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STATISTICAL OPTIMIZATION OF COMPRESSION COATED KETOPROFEN TABLET USING AMYLOSE/ETHYL CELLULOSE MIXTURE FOR COLONIC DELIVERY

Nirmala Devi[1*], Manju Sharma[2]

1. Shridhar University Pilani-Chirawa Road, Pilani-333 031 (Rajasthan)

2. Faculty of Pharmacy, Hamdard University,New Delhi

In the present study the effect of two independent factors (amount of ethyl cellulose in coating layer and coating level) on ketoprofen release from compression coated tablet in order to optimize coated tablet for colonic delivery. 3^2 factorial design was used for designing coated formulation. Amount of ethyl cellulose (X1) and coating level (X2) were selected as independent variables. The studied responses were drug release at 5 hr (Y1) and drug release at 10 hr (Y2). The core tablets were compression coated with different ratio of amylose and ethylcellulose. *In vitro* drug release study was carried out in pH1.2 for 2 hr, pH 7.4 for 3 hr and goat caecal medium for 5 hr. Drug release revealed that amount of ethyl cellulose and coating level have antagonistic effect on drug release. Multiple regression analysis was used for generation of polynomial equation and optimization of formulation. The optimized formulation consisted of ethyl cellulose (14.33 %) and coating level (318 mg) provided a release profile that is closed to estimated values. The model is found to be accurate and robust for optimization of compression-coated tablet for colonic delivery of ketoprofen.

Keywords: Colon drug delivery, factorial design, independent variables, multiple linear regression, optimization

INTRODUCTION

Colon targeted drug delivery provides better options in the treatment of many diseases of colon such as colon cancer, ulcerative colitis, chron's disease etc. Colon targeted drug delivery system also has improvised systemic absorption of polypeptides and many other drugs susceptible to enzymatic digestion in the upper gastrointestinal tract (1-4).

There are various approaches used for colon targeted drug delivery including time dependent, pH dependent, GI pressure dependent and colonic microflora enzyme activated systems. Time dependent approach is a less reliable option due to high inter-subject variability in gastric emptying time. The pH dependent approach has also poor colon specificity due to less difference in pH between small intestine and colon. Specific enzyme secretions by colonic microflora makes enzyme activated systems as the most reliable approach (5-7). In this orbit, most suitable carrier for colon targeted drug delivery is polysaccharides (8-12). Among these carriers, amylose is a viable one which is an unbranched linear polymer of glycopyranose units (α-1,4-D glucose) linked through α-D (1-4) linkage. It is resistant to pancreatic amylase but it degraded by the colonic bacterial enzyme (13). Amylose has been used as matrix and coating material for oral delayed released formulations intended for colon targeting (14-17). However, pure amylose takes up considerable amounts of water upon contact with aqueous body fluids. Due to the very permeable nature of the amylose, the drug has been released before the colon is reached. To overcome this drawback, water insoluble polymers are used with amylose for colon targeted drug delivery (18).

Pharmaceutical industry mostly used solvent coating techniques. But these techniques have several drawbacks such as time consuming nature; drug stability for heat labile and hydrolysis; costly and environment pollution. To overcome these, non solvent coating techniques were gained interest in recent years. Among non solvent techniques compression coating is the simple and cost effective. Now a day compression coating is often used for hydroscopic and heat sensitive drugs; to separate incompatible drugs in fixed dose combination and to modify drug release pattern.

Ketoprofen has been used as a model drug in the present study, which is a propionic acid class non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs are widely utilized in the treatment of chronic inflammatory bowel disease. Moreover, they showed an effective role in prevention and treatment of colitis and colon cancers (19-22). However, once they are administered orally, a large amount of the drug is absorbed from the upper GIT, and causes systemic side effects. Therefore, it is preferred to deliver the drug targeted to the colon.

Experimental design is an effective statistical tool to study simultaneously the effect independent formulation variables at

For Correspondence

nirmalapharma21@gmail.com

different levels on various responses. A growing body of published literatures indicated the application of factorial design approach for colon-targeted formulations by many researchers (7, 23, 24).

The present study aims at the preparation of an optimized compression coated tablet formulation and evaluation of the same for better colonic drug delivery. The study also deals with the effect of two variables on the release profile of ketoprofen by utilizing a factorial design approach to get an optimized formulation where the two variables mentioned, includes the amount of ethyl cellulose and the coating level.

MATERIALS AND METHOD

Ketoprofen was received as a gift sample from Alembic Pharmaceuticals; India. Glassy amporphus amylose was obtained from Shekharr Starch Pvt. Ltd.; Mumbai, India. All other materials such as microcrystalline cellulose, cross linked polyvinyl pyrrolidone, corn starch, magnesium stearate and ethyl cellulose were obtained from SD Fine Chemicals Ltd.; Mumbai, India.

Experimental Design

In this study, a 3^2 factorial design was used for optimisation of the formulation. The independent variables were percentage of ethyl cellulose (X1) and coating level (X 2). The dependent variables were percentage of drug release in 5 hr (Y1) and percentage of drug release in 10 hr (Y2). The formulation design along with values for variables is presented in the table 1 & 2.

Table 1: Factor and responses for experimental design

Independent variables (Factor)			Dependent variables (Response)
Level	**X1**	**X 2**	Y1 =Percentage Drug release at 5 hr (Q 5)
-1	0	300	
0	20	400	Y 2= Percentage Drug release at 10 hr (Q 10)
+1	40	500	

X1 – Ethyl cellulose content (%); X 2 – Coating level

The science behind the selection of the above dependent variables is supported by the fact that colon targeted delivery becomes successful when the release rate obeys a slow or nil profile in the upper GIT and a subsequent optimum release rate profile in the colonic environment. For a successful colonic drug delivery system, it is essential to release the drug in the colonic environment without any release in the upper GIT. A slow release in the upper GIT can also be acceptable to a considerable extent. Therefore, the dependent variables i.e. the time points (5 hr& 10 hr) are very much significant for the study design.

Table 2: Experimental Design lay out and observed Results

Std order	Formulation	Independent variables (Factor)		Dependent variables (Response)	
		X1	**X 2**	**Y1**	**Y2**
1	F 1	-1	-1	28.35	98.65
2	F 2	0	-1	16.32	70.39
3	F 3	1	-1	10.66	56.72
4	F 4	-1	0	26.61	94.08
5	F 5	0	0	15.89	62.98
6	F 6	1	0	8.83	48.02
7	F 7	-1	1	25.1	93.2
8	F 8	0	1	13.82	56.73
9	F 9	1	1	6.98	40.72

X1 indicates ethyl cellulose content (%); X2 indicates coating level (mg); Y1 indicates Percentage Drug release at 5 hr (Q 5); Y2 indicates Percentage Drug release at 10 hr (Q 10)

Preparation of ketoprofen containing core tablet

Ketoprofen core tablet was prepared by direct compression method. Each core tablet contained ketoprofen (50 mg), microcrystalline cellulose (60 mg) and sodium starch glycolate (5mg). Mixture of stearate (1 %) and talc (2 %) was added as lubricant. Core tablets (diameter 6 mm, average tablet weight 120 mg) were compressed within 6 mm of punches on cadmach 16 station compression machine under a common compression force of 3-4 kg/cm^2.The core tablets were evaluated for hardness, content uniformity, thickness, friability and dissolution performed in different dissolution medium (pH 1.2, 6.8, 7.4) .

Coating of core tablets

The core tablets were compression coated with different ratio of amylose and ethyl cellulose. Half amount of polymer blend placed in the die cavity; the core tablet positioned centrally in the die cavity. Die cavity was filled with remaining half amount of polymer blend. The coating material compressed around the core tablet within 12 mm of punches on cadmach 16-station compression machine under common compression force of 5-6 kg/cm^2.

In vitro drug release study

The integrity of compression-coated tablets of ketoprofen in physiological environment of upper gastrointestinal tract was evaluated by performing dissolution study under condition mimicking mouth to colon transit environment. The dissolution test was carried out using the USP XXXIII type II apparatus (paddle apparatus TDL 08 L; Electrolab India Pvt Ltd, Mumbai, India) with a rotation speed of 100 RPM in 0.1 N HCl (900 ml) for 2 hr. Then the dissolution medium was replaced with pH 7.4 Sorensen's phosphate buffer (900 ml) and further studied for the next 3 hrs. At specified time points, 5 ml sample was taken, suitably diluted and analyzed for ketoprofen content using HPLC method (25). To study enzymatic action of colonic bacteria on amylose, the dissolution study was continued in 900 ml of pH 6.8 phosphate buffer saline using goat caecal content up to 10 hr (26).

RESULT AND DISCUSSION

The ketoprofen core tablet was prepared by direct compression method. The average weight of core tablet was found to be 120±1.3 mg. The hardness was found to be in the range of 3-4 kg/cm^2. Weight loss in friability test was found to be less than 0.5 %, indicating compliance with acceptance limit. The mean drug content of core tablet was found to be 98.23±1.39 %.The core tablets were also found to comply with the disintegration test as the core tablet disintegrate within 15 minute.

The thickness of core tablet was found to be 1.64± 0,04 mm. The drug release profile (Fig 1) of core tablet in various medium (pH 1.2, 6.8, 7.4) shows no lag time. More than 75 % of the drug was found to be released within the initial 30 minutes.

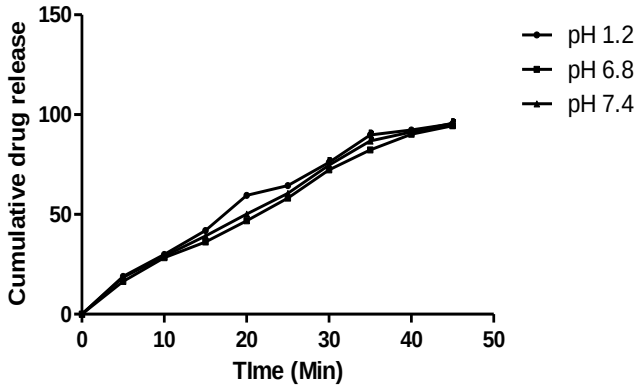

Figure 1. *In vitro* drug release profile of core tablet at pH 1.2, pH 6.8 & pH 7.4

Nine compression-coated formulations were suggested by 3^2 factorial designs for two dependent variables i.e., amount of ethyl cellulose (X1) and coating level (X2). The post compression parameters of compression coated tablets were presented in table 3.

The dissolution studies of coated tablets were carried out at pH 1.2 for 2 hr, pH 7.4 for 3 hr and pH 6.8 (goat cecal content) till 10 hr. The drug release profiles of coated tablets are shown in figure 2. At pH 1.2, coated formulations demonstrated very less drug release (below 6.5%). At pH 7.4 (simulated intestinal fluid), coated tablets demonstrated drug release not more than 20 %.The result showed a minimal drug release in physiological environment of stomach and small intestine. It is anticipated that presence of hydrophobic polymer (ethyl cellulose) in coating layer, prevents leaching of drug in to upper GIT medium.

The drug release of compression coated tablets in pH 7.4 for 3 hr (simulated intestinal fluid) revealed that formulations (F1, F 3, F7) containing only amylase shows drug release 28.35 %, 26.6 % and 25.1 % for F1, F 3 and F7 respectively. This indicated that single coating of amylose was not suitable to prevent drug release in small intestine. As the hydrophobic content (ethyl cellulose) added up to 20 % in coating layer (F 2, F 5, F 7) drug release at 5 hr was found to be decreased. Formulation F4, F 5 and F 8 released 16.32 %, 15.89 % and 13.83 % of drug at 5 hr respectively. On further increase in ethyl cellulose content from 20 % to 40 % in formulations (F3, F6, F9), drug release at 5 hr was found to be diminished to 10.66 %, 8.83 % and 6.93 % for F3, F6 and F9 respectively. This demonstrated that increase in hydrophobic content (ethyl cellulose) in coating layer, minimized drug release in small intestine.

Amylose swelled in presence of the medium, which leads to a disruption in the structure of coating layer and formulation of aqueous filled pores through which drug release can occur. Ethyl cellulose is insoluble in nature and poorly swellable in aqueous medium. Ethyl cellulose prevent water uptake and drug release in upper GIT. As coating level was increased, drug release in pH 7.4 was found to be diminished. This might be due to increase in diffusion path length.

Table 3: Post compression characteristics of various formulation (F 1-F 10)

Formulation code	Parameter				
	Thickness (mm)	Weight (mg)	Hardness (Kg/cm^2)	Friability (%)	Assay (%)
F 1	2.51±0.12	421.34±2.31	5.4±0.13	0.62±0.08	100.65±2.32
F 2	2.54±0.21	419.64±2.49	5.6±0.18	0.53±0.03	100.45 ± 2.93
F 3	2.58±0.11	420.72± 2.21	5.7±0.19	0.62±0.04	103.11±1.93
F 4	3.42±0.19	520.18± 3.42	5.9±0.21	0.64±0.08	101.33 ± 1.43
F 5	3.46±0.21	521.89±2.86	5.8±0.14	0.73±0.05	103.12±2.92
F 6	3.44±0.12	522.34±3.43	5.3±0.24	0.78±0.06	100.71 ±2.74
F 7	4.42±0.15	621.32±2.23	6.1±0.14	0.58±0.03	102.49 ± 1.84
F 8	4.39± 0.17	619.31±1.98	5.9± 0.12	0.73±0.02	100.21 ± 2.21
F 9	4.37±0.19	622.32±2.94	6.2 ± 0.15	0.81±0.06	100.68 ± 1.94
F 10	4.19±0.21	500.23±2.80	5.4±0.14	0.57±0.04	100.38 ± 2.12

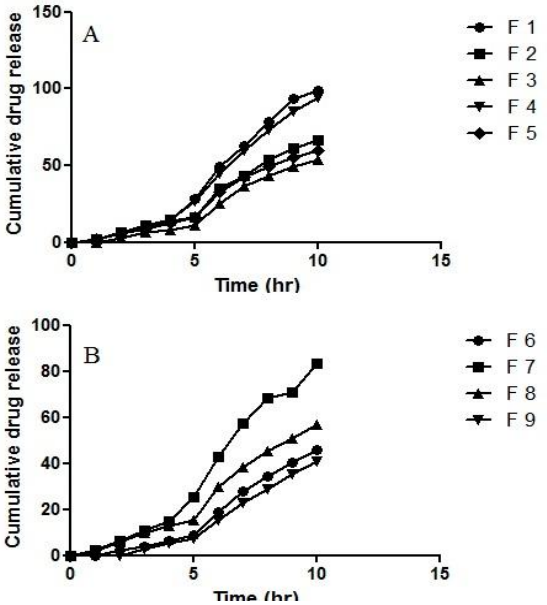

Figure 2 *In vitro* drug release profile of compression coated formulations in pH 1.2(2 hr), pH 7.4 (3 hr) and pH 6.8 containing goat caecal (5 hr)

Table 4: Coefficient and *p*-value of each factor, for response Y1 and Y2

Factor	Y1		Y2	
	Coefficient	*p*-value	Coefficient	*p*-value
X1	-8.93	<0.0001	- 23.41	<0.0001
X2	-1.57	0.0037	- 5.85	0.0009
X12	-0.11	**0.6765**	- 2.64	0.0166
X_1^2	2.41	0.0053	8.53	0.0016
X_2^2	-0.24	**0.5225**	1.04	**0.2671**

Significant factor ($p < 0.05$).All bold values have p- value > 0.05, hence considered insignificant.

Table 5: Result of Analysis of variance (ANOVA)

Source of Variation	Df	SS	MS	F	R^2	*p*-value
ResponseY1, Drug release at 5 hr (%)						
Model	3	505.10	168.37	1034.47	0.9984	< 0.0001
Residual	5	0.81	0.16			
Total	8	505.91				
ResponseY2, Drug release at 10 hr (%)						
Model	4	3667.49	916.87	644.36	0.9985	< 0.0001
Residual	4	5.69	1.42			
Total	8	3673.18				

DF indicates: degrees of freedom; SS, sum of squares; MS, mean of squares; F, fischer's ratio; R^2 , regression coefficient..

To determine the effect of colonic enzymes on ketoprofen release, dissolution studies were further carried out in pH 6.8 containing goat caecal content for next 5 hr. All the formulations demonstrated a rapid drug release in the initial hour. This could be due to degradation of amylose in the coat by bacterial amylase normally found in colon. The tablets only coated with amylose (F 1, F 4, F 7) showed rapid drug release in goat cecal medium and drug release was found to be 98.65 %, 94.02 % and 93.20 % at 10 hr respectively. Amylose was rapidly fermentable in the colon and ethyl cellulose is non fermentable in the colon. Therefore amylose and ethyl cellulose are unsatisfactory as sole coating agent and it is necessary to blend both in order to improve the mechanical properties of coat for successful colon targeted drug delivery(17). Drug release of tablets containing 20 % ethyl cellulose was found to be 70.39 %, 62.98 % and 56.73 % at 10 hr for F 2, F 5 & F 8

respectively. On further increasing ethyl cellulose content from 20 % to 40 %, the drug release was found to be decreased to 56.72 %, 48.02 % and 40.72 % at 10 hr for F 3, F 6 and F 9 respectively. It is suggested that ethylcellulose had prevented the disruption of coat thereby retarding the drug release.When the coat thickness increased, drug release in caecal content medium was found to be slower. A multivariate optimization was carried out in order to find optimum ethyl cellulose content and coating level to achieve a optimum colon targeted drug delivery from a compression coated tablets. The statistical analysis of the response was performed by one-way ANOVA using Design Expert software version 7.0.0. F test was used to evaluate individual response. To establish a relationship between independent and dependent variables, polynomial equation was generated using multiple linear regression analysis. The polynomial equation generated that fitted to data was as follow:

$$Y_i=b_0+b_1X_1+b_2X_2+b_{12}X_1X_2+b_{11}X_1^2+b_{22}X_2^2 \quad \text{..... (Eq.1)}$$

Where Y is the response (dependent variable), b_0 is the arithmetic mean of all the outcomes of 9 runs ; b_1, b_2, b_{12}, b_{11}, b_{12} are the coefficient computed from the observed experimental value; and X_1, X_2 stands for average result of changing one factor at a time from its low value to high value. The X_1& X_2 interaction term shows the response when two response simultaneously changed. The quadratic term (X_1^2 & X_2^2) were used to imitate non linearity of design space.The mathematical equations of responses are as follow:

$$Y_1 = +15.50 - 8.93X_1 - 1.57X_2 - 0.11X_1X_2 + 2.41X_1^2 - 0.24X_2^2 \quad \text{..... (Eq. 2)}$$

$$Y_2=+62.67 - 23.91X_1 — 5.85X_2 - 2.64X_1X_2 + 8.53X_1^2 - 1.04X_2^2 \quad \text{..... (Eq. 3)}$$

Table 4 shows the factor effect of model and respective P values for responses Y_1 and Y_2. A factor significantly affects the response, if p value is less than 0.05. X_1X_2 and X_2^2were found to be non-significant for response Y_1. For response Y_2, $X_2^2$was found to be insignificant. A backward elimination procedure by eliminating non significant term ($p > 0.05$) was adopted to fit the data in to different predictor equations.

The final equation of the responses is given below:

$$Y_1=+15.50 - 8.93X_1 - 1.57X_2 + 2.41X_1^2 \quad \text{..... (Eq. 4)}$$

$$Y_2=+63.37 - 23.41X_1 — 5.85X_2 - 2.64X_1X_2 + 8.53X_1^2 \quad \text{...(Eq. 5)}$$

Table 5 shows result of analysis of variance (ANOVA). Larger F value and high R square value indicated that models were significant and valid.

To validate the polynomial mathematical model, dissolution of four random formulations covering the entire range of independent variables were performed. For each of these formulations, value of X1 and X2 were substituted to estimate response Y1 and Y2. Table 6 shows the experimental condition of random formulations, predication and observed value of responses along with percentage prediction error. Linear correlation curve (Fig.3) between observed and predicated responses, establish a close agreement (r^2> 0.98). Robustness of mathematical model is demonstrated by significant value of r^2 and lower value of percentage predication error (-2.80- 3.61 for response Y1 & -2.46- 2.61 for response Y2.

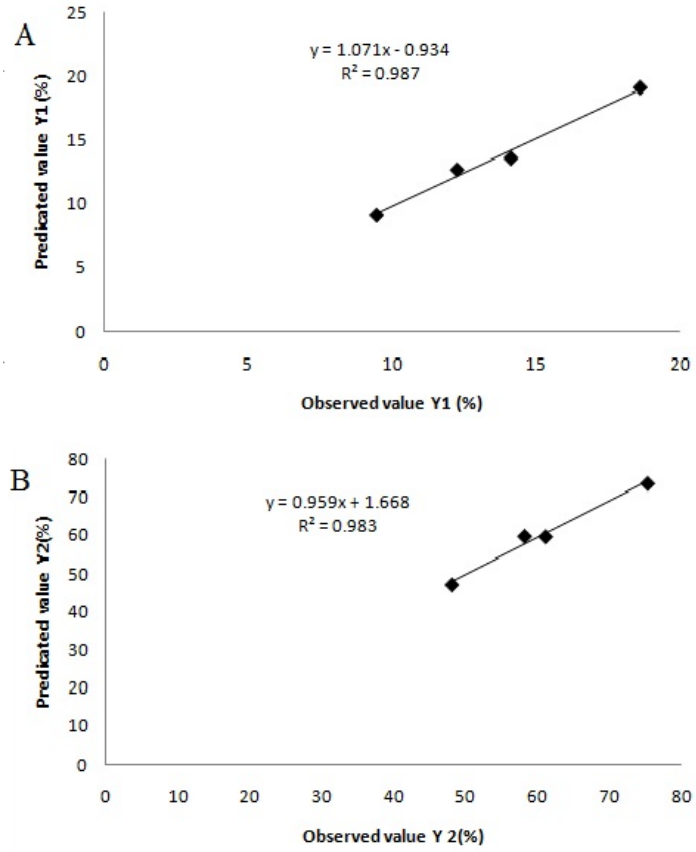

Figure 3. Linear correlation curve (A & B) between observed and predicated value for response Y1 (drug release at 5 hr) and Y2 (drug release at 10 hr)

The three dimensional response surface graphs were constructed by using quardratic model for graphical representation of effect of factors on each response. Figure 4 depicts the effect of two dependent variables on drug release at 5 hr (Q5).

Table 6: Comparison between observed and predicated value for response Y1 (Percentage Drug release at 5 hr) and Y 2 (Percentage Drug release at 5 hr) for different check points

Factors (Coaded)		Response	Observed value	Predicated value	Percent predication error
X1	X2				
0.30	- 0.50	Y 1	14.13	13.62	3.61
		Y 2	58.21	59.64	-2.46
-0.50	0.8	Y 1	18.63	19.15	-2.80
		Y 2	75.34	73.58	+ 2.33
0.8	0.4	Y 1	9.46	9.11	3.70
		Y 2	48.09	46.92	2.34
0.5	-0.8	Y 1	12.26	12.73	- 3.83
		Y 2	61.13	59.53	2.61

Percent predication error was calculated by using formula $\frac{(observed\ \ value - predicated\ \ value)}{Observed\ \ value} x\ 100$

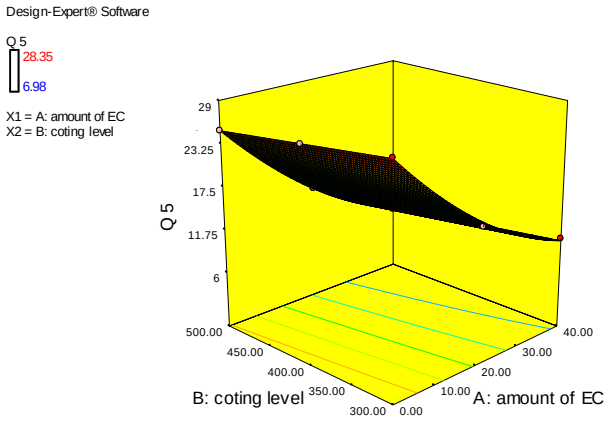

Figure 4. Response surface plot showing the influence of ethyl cellulose content (X1) and coating level (X2) on response Y1(drug release at 5 hr)

The figure shows that both factors have a negative effect on response Y_1 (Q5). This might be due to decrease permeability of coating layer as ethyl cellulose content increases. Diffusion path length also gets increased by increasing coating level. However, the effect of ethyl cellulose content is little more significant than coating level. This is also supported by polynomial equation for response Y_1 and table 4. Figure 5 shows a curvilinear relationship of response Y_2 with both the factors. This can be due to interaction between two variables, interpreting that each factor is inclining to change the effect of another factor towards the drug release in caecal content medium. Q 10 is decreased on increasing either of the factors. This may be due to decreased permeability and more torus path length because of the water insolubility of ethyl cellulose along with increase in coating thickness. The effect of ethyl cellulose content on Q 10 seems to be more than coating thickness. This is also supported by the polynomial equation for response Y_2 (Q10). This is in agreement with the fact that when coating material is soluble in dissolution medium, the coating level is not an effective factor for sustaining the drug release (27).

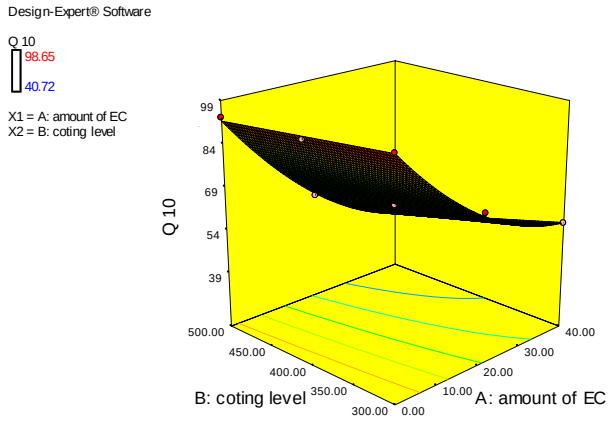

Figure 5: Response surface plot showing the influence of ethyl cellulose content (X1) and coating level (X2) on response Y2(drug release at 10 hr)

A numerical optimization technique was used to develop an optimized formulation. Constraints for responses are shown in table 7. The optimal values of factors were: ethyl cellulose content, 14.22 % and coating level 318.38 mg.

Table 7: Optimization of compression coated tablet

Constraints			
Name	**Goal**	**Lower limit**	**Upper Limit**
Amount of ethyl cellulose (%)	In range	0	40
Coating Level	In range	300	500
Cumulative drug release at 5 hr (%)	In range	6.98	20
Cumulative drug release at 10 hr (%)	Target ≥ 75	40.72	98.65

SOLUTION (F 10)				
Amount of ethyl cellulose (%)	Coating Level	Cumulative drug release at 5hr (%)	Cumulative drug release at 10 hr (%)	Desirability
14.22	318.38	19.4091	75	1.00

In vitro dissolution study of optimum formulation was performed. Figure 6 shows drug release pattern of optimum formulation.

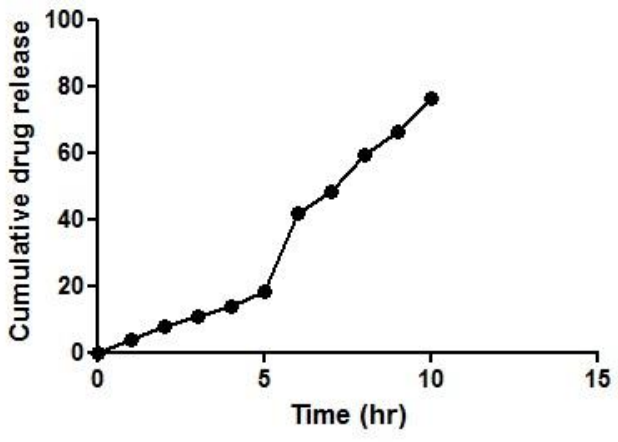

Figure 6 *In vitro* drug release profile of optimum formulation (F 10)

Table 8 shows a close agreement between predicated and experimental value.

Table 8: Predicated and observed responses of optimum formulation (F 10)

Response	Observed value	Predicated value	Percent predication error
Y 1	18.56	19.40	- 4.52
Y 2	76.31	75	1.71

Y1 indicates Percentage Drug release at 5 hr (Q 5); Y2 indicates Percentage Drug release at 10 hr (Q 10).

Percent predication error was calculated by using formula $\frac{(observed\ \ value - predicated\ \ value)}{Observed\ \ value} x\ 100$

CONCLUSION

It was concluded that factorial design was a successful tool for optimization of compression coated tablet based on amylose and ethyl cellulose in order to achieve colonic delivery. The optimized formulation containing ethyl cellulose (14.22 %) and coating level (318 mg) showed Q5 (18.56 %) and Q10 (76.31 %) , which were found to be close to the predicated values. Thus the developed coated tablet can be a reliable approach for colonic drug delivery.

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