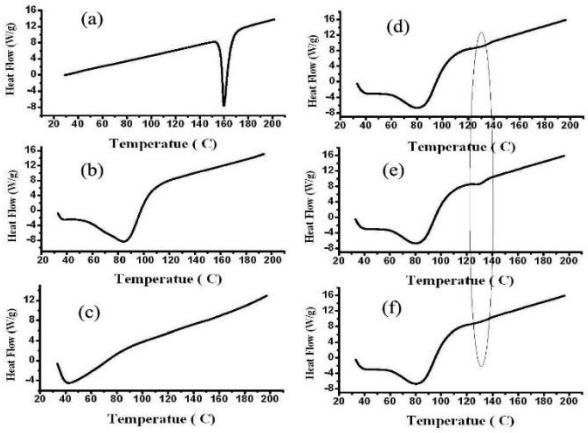
Figure 5. X- ray diffraction pattern of Indomethacin (a), βCD (b), PVP K30 (c), IDM: βCD: PVP K30 PM (d), ternary solid dispersion- CPF1 (e), CPF2 (f), CPF3 (g).



Differential scanning calorimetric analysis

The DSC scan of IDM (Fig. 6a) exhibited a sharp endothermic peak at 160°C corresponding to the melting point of the drug[4,19].

Figure 6. DSC thermograms of Indomethacin (a), βCD (b), PVP K30 (c), ternary solid dispersion- CPF1 (d), CPF2 (e), CPF3 (f).

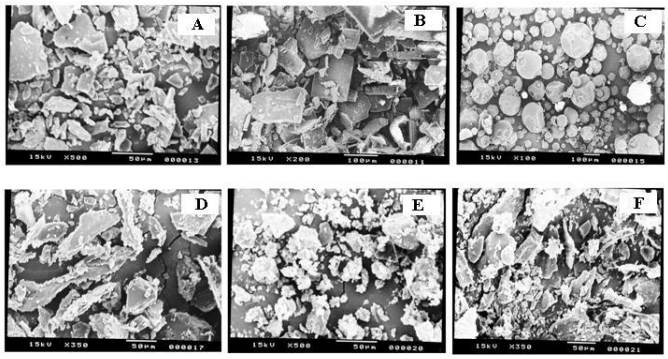


A large endothermic effect owing to the release of water was observed in βCD and PVP K30 (Fig. 6b&c) at 90°C and 42°C respectively. In the ternary solid dispersion, (Fig. 6d, e, f) a weak endothermic deflection was observed around 130° to 135°C which is assumed to be the modified melting point of drug in presence of molten carriers. This suggests that the drugs get diluted with the amorphous carrier in the molten state led to reduction of crystallinity. The lowering of melting point in the ternary system can be attributed to the incorporation of guest molecules in the βCD cavities and their thermal proportions shift to different temperatures within the temperature range where βCD decomposes.

Surface morphology

The SEM micrographs of IDM crystals exhibit plates with irregular borders (Fig. 7a) whereas βCD particles (Fig. 7b) are prism-shaped crystals apparently formed by plates assembled together resulting in a laminated crystal appearance. The pure PVP K30 (Fig. 7c) are irregularly rounded spheroids with cracks which may be due to the process employed for its polymerization[3]. A new solid phase (small size amorphous, lamellate particles) was observed in the ternary solid dispersion (Fig. 7d, e, f) indicates phase (crystalline) transform of drug. From this, it is clearly visible that the morphology is highly influenced by the processing variables.

Figure 7. SEM micrographs of Indomethacin (a), βCD (b), PVP K30 (c), ternary solid dispersion- CPF1 (d), CPF2 (e), CPF3 (f).



Optimization by desirability function

The optimized ternary solid dispersion of Indomethacin was made by choosing maximum responses which was based on the constraints of the processing parameters (vide Table 5).

Table 5 - The Criterion for numerical optimization

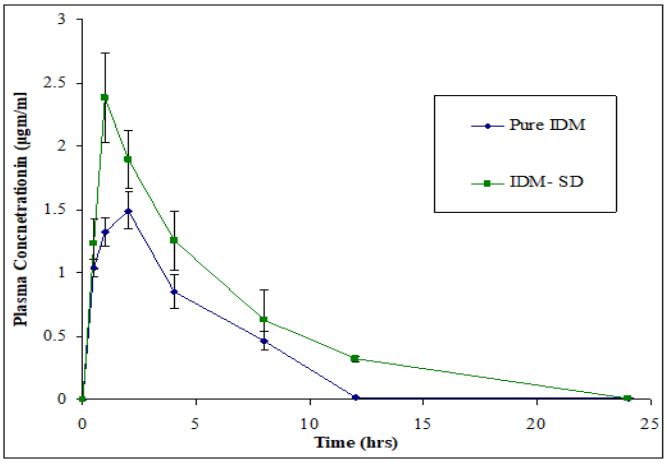
Parameter	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
βCD	is in range	300	700	1	1	3
PVP K30	is in range	100	500	1	1	3
Composite Solvent	maximize	-1	1	1	1	3
Sol_{aq} mg/mL	maximize	10.12	31.05	1	1	3
rel 5min (%)	maximize	45.67	95.2	1	1	3
Solutions (Optimum process parameters for desired goals)						
Number	βCD	PVP K30	Composite Solvent	Sol_{aq} mg/mL	rel 5min (%)	Desirability
1	448	500	1	29.46	95.2	0.974

With exhaustive grid search of response variables, the formulation composition with Indomethacin (=100mg); βCD (= 448mg); PVP K30 (= 500mg) and 50mL composite solvent (ethanol/water) (= 35/15) were found to fulfill the requisite of an optimum formulation. The optimized formulation has the aqueous solubility and release at 5 min of 29.5mg/mL and 95.2 %, respectively.

***In-vivo* study (Pharmacokinetic parameter)**

The profiles of the plasma concentrations of Indomethacin versus time after oral administration of pure drug and solid dispersions are depicted in Fig. 8.

Figure 8. Plasma concentration profile of Indomethacin after oral administration of optimized formulations (SD) and pure drug



At all time intervals, the plasma levels of the dispersed drug (in SD) are higher than that of the pure drug.

Table 6 Pharmacokinetic Parameters of Optimized Formulations and Pure Drug

Kinetic Parameter	Control (plain IDM)	Test (SD)
$AUC_{(0-t)}$ (µgm/mL h)	8.06 ± 0.362	14.14 ± 1.502
$AUC_{(0-inf)}$ (µgm/mL h)	8.1 ± 0.369	14.2 ± 1.468
T_{max} (h)	2*	1.33 ± 0.577*
C_{max} (µgm/mL)	1.49 ± 0.142	2.42 ± 0.286
K_{el} (h^{-1})	0.23 ± 0.002*	0.21 ± 0.022*
$t_{1/2}$ (h)	2.91 ± 0.025*	3.26 ± 0.339*

* Terms are not significant ($p<0.05$)

The t_{max} was strongly shortened in solid dispersion but not significant statistically ($p<0.05$). This decrease is directly related to the improvement in the absorption of the drug. Indomethacin was absorbed from the GI tract as rapidly as it dissolves and so, lowering of t_{max} is certainly due to the enhancement of drug (SD) dissolution rate. The pharmacokinetic parameters (C_{max}, AUC_{0-24h} and $AUC_{0-\infty}$) as obtained after statistical analysis confirmed that solid dispersion showed significant ($p<0.05$) enhancement in the bioavailability than that of pure drug (Table 6). Therefore, the enhanced bioavailability of Indomethacin in the presence of βCD: PVP K30 appeared to be related to the increase in solubility and dissolution rate of the drug. Similarly, the elimination rate constant (K_{el}) and half-life ($t_{1/2}$) values (Table 6) of Indomethacin in SD were not significantly different when compared to Indomethacin pure form ($p<0.05$). This indicated that $t_{1/2}$ and elimination characteristics of the drug were not altered in case of drug-carrier system. These results suggested that the enhanced solubility and

dissolution of Indomethacin obtained by solid dispersion technique could show the way to the improved oral bioavailability of Indomethacin.

CONCLUSION

It was verified that 'Central Composite Face Centered design' allowed systematic optimization of the ternary solid dispersion formulation by evaluating the most important factors on observed responses and investigating the relationship between factors by the response surface methodology. According to the criteria of desirability, mass of 100mg of Indomethacin, 448mg of βCD, 500mg of PVP K30 and 50mL of composite solvent (ethanol/water = 35/15) constitute the optimum formulation of Indomethacin ternary solid dispersion system. The use of SEM, DSC, XRD and FT-IR enabled us to thoroughly elucidate the solid-state interactions among the ingredients of ternary systems and appearance of new solid phases with partial amorphous state suggested the formation of ternary inclusion complex between drug and carrier. This interaction in ternary solid dispersion system can lead to important modifications in the physicochemical and biological properties of the guest molecule, such as the improvement on bioavailability that might eventually have relevant pharmaceutical potential.

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