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Central composite design for the formulation and optimization of a multi-unit potential colonic drug delivery system of budesonide for ulcerative colitis

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Budesonide is a potent glucocorticoid with high affinity for the glucocorticoid receptor, which is used for the treatment of inflammatory bowel diseases. Current oral formulations of budesonide present low efficacy against ulcerative colitis because of the premature drug release in the upper part of the gastrointestinal tract. The objective of this study was to develop a colon specific delivery system for budesonide to increase the efficacy in the treatment of ulcerative colitis using a statistical procedure. Pellets were prepared by powder layering of budesonide on nonpareils (0.5–0.6 mm) in a coating pan. Drug-layered pellets were coated with an inner layer of a combination of Eudragit® RL PO and RS PO and an outer layer of Eudragit FS in a fluidized-bed apparatus. Central composite design was used to study the effect of three independent variables. The independent variables selected were amount of Eudragit FS outer coating (X_1), proportion of Eudragit RL PO in the inner coating (X_2), amount of Eudragit RL PO–RS PO inner coating (X_3). Fifteen batches were prepared and evaluated for amount of drug released in 6 h (Y_1), amount of drug released in 12 h (Y_2). The proportion of the more hydrophilic polymer Eudragit RL PO had the most significant effect on drug release – higher proportion gave faster release; the amount of inner and outer coat did not have a significant effect on the rate of drug release at either 6 or 12 h in the range studied. The computer optimization process and contour plots predicted the levels of independent variables X_1, X_2, and X_3 (0.79, 0.69 and 0.35 respectively), for colon targeting.

1. Introduction

Aminosalicylates and glucocorticoids are the drugs of choice for the active phase of inflammatory bowel disesase (IBD) and immunosuppressants are usually used to establish, and importantly, maintain remission of IBD. Systemic glucocorticoids are currently being used for the treatment of mild, moderate and severe ulcerative colitis, though their severe adverse effects limit their use. Since IBD is characterized by local inflammation, targeting drugs directly to the site of injury has the benefit of lower adverse effects and more effective therapy. Different delivery systems have been developed for colon targeted therapy including time-dependent, pH-sensitive, pressure-controlled and microbially triggered systems. (Friend 2005; Ashford and Fell 1994; Rubinstein 1995; Van den Mooter and Kinget 1995; Watts and Illum 1997).

The present study dealt with the optimization of the colonic delivery system. Several variables usually need to be optimized during development of pharmaceutical products. and there approaches have been widely reported to simultaneously optimize multiple response variables. The first approach known as constrained optimization optimizes one response variable while placing constraints on the remaining response variables to keep them within acceptable limits. In the second approach, the contour diagrams of different response variables are superimposed. The last approach utilizes a desirability function that combines the responses into one measurement. The second approach using contour diagrams is probably the easiest to use if the number of response variables is equal to or less than three, and if all the responses are on the same scale on a graph. (Hileman et al. 1993; Abu et al. 1996; Bodea and Leucuta 1997; and Zhou et al. 1998)

In this work, central composite design was used to simultaneously study the effect of the three formulation variables of the colonic drug delivery system on two response variables. Central composite design and analysis of response surfaces were used because they are systematic and efficient methods to simultaneously study the effect of multiple variables and to find an optimum formulation. The three formulation variables studied were the amount of Eudragit FS outer coat, proportion of Eudragit RL in the inner coat, and the amount of Eudragit RL–RS inner coat. The two response variables studied were the amount of drug released in 6 h and the amount released in 12 h. Response surfaces were generated and the formulation was optimized by superimposing the contour plots. The response variable, amount of drug released in 12 h, was maximized while applying a constraint to the model for the other variable, amount of drug released in 6 h.

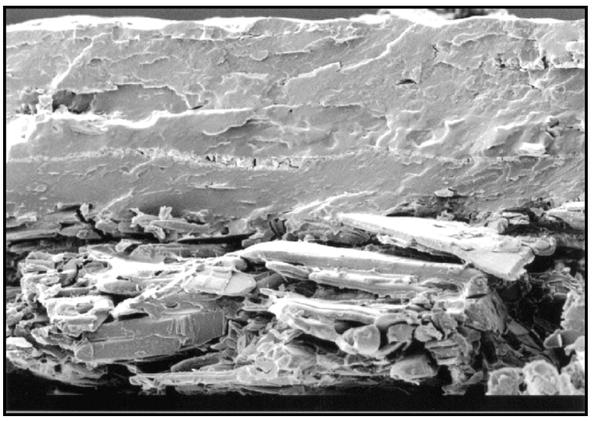

Fig. 1: SEM picture of the coatings (8% inner coating and 30% outer coating) of Budesonide pellet (magnification 2500 X)

Budesonide is a potent glucocorticoid with high local anti-inflammatory effect and low systemic bioavailability due to the result of extensive first pass metabolism. Budesonide is available in two controlled-release oral dosage forms, Budenofalk® and Entocort® (Fedorak and Bistritz 2005). These two formulations deliver the drug to the ileum and ascending colon and only a small fraction of the active molecule is released in transverse and descending colon and consequently they are less effective in the treatment of ulcerative colitis (Edsbäcker et al. 2003). On the light of above considerations, designing and developing a system which could deliver budesonide to the colon seems imperative. Eudragit RL PO is more hydrophilic than Eudragit RS PO, and the release of most drugs is faster from Eudragit RL PO than from Eudragit RS PO, hence the pellets can be coated with different combinations of Eudragit RL PO and RS PO to provide various degrees of sustained-release of the drug. Eudragit FS30D dissolves at pH 6.8; as the pH in distal ileum is reported to be 7–8, it is expected that Eudragit FS30D will dissolve in that region, and can be used to control the site of release of a pellet system previously coated with a Eudragit RL–RS layer for sustained-release of a drug in the colon. (Rohm 1999) Pellets were chosen for development because multi-unit delivery systems are statistically more reliable than single-unit delivery systems (Li et al. 1995; Amighi et al. 1998).

2. Investigations, results and discussion

2.1. Characterization of coating

Scanning electron micrograph (SEM) pictures of the pellets from all the 16 experimental batches were taken to characterize the coating. The uniformity and homogeneity of both the inner and outer coats can be observed in Fig. 1; it is the SEM of a pellet containing 8% inner coating and 30% outer coating. A thin porous layer of Aerosil can be spotted between the inner and outer coating in the picture.

2.2. Fitting of data to the model

Dissolution profiles of the 16 formulations prepared using the experimental designs are shown in Fig. 2. The model was fitted to the release data simultaneously for both the responses. The quality of fit of the model for Y_1 is shown in Fig. 3 by plotting predicted vs. observed values. The model was acceptable for both the responses as the observed values for the drug release were within 95% of the predicted values.

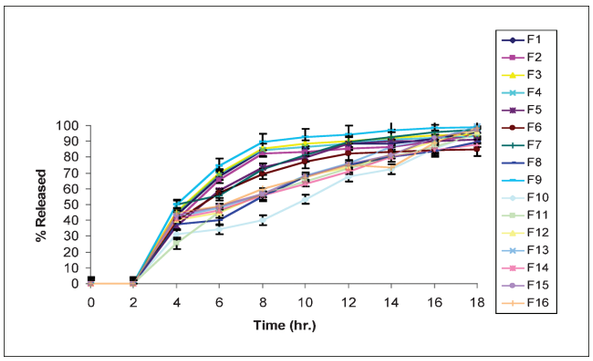

Fig. 2: Release of budesonide from the 16 batches of coated pellets prepared using the experimental design. For the composition of batches, refer to Table 2

2.2.1. Examination of the equations and coefficients

The equations representing the quantitative effect of the formulation variables at the level of 20% outer coating on the responses Y_1 and Y_2 are shown below:

$$Y_1 = 39.483 + 4.34X_2 - 0.527X_2^2 - 0.469X_3 - 0.0578X_3^2 + 0.0189X_1X_2 - 0.0209X_1X_3 + 0.0174X_2X_3 - 22.875 \quad (1)$$

$$Y_2 = 79.857 + 0.851X_2 - 0.0127X_2^2 - 0.0447X_3 - 0.0440X_3^2 + 0.0120X_1X_2 - 0.0720\ X_1X_3 + 0.0254X_2X_3 - 7.574 \quad (2)$$

Coefficients with one factor represent the effect of that particular factor while the coefficients with more than one factor and those with second order terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Positive sign in front of the terms indicates a synergistic effect while negative sign indicates an antagonistic effect upon the factors.

It can be concluded from the equations that X_2 (proportion of Eudragit RL PO in the inner coat) had the largest synergistic effect on both the responses. The effect of the quadratic term of X_2 was also significant. The effects of X_1 (amount of outer coat), X_3 (amount of inner coat), and the interaction among the factors were statistically insignificant.

2.3. Analysis of response surfaces and fitted data

All the 16 batches prepared using statistical design showed that the integrity of enteric coating was evident because of less than

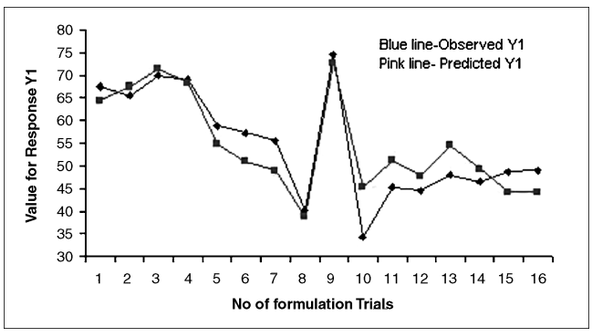

Fig. 3: Predicted and observed values of Y1 for the 16 batches prepared using experimental design

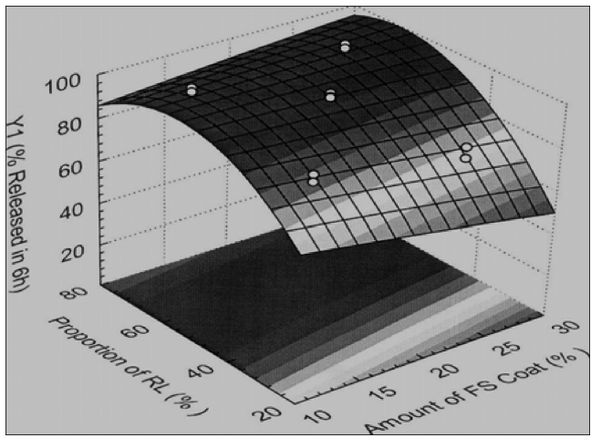

Fig. 4: Effect of the proportion of Eudragit RL PO and the amount of Eudragit FS (FS) coat on Y_1

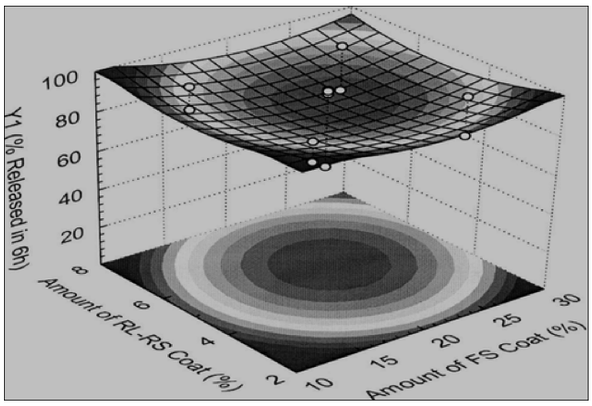

Fig. 5: Effect of the proportion of Eudragit RL PO and the amount of Eudragit RL PO–RS PO coat on Y_1

one percent drug release in pH 1.2 dissolution media. Moreover, all the batches released less than 1% drug in pH 6.5 dissolution media indicating that even 10% Eudragit FS coating can be potentially used for delivering majority of the drug to the colon. This is significant because, if the objective of colonic delivery can be achieved with a lower coating level of the polymer, it leads to lower cost, reduction in processing time, and lower weight and smaller size of the final dosage form.

Three dimensional response surfaces depicting the effects of three formulation variables X_1, X_2, and X_3 on the response variable Y_1 are shown in Figs. 4–6. The formulation variables had a similar effect on Y_2, however, the effect on Y_2 was less pronounced as compared to the effect on Y_1. The rate of release of budesonide increased with an increase in the proportion of Eudragit RL PO in the inner layer. Eudragit RL PO and Eudragit RS PO are water-insoluble, diffusion-release polymers over the entire pH range. Eudragit RL PO has more hydrophilic quaternary ammonium groups than Eudragit RS PO; this leads to higher hydration and increased permeability of the coating. Higher permeability of the coating because of higher proportion of Eudragit RL results in faster drug release.

The effect of the amount of Eudragit FS coat in the range studied was not statistically significant. As can be seen from the release curves in Fig. 2, the formulations containing higher amount of Eudragit FS coating released the drug after a lag time compared to the formulations that have lower amount of Eudragit FS coating. However, because of faster ionization of the carboxyl groups of Eudragit FS at pH 7.5, the lag times are too small (15–30 min) to make the effect of Eudragit FS statistically significant.

The effect of the amount of Eudragit RL PO–RS PO inner coating in the range of 2–8% was also statistically insignificant. This gives more flexibility to the formulators as they can chose the minimum amount of coating from this range that gives them reproducible coating of the batches. While the SEM pictures of all the 16 batches of experimental design revealed a uniform Eudragit RL–RS coating, in general, a 2% coating level is considered too small to provide homogenous and uniform coating, primarily because of the relatively short time spent by the charge load in the fluidizing chamber. On the other hand, very high coating levels lead to longer processing time and escalation of cost. In order to strike a balance between the coating uniformity of the batch and the processing time, the formulator may decide to choose 3–5% inner coating to ensure the uniformity of coating in a batch without appreciably increasing the processing time.

It must be mentioned that in the present study, the effect of the thickness of inner Eudragit RL PO– RS PO coat was insignificant probably because of the narrow range (2–8%) of coating level studied. At higher coating levels, the effect might be more pronounced.

2.4. Optimization of the formulation

The values of the constraints were decided after careful consideration of the transit time of dosage forms through the gastrointestinal (GI) tract, especially the residence time in the colon. Since the outer coat of the colonic delivery system used in this study is pH-dependent and not time-dependent, the variability in time for the colonic arrival of the delivery system will not significantly affect the effectiveness of the system and is hence, not important for the purpose of formulation optimization. Compared to the other regions of the GI tract, the mean colonic residence time is highly variable and has been reported to range from 10–36 h (Mrsny 1992). However, since the main function of the colon is absorption of water, the viscous consistency of colonic contents increases appreciably as one move down the colon; this may impede the drug release from a dosage form as time progresses. Hence, a time value of 12 h was considered reasonable for 85–100% removal of drug from the delivery system in the colon. A value of 50–65% for the amount of drug released in 6 h (Y_1) combined with the value of 85–100% for the amount of drug released in 12 h (Y_2) would ensure sustained and complete release of drug in the colon. Optimization was performed for the response Y_1 and Y_2 by applying constraints on both the responses. In optimization, (Fig. 7) desirability 1.0

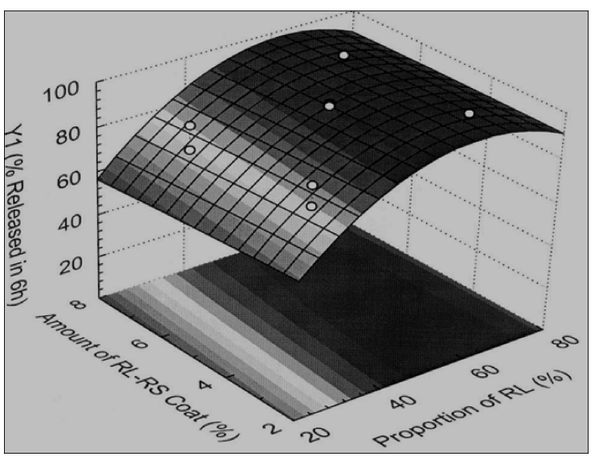

Fig. 6: Effect of the amount of Eudragit FS (FS) coat and amount of Eudragit RL PO–RS PO coat on Y_1

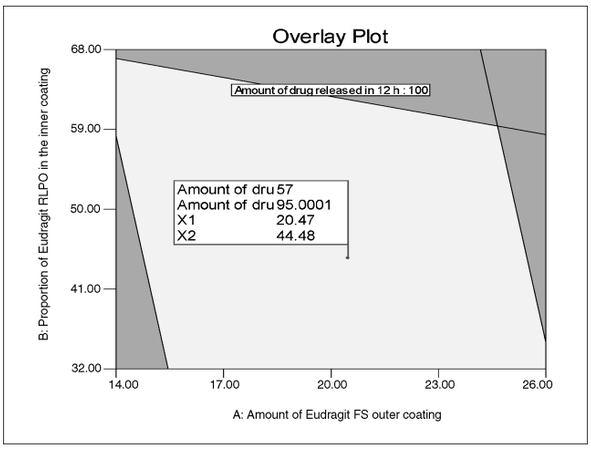

Fig. 7: Overlay plot for optimization

indicated optimum formulation was achieved at 20.47 % of (X_1), 44.48% of (X_2) and 2.71% of (X_3).

2.5. Checkpoint analysis

Three checkpoint batches were prepared and evaluated for Y_2, as shown in Table 1. Results indicate that the measured Y_2 values were as expected. When measured Y_2 values were compared with predicted Y_2 values using Student's t-test, the differences were found to be not significant. Thus, we can conclude that the obtained mathematical equation is valid for predicting Y_2
Since all the observed values for dissolution were within 95% confidence level of the predicted values, it was concluded that the optimal surface was chosen correctly and that the model has satisfactory predictive power.

3. Experimental

3.1. Materials

Budesonide was a gifted from Cadila Healthcare ltd., Ahmedabad, India. Eudragit® RL PO, RS PO0 and Eudragit FS30D were gifted from Alembic Ltd. Baroda, India. All other chemicals were of reagent grade.

3.2. Methods

3.2.1. Preparation and coating of pellets

Pellets were prepared by powder layering of budesonide on nonpareils (nuclei) in a conventional coating pan (Erweka, Germany). Excipients of the powder layering composition were sieved and mixed. Binder solution (aqueous solution of polyvinyl pyrrollidone) was continuously sprayed on the moving nonpareils by means of a peristaltic pump and a spray-nozzle. At fixed intervals, fixed amounts of the powder composition were layered onto the particles. The drug-loaded pellets were dried in an oven at 40 °C for 24 h after which sieve analysis was done and the fraction of 0.8–1.0 mm was separated for coating. In order to prevent the batch-to-batch variability of the drug-layered pellets from affecting the different batches of coated pellets, the sieve-cuts of 0.8–1.0 mm from several batches of drug-layered pellets were pooled together and blended and the pellets for coating were taken out from this bulk.
For the inner coat, the pellets were coated with a combination of Eudragit RL PO –RS PO in a fluidized bed coating apparatus (GPCG 1.1, Glatt, Germany). After the coating, the pellets were gently fluidized for about 5 min after which they were cured in an oven for 24 h at 40 °C.
For the outer coat, the cured pellets containing inner coat of Eudragit RL PO–RS PO were further coated with Eudragit FS30D in the fluidized-bed processor. After the coating, the pellets were gently fluidized for about 5 min after which they were again cured in an oven for 24 h at 40 °C. To prevent the coated pellets from sticking together, 0.5% Aerosil 200 was added to the finished product after both inner and final coatings.

3.2.2. Experimental design

A randomized rotatable Central composite design was implanted for the optimization of multi-unit potential colonic drug delivery system. According to model it contains four full factorial design points, four axial points and three centre points. Higher and lower levels of each factor were coded as +1 and -1 respectively, and the mean value as 0. The selected factor levels are summarized in Table 2. The centre points were repeated three times to estimate pure experimental uncertainty at the factor levels.
This statistical design provided an empirical second order polynomial equation used for the prediction of the effect of formulation variables on the release characteristics using a smaller number of experimental runs. In this approach, each experimental response Y can be represented by a quadratic equation of the response surface.

$$Y_i = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3 + b_{11} X_1^2 + b_{22} X_2^2 + b_{33} X_3^2$$

This equation enables the simultaneous investigation of the effect of each factor and their interaction over the experimental responses.
The modeling was performed using SPSS (Version 8.0) with a backward, stepwise linear regression technique and significant terms ($P < 0.05$) were chosen for final equations. Response surface plots and contour plots resulting from equations obtained by DESIGN EXPERT 8.0.1 (STAT-EASE).
The matrix of the experimental plan and the composition of the 16 batches are shown in Table 3.
The following three independent variables at five levels each in the range indicated below:

Amount of Eudragit FS outer coating (X_1):10–30%
Proportion of Eudragit RL PO in the inner coating (X_2): 20–80%

Table 1: Checkpoint batches with predicted and measured Y_2

Batch code	X_1	X_2	X_3	Y_2	
				Measured[a]	Predicted
1	0	−0.5	0.5	91.19 ± 0.4	90.49
2	0.5	0	−0.5	98.65 ± 0.3	99.47
3	−0.5	0.5	0	83.79 ± 0.1	80.46
Optimum batch	0.79 (20.47 %)	0.65 (44.48%)	0.39 (2.71%)	95.00 ± 1.8	96.46

[a] Mean ± SD, n = 3

Table 2: Factors and their corresponding levels implemented for the construction of CCD

Factor	Factor level				
	−1.41	−1	0	1	1.41
Amount of Eudragit FS outer coating (X_1):10–30%	10	14	20	26	30
Proportion of Eudragit RL PO in the inner coating (X_2): 20–80%	20	32	50	68	80
Amount of Eudragit RL PO–RS PO inner coating (X_3): 2–8%	2	3	5	7	8

Table 3: Composition of 16 batches prepared using central composite design with measured response[a]

Batch No.	Amount of FS Coat, X_1 %	Proportion of RL PO, X_2 %	Amount of RL PO–RS PO coat, X_3 (%)	Amount of drug released in 6 h (Y_1)	Amount of drug released in 12 h (Y_2)
1	−1	−1	−1	67.59	88.49
2	−1	−1	1	65.49	85.47
3	−1	1	−1	69.89	97.49
4	−1	1	1	68.89	98.49
5	1	−1	−1	58.69	98.59
6	1	−1	1	57.19	92.19
7	1	1	−1	55.49	97.49
8	1	1	1	40.26	89.49
9	−1.41	0	0	74.59	97.49
10	1.41	0	0	34.16	95.49
11	0	−1.41	0	45.19	72.94
12	0	1.41	0	44.59	96.57
13	0	0	−1.41	47.89	99.59
14	0	0	1.41	46.59	88.09
15	0	0	0	48.75	97.19
16	0	0	0	49.06	98.26

[a] FS – Eudragit FS, RL – Eudragit RL, and RS – Eudragit RS

Amount of Eudragit RL PO –RS PO inner coating (X_3): 2–8%

These three formulation variables were found important for drug and their range was chosen based on the preliminary studies done in our lab and the previous literature reports.

3.2.3. Content uniformity

The quantitative determination of BUD was performed by HPLC. A Shimadzu HPLC system with UV-Visible detector: SPD-10A, liquid chromatogram: LC-10AD, integrator: C-R6A, injector (20 μL): 6E (Shimadzu, Japan). The analysis was carried out using Hypersil C18 column (Thermo Electron Corporation) with dimensions = 150 × 4.6 mm and particle size = 5 μm at a wavelength of 247 nm. The volume of injection was 20 μL. The mobile phase consisted of acetonitrile–water (40:60). The flow rate was 1.5 mL/min.

3.2.4. Determination of the response variables

Dissolution studies were carried out in a USP XXIII dissolution apparatus I in 900 ml medium at 37 ^{0}C at a rotation speed of 100 rpm. Accurately weighed pellets containing the equivalent of 10 mg of budesonide were transferred to the dissolution medium. At predetermined intervals, the samples were taken from the vessel and analyzed by a Shimadzu HPLC system with UV-Visible detector: SPD-10A, liquid chromatogram: LC-10AD, integrator: C-R6A, injector (20 μL): 6E (Shimadzu, Japan). For simulating conditions of the GI tract, dissolution tests were carried out in media with pH 1.2 (HCl 0.1N), pH 6.5, 6.8 and 7.2 (phosphate buffer). Samples were introduced into each medium, separately. Dissolution test was performed for 2 h for acidic stage (pH 1.2) and 10 h in the other media. The most promising formulation was tested under the continuous dissolution based on generally accepted GI transit times, i.e., 2, 1, 2 and 1 h for the stomach, proximal part of small intestine, lower part of small intestine and terminal ileum at the media with pH 1.2, 6.5, 6.8 and 7.2, respectively**.** The release data was plotted against time and both the response variables, the amount of drug released in 6 h (Y_1) and the amount of drug released in 12 h (Y_2) were determined from the graph.

3.2.5. Checkpoint analysis

A checkpoint analysis was performed to confirm the role of the derived polynomial equation and contour plots in predicting the responses. Values of independent variables were taken at 3 points, 1 from each contour plot, and the theoretical values of Y_2 were calculated by substituting the values in the polynomial equation. Budesonide pellets were prepared experimentally at 3 checkpoints, and evaluated for the responses.

3.2.6. Optimization data analysis

The calculation for optimized formulation was carried using software, DESIGN EXPERT 8.0.1 (STAT-EASE). Polymers used for colon targeting should be able to withstand the lower pH values of the stomach and of the proximal part of the small intestine and also be able to disintegrate at the neutral to slightly alkaline pH of the terminal ileum (Chourasia and Jain 2003).

The two response variables studied along with their constraint values are listed below:

Amount of drug released in 6 h (Y_1): $50 \leq Y_1 \leq 65$
Amount of drug released in 12 h (Y_2): $85 \leq Y_2 \leq 100$

These response variables were chosen because of their bearing on the effectiveness of the delivery system for colonic delivery.
The optimized formulation was obtained by applying constraints (goals) on dependent (response) and independent variables (factors). The models were evaluated in terms of statistically significant coefficients and R^2 values. Various feasibility and grid searches were conducted to find the optimum parameters. Various 3-D response surface graphs were provided by the Design Expert software. The optimized checkpoint formulation factors were evaluated for various response properties. The resultant experimental values of the responses were quantitatively compared with the predicted values to calculate the percentage prediction error.

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