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Original research paper

Box-Behnken experimental design in the development of pectin-compritol ATO 888 compression coated colon targeted drug delivery of mesalamine

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The aim of this study was to investigate the combined influence of 3 independent variables in the compression coated tablet of mesalamine for ulcerative colitis. A 3--factor, 3-level Box-Behnken design was used to derive a second order polynomial equation and construct contour plots to predict responses. The independent variables selected were: percentage of polymers (pectin and compritol ATO 888) in compression coating (X_1), coating mass (X_2) and coating force (X_3). Fifteen batches were prepared and evaluated for percent of drug released in 5 h (Y_5), time required for 50 % mesalamine to dissolve (t_{50}) with rat cecal (RC) content and without rat cecal content (t_{50}), percent of drug released in 24 h in the presence of rat cecal content (Y_{24} with RC). Transformed values of independent and dependent variables were subjected to multiple regressions to establish a full-model second-order polynomial equation. *F* was calculated to confirm the omission of insignificant terms from the full-model equation. The computer optimization process and contour plots predicted the levels of independent variables X_1, X_2, and X_3 (0, 0.2 and –0.15, respectively) for colon targeting and total percent of drug released up to 24 h.

Keywords: mesalamine, compression coated tablet, colon targeting, drug release study, compritol ATO 888, pectin, Box-Behnken experimental design

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A novel oral colon-specific drug delivery system (CDDS) has been developed as one of the site-specific drug delivery systems. This delivery system, by means of combining one or more controlled release mechanisms, hardly releases the drug in the upper part of the gastrointestinal (GI) tract, but rapidly releases drug in the colon following oral administration. The necessity and advantage of CDDS have been recognized and reviewed recently (1–3). Owing to CDDS specifically delivering the drug to the colon, many bene-

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fits would be acquired in terms of improved safety and reduced toxicity when treating local or systemic chronic diseases. First, in treating localized colonic diseases, *i.e.* ulcerative colitis, Crohn's disease, constipation, *etc.* (1), CDDS would be advantageous when a delay in absorption is desirable from the therapeutic point of view, as well as in the treatment of diseases that have peak symptoms in the early morning and that exhibit circadian rhythms, such as nocturnal asthma, angina and rheumatoid arthritis (4, 5).

Pectins are non-starch, linear polysaccharides extracted from the plant cell walls. They are predominantly linear polymers of mainly α-(1–4) linked D-galacturonic acid residues interrupted by 1,2-linked L-rhamnose residues. These polysaccharides remain intact in the physiological environment of the stomach and the small intestine, but are degraded by the bacterial inhabitants of the human colon (6, 7). Being soluble in water, pectin is not able to shield its drug load effectively during its passage through the stomach and small intestine. It was found that a coat of a considerable thickness was required to protect the drug core in simulated *in vivo* conditions (8, 9). Hence, the focus was shifted to the development of such pectin derivatives that were less water soluble but were degradable by the colonic microflora.

Glyceryl behenate (Compritol ATO 888) is a waxy material, originally introduced as a lubricant for tablets, which has recently had a wide application as a sustained-release excipient (10).

Mesalamine is an active ingredient of agents used for the long-term maintenance therapy to prevent relapses of Crohn's disease and ulcerative colitis (11). It is thus of tremendous importance to deliver mesalamine locally in order to reduce the influence of systemic drug absorption causing adverse effects and drug loss lowering the probability of therapeutic success. Hence, selective delivery of mesalamine into the colon is required.

The purpose of this study was to develop and evaluate a compression coated tablet containing mesalamine as the core and a pectin-compritol ATO 888 mixture as the coat layer based on the GI transit time concept. In the work reported here, a Box-Behnken design was used to optimize compression coated mesalamine tablets containing pectin and compritol ATO 888 as a compression coating polymer. The independent variables selected were: percentage of polymers (pectin and compritol ATO 888) in compression coating (X_1), coating mass (X_2), and coating force (X_3) to evaluate their separate and combined effects on the amount of drug released after 6 h (Y_6), time required for 50 % mesalamine to dissolve (t_{50}) with pectinase and without pectinase (t_{50}), percent of drug released after 24 h in the presence of pectinase (Y_{24}).

EXPERIMENTAL

Materials

Mesalamine USP was a gift from Bec Chemicals Ltd., India, pectin was obtained from National Chemicals, India, Compritol ATO 888 was a gift from Colorcon Ltd., India. All other materials were of reagent grade.

Methods

Preparation of 5-ASA core tablets. – Mesalamine was dry mixed with polyvinyl pyrollidone K-30 (PVP K-30) and water was added to granulate. Wet granules were sieved through a 1-mm screen and dried overnight at 40 °C. After adding 0.25 % magnesium stearate as a lubricant, tablets containing 100 mg of the drug were compressed using a Cadmach single punch tablet machine (M/S. Cadmach Machinery Co. Pvt. Ltd, India) with 6-mm flat faced punches. Tests such as mass variation, crushing strength, friability, thickness, and dissolution were performed on the core tablets.

Box-Behnken experimental design. – A Box-Behnken statistical design with 3 factors, 3 levels, and 15 runs was selected for the optimization study (12). The experimental design consists of a set of points lying at the midpoint of each edge and the replicated center point of a multidimensional cube. Independent and dependent variables are listed in Table I. The polynomial equation generated by this experimental design (using Sigma Plot 11) is as follows:

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

where Y_i is the dependent variable, b_0 is the intercept, b_1 to b_{33} are the regression coefficients, and X_1, X_2 and X_3 are the independent variables selected from preliminary experiments. The experimental design is summarized in Table II.

Compression coating of core tablets. – Mesalamine core tablets were placed in the 10-mm die cavity of a laboratory hydraulic press. Depending on the design, 100 % pectin, 80 %

Table I. Variables in Box-Behnken design

Independent variable	Level		
	Low	Medium	High
X_1 = percentage of polymers (pectin-compritol ATO 888) in compression coating (%)[a]	60-40	80-20	100-00
X_2 = coating mass (mg)[b]	250	350	450
X_3 = coating force (kN)	20	30	40
Transformed value	–1	0	1
Dependent variable			
Amount of drug released after 5 h (Y_5)			
Dissolution rate with RC (t_{50})			
Dissolution rate without RC (t_{50})			
Amount of drug released after 24 h in the presence of rat cecal content (Y_{24} with RC)			

RC – rat cecal content

[a] Indicates 60, 80, 100 % pectin and 40, 20, 0 % Compritol ATO 888.

[b] 50 mg Avicel PH 101 was incorporated in coating material to provide good compressibility.

Table II. Box-Behnken experimental design with responses[a]

Batch No.	X_1	X_2	X_3	Y_5	t_{50} (with RC) (h)	t_{50} (without RC) (h)	Y_{24} (with RC)
1	0	–1	–1	15.29	8.0	8.1	99.96
2	0	–1	1	0	14.4	15.3	85.69
3	0	1	–1	8.87	10.2	12.5	99.45
4	0	1	1	0	15.2	19.3	79.85
5	–1	0	–1	0	16.2	22.1	76.59
6	–1	0	1	0	19.0	26.4	65.49
7	1	0	–1	30.29	7.1	7.2	99.97
8	1	0	1	12.39	8.4	9.1	99.60
9	–1	–1	0	0	17.5	20.2	68.98
10	–1	1	0	0	18.0	20.0	70.26
11	1	–1	0	22.39	7.3	7.5	99.99
12	1	1	0	20.19	8.0	8.2	99.90
13	0	0	0	0	11.2	12.2	99.98
14	0	0	0	0	11.0	12.3	100.2
15	0	0	0	0	11.3	12.1	99.85

RC – Rat cecal content.
[a] All values are mean values obtained from the regression analysis.

pectin/20 % compritol ATO 888 and 60 % pectin/40 % compritol ATO 888 combinations were used for the outer shell compression coating. Coating forces were 20, 30 or 40 kN, and the coat mass was 250, 350 or 450 mg. To prepare coated tablets, half of the coating material was placed in a concave die (10 mm), the core tablet was carefully positioned in the centre of the die and the remaining coat material was added. The coat was compressed around the core at different compression forces (13).

Thickness was measured with a vernier caliper (SV-03, E-Base Measuring Tools, Taiwan). The hardness and friability of the core tablets and of the compression-coated tablets were determined using a hardness tester (Monsanto Tablet Hardness Tester, Mht-20, Campbell Electronics, India) and the Roche Friabilator (Type EF2, Eletrolab, India), respectively. The mass variation test was done by weighing 20 tablets individually, calculating the average mass and comparing the individual tablet mass to the average. Dissolution rates in different media were recorded on the compression coated tablets.

In vitro *drug release.* – Drug release studies (n = 3) were conducted under conditions mimicking mouth-to-colon transit (14–16). The dissolution medium consisted of 900 mL 0.1 mol L^{-1} HCl for 2 h, replaced by 900 mL Sorensen's phosphate buffer, pH 7.4 for 3 h, kept at 37 ± 0.5 °C and stirred at 100 rpm, using a USP (17) dissolution apparatus type II (TDT-06Lplus, Electrolab, India). Samples (5 mL) were withdrawn at the end of specified periods (2 h and 5 h), filtered using membrane filters (0.45 μm) and assayed spectrophotometrically (UV-1601, Shimadzu, USA) for mesalamine at 301.5 nm in 0.1 mol L^{-1}

HCl and at 334.5 nm in pH 7.4 buffer. To assess the susceptibility of the prepared mesalamine delivery systems to the enzymatic action of colonic bacteria, drug release studies were continued in phosphate buffer saline (PBS) pH 6.8 in the absence (control) and in the presence of rat cecal contents, since these are known to have similar contents, to those of human intestinal microflora (18). The studies were carried out using USP dissolution apparatus 1 (100 rpm, 37 °C) with slight modifications. A glass beaker (250 mL) containing 100 mL PBS pH 6.8 was immersed in the flask of the dissolution apparatus with water at 37 ± 0.5 °C. After completing the dissolution in 0.1 mol L^{-1} HCl (2 h) and phosphate buffer, pH 7.4 (3 h), baskets containing the tablets under study were immersed in the PBS medium and the release study was continued for up to 24 h. Samples (5 mL) were withdrawn at different time points (6, 8, 12 and 24 h), filtered using membrane filters (0.45 μm) and assayed spectrophotometrically for mesalamine at 299 nm in PBS pH 6.8. The same volume of fresh dissolution medium was added to restore the initial volume of the dissolution medium after each sample withdrawal. Experiments were carried out in triplicate.

Preparation of rat cecal content medium. – Before starting experiments on animals, the experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee (IAEC) of Anand Pharmacy College, Anand, India, and was approved. Susceptibility of the compression coated tablet to the enzymatic action of colonic bacteria was assessed by performing drug release in the medium contacting rat cecal content. Cecal material was collected from male albino rats weighing 150–200 g maintained on a normal diet, but the cecal enzyme production was induced by giving orally 1 mL of 2 % (*m/V*) dispersion of pectin for 7 days (administered directly into the stomach using teflon tubing). Thirty minutes before the commencement of drug release studies, four rats were killed and their abdomens were opened, the cecai was isolated, ligated at both ends, cut loose and immediately transferred into PBS previously bubbled with carbon dioxide. The cecal bags were opened and their contents were individually weighed, pooled and then suspended in PBS to give a final cecal dilution of 4 % (*m/V*).

The dissolution study was continued in 100 mL of the above made rat cecal media after 5 h. This was done with slight modification in the experimental set-up of the USP dissolution test apparatus. A beaker of 250 mL capacity containing 100 mL of PBS (pH 6.8) with rat cecal content was placed suitably in the dissolution vessel having water maintained at 37 ± 0.5 °C, which in turn was kept in the water bath of the apparatus. The study was continued from the 6^{th} h to 24^{th} h and the samples were withdrawn at different intervals (6, 8, 12 and 24 h) for analysis and each time replaced with fresh PBS containing rat cecal material bubbled with CO_2. The withdrawn samples were diluted with PBS and centrifuged. The supernatant was filtered through membrane filters (0.45 μm) and the filtrate was analyzed for mesalamine content 299 nm.

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_{24} with RC were calculated by substituting the values in the polynomial equation. Compression coated tablets were prepared experimentally at 3 checkpoints, and evaluated for the responses.

Optimum formula. – After developing the polynomial equations for the responses Y_5, t_{50} with RC and Y_{24} with RC with the independent variables, the formulation was optimized for the response Y_{24} with RC. Optimization was performed to find out the level of independent variables (X_1, X_2, and X_3) that would yield a maximum value of Y_{24} with RC with a minimum value of Y_5.

RESULTS AND DISCUSSION

An attempt was made to minimize drug release in the physiological environment of the GIT and to ensure maximum drug release in the colon by applying pectin as a compression coat over the mesalamine core tablets. It was found from the *in vitro* dissolution study that pectin alone cannot give a hard coat, so Compritol ATO 888 was added because it retards the release until polymer solubilization takes place at the higher pH of the colon. From Table II it was found that independent variable, X_1 (percentage of polymers, in compression coating) at high level medium level of (80-20) (100-0) could the release drug into the physiological stomach and intestine environment.

Data analysis

All batches of compression coated tablets were evaluated for Y_5, t_{50} with RC and Y_{24} with RC. Transformed values of all the batches, along with their results are shown in Table II. Formulations 2, 4, 5, 6, 9, 10, 13, 14 and 15 had the lowest Y_5 (0 %). Table III shows the observed and predicted values with residuals and percent error of responses for all batches. The Y_5 (dependent variable) obtained at various levels of the 3 independent variables (X_1, X_2, and X_3) was subjected to multiple regression to yield a second-order polynomial equation (full model):

$$Y_5 = 10.66\ X_1 - 1.07\ X_2 - 5.26\ X_3 - 0.55\ X_1X_2 - 4.475\ X_1X_3 + 1.61\ X_2X_3 + 7.63\ X_1^2 + 3.0\ X_2^2 + 3.03\ X_3^2 \quad (2)$$

The value of the coefficient of determination (R^2) of Eq. (2) was found to be 0.9952, indicating good fit. The Y_5 values measured for the different batches showed wide variation (*i.e.*, values ranged from a minimum of 0 to a maximum of 30.29). The results clearly indicate that the Y_5 value is strongly affected by the variables selected for the study. This is also reflected in the wide range of values for coefficients of the terms of Eq. (2) The main effects of X_1, X_2, and X_3 represent the average result of changing 1 variable at a time from its low level to its high level. The interaction terms (X_1X_2, X_1X_3, X_2X_3, X_1^2, X_2^2, and X_3^2) show how Y_5 changes when 2 variables are simultaneously changed. The negative coefficients for 2 independent variables, X_2 and X_3, indicate an unfavorable effect on Y_5, while the positive coefficients for the interactions between 2 variables X_2X_3 indicate a favorable effect on Y_5. The significance level of coefficients b_2 and b_{12} was found to be $p = 0.0510$ and 0.4, respectively, hence it was omitted from the full model to generate the reduced model. Coefficients b_1, b_3, b_{13}, b_{23}, b_{11}, b_{22} and b_{33} were found to be significant at $p < 0.05$; hence they were retained in the reduced model. The reduced

Table III. Testing the model in portions[a]

Regression	*Df*	For Y_5				F_{calc} = 3.697 F_{table} = 5.79
		SS	*MS*	R^2	*p*	
FM	9	1487.09	165.23	0.9952	2.89E-05	*Df* = (2,5)
RM	7	1476.59	210.94	0.9882	3.24E-06	
Residual						
FM	5	7.12	1.42			
RM	7	17.62	2.51			
Regression	*Df*	For t_{50} (with RC)				F_{calc} = 3.3841 F_{table} = 4.82
		SS	*MS*	R^2	*p*	
FM	9	237.65	26.40	0.9708	0.0025	*Df* = (8, 5)
RM	1	199.00	199.00	0.8129	0.0000043	
Residual						
FM	5	7.14	1.428			
RM	13	45.80	3.52			
Regression	*Df*	For Y_{24} (with RC)				F_{calc} = 4.31 F_{table} = 4.88
		SS	*MS*	R^2	*p*	
FM	9	2539.88	282.20	0.9727	0.0021	*Df* = (7, 5)
RM	2	2110.51	1055.25	0.8083	0.000049	
Residual						
FM	5	71.13	14.23			
RM	12	500.50	41.70			

model was tested in portions to determine whether the coefficients b_2 and b_{12} contributed significant information for the prediction of Y_5. The results of testing the model in portions are shown in Table III.

The critical value of $F = 5.79$ ($p = 0.05$) is higher than the calculated value ($F = 3.69$). It may be concluded that the interaction terms b_2 and b_{12} do not contribute significantly to the prediction of Y_5 and the low coefficients for these terms in *Eq.* (2) indicate that these terms contribute the least to the prediction of Y_5. Hence, these terms are omitted from the full model to obtain a reduced second-order polynomial equation [Eq. (3)] by multiple regressions of Y_5 and the significant terms ($p < 0.05$) of Eq. (2):

$$Y_5 = 10.66\ X_1 - 5.26\ X_3 - 4.475\ X_1X_3 + 1.61\ X_2X_3 + 7.63\ X_1^2 + 3.0\ X_2^2 + 3.03\ X_3^2 \quad (3)$$

This implies that the effects of the percentage of polymers (pectin-compritol ATO 888) in compression coating and the coating force are significant, as it is evident from

their high coefficients. The results of multiple linear regression analysis (reduced model) reveal that, on increasing the percentage of polymers (pectin-compritol ATO 888) in compression coating, an increase in Y_5 is observed; the coefficient b_1 bears a positive sign.

Time required for 50 % mesalamine to dissolve with RC (t_{50}) (h) was found to be in the range of 7.0 to 19.0 h. A polynomial equation was also developed for time required for 50 % mesalamine to dissolve with RC (t_{50}) (h):

$$t_{50}\ (\text{with RC}) = 11.17 - 4.99\ X_1 + 0.52\ X_2 + 1.94\ X_3 + 0.05\ X_1X_2 - 0.375\ X_1X_3 - 0.35\ X_2X_3 + 1.13\ X_1^2 + 0.40\ X_2^2 + 0.37\ X_3^2 \quad (4)$$

The value of R^2 from Eq. (*4*) was found to be 0.9708, indicating good fit. Among the independent variables selected and their interactions, only X_1 was found to be significant ($p < 0.05$), indicating a major contributing effect of X_1 on t_{50} with RC. The negative coefficients for independent variable X_1 indicate an unfavorable effect on t_{50} with RC, while the positive coefficients for independent variables X_2 and X_3 indicate a favorable effect on t_{50} with RC. The interactions between two variables X_1X_2 indicate a favorable effect on t_{50} with RC. The coefficient b_1 was the only coefficient found to be significant ($p < 0.05$); hence it was retained in the reduced model. The reduced model was tested in portions to determine whether the entire coefficient contributes significant information for the prediction of t_{50} with RC. The results testing the model in portions are shown in Table III.

The critical value of $F = 4.82$ ($p = 0.05$) is again higher than the calculated value (F = 3.3841). It may be concluded that none of the interaction terms except b_1 contribute significantly to the prediction of t_{50} with RC and the low coefficients for these terms in Eq. (*2*) indicate that these terms contribute the least to the prediction of t_{50} with RC. Hence, these terms were omitted from the full model to obtain a reduced first-order binomial Eq. (*5*):

$$t_{50}\ (\text{with RC}) = 12.18 - 4.99\ X_1 \quad (5)$$

This implies that the main effect of the percentage of polymers (pectin-compritol ATO 888) in compression coating is significant, as it is evident from the high coefficient. The results of multiple linear regression analysis (reduced model) reveal that, on increasing the percentage of polymers (pectin-compritol ATO 888) in compression coating, a decrease in t_{50} with RC is observed; the coefficient b_1 bears a negative sign. It was concluded that if at higher level of X_1 (pectin-compritol ATO 888: 100-0), time for 50 % of drug released compression coated tablets was decreased. Thus, it was concluded that compritol ATO 888 could control drug release from the compression coated tablets. It was concluded from Table II that at medium level of X_1, 50 % drug was released in 8 to 14 h. From Eq. (*4*), it was found that coefficients for X_2 and X_3 were positive *i.e.* if the coat mass and compression force were increased, time for 50 % drug release increased. It was concluded that the coat mass and compression force can also control drug release from compression coated tablets. Y_{24} (with RC) was found to be in the range of 65.0 to

100.0 %. A polynomial equation was also developed for the amount of drug released after 24 h in the presence of rat cecal content (Y_{24} with RC):

$$Y_{24}\ (\text{with RC}) = 100.01 + 14.76\ X_1 - 0.645\ X_2 - 5.67\ X_3 - 0.34\ X_1X_2 + 2.68\ X_1X_3 - 1.33\ X_2X_3 - 10.52\ X_1^2 - 4.70\ X_2^2 - 4.07\ X_3^2 \quad (6)$$

The value of R^2 from Eq. (*6*) was found to be 0.9727, indicating good fit. Among the independent variables selected and their interactions, only X_1 and X_1^2 were found to be significant ($p < 0.05$), indicating a major contributing effect of X_1 on Y_{24} (with RC). The negative coefficients for independent variables X_2 and X_3 indicate an unfavorable effect on Y_{24} (with RC), but the value of coefficient of X_2 was much lower than X_3, so X_3 alone has a major but unfavorable effect on Y_{24}. The interactions between variables X_1X_3 indicate a favorable effect on Y_{24} (with RC). Coefficients b_1 and b_{11} were found to be significant at $p < 0.05$; hence they were retained in the reduced model. The reduced model was tested in portions to determine whether the entire coefficient contributes significant information for the prediction of Y_{24} with RC. The results of testing the model in portions are shown in Table III.

The calculated value ($F = 4.31$) was lower than the critical value ($F = 4.88$), leading to the conclusion that none of the interaction terms except b_1 and b_{11} contribute significantly to the prediction of Y_{24} with RC. Hence, these terms were omitted from the full model to obtain a reduced second-order polynomial Eq. (*7*):

$$Y_{24}\ (\text{with RC}) = 94.99 + 14.76\ X_1 - 9.90\ X_1X_1 \quad (7)$$

This implies that the main effect of the percentage of polymers (pectin-compritol ATO 888) in compression coating is significant, as it is evident from the high coefficients. The results of multiple linear regression analysis (reduced model) reveal that, on increasing the percentage of polymers (pectin-compritol ATO 888) in compression coating, an increase in Y_{24} (with RC) is observed; the coefficient b_1 bears a positive sign. It was concluded that at higher level of X_1 (pectin-compritol ATO 888: 100-0), 100 % of drug was released from compression coated tablet within 24 h. From Table II it was concluded that at low level of X_1, 100 % drug was not released in 24 h. From Eq. (*6*) it was found that coefficients for X_2 and X_3 were negative, *i.e.*, if coat mass and compression force were increased, drug release was decreased. From Table II it was concluded that medium level of X_1 (pectin-compritol ATO 888: 80-20) was selected for targeting compression coated tablets into colon without releasing the drug in the stomach and intestine. It was also concluded that compression force can also affect drug release during its transit from mouth to colon but not coat mass. Medium level of compression force was selected (formulations 13–15) because around 100 % drug was released in 24 h.

The relationship between the dependent and independent variables was further elucidated by constructing contour plots. The effects of X_1 and X_3 with their interactions on Y_5 at a fixed level of X_2 (medium level) are shown in Fig. 1.

The plots were found to be nonlinear, indicating a nonlinear relationship between X_1 and X_3. It was determined from the contour plot that a lower value of Y_5 (0 %) could

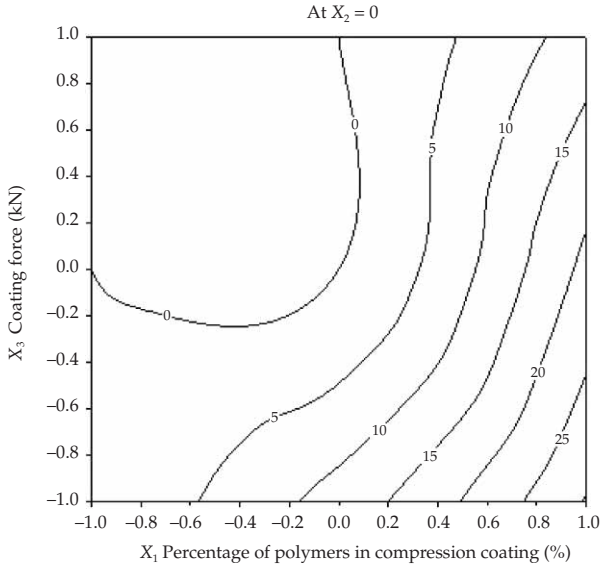

Fig. 1. Contour plot showing the effect of percentage of polymers (pectin-compritol ATO 888) in compression coating (X_1) and coating force (X_3) on the percent of drug released after 5 h (Y_5).

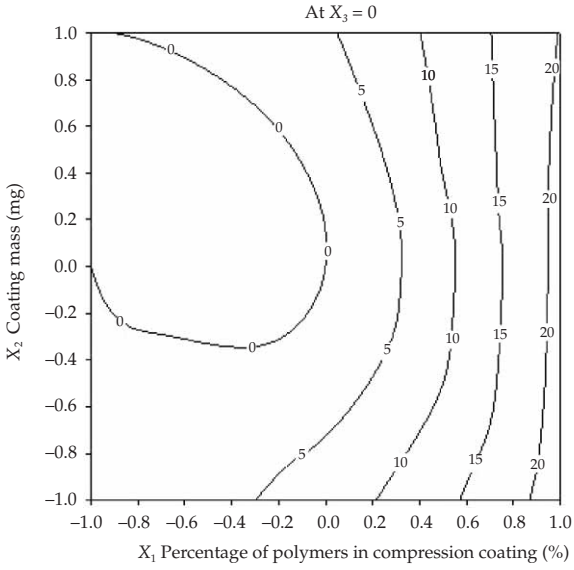

Fig. 2. Contour plot showing the effect of percentage of polymers (pectin-compritol ATO 888) in compression coating (X_1) and coating mass (X_2) on the percent of drug released after 5 h (Y_5).

be obtained with an X_1 level range from –0.1 to 0.0 and an X_3 level range from 0.0 to 1.0. It is evident from the contour that the low level of both X_1 and X_3 favors Y_5. When the coefficient values of the two key variables, X_1 and X_3, were compared, the value of variable X_1 (b_1 = 10.66) was found to be higher, indicating that it contributed the most to predicting the Y_5. The negative effects of X_3 on Y_5 may be attributed to the coating force of compression coated tablets and it was concluded that at higher coating force, core tablets remain intact up to 5 h. Fig. 2 shows the contour plot drawn at a 0 level of X_3. The contours of all Y_5 values were found to be curvilinear and indicated that a low value of Y_5 (0 %) can be obtained for a combination of the two independent variables, the X_1 level in the range of –1.0 to –0.3, and the X_2 level in the range of 0.0 to 1.0.

The effects of X_1 and X_3 with their interaction on Y_{24} at a fixed level of X_2 (medium level) are shown in Fig. 3. The plots were found to be nonlinear, indicating a nonlinear relationship between X_1 and X_3. It was determined from the contour plot that a higher value of Y_{24} (100 %) could be obtained with an X_1 level range from 0.0 to 1.0 and an X_3 level range from –1.0 to 0.0. It is evident from the contour that the medium level of both X_1 and X_3 favors Y_{24}.

The effects of X_1 and X_2 with their interaction on Y_{24} at a fixed level of X_3 (medium level) are shown in Fig. 4. The plots were found to be nonlinear indicating a nonlinear relationship between X_1 and X_2. It was determined from the contour plot that a higher value of Y_{24} (100 %) could be obtained with an X_1 level of 1.0 and an X_2 level range from –1.0 to 0.0. It is evident from the contour that the medium level of both X_1 and X_2 favors Y_{24}.

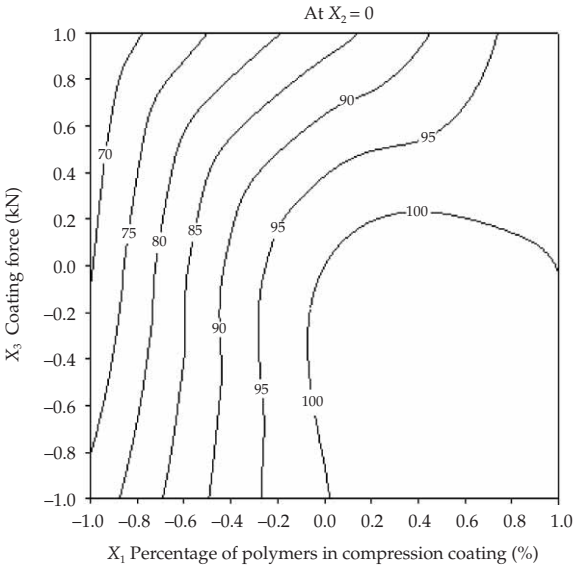

Fig. 3. Contour plot showing the effect of percentage of polymers (pectin-compritol ATO 888) in compression coating (X_1) and coating force (X_3) on the percent of drug released after 24 h in the presence of rat cecal content (Y_{24} with RC).

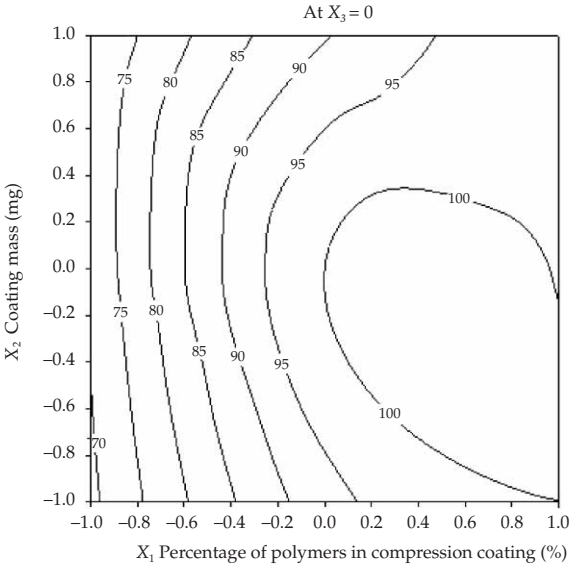

Fig. 4. Contour plot showing the effect of percentage of polymers (pectin-compritol ATO 888) in compression coating (X_1) and coating mass (X_2) on the percent of drug released after 24 h in the presence of rat cecal content (Y_{24} with RC).

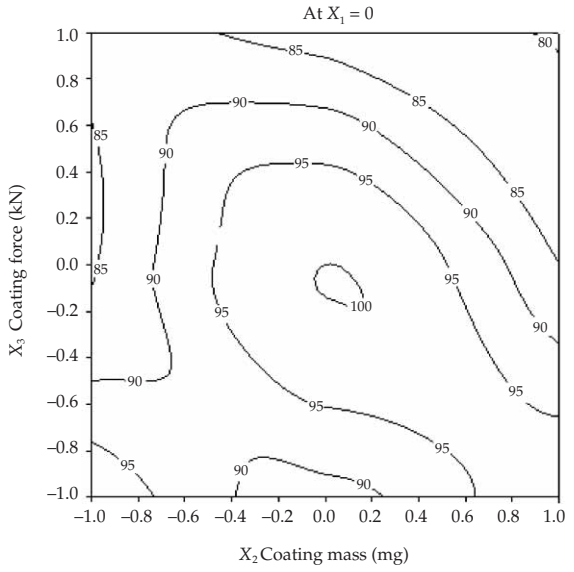

Fig. 5. Contour plot showing the effect of coating mass (X_2) and coating force (X_3) on the amount of drug released after 24 h in the presence of rat cecal content (Y_{24} with RC).

Table IV. Observed and predicted values with residuals of the response of Y_{24} (with RC)

Batch No.	Observed Y_{24} (with RC)	Predicted Y_{24} (with RC)	Residual	Error (%)
1	99.96	96.12	3.84	3.84
2	85.69	87.64	–1.96	2.28
3	99.45	97.49	1.96	1.97
4	79.85	83.69	–3.85	4.82
5	76.59	78.91	–2.32	3.03
6	65.49	62.41	3.08	4.70
7	99.97	103.07	–3.1	3.10
8	99.60	97.29	2.31	2.32
9	68.98	70.33	–1.36	1.96
10	70.26	69.72	0.54	0.76
11	99.99	100.53	–0.54	0.55
12	99.90	98.56	1.34	1.34
13	99.98	100.01	–0.03	0.03
14	100.20	100.01	0.19	0.19
15	99.85	100.01	–0.16	0.16

The effects of X_2 and X_3 with their interaction on Y_{24} at a fixed level of X_1 (medium level) are shown in Fig. 5. From Figures 1–5, the following observations can be made. All contour plots for a high value of Y_5 and Y_{24} were found to be nonlinear. This signifies that there is no direct linear relationship between the selected independent variables. A low value of Y_5 can be obtained for low and medium levels of X_1 but the low level of X_1 does not favor Y_{24}. Thus, medium level of X_1 was selected. Similarly, at higher level of X_3, the drug was not released up to 24 h, so the medium level of X_3 was selected.

Table IV shows the observed and predicted values with residuals and percent error of responses for all batches for Y_{24} with RC.

Checkpoint analysis

Three checkpoint batches were prepared and evaluated for Y_{24}, as shown in Table V. Results indicate that the measured Y_{24} values were as expected. When measured Y_{24} values were compared with predicted Y_{24} 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_{24}.

Optimum formula

After studying the effect of the independent variables on the responses, the levels of variables that give the optimum response were determined. It is evident from the polynomial equation and contour plots (Figs. 4 and 5) that a high level of X_1 could not target

Table V. Checkpoint batches with predicted and measured Y_{24} *(with RC)*

Batch code	X_1	X_2	X_3	Y_{24} (with RC)	
				Measured[a]	Predicted
1	0	–0.5	0.5	93.26 ± 0.26	95.69
2	0.5	0	–0.5	100.26 ± 1.20	102.36
3	–0.5	0.5	0	82.59 ± 1.40	88.58

[a] Mean ± SD, n = 3.

the compressed tablet to the colon and a low level of X_1 could not release the drug in 24 h. Hence, the medium level was selected as optimum for X_1, since up to this level a low value of Y_5 (0 %) can be obtained. The optimum formulation is one that targets compressed tablets to the colon (Y_5 = 0 %), 50 % of the drug releases within 8 h after reaching the colon (t_{50} with RC = 11 h) and the total amount of drug releases within 24 h in a controlled manner, Y_{24} (with RC) = 100 %. Using a computer optimization process and the contour plot shown in Fig. 8, we selected levels of 0.2 and –0.15 for both X_2 and X_3, respectively, which give the theoretical values of 0 % and 100.47 % for Y_5 and Y_{24}, respectively. Hence, a 0 level (80-20) for percentage of polymers (pectin-compritol ATO 888) in compression coating (X_1), a 0.2 level of coating mass (X_2), and a –0.15 level of coating force (X_3) were selected as optimal. To prove this, a fresh formulation was prepared at optimum levels of the independent variables, and the resultant colon targeted compressed tablets were evaluated for the responses. The observed values of Y_5 and Y_{24} with RC were found to be 0.7 % and 99.99 %, respectively, which were in close agreement with the theoretical values.

CONCLUSIONS

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 use fulness 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 optimum controlled release colon targeted formulation of mesalamine with desired properties.

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S A Ž E T A K

Box-Behnkenov eksperimentalni dizajn u izradi pektin-kompritol ATO 888 obloženih tableta za ciljanu isporuku mesalamina u kolon

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Cilj rada bio je ispitati utjecaj tri nezavisne varijable u obloženim tabletama mesalamina za ulcerativni kolitis. 3-faktorijalni, Box-Behnkenov dizajn na 3 nivoa upotrijebljen

je za dobivanje polinomske jednadžbe drugog reda i konstruiranje konturnih krivulja za predviđanje odgovora. Izabrane nezavisne varijable bile su: udio polimera (pektin i kompritol ATO 888) u oblaganju kompresijom (X_1), masa tvari za oblaganje (X_2) i sila za oblaganje (X_3). Izrađeno je petnaest pripravaka kojima su ispitani sljedeći parametri: udio lijeka oslobođenog nakon 5 h (Y_5), vrijeme potrebno za otapanje 50 % mesalamina (t_{50}) uz i bez prisutnosti crijevnog sadržaja štakora (RC), postotak oslobođenog lijeka tijekom 24 h u prisutnosti crijevnog sadržaja (Y_{24} uz RC). Transformirane vrijednosti nezavisnih i zavisnih varijabla podvrgnute su višestrukoj regresijskoj analizi da se odredi polinomska jednadžba drugog reda potpunog modela. *F* vrijednost izračunata je da se potvrdi izostavljanje neznačajnih članova iz jednadžbe potpunog modela. Kompjutorski optimizirani proces i krivulje predviđaju nezavisne varijable X_1, X_2 i X_3 (0, 0,2, odnosno –0,15) za ciljanu isporuku u kolon i ukupni postotak lijeka oslobođenog tijekom 24 h.

Ključne riječi: mesalamin, obložene tablete, ciljana isporuka u kolon, oslobađanje lijeka, kompritol ATO 888, pektin, Box-Behnkenov eksperimentalno dizajniranje

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