Formulation and *In-Vitro* evaluation of Ibuprofen tablet for colonic delivery: Optimization of Formulation variables Using Box-Behnken Design

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ABSTRACT

The aim of the study was to develop a pulsatile drug release system of Ibuprofen for colon with a pre-determined lag time of about 6 hour using Ethyl Cellulose as a coating material, Polyethylene Glycol 6000 as a channelling agent and Potassium Chloride as an osmotic agent. A 3-factor, 3- level Box- Behnken design was used to construct polynomial models correlating the dependent and independent variables. Independent variables were potassium chloride, ethyl cellulose coating thickness and polyethylene glycol. The cumulative percentages of drug released at 3rd, 4th 5th, 6th and 7th hour were selected as dependent variables. A second order polynomial equation fitted to the data was used to optimize the independent formulation variables. Based on Box-Behnken experimental design, different release profiles were obtained. The optimized formulation containing 16.027 g potassium chloride in the batch size of 1000 tablets of 400 mg each was prepared. Similarly, the coating solution per litre containing 75 g ethyl cellulose and 0.16 g polyethylene glycol 6000 was prepared and applied to achieve 5 % w/w gain in the core tablet. It provided a release profile which was very close to the targeted release profile, where the calculated values of similarity factor (F_S) and dissimilarity factor (F_D) were 85.958 and 38.663 respectively.

The optimum formulation using Box-Behnken experimental design showed that low levels of the independent variables were in the optimum zone and had the potential for time controlled pulsatile delivery of Ibuprofen.

Keywords: Ibuprofen, Pulsatile, Box Behnken experimental design, Ethyl cellulose, Polyethylene Glycol

1. INTRODUCTION

Over the last few years, the colon has received considerable attention to treat colonic diseases locally and utilize its high absorption capacity for specific drugs. Several oral colon-specific drug delivery system (CDDS) are being developed as one of the site-specific drug delivery systems [1]. Targeted drug delivery into the colon is highly desirable for local treatment of a variety of bowel diseases such as ulcerative colitis, crohn's disease, amoebiasis, colonic cancer, local treatment of colonic pathologies, and systemic delivery of protein and peptide drugs [2].

Diseases where a constant drug levels are not preferred, but needs a pulse of therapeutic concentration in a periodic manner acts as a push for the development of Pulsatile Drug Delivery System (PDDS). In these systems, there is rapid and transient release of certain amount of drug molecules within a short time period immediately after a predetermined off release period [3]. Ethylcellulose (EC) is one of the most widely used water insoluble polymers in pharmaceutical coating because it is non toxic, non-allergenic, non-irritant and a good film former. EC is soluble in chloroform and alcohol and has very inert physiological action. Thus, this polymer can be applied as organic solutions or aqueous dispersions [4].

Different types of release mechanisms have been reported in the literature for EC polymer coated solid dosage forms including drug diffusion through intact macromolecular networks, crack formation and subsequent drug release through water-filled pores, drug dissolution, water penetration into the pellets, polymer swelling and/or (partial) dissolution, and osmotic effects generated by the pellet core [5].

Ibuprofen was used as model drugs because it is absorbed at different sites in the human gastrointestinal tract and to avoid gastrointestinal discomfort associated with Ibuprofen therapy. Ibuprofen is well absorbed throughout the colon. The half elimination half-life of model drug is brief, about 2 hours.

2. MATERIALS AND METHODS

2.1 Materials

Ibuprofen was received as a gift sample from Time Pharmaceuticals Pvt. Ltd, Mukundapur, Nawalparasi, Nepal. Sodium Starch Glycolate (SSG), Croscarmellose Sodium, Polyvinyl Pyrollidone K30, Magnesium Stearate, Aerosil, Potassium Chloride (KCl), Lactose, Ethyl cellulose, Polyethylene glycol 6000, Methylene Chloride, Isopropyl Alcohol and Cellulose Acetate Phthalate were obtained from Deurali Janta Pharmaceuticals Pvt. Ltd, Kathmandu, Nepal.

2.2 Methods

Box Behnken experimental design

A three-factor, three-level Box-Behnken statistical design was employed for the optimization procedure using Statgraphics Centurion software Version XV. The investigated factors (independent variables) were KCl (X_1) in the core tablet, EC coating thickness (X_2) and PEG 6000 content (X_3) in the coating composition. The levels of these factors were determined from the preliminary trial. The cumulative percentages of drug released at 3, 4, 5, 6 and 7 hr (Y_3, Y_4, Y_5, Y_6 and Y_7, respectively) were selected as dependent variables and is shown in **Table I**.

Preparation of Ibuprofen Core Tablet

Core tablet consisting of Ibuprofen (100 mg) was prepared. All the materials were wighed for the batch size of 1000 Tablets. Ibuprofen, crosscarmellose sodium and lactose were mixed in a polythene bag for 10 min manually. The granulating fluid was prepared by heating 120 ml of water to 45^{o}C. PVP K30 was added to this and was cooled to room temperature. The granulating fluid was added in the pre-mix powder and a wet mass was formed. The wet mass was passed through sieve number 10 and the resulted granules were dried at 50 ^{o}C upto residual humidity content 2-3% and sieved, by selecting sieve number 16. The dried granules obtained after passing through sieve number 16 was mixed with SSG, KCl, magnesium stearate and aerosil in a polybag for 10 min manually.

A superdisintegrant, SSG, was incorporated extragranulary to assist the breakdown of tablets into smaller granules or fragments and thus, ensure more uniform distribution of Ibuprofen throughout the colon. Wet granulation technique, although more time consuming than direct compression was employed in this study because the drug has poor flowability and compressibility.

After analyzing the granules, it was compressed using round, biconvex die punch of diameter 10.95±1 mm in a 10 station rotary compression machine at room condition of 20 ±2°C and 45 ±5 % RH. The weights of the tablets were set at 400 mg ± 5%, with thickness of 4.95±0.5 mm. The tablet weight, thickness, hardness, friability, drug content and disintegration time were checked.

Coating of the prepared tablets

In order to optimize the pulsatile release formulation with predetermined lag time of about 6-7 h, and to resist the prolonged contact with the strongly acidic gastric fluid but to dissolve in the mildly acidic or neutral intestinal environment, the Box-Behnken experimental design (**Table II**) was proceeded with coating of the core tablets with hydrophobic polymer i.e. EC and channeling agent PEG 6000 at three different concentrations (5%, 10% and 15%) followed by coating with Cellulose Acetate Phthalate.

Coating of the core tablets with Ethyl Cellulose

Core tablets was loaded in a coating pan and coated at the speed of 55±5 r/m maintaining inlet temperature 45±5 °C; coating bed temperature 25±2 °C; compressed air pressure 2.0 kg/cm^2; spray gun nozzle diameter 1 mm. The coating solution was prepared by dissolving 400 gm of EC and 0.75 gm of PEG 6000 in 3 L of methylene chloride and 1 L of isopropyl alcohol with continuous gently stirring. The coating was performed and tablets were collected at gain weight of 5, 10, and 15% on core tablets.

Coating of coated EC tablets with CAP

A 50 g of CAP was dissolved in a litre of acetone solution and was sprayed in the EC coated tablet (5% CAP solution).

Characterization of Core Tablets

The prepared tablets obtained from each experiment were evaluated regarding weight variation, hardness, thickness and diameter, friability and assay. For the determination of weight variation, 20 tablets were randomly sampled and their individual weights were taken in a digital balance. The hardness of 10 tablets (uncoated) was measured using Tablet hardness tester, Electrolab. The individual thickness and diameter of 10 tablets were randomly sampled and were measured using digital vernier caliper. Friability was determined by taking 20 tablets (uncoated) in Friability tester (Model Electrolab Friability USP EF 1W) for 4 min at 25 r/m. For estimating assay, twenty tablets were weighed and crushed in a mortar and pestle. Powder containing about 0.5 g of Ibuprofen was extracted with 60 ml of chloroform for 15 min and was filtered through a Whatman filter paper No. 42. The obtained residue was washed with three quantities, each of 10 ml of chloroform. The filtrate

was gently evaporated to dryness in a current of air. The residues was then dissolved in 100 ml of ethanol (95%), previously neutralized to phenolphthalein solution, and was titrated with 0.1 M sodium hydroxide using phenolphthalein solution as indicator. One ml of 0.1 M sodium hydroxide was equivalent to 0.02063 g of $C_{13}H_{18}O_2$ [6].

Dissolution study for coated tablets

Dissolution study was carried out in dissolution apparatus USP Type- II at 37±2°C with a rotation speed of 100 rpm using 900 ml of phosphate buffer, pH 6.8 till 9-10 h. Ten ml of the sample was withdrawn at an interval of an hour and equal volume of fresh medium was replaced into the dissolution jar after each sampling. The withdrawn sample was subjected to filter and the filtered sample was then used for quantitative analysis using UV Spectrophotometric method. Cumulative percentage of drug released from the tablets were calculated and plotted as a function of time.

A UV Spectrophotometric method of quantitative analysis was developed and validated for the dissolution medium.

Validation of Analytical Method

UV Visible-Spectrophotometric method for dissolution studies was developed and validated. Validation of dissolution method was done in terms of linearity curve, specificity, accuracy and precision.

Determination of Absorption maxima

A 100 mg of Ibuprofen was weighed accurately and was dissolved in 5 ml of methanol and the volume was made to 100 ml with phosphate buffer, pH 6.8 in 100 ml volumetric flask and was labeled as a stock solution. Two ml of stock solution was transferred into 100 ml volumetric flask and was diluted upto 100 ml with phosphate buffer, pH 6.8. The resulting solution was labelled as standard working solution. The spectrum of this solution was run in 200-400 nm range in UV-visible spectrophotometer. The λ max of Ibuprofen was found to be 223 nm.

Linearity Curve

From working standard solution, 2 ml, 4 ml, 6 ml, 8 ml and 10 ml were withdrawn and diluted to 10 ml with phosphate buffer, pH 6.8 in 10 ml volumetric flask to get concentration of 4 µg, 8 µg, 12 µg, 16 µg and 20 µg respectively. The absorbance of each solution was measured by UV visible spectrophotometer at 223 nm using phosphate buffer, pH 6.8 as a blank. The curve of absorbance versus concentration was then plotted. Linear equation and correlation coefficient (R^2) of the curve were determined.

Specificity

Spectrum was run for both the standard and test sample in phosphate buffer, pH 6.8 to see whether graphs of the excipients interferes the graph of the active ingredient or not.

Accuracy and Precision

To determine the accuracy and precision, known amount of Ibuprofen such as 17.5 mg, 25 mg, and 32.5 mg (each n=3 of Ibuprofen) was added to the tablet calculating weight of placebo mixture and analyzed in phosphate buffer, pH 6.8. Three replicates at each concentration were assessed in the dissolution medium and % recovery was calculated after obtaining the calculated value.

Data Analysis

The obtained data were statistically evaluated using Statgraphics Centurion Version XV. The drug release profiles in terms of achieving lag time with osmotic agent KCl and different amount of EC and channeling agent PEG 6000 in the coating formulations was analyzed with the hypothesis that there is no significant effect in lag time with varying level of KCl in the core tablet and EC and PEG 6000 in the coating solution, at 95% of confidence interval. Each response variable were evaluated with respect to the independent factors and their relation were presented in the form of Equation (1)

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (1)$$

where Y_i is the measured response associated with each factor level combination, b_0 is an intercept, b_1 to b_{33} are the estimated regression coefficients computed from the observed experimental values of Y_i, and X_1, X_2 and X_3 are the coded levels of independent variables. The terms X_i X_i and X_i^2 (i=1, 2, or 3) represent the interaction and quadratic terms, respectively[25]. Analysis of Variance (ANOVA) of the mathematical relationships was performed and selected on the basis of highest R_a^2 value, and significant effect ($P < 0.05$). Coefficients of each estimate i.e. independent and interacting variables on the equation were also evaluated using ANOVA for their significant effect ($P < 0.05$) and nature of the effect (positive or negative) on the response variable.

Multiple Response Optimization was used for optimization the amount of EC, PEG 6000 and KCl in the formulation at desired lag time of the dosage form. A 3D response plots was constructed and one final formulation corresponding to the predicted optimum lag time, coating level, PEG 6000 concentration and

amount of KCl was determined. Subsequently, the resultant experimental data of the response properties was quantitatively compared with those of the predicted values.

Similarity and dissimilarity test

$$F_s = 50\ Xlog\left\{\left[1 + \left(\frac{1}{n}\right)\sum_{j=1}^{n}(Rj - Tj)^2\right]^{-0.5} X\ 100\right\} \text{.....} \quad (2)$$

Similarity (F_S) and dissimilarity (F_D) test, a model independent study of dissolution profiles was used and compared in the cumulative percentage drug release for the predicted and observed drug release.

$$F_d = \left[\frac{\sum_{i=1}^{n}(Rj-Tj)}{\sum_{i=1}^{n}Rj}\right]X100 \text{.....} \quad (3)$$

3. RESULTS AND DISCUSSION

Characterization of core tablets

All the prepared tablets were found to be of good quality with acceptable physical characteristics. The average weight of the uncoated tablet received was found slightly less than the actual theoretical values, but it lies within the pharmacopeial limit (±5% of average weight). The thickness range was found to be in between 4.94 mm to 5.03 mm, while the diameter range was found to be in between 10.98 mm to 11.00 mm. Similarly, the hardness range was found to be in between 4.55 Kg/cm^2 to 4.73 Kg/cm^2 in all the formulation indicating good mechanical strength having a capability to withstand physical and mechanical stress condition while handling and transportation. The friability of all the formulation was less than 1% which meets with the Indian Pharmacopoeia limits. All formulations tablets were disintegrated before 3 minutes.

The assay values of all the formulated tablets were found in between 94.86% to 98.11%. Assay difference was due to the weight variation as well as process errors while mixing.

Validation of Analytical Method

Linearity

Absorbance was measured by UV Visible Spectrophotometer at 223 nm of the concentration of 4 µg, 8 µg, 12 µg, 16 µg and 20 µg respectively. From the working standard solution, the curve was plotted by taking absorbance on y-axis and concentration on x-axis (Figure 1). The graph shows that there is linear relationship between concentration and the absorbance with correlation coefficient (R^2) 0.9994 and regression Equation (4).

$$\boldsymbol{y = 0.044x + 0.014} \text{.....} \quad (4)$$

Specificity

After scanning the active ingredient and excipients in the range of 200-400 nm, in the UV-visible spectrophotometer, a prominent peak was observed by the active ingredient at 223 nm in phosphate buffer, pH 6.8 while a flat line was observed in the placebo at the same range of wavelength.

Accuracy and Precision

To determine the accuracy and precision, 17.5 mg, 25 mg and 32.5 mg (each n=3) of Ibuprofen was added to the tablet, calculating weight of placebo mixture and analyzed in the phosphate buffer, pH 6.8 and the absorbance was taken at 223 nm using UV- Visible spectrophotometer.

This method found to be accurate as the recovery values lie within the limit of 98.00 to 102.00% with a lower limit of 98.17% and upper limit of 100.44% while the relative standard deviation (RSD) was calculated and was found to be 0.927%. Thus, the method of analysis was found to be accurate and precised one.

***In-Vitro* Dissolution Study**

For experimental designing, total 15 different formulations were prepared using 3^3 (Three factors at three levels) full factorial orthogonal design. The *In-vitro* dissolution test was carried out for all 15 different formulations in phosphate buffer, pH 6.8 for 7 hr. The cumulative percentage drug release obtained from the formulated tablets are given in **Table III**.

The minimum lag-time of 4 hr was observed for formulation F3, while lag time of 5 hr was observed for formulation F4 and F9. The formulations F3, F4 and F9 showed a burst release of the drug after their lag time. The formulation F5 showed a lag time of 5 hr but a burst release of the drug was not observed after its lag time. Though F3 had same EC coating thickness as compared to compared to F4, F5 and F9, minimum lag time obtained may be due to the non uniformity coating as well as high level of pore forming agent in formulation. With high level of PEG 6000 in the coating solution, more pores may have formed in the rupturable layer as the water soluble plasticizer gets dissolved with the dissolution medium as a result of which dissolution medium diffuses inside the drug core. The core tablet has osmotic agent (KCl) and swelling agent containing a superdisintegrant (SSG) to facilitate in imbibing the dissolution medium inside the core tablet. The medium swell to create a pressure for the outer barrier layer to rupture and the drug releases instantly. The lag time was not observed for the rest of other formulations i.e. for 10% EC coating and 15% EC coating. Thus the

dissolution profile for different 15 formulations showed that increasing the coating thickness cause the drug retardation.

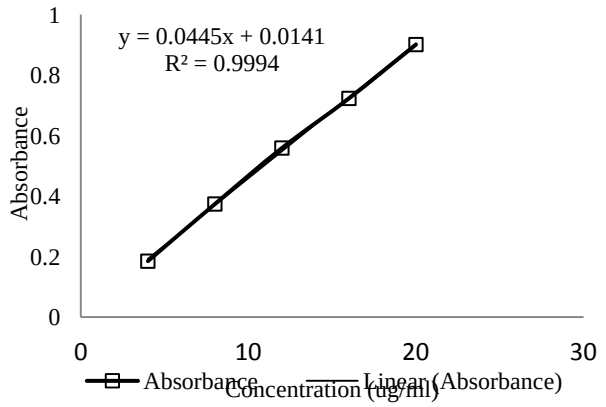

Figure I. Standard calibration curve of Ibuprofen in phosphate buffer, pH 6.8.

Determination of the Regression Model and statistical evaluation

For experimental designing, total 15 different formulations were prepared using 3^3 (Three factors at three levels) full factorial orthogonal design. The independent variables and dependent variables used in the design are listed in Table 1. The formulations were evaluated with 5 response variables, which are cumulative % drug release in 3^{rd} hr, 4^{th} hr, 5^{th} hr, 6^{th} hr and 7^{th} hr respectively. (Y_3 to Y_7 dependent variables).

The experimental run for 15 formulations is summarized in **Table II**.

The result obtained for response variables from the experiment was statistically analyzed and the mathematical relationships for independent factors and each response variable were obtained by regression analysis using statistical package (Statgraphics version XV). The obtained relationship were in the form of Equation (1). The selection of the best fitting mathematical model involving the individual main efforts and interaction factors was based on the comparison of some statistical parameters including the multiple regression coefficient (R^2), adjusted multiple regression coefficient (R_a^2), and the mean absolute error (MAE), provided by the Statgraphics Centurion software version XV. Among the different mathematical relationship shown in Table 4, response Y_7 was chosen because it had the smallest value of MAE and largest values of adjusted R_a^2 indicating good fit. MAE indicates how well the model fits the data. The smaller the MAE statistic is, the better the model fits to the data points [7].

Regression coefficients and their estimates of regression model for responses Y_3 to Y_7 are given in Table 4. The coefficient estimate and standardized main effects (SME) values in the form of a polynomial equation for the responses are given in Table VI. SME values were calculated by dividing the main effects by the standard error of the main effects [7].

From **Table VI**, it could be inferred that EC coating thickness (X_2) and PEG 6000 (X_3) were significant in controlling the release of Ibuprofen throughout the dissolution time ($p \leq 0.05$). In addition, EC coating thickness (X_2) showed the largest SME (-2.66, -2.050, -3.729, -21.398 and -195.41 at Y_3, Y_4, Y_5, Y_6 and Y_7 respectively) indicating that EC coating thickness (X_2) was the main influential factor on pulsatile drug release from the tested tablets in the whole stages of Ibuprofen *in-vitro* release studies.

As evident from Table VI, the effect of KCl did not show any estimated effect for response variable Y_3-Y_7. This could be attributed to the fact that the spontaneous movement of a solvent from a solution of lower solute concentration to a solution of higher solute concentration through an ideal semipermeable membrane, which is permeable only to the solvent but impermeable to the solute could be retarded by the EC coating thickness.

Optimization of Drug Release and Validation of Optimized Formulation

After generating the polynomial equations relating the dependent and independent variables, the release profile was optimized for the responses Y_3 to Y_7. The desirability function was evaluated at each point in the design. Among the design points, the maximum desirability was found to be achieved at run 4. The optimum value of optimize desirability was found to be 0.777. To find the combination of factors which achieves the overall optimum desirability, the desirable range of these responses was restricted to the low and high value along with their goals, which are listed in Table V. The optimum values of the variables were obtained by graphical and numerical analysis using statgraphics centurion software (version XV) and based on criterion of desirability.

As KCl being an insignificant factor, any value for KCl within the range should give the same result for different variable. So, statgraphics centurion software (version XV) gave a value of KCl in optimization

formulation which is possibly a least value in the range and can be comfortably adjusted in the formulation. Figure II represents an estimated response surface plot showing the optimized parameters suggested by the statgraphics centurion software (version XV) to get the responses in the required range. Constraints used for optimization, formula for optimized formulation and predicted and observed responses for the optimized formulation are given in **Table V**. To verify the predictability, a new formulation was prepared at the optimum levels of the independent variables, and results were observed for responses Y_3 to Y_7. The responses for optimized formulation were evaluated against the predicted responses. The observed values of Y_3 to Y_7 were in a very close agreement to the predicted one. By this the validity of the optimization procedure was proven. **Table VI** shows that the optimized formulation prepared according to computer-determined levels exhibited a release profile which was close to that of the ideal targeted release profile. Additionally, these dissolution profiles were compared using two mathematical model independent similarity test (F_S) and dissimilarity test (F_D). From this, the formulation that was similar to the optimized formulation was compared. The value of similarity (F_S) and dissimilarity (F_D) of the predicted and observed dissolution profile obtained was 85.958 and 38.663 which clearly indicates that the drug release profile of observed and predicted dissolution profile are identical as the similarity value (F_S) is greater than 50 and thus confirms the practicability of the model.

The dissolution profile for optimized batch showed that the dosage form was intact within 5th hour of the study, then a crack gradually developed at the edges of the tablets with minimal release of the drug from the ruptured edge till the 5th hour and during the mid of the 5th hour, there was abrupt release of the drug through the ruptured edges with almost complete burst release in the 6th hour of the dissolution study.

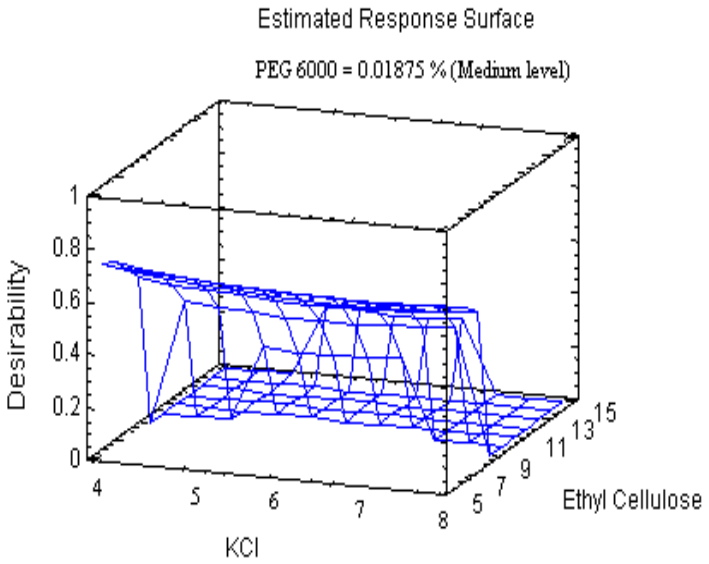

Figure II. Estimated Response Surface plot for optimized variables

4. CONCLUSION

Optimization of a colon targeted formulation is a complex process that requires a large number of variables and their interactions to be considered. The present study conclusively demonstrates the usefullness of a Box-Behnken design in optimization of colon targeted formulations. The derived polynomial equations and contour plots aid in predicting the values of selected independent variables for preparation of the optimium pulsatile release colon targeted formulation of Ibuprofen with desired properties. Thus, for the optimization formulation, the optimum formulation using the contour plot showed that low levels of the independent variables were in the optimum zone and had the potential for time controlled pulsatile delivery of Ibuprofen (release after 6 hr of drug intake).

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Independent Variables	Level		
	Low	Medium	High
Tranformed values	-1	0	+1
X_1 = KCl (%)	4	6	8
X_2 = Thickness (%)	5	10	15
X_3 = PEG 6000 (%)	0.015	0.01875	0.0225
Dependent variable			
Amount of drug released after 3 hr. (Y_3)			
Amount of drug released after 4 hr. (Y_4)			
Amount of drug released after 5 hr. (Y_5)			
Amount of drug released after 6 hr. (Y_6)			
Amount of drug released after 7 hr. (Y_7)			

Table I: Variables in box-behnken design.

Batch No. (Batch Size:1000 Tabs)	KCl (%)	EC coating thickness in each tablet (%w/w)	PEG in coating solution (%)
F1	6	10	0.01875
F2	8	15	0.01875
F3	6	5	0.0225
F4	4	5	0.01875
F5	6	5	0.0150
F6	6	15	0.0225
F7	8	10	0.0150
F8	6	10	0.01875
F9	8	5	0.01875
F10	8	10	0.0225
F11	4	15	0.01875
F12	4	10	0.0225
F13	6	15	0.0150
F14	4	10	0.0150
F15	6	10	0.01875

Table II: Box-behnken experimental design

Time (hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
3	1.72	1.91	3.69	1.31	2.5	1.31	0.78	1.72	5.43	1.01	0.34	2.31	1.003	3.18	0.78
4	1.06	3.88	6.53	1.63	3.25	2.19	1.03	1.06	3.25	3.05	0.37	4. 83	0.91	2.19	1.03
5	2.07	3.14	83.43	18.51	9.36	2.33	2.2	2.07	18.32	2.98	0.51	2.07	0.96	1.24	2.29
6	2.18	3.55	95.97	95.01	65.37	2.47	2.14	2.18	97.93	2.18	0.65	2.18	0.76	2.53	4.60
7	5.84	5.12	96.77	97.59	92.82	2.39	4.89	5.84	-	4.33	-	5.84	0.62	2.87	5.59

Table III: Dissolution profiles of the different formulations

Regression model	R^2	(R_a^2) (adjusted)	MAE
$Y_3 = 0.199617 + 0.159375X_1 + 0.173325X_2 - 0.0159375X_1X_2$	41.31	31.53	0.84
$Y_4 = 33.1152 - 0.829519X_2 - 79.0474X_3 + 0.0323385X_2^2 + 57.8205X_3^2$	61.62	46.28	0.78
$Y_5 = 26.4987 + 5.22031X_1 + 3.15833X_2 - 156.097X_3 - 0.108757X_1^2 + 0.597483X_2^2 - 24.2333X_2X_3 + 308.426X_3^2$	82.67	69.68	6.81
$Y_6 = 90.305 - 2.78X_1 - 35.5221X_2 + 418.689X_3 + 0.0579167X_1^2 + 1.70367X_2^2 - 9.63X_2X_3 - 197.148X_3^2$	98.83	97.96	3.41
$Y_7 = 193.415 - 0.306042X_1 - 44.2493X_2 + 216.608X_3 + 0.023431X_1^2 - 0.0267083X_1X_2 - 0.735417X_1X_3 + 1.80668X_2^2 - 0.726667X_2X^3 - 123.278X_3^2$	99.99	99.98	0.29

Table IV: Regression coefficients and their estimates of regression model for responses y_3 to y_7.

Independent Variable		Low, -1	Medium, 0	High, +1	Optimum	
Potassium chloride (X_1)		4%	6%	8%	4.006%	
EC Coating thickness (X_2)		5.0%	10%	15%	5.0%	
PEG 6000 (X_3)		0.015%	0.01875%	0.0225%	0.016275%	
		Constrains			**Optimized**	
Dependent/Response Variable		Low	High	Goal	Predicted	Observed
% of drug release in 3rd hour	Y_3	0.34	5.43	Maintain	2.34	1.47
% of drug release in 4th hour	Y_4	0.37	6.53	Maintain	2.81	2.36
% of drug release in 5th hour	Y_5	0.51	83.43	Maintain	10.20	11.81
% of drug release in 6th hour	Y_6	0.65	97.93	Maximize	83.30	80.75
% of drug release in 7th hour	Y_7	0.62	100.00	Maximize	95.04	96.82

Table V: Variables, constraints and output of experimental designing.

		X_1	X_2	X_3	X_1^2	X_2^2	X_3^2	X_1X_2	X_1X_3	X_2X_3
Y_3	**CE**	-	-2.09	-	-	-	-	-1.27	-	-
	P-value	-	0.02	-	-	-	-	-0.272	-	-
	SME	-	-2.66	-	-	-	-	-1.15	-	-
Y_4	**CE**	-	-1.82	2.30	-	1.61	2.6	-	-	-
	P-value	-	0.067	0.027	-	0.244	0.074	-	-	-
	SME	-	-2.05	2.58	-	1.23	1.98	-	-	-
Y_5	**CE**	-	-30.67	19.26	-13.92	29.87	13.87	-	-	-36.3
	P-value	-	0.005	0.047	0.283	0.038	0.284	-	-	0.014
	SME	-	-3.72	2.34	-1.14	2.46	1.14	-	-	-3.12
Y_6	**CE**	-	-86.71	8.000	7.41	85.18	-8.87	1.19	-	-14.4
	P-value	-	0	0.083	0.249	0.000	0.175	0.035	-	0.035
	SME	-	-21.39	1.97	1.24	14.27	-1.48	-	-	-2.52
Y_7	**CE**	-	-93.01	2.03	2.99	90.33	-5.54	-2.13	-1.76	-1.09
	P-value	-	0.000	0.004	0.005	0.000	0.000	0.036	0.029	0.12
	SME	-	-195.4	4.93	4.60	138.76	-8.52	-2.84	-3.027	-1.869

Standardized main effects (SME) were calculated by dividing the main effect by the standard error of the main effect.
CE: Coefficient estimate

Table VI: Standardized main effects of the factors on the responses (y_3-y_7)