



OPTIMIZATION AND CHARACTERIZATION OF COLON TARGETED DRUG DELIVERY SYSTEM USING COMPRESSION COATING TECHNIQUE

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ABSTRACT

Objectives: The impetus of the present study was to develop colon specific drug delivery system using compression coating technique by Box-Behnken design for the treatment of inflammatory bowel disease (IBD). **Experimental methods:** A colonic drug delivery with a new concept based on a combination of time-, pH-, and enzyme-controlled system was developed. To begin with the methodology core tablet of Mesalamine with lower strength (50 mg) and Spray dried Chitosan acetate were prepared. A Box-Behnken design was used to derive polynomial equation and construct contour plots to predict responses. Critical process parameters (CPPs) were taken as concentration of Spray dried Chitosan (X1), Percentage ratio of HPMC K15M: Guar gum (X2) and Coating Mass (X3). Seventeen batches were prepared as per the Design expert 8.0.7.1 and Critical quality attributes (CQAs) were taken as: Cumulative percentages of drug release at 5th hr (Y1), Time to dissolve the drug for 50% (Y2) and Cumulative percentages of drug release at 14th hr (Y3).. The computer optimization process and contour plots predicted the levels of independent variables X1, X2, and X3 (respectively) and total percent of drug released up to 14 hr. The optimized batch is further evaluated for In vivo drug release study. **Results:** Based on Box-Behnken experimental design, different release profiles were obtained. The optimized formulation containing Chitosan acetate: HPMC K4M: guar gum at 23.76: 51.82: 24.42% weight ratio and the this optimized batch shows 44.61% and 93.75% drug release in absence and presence of Rat cecal respectively.

KEYWORDS: Compression coated tablets, Box-Behnken design, Mesalamine, Chitosan acetate, Hydroxy-propyl methyl cellulose (HPMC) K15M.

INTRODUCTION

A colon-specific drug delivery system (CDDS) by compression coating which is the absolute dry coating without solvent and heat use has been developed as one of the novel targeted site specific drug delivery systems. This delivery system possess the combination of one or more controlled release mechanisms such as time, pH controlled and enzyme controlled mechanism and hardly releases the drug in the upper part of the gastrointestinal (GI) tract, but rapidly releases drug in the colon following oral administration. Owing to CDDS specifically delivering the drug to the colon, it is used to treat seriousness from constipation & diarrhoea to the debilitating inflammatory bowel diseases (IBD) (ulcerative colitis & Crohn's disease) through to colon carcinoma which is two third cause of cancer in both man & women.^[1,2,3]

Guar gum is a galactomannan and consists of linear chains of (1,4)- β -D-mannopyranosyl units with α -D-galactopyranosyl units attached by (1,6) linkages. The ratio of D-galactose to D-mannose is between 1: 1.4 and

1: 2. 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. Being soluble in water, guar gum 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 with other control released ingredients was required to protect the drug core in simulated in vivo conditions. Hence, the focus was shifted to the development of such a dosage form which has intact shielding.^[4]

Moreover, Chitosan is also being developed as rate controlling natural poly saccharide due to a wide range of molecular weights its low toxicity and biodegradability was used in the form of Spray dried acetate salt in above mentioned formulation and HPMCK15 was included in preparation of coating layer to control the solubility of Guar gum and premature drug release in stomach and small intestine.^[5]

Mesalamine is used as chronotherapeutic agent. To overcome the influence of systemic drug absorption causing adverse effects, the drug is required to deliver directly into the colon.^[6]

The purpose of this study was to develop, optimize and evaluate a compression coated tablet containing Mesalamine as the core tablet and a Guar gum, Chitosan acetate and HPMC K15 M mixture as the coat layer based on the GI transit time concept and microfloral degradation. In the present study, a Box-Behnken design was used to optimize compression coated Mesalamine tablets. The independent variables selected were: concentration of Spray dried Chitosan (X1), Percentage ratio of HPMC K15M: Guar gum (X2) and Coating Mass (X3) and dependent variable as: cumulative percentages of Drug release at 5th hr (Y1), time to dissolve the drug for 50% (Y2) and cumulative percentages of Drug release at 14th hr (Y3).

MATERIALS

Mesalamine was obtained by Zydus Cadila, Ahmedabad, India., Chitosan was obtained Cognis Ltd. (BASF), Germany. HPMC K15M and Guar gum from Colorcon Asia Pvt Ltd., Goa, India and Astron Chemicals, Ahmedabad, India respectively.

METHOD

1. Preparation of spray dried Chitosan acetate^[7]

Low molecular weight Chitosan was chosen for its better solubility and ease of degradation by colonic enzymes. Chitosan was dissolved in an aqueous acetic acid solution (1% v/v) to make a 3.5% w/w solution. The solution was sprayed at an inlet temperature of $125 \pm 2^\circ\text{C}$ and feed rate of 3ml/hr using a spray dryer. The obtained powder was used for compression coating.

2. Drug-Polymer Compatibility Study

2.1 Fourier Transform Infrared Spectroscopy^[8]

I.R. spectra of Mesalamine and mixture of Mesalamine, CSA and guar gum were monitored as KBr tablet using a Shimadzu – 8700 FTIR spectrophotometer.

2.2 Differential Scanning Calorimetry^[8]

Thermal analysis using a DSC method was carried out on Mesalamine and physical mixture of Mesalamine, CSA and guar gum employing differential scanning calorimeter (Mettler Toledo DSC822e, USA).

3. Preparation of compression coated tablets of mesalamine

3.1 Preparation of Core tablets of Mesalamine

Core tablets of Mesalamine were prepared by wet granulation method. The composition of core tablets containing the strength 50 mg of Mesalamine is depicted in Table no. I. First the drug was mixed with the

determined quantities of Micro crystalline cellulose (MCC) and Starch. MCC was used as a diluent and Starch as a disintegrant. The mixture was subjected to wet massing by using PVP K30 as a binder in Isopropyl alcohol. Granules were prepared by passing the wet mass through the sieve #10. Granules were dried in a hot air oven at 45°C for 30 min. Dried granules were passed through sieve #22 and #44. Magnesium Stearate and Talc were added to granules as a lubricant and a glidant, respectively. The granules ready for compression were evaluated for angle of repose, Carr's index, and Hausner ratio. Granules were compressed at a fixed compression force using a Tablet rotary press machine (10 Station RSB4-1, Rimek Machinery, Ahmedabad, India.) with a suitable diameter die and punch set. (i.e. 7 mm for Core tablet). The prepared core tablets were evaluated for weight variation, friability, hardness and drug content.

Table I. Composition of Core tablets of Mesalamine.

Ingredients	Core
Mesalamine	50 mg
Microcrystalline Cellulose (q.s.)	27 mg
PVP K30	10 mg
Starch	10 mg
Talc	2 mg
Magnesium Stearate	1 mg

4. Box-Behnken experimental design^[11]

A Box-Behnken statistical design with 3 factors, 3 levels, and 17 runs was selected for the optimization study. Independent and dependent variables are listed in Table II. The polynomial equation generated by this experimental design is as follows:

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

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.

5. Compression coating of core tablets

Each core tablet containing 50 mg mesalamine was compression coated with the dry blend of HPMC K15M, Chitosan acetate and Guar gum at different proportion using a rotary press machine (10 Station RSB4-1, Rimek Machinery, Ahmedabad, India.) with a 10 mm diameter and punch set. Coating forces applied was 30 kN. 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.

Table II. Variables in Box-Behnken design.

Independent variable	Level		
	Low	Medium	High
X1 = percentage of Chitosan	10	20	30
X2 = percentage of polymers (HPMC K15M: Guar gum) in compression coating (%)	50:40	60:20	70:00
X2 = coating mass (mg)	250	350	450
Dependent variable			
Amount of drug released after 5 h (Y5)			
Dissolution rate (t50)			
Amount of drug released after 24 h			

6. Evaluation Parameters

6.1 Weight variation test

Compression coated tablets (10) and core tablets (10) were weighed and the average weight was calculated. Then percentage deviation of each tablet's weight was calculated from average weight. (Indian Pharmacopoeia, 2007).

6.2 Determination of drug content

The core tablets were tested for drug content. The tablets were crushed and finely powdered, and the quantity of powder equivalent to 50 mg of mesalamine were accurately weighed and transferred to 50 ml volumetric flask containing 50 ml of phosphate buffer, pH 6.8. The resultant solution was further diluted to obtain the final concentration of 10 µg/ml. The solutions were filtered through 0.45 µm membrane filter and analyzed for the content of mesalamine using UV double beam spectrophotometer. (UV-1650 PC, Shimadzu, USA).

6.3 Hardness

Monsanto hardness tester was used for the determination of the hardness. The force of the fracture was recorded. In this work, for each formulation the hardness of 6 tablets (Compression coated tablets, Core tablets) were evaluated.

6.4 Friability

Friability of the tablets was determined using Roche friabilator (Electrolab, Mumbai). The friability (F) is given by the formula:

$$F = (W_0 - W / W_0) \times 100$$

Where, W_0 is the weight of the tablets before the test and W is the weight of the tablet after the test.

6.5 In-vitro drug release study^[9]

In-vitro releases of mesalamine from core compression coated with Hydroxy propyl methyl cellulose K15M: Chitosan acetate: Guar gum at various % weight ratios were studied using USP apparatus 2 (Electrolab, TDT-08L, USA). At first, the drug dissolution was determined in 0.1 N HCl for 2 hr (stage I). Afterwards, each tablet was transferred to, pH 6.8, phosphate buffer and run for 4 hr (stage II) and then in acetate buffer pH 5.0 / phosphate buffer pH 7.4 until 14 hr (stage III, during the 6th to the 14th hour from the beginning). The amount of drug released was analyzed by UV spectrophotometry (UV-1650 PC, Shimadzu). In vitro dissolution study was also carried out in the presence of rat cecal content because micro flora of rat cecal content is very identical to human's colonic micro flora to check the effect of the colonic microflora on the release of drug from the dosage form.

Table III. Box-Behnken experimental design with responses.

Batch	X1	X2	X3	Y1	Y2	Y3
1	0.000	-1.000	1.000	0	7.5	92.2
2	-1.000	0.000	1.000	6.3	9	82.3
3	1.000	0.000	-1.000	19.5	5.5	98.11
4	0.000	1.000	-1.000	26.3	8.5	85.32
5	0.000	0.000	0.000	8.6	7.5	93.4
6	-1.000	-1.000	0.000	0	7.5	95.3
7	0.000	1.000	1.000	19.5	9	81.2
8	0.000	-1.000	-1.000	0	7	92.3
9	0.000	0.000	0.000	8.2	7	93.4
10	0.000	0.000	0.000	8.3	7.5	94.5
11	0.000	0.000	0.000	8.3	7.5	93.2
12	-1.000	0.000	-1.000	14.5	7	84.5
13	1.000	-1.000	0.000	0	7.5	98.23
14	1.000	0.000	1.000	13.6	6.5	92.59
15	1.000	1.000	0.000	28.3	7	93.9
16	-1.000	1.000	0.000	24.3	8	75.2
17	0.000	0.000	0.000	7.9	7.5	93.2

*All values are mean values obtained from regression analysis.

7. Statistical analysis

Statistical analysis of the Box-Behnken design batches was performed by multiple regression analysis using Microsoft Excel. To evaluate the contribution of each factor with different levels to the response, the two-way analysis of variance (ANOVA) was performed using the DESIGN EXPERT 8.0.1 (STAT-EASE) demo version software. To graphically demonstrate the influence of each factor on the response, the response surface plots were generated using the DESIGN EXPERT 8.0.1 (STAT-EASE) demo version software. Variables were described in Table no. II and full design with responses in Table no. III.

8. Checkpoint Analysis

A checkpoint analysis was performed to confirm the role of the derived polynomial equation and contour plots in predicting the responses. Compression coated tablets were prepared experimentally at 1 checkpoint and evaluated for the responses.

Table IV. Constraints for responses and factors.

Name	Goal	Lower Limit	Upper Limit
A: Concentration of Chitosan	Range	-1	+1
B: Ratio of HPMC K15M: Guar Gum	Range	-1	+1
C: Coating Mass	Range	-1	+1
% release of drug at 5 th h	Target 0	-----	-----
Time for 50% drug release, $t_{50\%}$	Range	6	8
% release of drug at 5 th h	Range	90	100

10. Evaluation of optimized formula

10.1 Preparation of rat cecal content medium^[10]

Wistar rat weighing 150–200 g and maintained on a normal diet (soaked gram) were used. Forty-five-minute before the commencement of drug release studies, four rats were killed by spinal traction. The abdomen was opened, the cecal was traced, ligated at both the ends, dissected, and immediately transferred into pH 6.8 buffer previously bubbled with carbon dioxide. The cecal bags were opened, their contents were individually weighed, pooled, and suspended in the buffer continuously bubbled with carbon dioxide. These were finally added to the dissolution media to give a final cecal dilution of 2 % w/v. All the above procedures were carried out under carbon dioxide in order to maintain anaerobic condition.

10.2 Drug release profile comparison of optimized batch in present and absence of Rat cecal.

The optimized formulation was compared for the drug release profile. The amounts of drug released from the was compared in presence and in absence of Rat cecal.

11. Kinetic analysis to dissolution data

Model Dependent Approach

1) Zero order release

Zero order release kinetics refers to the process of constant drug release from a drug delivery device such as oral osmotic tablets, transdermal systems, matrix tablets

9. Optimization Data Analysis

The computation for optimized formulation was carried using software, DESIGN EXPERT 8.0.1 (STAT-EASE). The optimized formulation was obtained by applying constraints (goals) on dependent (response) and independent variables (factors). Constraints for responses and factors are shown in Table IV.

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.

with low-soluble drugs and other delivery systems. In its simplest form, zero order release can be represented as

$$Q = K_0 t$$

Where,

Q = the amount of drug release at time t , K_0 = the Zero order release constant

t = the time required for drug release

The plot made: cumulative % drug release vs. time (zero order kinetic model).

2) Korsmeyer and peppas release mechanism

Korsmeyer et al (1983) derived a simple relationship which described drug release from a polymeric system. To find out the mechanism of drug release, first 60% drug release data was fitted in Korsmeyer–Peppas model:

$$\text{Log } (M_t/M_\infty) = \text{Log } K + n \text{ Log } t$$

$$\frac{M_t}{M_\infty} = K t^n$$

Where, M_t/M_∞ is the fractional drug release at time t K is a kinetic constant incorporating structural and geometrical characteristic of the drug/polymer system (devices). n is diffusion exponents that characterizes the mechanism of drug release, t is the time. A plot of log

(Mt/ M_∞) versus log t gives straight line of gradient n and an intercept of log k. n value showing different release mechanism described in Table no. V.

Table V. n value showing different release mechanism.

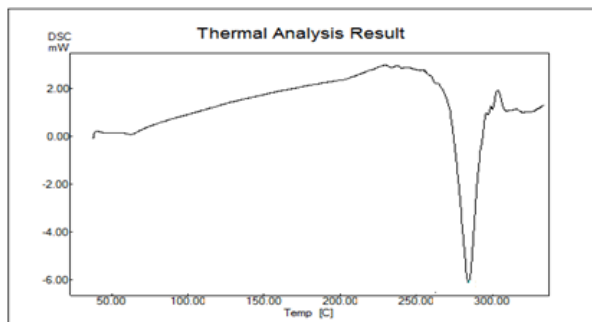
Diffusion exponent (n)	Overall solute diffusion mechanism
0.45	Fickian diffusion
0.45 < n < 0.89 Anomalous	(Non-Fickian) diffusion
0.89	Case-II transport
n > 0.89	Super case-II transport

Table VI. Characteristic functional groups peaks in mesalamine and physical mixture.

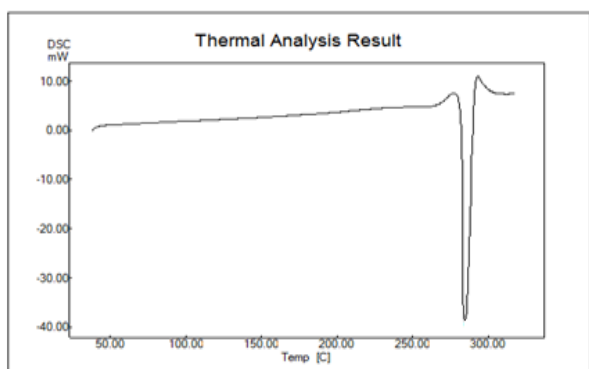
Functional Group	Amino(NH ₂)	Carboxylic(COOH)	Phenolic(OH)
Mesalamine	3422 cm ⁻¹	1651 cm ⁻¹	3086 cm ⁻¹
Physical mixture	3434 cm ⁻¹	1649 cm ⁻¹	3090 cm ⁻¹

1.2 Differential scanning calorimetry (DSC)

The DSC patterns of pure Mesalamine and physical mixture with polymers are shown in figure II. Pure Mesalamine showed a sharp endotherm at 283.3°C corresponding to its melting point (280-284). There was a negligible change in the melting endotherms of the physical mixture compared to pure drug. Physical mixture showed sharp endotherm in the range of 282.1, which is corresponding to the melting point of pure Mesalamine. Diagram were shown in Fig. 1(a) and (2).



(a)



(b)

Fig. 1: DSC thermogram of (a) pure Mesalamine, (b) mixture of Mesalamine, Chitosan acetate and Guar gum.

RESULT AND DISCUSSION

1. Drug polymer compatibility study

1.1 FTIR study

The possible interactions between the drug and the polymers were studied by FTIR spectroscopy. The details of reading were shown in Table no. VI and no any sifting of characteristic absorption bands of mesalamine was observed in IR spectra of physical mixtures produced with polymer.

2. Core tablets of mesalamine

2.1 Physical characterization of granules for core tablets

In this study, flow properties of granules were evaluated by determining Carr's index and angle of repose. According to the literature data, excellent flow properties are seen for granules with compressibility (Carr's) index, Ci between 15 and 25. The compressibility index of granules for all tablets was ranged between 16 to 18. Thus, it indicates suitability of tableting. Hausner's ratio was between 1.19 to 1.21 which indicated good flow property of granules. Angle of repose was ranged between 26 to 28 indicated that granules had good flow properties. Results were shown in Table no. VII

Table VII. Physical characterisation of granules for core tablets

Evaluation Parameters	Core Tablets
Bulk Density (gm/cm ³)	0.281 ± 0.005
Tapped Density (gm/cm ³)	0.335 ± 0.003
Carr's index	16.222 ± 0.509
Hausner's ratio	1.194 ± 0.007
Angle of repose (°)	26.594 ± 0.550

*Values are mean ± S.D of n=3

2.2 Physicochemical Characteristics of Core Tablets

As per Table VII, in determinations of tablet weights, all core tablets were found to be within pharmacopoeia limits (i.e. less than 5% for uncoated tablet). The hardness value of the core tablets was within the range of 3.5-5.00 kg/cm², within pharmacopoeia limits. Friability value of all formulations was less than 1%. Small values in friability imply much less friability during transportation. Good uniformity in drug content was found among all batches of core tablets. Tablets showed acceptable drug content values (i.e. between 98-102%).

Table VIII. Physicochemical characteristics of core tablets.

Batch	Weight variation	Hardness (kg/cm ²)	% Friability	% Drug content
Core Tablet	100.3 ± 2.09	4.5 ± 0.06	0.28 ± 0.02	98.57 ± 0.48

*Values are mean ±S.D of n=3.

3. Compression coated tablets containing core tablets (50 mg mesalamine)

Physicochemical Characteristics of compression coated tablets of preliminary batches

In determination of weight variation, compression coated tablets of all batches were found to be within

pharmacopoeia limits (i.e. less than 5% for uncoated tablet). The hardness value of the tablets of all batches was within the range of 3.5-5.00 kg/cm², within pharmacopoeia limits. Friability value of all formulations was less than 1%. Small values in friability imply much less friability during transportation as per Table no. IX.

Table IX. Physicochemical Characteristics of compression coated tablets of preliminary batches B1 – B5.

Batch No.	Weight variation	Hardness	%Friability
B1	300.10 ± 3.51	4.0 ± 0.15	0.62 ± 0.05
B2	300.25 ± 2.43	3.9 ± 0.10	0.75 ± 0.06
B3	299.90 ± 3.46	3.7 ± 0.10	0.78 ± 0.04
B4	301.50 ± 3.82	3.6 ± 0.06	0.81 ± 0.03
B5	301.70 ± 2.81	3.5 ± 0.06	0.89 ± 0.04
B6	300.25 ± 2.43	3.7 ± 0.10	0.78 ± 0.04
B7	299.90 ± 3.46	3.6 ± 0.06	0.81 ± 0.03
B8	301.50 ± 3.82	3.5 ± 0.06	0.89 ± 0.04
B9	300.10 ± 3.51	4.0 ± 0.15	0.62 ± 0.05
B10	300.25 ± 2.43	3.9 ± 0.10	0.75 ± 0.06
B11	299.90 ± 3.46	3.7 ± 0.10	0.78 ± 0.04
B12	301.50 ± 3.82	3.5 ± 0.06	0.78 ± 0.04
B13	300.10 ± 3.51	3.7 ± 0.10	0.81 ± 0.03
B14	300.25 ± 2.43	3.6 ± 0.06	0.89 ± 0.04
B15	300.25 ± 2.43	3.5 ± 0.06	0.78 ± 0.04
B16	299.90 ± 3.46	3.7 ± 0.10	0.75 ± 0.06
B17	301.50 ± 3.82	3.6 ± 0.06	0.78 ± 0.04

*Values are mean ±S.D of n=3.

4. Box-Behnken design

The concentration of Chitosan Acetate(X1), ratio of HPMC K15M (X2) to Guar gum and Mass of coating material(X3), were selected as independent variables in Box-Behnken design. The % Release at 5th h (Y1), t_{50%} (Y2), % Release at 14th h (Y3) were taken as responses. Based on the experimental design, the factor combinations yielded different mean responses. Table (3) summarizes the experimental runs, their factor combinations and the levels of experimental units in the study as well all the responses. In order to determine the levels of factors which yielded optimal % release, mathematical relationships were generated between the dependent and independent variables.

$$\% \text{ Release of Drug at 5th hr} = +8.26 + 2.04 * A + 12.30 * B - 2.61 * C + 1.00 * A * B + 0.58 * A * C - 1.70 * B * C + 3.46 * A^2 + 1.43 * B^2 + 1.76 * C^2$$

$$\text{Dissolution rate for 50\% drug release (t}_{50}\text{\%)} = +7.40 - 0.94 * A + 0.69 * B + 0.50 * C + 0.3 * A * B + 0.25 * A * C + 0.000 * B * C - 0.76 * A^2 + 0.24 * B^2 + 0.36 * C^2$$

$$\% \text{ Release of Drug at 14th hr} = +93.54 + 5.69 * A - 5.30 * B -$$

$$1.49 * C + 3.94 * A * B - 0.83 * A * C - 1.00 * B * C - 0.63 * A^2 - 2.25 * B^2 - 3.53 * C^2.$$

From the results of multiple regression analysis, it was found that the dependent variables, all responses dependent on the independent variables ($p < 0.05$). The correlation coefficients indicate a good fit. Polynomial equations can be used to draw a conclusion after considering the magnitude of the coefficient and the mathematical sign it carries (positive or negative).

There was no drug release observed at first 2 h for all formulations in stage I, simulated gastric fluid (pH 1.2). Drug release data showed there was no burst effect observed at initial hours, after changing pH from 1.2 to 6.8, stage II (simulated intestinal fluid). In stage III of dissolution, the dissolution media was changed from pH 6.8 to pH 5.0, acetate buffer (Simulated colonic fluid). The polymer blends used as compression coat, controlled the drug release by providing lag times varying from 5 to 8 h. As the amount of Chitosan acetate increased, the rate of drug release increased but the lag time decreased. After 5 h, high drug release was observed in B15 formulation, which may be due to the higher

concentration of Chitosan acetate as it gets dissolved rapidly in acidic condition of colonic fluid leading to fast erosion of compression coat and also due to thin compression-coating layer. The formulation should provide drug release after 5-6 h to target the drug to colon. The lower percentage of drug release for some batches may be due to higher concentration of HPMC K15M and lower concentration of Chitosan acetate and higher thickness of compression coat, suggesting the slower dissolution of the compression coat. B1, B6, B8 and B13 provided the appropriate lag time of 5 hr for drug release.

Chitosan acetate also formed a swollen gel which partly dissolved in 0.1 N HCl during the first 2 h but the dissolution of Chitosan acetate was decreased after transferring to the higher pH medium in the stage II (pH 6.8). In the stage III (pH 5.0, acetate buffer), the drug was released over 98% within 14 hr. The increasing rate of drug release in this stage was attributed to the more rapid dissolving of Chitosan acetate especially in the colonic acid medium.

4.1 Three-dimensional (3D) response surface plots:

In Table no. X, three-dimensional (3D) plots for the measured responses were formed, based on the model polynomial functions to assess the change of the response surface. Also the relationship between the dependent and independent variables can be further understood by these plots (Baumgartner *et al.*, 2000). Since the model has more than two factors, one factor was constant for each diagram. Response surface plots with contour below for responses Y1, Y2 and Y3 are further presented in figures (3–5) GG: HPMC K15M seems to be a better option for colon specific drug delivery because the compression coat in this case consists of a relatively reduced concentration of degradable polysaccharide which will facilitate a faster drug delivery to the colon.

Table X. Responses of Box-Behnken Design.

Batch	% Release at 5 th h(Y1)	t ₅₀ %(Y2)	% Release at 14 th h(Y3)
B1	0	7.5	92.2
B2	6.3	9	82.3
B3	19.5	5.5	98.11
B4	26.3	8.5	85.32
B5	8.6	7.5	93.4
B6	0	7.5	95.3
B7	19.5	9	81.2
B8	0	7	92.3
B9	8.2	7	93.4
B10	8.3	7.5	94.5
B11	8.3	7.5	93.2
B12	14.5	7	84.5
B13	0	5	98.23
B14	13.6	6.5	92.59
B15	28.9	7	93.9
B16	24.3	8	75.2
B17	7.9	7.5	93.2

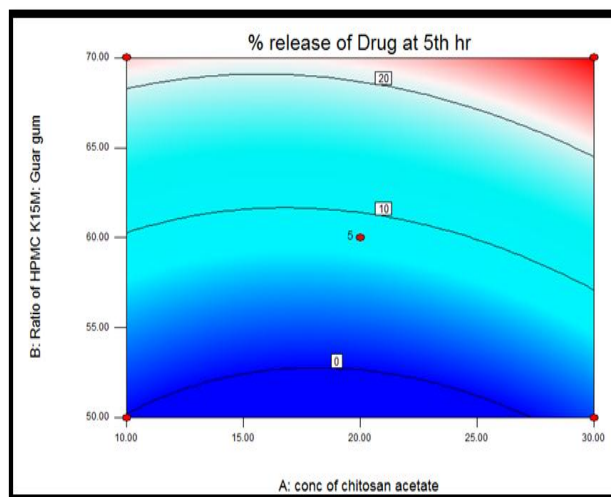


Fig. 2: contour plot for Response Y1.

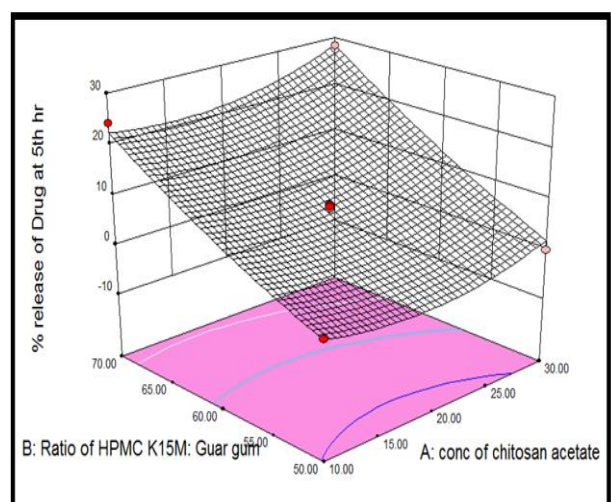


Fig. 3: Response surface plot (3D) with contour below showing the effect of X1, X2 and X3 on Y1 response.

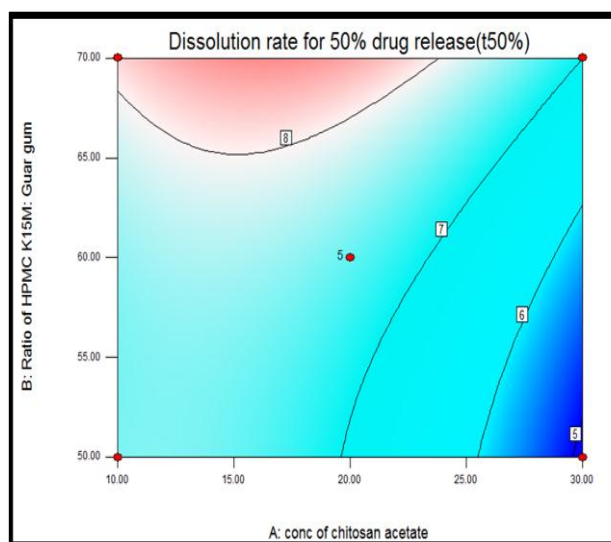


Fig. 4: contour plot for Response Y2.

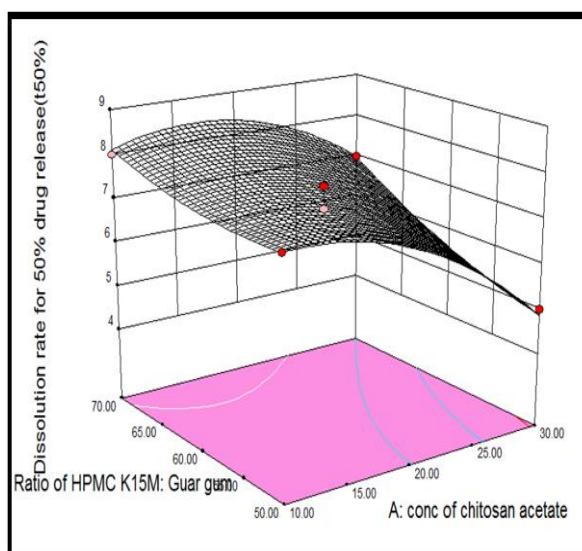


Fig. 5: Response surface plot (3D) with contour below showing the effect of X1, X2 and X3 on Y2 response.

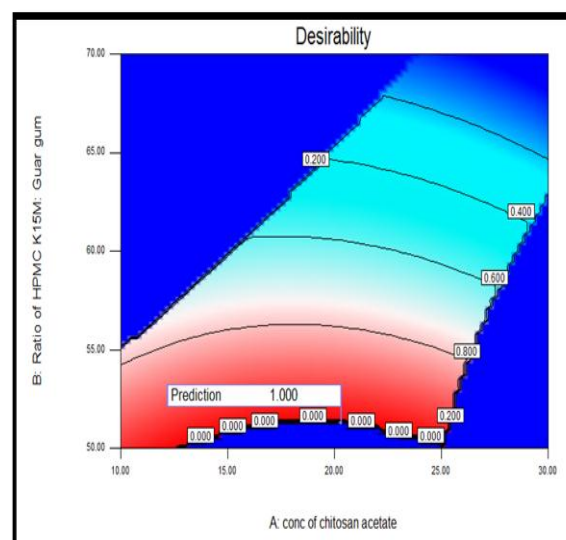


Fig. 8: Countour plot for optimization.

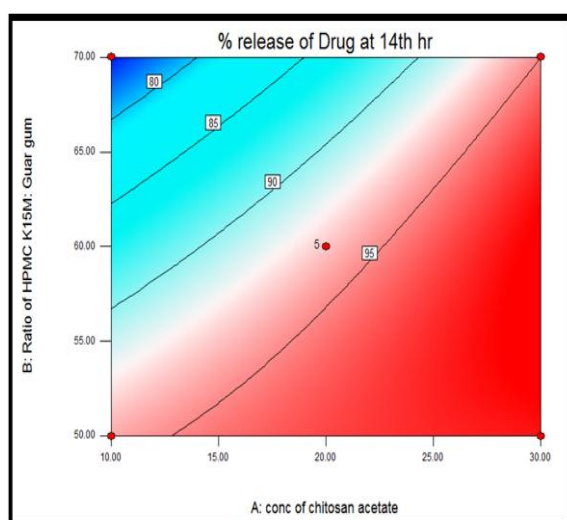


Fig. 6: contour plot for Response Y3.

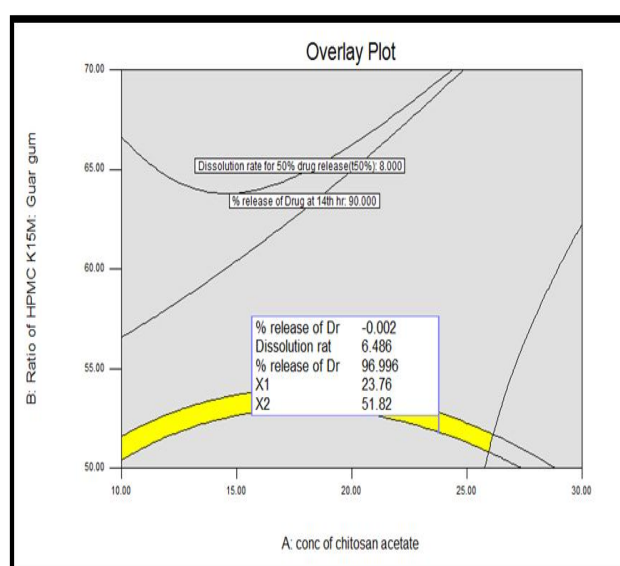


Fig. 9: Overlayplot for optimization.

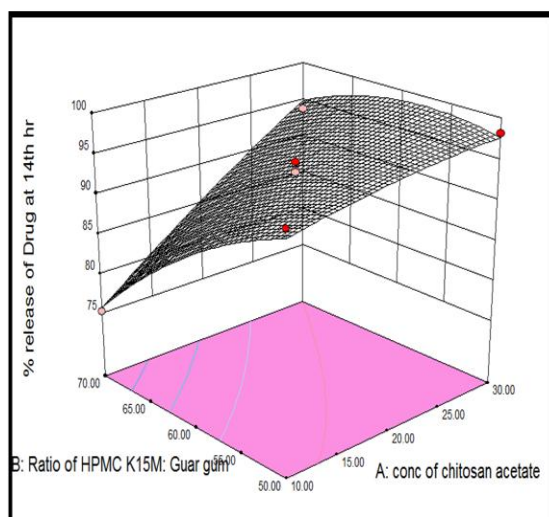


Fig. 7: Response surface plot (3D) with contour below showing the effect of X1, X2 and X3 on Y3 response.

4.2 Formulation Optimization

For the optimization of compression coated tablets of Mesalamine, constraints were fixed for all factors and response (Table II). Constraints were set according to formulation of tablets using appropriate amount of excipients, which would give desired response values. In the present study our aim was % release at 5th hour should be less than 0%, t50% should be 6-8 hours and % release at 14th hour should be 90-100%. Table no. XI shows Optimized Formula with predicted and Measured Responses In optimization (Fig. 8) desirability 1.000 indicated optimum formulation was achieved at of 23.6 X1, 51.82 of X2 and 375.15 of X3.

Validation of optimization technique done by preparing check-point batch and response were evaluated. The responses value observed in checkpoint batch was very near to optimized batch.

Table XI. Optimized Formula with predicted and Measured Responses.

X1	X2	X3	Y1		Y3	
			Predicted	Measured	Predicted	Measured
23.76	51.82 :24.42	357.1	1.33	0.00	96.99	95.21

5. In-vitro drug release study of optimized batch (B4) in presence of rat cecal content

Above prepared showed optimum drug release in the colonic acid condition of IBD. But the same batch should show similar lag time and drug release profile in variable colonic condition of IBD, i.e. pH 7.4 and presence of colonic microflora. Dissolution testing was carried out using USP Apparatus 2 at 50 rpm, in 200 ml of medium at 37°C. For the formulation B4, first 2 hr of exposure in 0.1N hydrochloric acid (pH 1.2) was carried out followed by dissolution in phosphate buffer of pH 6.8 for 4 hr. Then the same was subjected to the dissolution in phosphate buffer, pH 7.4 containing 2% of the rat cecal

content up to 14 hr. Anaerobic condition was maintained by continuous CO₂ flux. The samples were analyzed using UV-visible spectroscopy at 330 nm. To access the susceptibility of guar gum in compression coat to the colonic micro flora the drug release studies were carried out in the presence of rat cecal contents because of its similarity to human intestinal micro flora. It was observed that the optimized formulation showed a lag time of 6 hr. In presence of rat cecal content it released above 93% Mesalamine within 14 hr. In the absence of rat cecal content, the same formulation showed a lag time of 7 hr and released only 45% of the drug within 14 hr, at pH 7.4 as shown in Table no. XII.

Table XII. Percentage Cumulative drug release (% CDR) with respect to time of Optimized Batch in presence and in absence of rat cecal content.

Time (hr)	% CDR	
	Presence of rat cecal content	Absence of rat cecal content
1	0.00	0.00
2	0.00	0.00
3	0.00	0.00
4	0.00	0.00
5	0.00	0.00
6	0.00	0.00
7	9.42 ± 3.25	0.00
8	21.94 ± 6.46	0.00
9	36.36 ± 4.95	0.00
10	47.41 ± 5.49	4.63 ± 2.56
11	60.52 ± 5.96	14.16 ± 5.43
12	72.18 ± 3.64	26.27 ± 3.29
13	84.39 ± 3.87	36.16 ± 5.91
14	93.75 ± 2.85	44.61 ± 3.69

*Values are mean ±S.D of n=3.

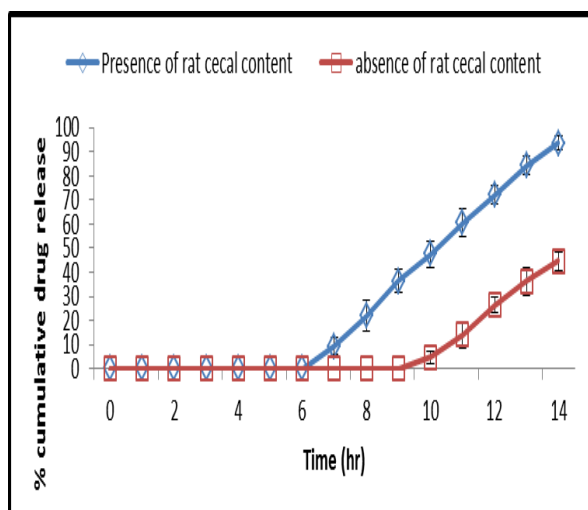


Fig. 10: Dissolution behaviour of Optimized Batch in presence and in absence of rat cecal content.

5.1 Kinetic analysis

The release kinetics was determined by using mathematical models of first order, zero order kinetic, Higuchi's model of diffusion and korsmeyer peppas model. The kinetic investigations of the release profile gave us useful insight into the mechanism of drug release from the compression coated tablets. Table XIII depicts the different release kinetic models for the optimized batches of compression coated tablets of mesalamine. All the tablet formulations followed the zero order release as their r^2 values were between 0.9748 and 0.9921. The mechanisms of drug release were non-Fickian diffusion (super case-II), since they fitted well with Korsmeyer–Peppas models (r^2 in the range of 0.9684–0.9913 with n value above 1). This indicated that drug release depended on swelling, relaxation and erosion of polymers with zero order release kinetics.

Table XIII. Regression coefficient (R^2) values of drug release data obtained from various kinetic models and n value according to Korsmeyer-Peppas.

Zero order	First order	Higuchi model	Korsmeyer-Peppas model	
R^2	R^2	R^2	R^2	N
0.9921	0.9063	0.9413	0.9913	1.3729

CONCLUSION

This study illustrated a novel time, pH, and enzyme-controlled colon specific drug delivery system of Mesalamine using compression coating technique. The results from this study clearly showed the potential of a new combination coating material of Chitosan acetate, HPMC K15M and guar gum for colonic drug delivery. These compression-coats at the appropriate mixing ratio (Chitosan acetate: HPMC K4M: guar gum at 23.76: 51.82: 24.42% weight ratio) were capable of retarding the release of Mesalamine until the dosage forms reached the colon. The system was able to release the drug specifically in the colon over a specific period of time in the variable conditions IBD.

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