Design, Optimization and *In vitro* Evaluation of Mesalazine 400 mg Delayed Release Tablet for Colon Specific Delivery

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ABSTRACT: Ulcerative colitis is a chronic inflammatory disease and patients would get more benefit if the drug is given directly to the colon. Mesalazine is intended to deliver to the colon for treating ulcerative colitis. Here, we aimed to design and optimize mesalazine 400 mg delayed release tablet for colon-specific delivery using a quality by design (QbD) approach. The tablet was first formulated as an optimized core tablet and then coated with Eudragit S 12.5 for ensuring colonic delivery. The experimental design for the core tablet was constructed using a 3^2 full factorial design, where the percentages of sodium starch glycolate (SSG) and polyvinylpyrrolidone (PVP K-30) were independent variables and the tablet hardness (kg/cm^2) & cumulative percentage of drug release into phosphate buffer at pH 7.2 after 1.5 hours were treated as responses. Responses obtained from the initial exploratory formulations were evaluated to develop an optimized formulation to have a hardness value of 7-8 kg/cm^2 and the maximum amount of drug release at pH 7.2 buffer. The optimized formulation involved the use of SSG and PVP K-30 at 3.05% and 1.69%, respectively. Hardness and cumulative percent of drug release obtained for the optimized core tablet were 7.8 kg/cm^2 and 91.76%, respectively. The compatibility of drug and excipients was studied utilizing XRD, FTIR and TGA. The optimized core tablet was then coated with eudragit S 12.5 to deliver the drug selectively to the colon and further assessed for its *in vitro* dissolution. Dissolution studies indicated that coated tablets with a weight gain of 7.4% exhibited the maximum cumulative percent of drug release (91.19 ± 0.11%), with a zero-order drug release profile (R^2 = 0.943). A stability study performed according to ICH Q1A (R2) guidelines at accelerated storage conditions identified that there was no significant change in drug content over the storage period, indicating the stability of the formulated tablet batches. All these data obtained here suggest that the mesalazine tablet developed through the QbD approach offers excellent physical properties and drug release profile and, therefore, could be recommended for commercial manufacturing.

Key words: Mesalazine, quality by design, colon specific delivery, eudragit S 12.5, ulcerative colitis, delayed release tablet.

INTRODUCTION

Mesalazine, an anti-inflammatory agent chemically known as 5-aminosalicylic acid, is recommended for the management of mild to moderate ulcerative colitis (UC).[1] UC is a form of inflammatory bowel disease (IBD), which causes swelling, ulcerating and loss of function of the large intestine, and can lead to colon cancer.[2] Although there are many empirical approaches (i.e., bed rest, high protein diet, opioids, rectal installation and so on), they are not so effective to treat UC patients.[3] Thus, a site-specific, highly effective and scientifically approved approach is always desired.

Mesalazine has drawn particular attention recently as it can be delivered to the colon to treat the disease.[4] The drug acts topically on the colonic mucosa and can be administered orally or rectally.[5] Following oral administration of conventional capsules or uncoated tablets of mesalazine, the drug is absorbed extensively from the proximal part of the GI tract.[6] Therefore, to achieve a local effect in lower

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portions of the GI tract, several methods have been developed to deliver mesalazine effectively in the colon, which include: a prodrug based system, enteric-coated formulation, and extended-release formulation.[7]

Sodium starch glycolate has been widely used as disintegrating agent in tablet formulation which provides its action through swelling mechanism.[8,9] Disintegration of tablet particles plays a pivotal role for release of drugs. It was reported that at higher concentration of sodium starch glycolate, it slows down drug releases due to gelling and viscosity producing effects.[10] At the same time hardness of the tablet also plays an important role for drug release. A higher value of hardness imparts in slower release of drug particles due to slower penetration of medium into the compact tablet mass.[11] In contrast, a lower value of hardness can cause rapid disintegration of particles which will result in formation of gel surrounding the disintegrated drug particles.[11] In wet granulation technique hardness of the tablet depends on the amount of granulating agent used in the formulation.[12,13]

After initiation of the term quality by design (QbD) by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) in 2009, it has gained much popularity in pharmaceutical development.[14] QbD offers a logical approach that proposes analytical and risk-management methodologies for the design, development and manufacturing of new medications.[15,16] The QbD approach is based on the principle of continuous improvement and helps to establish a design space within which robust processes are always obtained.[17] It provides a clear concept of the process parameters and their possible influences on drug formulation. Therefore, the possibility of unexpected batch failure is minimised and the consistency from batch to batch can be maintained by incorporating quality into the process.[15]

To obtain QbD, Design of Experiment (DoE) can be done in many ways, such as full factorial design, Placket Burman design, Taguchi's design, response surface methodology and so on.[18] Factorial design is one of the tools of QbD which is commonly used for statistical optimization of pharmaceutical formulations.[19] A full factorial design involves testing every possible combination of factors with their levels. As a result, interactions of the factors can be easily captured and the effect of factors on response can be determined.[19]

The current study focuses on developing and optimizing a mesalazine tablet dosage form for maximizing colon-specific delivery using the QbD approach. To achieve the target, a computer-aided optimization tool (i.e., Design Expert® software) was used and a two-factor with three-level full factorial design was employed. The effects of polyvinylpyrrolidone K-30 (PVP K-30) and sodium starch glycolate (SSG) on tablet hardness and cumulative percent of drug release from the core tablet of mesalazine were also assessed. Mathematical models (i.e. linear, quadratic and cubic) and response surface analysis were also performed to statistically optimize these formulation factors, thereby obtaining a desired formulation.

MATERIALS AND METHODS

Chemicals and reagents. Each enteric-coated tablet contained mesalazine 400 mg (BEC Chemicals, India, and assay by HPLC 100.5%) as an active ingredient. Sodium starch glycolate (SSG), polyvinylpyrrolidone K-30 (PVP K-30, average molecular weight of 40,000) and lactose monohydrate were from LobaChemie, India and used as additives for core tablet preparation. Talc (Merck KGaA, Germany) and colloidal silicon dioxide (Degussa AG, Germany) were also used to aid the preparation. For enteric coating, eudragit S 12.5 (Evonik Rohm GmbH, Germany), iron oxide red (Colorcon Asia Pvt. Ltd, India), iron oxide yellow (Colorcon Asia Pvt. Ltd, India), dibutylsebacate (Merck, India) and acetone (Scharlau, Spain) were used. Distilled water required for the experiment was prepared using a Barnstead Fistreem Distiller (Lake Balboa, California). All the formulation ingredients were generously donated by the UniMed UniHealth Pharmaceuticals Limited (Dhaka, Bangladesh).

Preformulation study. Before preparing the mesalazine tablet, preformulation study was performed which included Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) analysis, thermogravimetric analysis (TGA) and scanning electron microscopy (SEM). For the FTIR study, the IR spectrum of the active ingredient (mesalazine), and a mixture of mesalazine and excipients (1:1) were recorded using an FTIR spectrophotometer (Perkin Elmer LS55, Waltham, MA). The sample scanning was performed in the range of 4000-400 cm^{-1} at room temperature. Thermogravimetric analysis of mesalazine was performed to determine the thermal stability of the drug. The analysis was done in the temperature range of 0-600°C in a nitrogen atmosphere with a TGA-50H detector. For the XRD study, XRD diffractograms were recorded for active mesalazine, the mixture of mesalazine and excipients (1:1), and finely crushed tablets using a scintillation counter detector with a scanning range of 10-70 deg. A scanning electron microscopy (SEMTech Solutions, North Billerica, MA) study was also performed for mesalazine granules of optimized formulation.

Preparation of mesalazine core tablet. Mesalazine core tablets were prepared following the wet granulation technique. To prepare the granulating solution, PVP K-30 was first dissolved in purified water at 70-80°C temperature and then cooled down to 50°C. Mesalazine, lactose monohydrate (as a filler) and half of the total required amount of SSG were passed through mesh #30 screen and dry mixed for 10 min. The prepared granulating solution was then added to it and mixed homogeneously. The wet mass was then dried at 50-55°C (WTC BINDER, Germany) to obtain a target moisture content of 2-2.5%. Coarse dried granules were then passed through the mesh #20 screen. After that, the screened granules, and the rest amount of SSG and talc (as an anti-adherent) were passed through mesh #30 screen and blended for 10 min. Colloidal silicon dioxide (as a glidant) and magnesium stearate (as a lubricant) were passed through mesh #30 and mixed well for 2 min. Finally, the core tablets were prepared using a Clit 8 station tablet press machine with a 14.5x 5.7 mm capsule shaped punch. Table 1 shows the composition of the mesalazine core tablet.

Table 1. Composition of mesalazine 400 mg core tablet.

Ingredients	Amount (mg/Tab)	Amount (%/Tab)
Mesalazine	400	76.19
SSG	10.5 - 21.0	2-4
PVP K-30	5.25 - 15.75	1-3
Talc	10.4	1.98
Magnesium stearate	6.2	1.18
Colloidal silicon dioxide	2.6	0.5
Lactose monohydrate	q.s.	q.s.
Total	525	100

Experimental design for mesalazine core tablets. The investigation of the effect of factors, the content of super disintegrant SSG (X_1) and binder PVP K-30 (X_2) on hardness (Y_1) and cumulative percentage of drug release from the core tablet (Y_2), the two responses selected for this study, has been carried out using a two-factor with three-level (3^2) full factorial design. Factors were categorised as low, mid and high levels (Table 2).

Table 2. Factors and responses for formulating mesalazine core tablets.

Factors	Unit	Type	Coded values			Actual values (%)		
			Low	Mid	High	Low	Mid	High
SSG (X_1)	%	Numeric	-1	0	1	2	3	4
PVP K-30 (X_2)	%	Numeric	-1	0	1	1	2	3
Responses						Constraints		
Hardness (Y_1)						7-8 kg/cm^2		
Cumulative % of drug release (Y_2)						Maximum		

Based on 3^2 full factorial designs, nine formulations were generated by Design Expert® software (Version 12, Stat-Ease Inc., USA) and responses obtained from these formulations are highlighted in table 3. In several prior studies, a hardness value between 5 to 7 kg/cm^2 was used as optimum amount for developing tablet formulations.[20,21] However, in current study as the formulated core tablets will be subjected to coating process, a hardness value of 7-8 kg/cm^2 was desired so that the tablets can withstand the coating stress. Since the intention of the formulation was to deliver the drug molecule effectively at colon, it was intended to have a maximum amount of drug release which was considered as response 2. Hardness study was performed on 6 tablets from each batch using Monsanto hardness tester. For cumulative percent of drug release, average release of 6 tablets from each batch was determined. The responses were evaluated using ANOVA, Fit statistics and a 3D response surface plot. The optimization of the formulation was done to obtain hardness and drug release profile within the set constraints (that is, hardness: 7-8 kg/cm^2 and drug release: maximum). All the levels and constraints were determined from sufficient preliminary trials.

Table 3. Formulations obtained from 3^2 full factorial design and observed responses.

Formulation	Factors		Responses (mean ± standard deviation, n=6)	
	X_1 (%)	X_2 (%)	Y_1 (kg/cm^2)	Y_2 (%)
F1	3	3	11.6 ± 0.21	88.57 ± 0.16
F2	4	2	9.2 ± 0.33	86.75 ± 0.13
F3	3	2	8.5 ± 0.17	90.27 ± 0.18
F4	2	3	11.8 ± 0.25	86.57 ± 0.21
F5	3	1	5.6 ± 0.13	89.95 ± 0.14
F6	2	2	9.4 ± 0.24	88.82 ± 0.14
F7	2	1	5.8 ± 0.18	89.58 ± 0.21
F8	4	3	11.5 ± 0.26	84.54 ± 0.16
F9	4	1	5.5 ± 0.11	85.58 ± 0.29

Evaluation of the physical properties of granules. Prepared granules were evaluated in terms of bulk density (D_b), tapped density (D_t), Carr's index (I), Hausner ratio (H) and angle of repose (θ), which were defined as follows:

D_b = Mass of powder / bulk volume of powder

D_t = Mass of powder / tapped volume of powder

$I = \{(D_t - D_b) \times 100\} / D_t$

$H = D_t / D_b$

θ = $\tan^{-1}$ (height of the powder cone/radius of that powder cone)

Carr's index of ≤21 indicates fair powder flow, whereas a value of 5-15 denotes excellent flow property. Additionally, the Hausner ratio and angle of repose of ≤1.25 and ≤30°, respectively, denote the fair flow property of the granules.

Optimization of the core tablet. Optimization of the core tablet was done using Design-Expert® software. Out of nine formulations, the one with a desirability value of 1.00 was selected to obtain the expected responses. The predicted error was then determined using experimental values and predicted values.

Enteric coating of the prepared core tablets. The optimized core tablet was then coated with different concentrations of eudragit S 12.5 using Solace Auto Coater (3L). During coating operation, the applied inlet and exhaust temperatures were 50-55°C and 40-55°C, respectively. Air pressure of 1-1.2 kg/cm^2, pan speed of 1-5 rpm and spray rate of 6 gm/min were used. A mixture of water (10% w/w) and acetone (90% w/w) was used as a solvent for preparing the coating dispersion, with the concentration of 5% w/w. After coating, the percentage of weight gain were found to be 5% (B1), 5.8% (B2), 6.6% (B3), 7.4% (B4), 8.2% (B5) and 9% (B6). Table 4 describes the composition of the coating for six batches (B1 – B6).

Physical evaluation and drug release kinetics studies. Formulated core and coated tablets were assessed for length, width, thickness, weight, hardness and friability. *In vitro* drug release study was performed by fitting the dissolution profile in five different kinetics models, i.e., Zero-order ($C = k_0 t$), First order ($\log C = \log C_0 - k_1 t/2.303$), Higuchi

($Q_t = K_h t^{1/2}$), Korsmeyer-Peppas ($\log Q_t/Q_\infty = \log k_{kp} + n \log t$) and Hixson-Crowell ($Q_0^{1/3} - Q_t^{1/3} = K_{hc}t$), where C_0 and C are concentrations of drug dissolved at initial time (t=0) and time t, respectively; Q_0, Q_t and Q_∞ are the initial quantity of drug in the tablets, drug dissolved at time t and drug dissolved at infinite time, respectively; K_0, K_1, K_h, K_{kp} and K_{hc} are respective rate constants. A model with the maximum value of regression coefficient (R^2) was treated as the best-fitted model.

Table 4. Composition of coating dispersion for different batches of mesalazine tablets (Quantities are expressed as mg/Tab).

Ingredients	Batch no.					
	B1	B2	B3	B4	B5	B6
Eudragit S 12.5	109.2	124.8	140.4	156	171.6	187.2
Talc	6.2	6.82	7.68	8.53	9.38	10.23
Iron oxide red	2.9	3.31	3.73	4.14	4.55	4.96
Iron oxide yellow	0.53	0.61	0.68	0.76	0.84	0.91
Dibutylsebacate	3.8	4.34	4.89	5.43	5.97	6.48
Vehicle (acetone: purified water, 9 : 1)	q.s. to make the coating dispersion of 5% w/w					
% weight gain	5	5.8	6.6	7.4	8.2	9

As the core tablet lacks enteric coating, dissolution conditions with an acid stage and pH 6.0 buffer (stage 1), as described in the United States Pharmacopeia (USP) for mesalazine modified-release tablets, were not considered during its drug release kinetics study. Accordingly, the drug release of the core tablet was performed using 900 ml phosphate buffer (pH 7.2) for 1.5 h with USP type 2 apparatus (Electrolab, India) at 50 rpm. The drug release study of the coated tablet was performed according to the USP specifications (USP Monographs: Mesalamine Delayed-Release Tablets).[22] From each coated tablet batch, 6 tablets were studied for determining the drug release profile. Acceptance criteria specify that average drug release at both acid and stage 1 buffer (pH 6.0) should not be more than 1% and that of at stage 2 buffer (pH 7.2) should not be less than 80%.

Stability study. Stability study was done through checking the tablet physically as well as determining the drug content through a validated RP-HPLC method at initial, after 3 and 6 months respectively at accelerated storage conditions (40°C ± 2°C and 75% ± 5% RH) following ICH Q1A (R2) guidelines. The assay was performed utilizing a reversed-phase C18 column (5µm, 150 ×4.6 mm) supported by a photodiode array plus (PDA+) detector with detection at 214 nm and mobile phase of pH 7.4 phosphate buffer: methanol at a ratio of 63.5: 36.5 (v/v) with a flow rate of 1.1 ml/min.[23,24]

RESULTS AND DISCUSSION

Preformulation study. FTIR spectrum of active mesalazine, mesalazine with core tablet excipients and mesalazine with both core and tablet coating excipients denotes that there were no significant chemical interactions between drug molecule and the excipients, as the most reactive functional groups of mesalazine, such as carboxylic group (-COOH) at 3000-2500 cm^{-1}, N-H bond peak at 1445.6 cm^{-1}, and trisubstituted aromatic ring peak at 807.5 & 772 cm^{-1} were observed unaltered in the drug-excipients mixture (Figure 1a, 1b and 1c). Moreover, the XRD study also revealed no significant interaction between drug and excipients, as the characteristic peaks of mesalazine retained the crystalline nature in the crushed tablet powder (Figure 1d, 1e and 1f). TGA thermogram of mesalazine signifies that mesalazine was thermally stable at more than 100°C temperature, indicating its compatibility to the high temperature associated with the wet granulation process (Figure 1g). Furthermore, the SEM study indicated a homogenous distribution of the granules (Figure 2a and 2b).

Evaluation of physical characteristics of the granules. Results of Carr's index (I), Hausner ratio (H) and angle of repose (θ) obtained from nine experimental runs are presented in table 5. From the values, it was found that formulation F9 had poor passable flow properties and all other formulations were of good to a fair standard.

Table 5. Flow properties of the tablet formulations (F1 – F9).

Formulation	D_b (gm/ml)	D_t (gm/ml)	H	I	θ
F1	0.439±0.07	0.546±0.01	1.24±0.05	19.59±0.04	26.75±0.02
F2	0.431±0.05	0.518±0.07	1.20±0.04	16.67±0.06	25.05±0.08
F3	0.442±0.04	0.531±0.08	1.20±0.04	16.67±0.03	22.85±0.07
F4	0.455±0.03	0.538±0.02	1.18±0.07	15.42±0.01	24.18±0.03
F5	0.446±0.02	0.525±0.07	1.17±0.03	15.04±0.06	27.25±0.04
F6	0.437±0.06	0.503±0.08	1.15±0.06	13.12±0.04	24.48±0.05
F7	0.435±0.05	0.529±0.05	1.21±0.03	17.76±0.04	23.38±0.03
F8	0.426±0.02	0.486±0.04	1.14±0.02	12.34±0.07	22.61±0.05
F9	0.427±0.08	0.552±0.03	1.29±0.06	22.64±0.05	32.28±0.04

Physical evaluation of mesalazine tablets. The core tablets obtained after compression were observed to have maximum friability of 0.53%, which falls well below the set limit (1%). The observed range of core tablet weight was 521.9 - 528.2 mg, with a variation of less than 10% for each batch, indicating a satisfactory core tablet weight. The variation in length, width and thickness were also found to be within the limit (≤10%), and the calculated ranges were found to be 1 - 8%. For coated tablets, hardness was found within 12.2 - 14.2 kg/cm^2, with the maximum observed friability of 0.16%. The weight of the tablets varied from 3 - 8%, and the variation in length, width and thickness were within 1 - 6%. The formulated core and coated tablets are represented at figures 2c and 2d.

Analysis of responses. Effects of percentage of SSG (X_1) and PVP K-30 (X_2) in tablet composition on hardness (Y_1) and the cumulative percentage of drug release from the core tablet (Y_2) in pH 7.2 phosphate buffer medium were studied. The statistical analysis of the study is presented in table 6, and the response surface plots (3D) indicating the effect of X_1 and X_2 on both Y_1 and Y_2 are shown in figure 3. The error calculated from predicted and experimental observations is mentioned in table 7.

In the case of hardness (response 1), a linear model is proposed, and the predicted R^2 value (0.973) is in good agreement with the adjusted R^2 value of 0.982, as the difference between them is ≤ 0.2. The signal to noise ratio as measured by adequate precision is of 30.632 in the linear model, indicating an adequate signal as the value of ≥ 4 is desirable. The model F-value of 215.47 and *p*-value of < 0.0001 assure that the proposed mathematical model is significant. For cumulative percentage of drug release (response 2), the quadratic model is suggested. The predicted R^2 (0.838) is found to be also in reasonable agreement with the adjusted R^2 (0.964). The adequate precision was 17.395, which indicates an adequate signal. The model F-value of 43.76 and *p*-value of < 0.0053 imply that the proposed model was significant (Table 6).

Drug release kinetics study. Drug release kinetics studies for the coated tablets were performed according to USP specifications. Drug release from batch B1, B2 and B6 did not comply with USP specifications for modified release mesalazine tablets, as they either crossed the threshold of NMT 1% release for both acid and stage 1 buffer or constrained to NLT 80% release for stage 2 buffer, and thus they were not considered for further evaluations. Interestingly, batch B4 showed the

maximum amount of drug release in pH 7.2 buffer (91.19%) which was even higher than the commercial mesalazine tablet (88.41%) (Table 8).

The kinetics study of batches B3-B5 and the commercial product indicated that batch B4 tends to follow zero order drug release profile with the highest R^2 value of 0.943, whereas the commercial product appears to follow the first order release kinetics (R^2 = 0.933) (Table 9 & Figure 4 a-e).

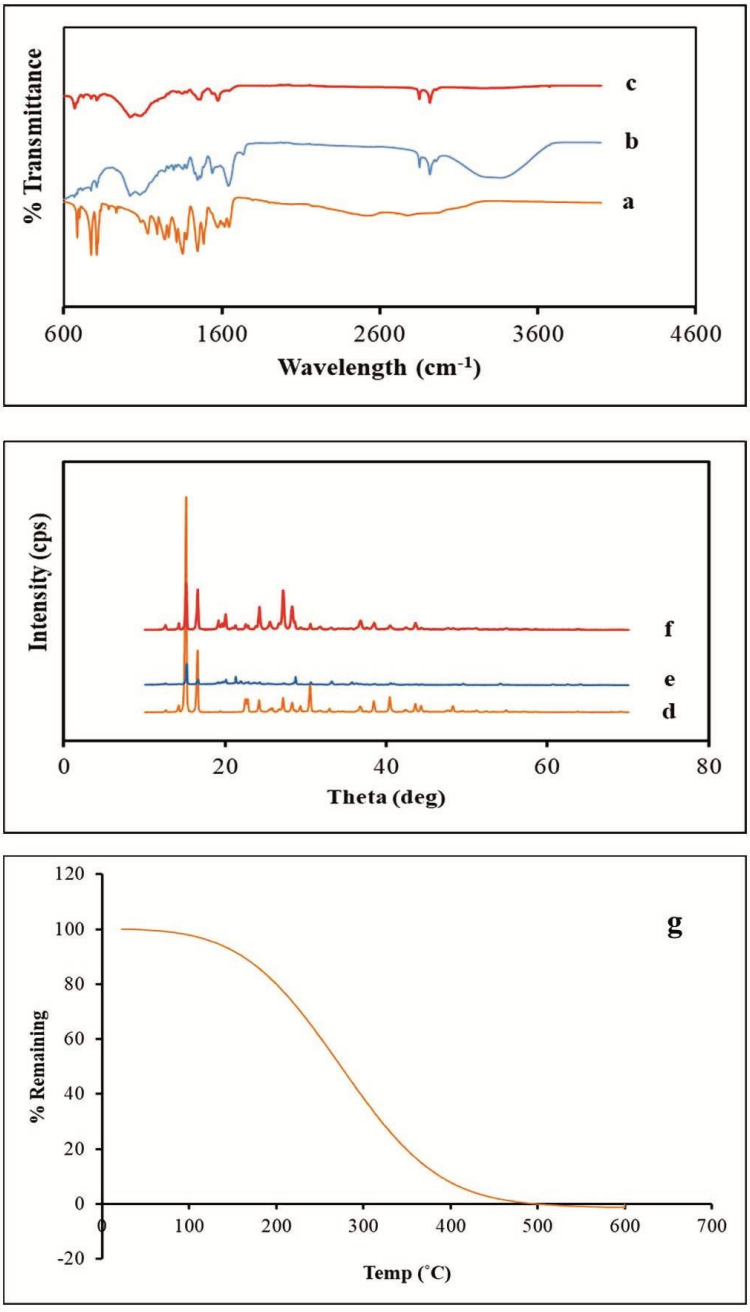

Figure 1. (a) FTIR spectrum of pure mesalazine. (b) FTIR spectrum of mixture of mesalazine and core tablet excipients. (c) FTIR spectrum of mixture of mesalazine and both core tablet and coating excipients. (d) XRD diffractogram of pure mesalazine. (e) XRD diffractogram of mesalazine-core tablet excipients. (f) XRD diffractogram of mesalazine-both core tablet and tablet coating excipients. (g) TGA thermogram of pure mesalazine.

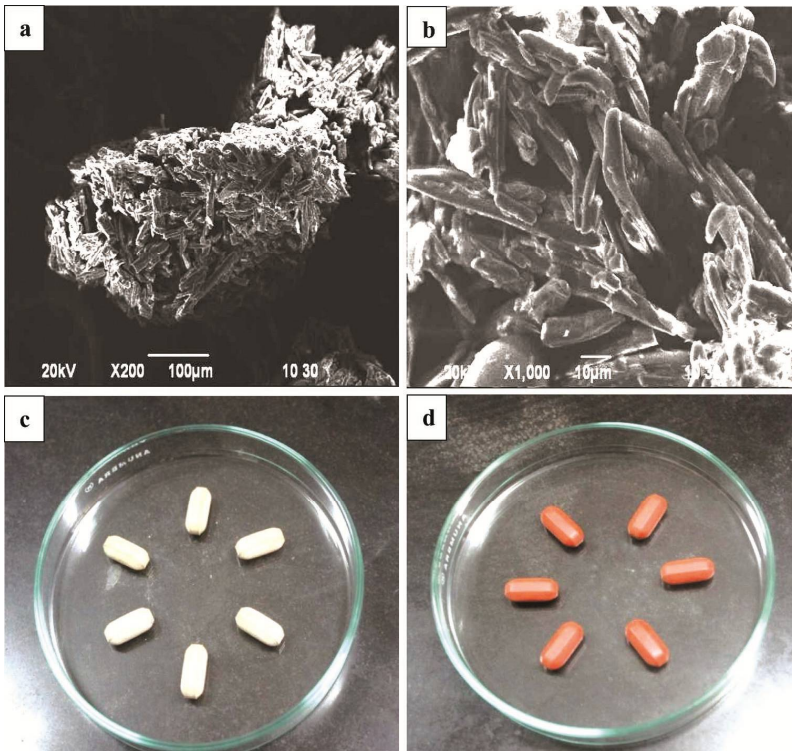

Figure 2. (a) SEM image of granules (200X magnification). (b) SEM image of granules (1000X magnification). (c) Image of formulated core tablets. (d) Image of formulated coated tablets.

Table 6. Model summary statistics, ANOVA, fit statistics and regression equation of responses.

Model summary statistics				
	Y_1		Y_2	
Source	Adjusted R^2	Predicted R^2	Adjusted R^2	Predicted R^2
Linear	0.982	0.973	0.294	-0.2027
2FI	0.978	0.965	0.199	-1.5961
Quadratic	0.987	0.965	0.964	0.8378
Cubic	0.963	0.169	0.995	0.8964
ANOVA				
	Y_1		Y_2	
Source	F-value	P-value	F-value	P-value
Model	215.47	< 0.0001	43.76	0.0053
X_1	0.8496	0.3922	72.02	0.0034
X_2	430.09	< 0.0001	32.37	0.0108
$X_1.X_2$			6.39	0.0856
X_1^2			90.65	0.0025
X_2^2			17.37	0.0251
Residual			43.76	0.0053
Cor Total			72.02	0.0034
Summary of fit statistics for responses				
Parameters		Y_1	Y_2	
Std. Dev.		0.354	0.389	
Mean		8.77	87.85	
C.V. %		4.04	0.445	
Adeq Precision		30.632	17.395	
Regression equation				
Y_1		+3.166 -0.133 X_1 +3.00 X_2-30		
Y_2		+48.881 +22.325 X_1 +4.079 X_2 -1.147 X_1 * X_2-30 -2.97 X_1^2 -0.86 X_2^2		

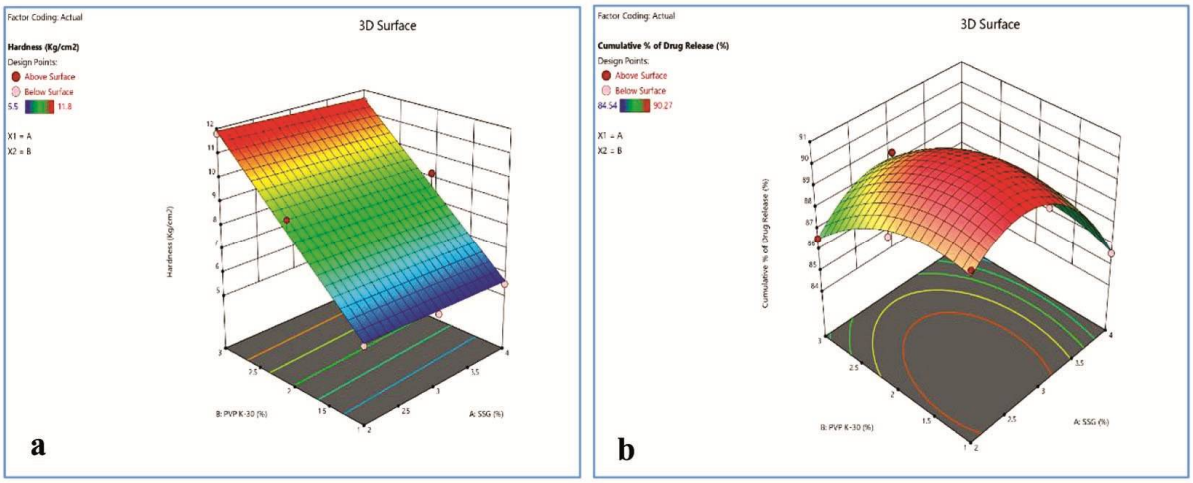

Figure 3. Response surface plot showing the effect X_1 and X_2 on (a) hardness (Y_1) and on (b) cumulative percentage of drug release (Y_2).

Table 7. Determination of predicted error (%) from optimized formulation.

Components	X_1 (%)	X_2 (%)	Y_1 (kg/cm^2)	Y_2 (%)
Predicted values	3.08	1.69	7.81	90.38
Experimental values	3.05	1.69	7.80	91.76
*Predicted error (%)			-0.13	1.52

*Predicted error = (Experimental value – Predicted value)*100 / Predicted value.

Stability study. Physically tablets of batches B3, B4 and B5 were found stable after 3 months and 6 months kept at accelerated storage condition of 40°C ± 2°C and 75% ± 5% RH following ICH guidelines. For assay, samples were taken from randomly selected 6 tablets at initial (T_0), after 3 months (T_3) and 6 months (T_6) period for each batch. Assay results of tablets from the batch B3 showed standard deviation of 0.56-0.86, for batch B4 it was 0.31-0.58 and for batch B5 it was 0.14-0.47. One-way ANOVA study of the mean drug contents of batch B3 at initial, after 3 months and after 6 months showed a *p*-value of 0.85 with an alternative hypothesis that all means are not equal at a significance level of 0.05. This high value of *p* suggests that there was no significant difference between the mean drug contents of tablets of batch B3 at initial, after 3 months and after 6 months storage at accelerated conditions. Similarly, for batches B4 and B5 the *p*-values were 0.67 and 0.22, respectively, which also denote that there were no significant differences between the mean drug contents of batches B4 and B5 during their storage at accelerated condition (Table 10).

Table 8. Cumulative percentage of drug release from coated tablet (B1 to B6) in different dissolution mediums.

Batch	% Weight gain/tablet	Cumulative % of drug release (mean ± standard deviation, n=6)		
		Acid stage (0.1 N HCl, 2 hr)	Buffer stage 1 (pH 6.0 PB, 1 hr)	Buffer stage 2 (pH 7.2 PB, 1.5 hr)
B1	5	0.18 ± 0.07	1.11 ± 0.13	89.97 ± 0.14
B2	5.8	0.15 ± 0.02	1.05 ± 0.09	92.95 ± 0.21
B3	6.6	0.12 ± 0.02	0.53 ± 0.06	86.50 ± 0.12
B4	7.4	0.10 ± 0.05	0.43 ± 0.02	91.19 ± 0.11
B5	8.2	0.07 ± 0.01	0.46 ± 0.08	83.92 ± 0.17
B6	9	0.06 ± 0.03	0.40 ± 0.03	76.82 ± 0.15
CP	-	0.09 ± 0.04	0.58 ± 0.02	88.41± 0.14

*PB = Phosphate buffer, CP = Commercial product.

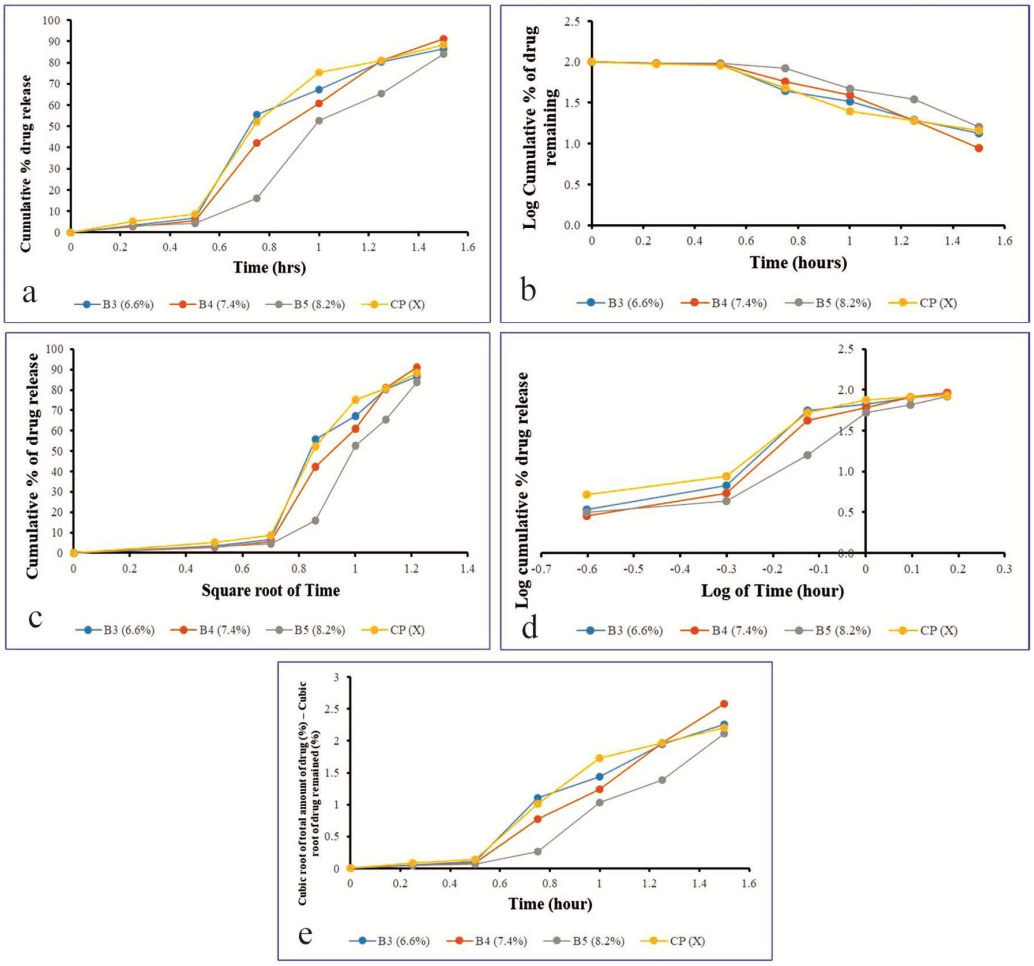

Figure 4. (a) Zero order plot. (b) First order plot. (c) Higuchi plot. (d) Korsmeyer-Peppas plot. (e) Hixson-Crowell plot.

Table 9. R^2 values for batches B3-B5 and CP.

Mathematical model	Formulations			CP
	B3	B4	B5	
Zero order	0.914	0.943	0.904	0.906
First order	0.938	0.886	0.837	0.933
Higuchi	0.789	0.774	0.699	0.507
Korsmeyer-Peppas	0.90	0.923	0.916	0.899
Hixson-Crowell	0.940	0.924	0.870	0.930

Table 10. Stability study of tablets of batches B3, B4 and B5.

Parameters	B3			B4			B5		
	T_0	T_3	T_6	T_0	T_3	T_6	T_0	T_3	T_6
Mean drug content	99.54	99.37	99.72	100.03	99.82	99.69	100.13	99.55	99.73
Standard deviation	0.86	0.76	0.56	0.58	0.31	0.44	0.14	0.47	0.39
P-value		0.85			0.67			0.22	

The developed mesalazine formulation is designed through a systematic approach which will enable the formulator to design the formulation within a design space for achieving optimum responses.[8-12] For both the responses cubic model was aliased. This is due to the fact that factorial design is a small RSM design which causes difficulty to estimate cubic models. From the acquired result it is found that percentage of disintegrant SSG (X_1) has no significant impact on hardness (Y_1) of the core tablet, while the percentage of binder PVP K-30 (X_2) has a very significant role on hardness (Y_1) of the core tablet. Figure 3a shows that hardness of the core tablet increases linearly with the increase of the amount of PVP K-30. This might be due to fact that with the increase of the amount of PVP K-30 in formulation, binding between particles becomes more compact.[13] In case of cumulative percentage of drug release at pH 7.2 phosphate buffer (Y_2), both percentages of SSG and PVP K-30 play a significant role. Figure 3b shows how the percentages of these two components affect the drug release from the formulated core tablet. It was observed that the relationship between the percentages of SSG and PVP K-30 with cumulative amount of released drug is quadratic rather than linear. Further analysis revealed that the percentage of SSG has a positive role on the amount of drug release but, as the relationship is quadratic, there also exist a squared form of the amount of SSG and this value affects negatively to the amount drug released (Table 6). These findings support the earlier findings of using SSG slows down the drug release profile due to gelling effect.[8] Another component PVP K-30 also acted in a quite similar way, though at a very minimal extent. The increase in amount of PVP K-30 in formulation initially shows a small increase in drug release but eventually lead to reduction of drug release, as the squared form of variable X_2 appeared to deter the release (Figure 3b and Table 6). This might be due to the fact that at higher concentration PVP K-30 imparts in forming compact tablet mass with less porosity in surface which ultimately slows down the penetration of water into the tablet.[11] The mutual effect of the percentage of SSG and PVP K-30 on drug release was appeared to be statistically insignificant, as the model term product of factors X1 and X2 found to have a *p*-value > 0.05. Based on the effects of the percentage of SSG and PVP K-30 on hardness and cumulative amount of drug release, the formulation for core tablet was then optimized to achieve a hardness value of 7-8 kg/cm^2 and maximum amount of drug release. The model predicted a formulation containing 3.08% of SSG (X1) and 1.69% of PVP K-30 (X2) to meet these constraints. This might be because at this level the SSG can play its best role for disintegrating the particles in the formulated core tablet and PVP K-30 might provide the desired level of hardness. Also, at this level PVP K-30 might aid the disintegrant through swelling action.[8] The experimental formulation for optimization contained 3.05% of SSG and 1.69% of PVP K-30 in the core tablet. The predicted error obtained for response 1 (Y_1) was -0.13% and that of for response 2 (Y_2) was 1.52% (Table 7). Thus, the errors in response prediction by the model in both cases were within the limit (±2%). The coating trial was done with six different coating compositions and evaluation of the coated tablets revealed that batches B1 and B2 caused drug release of more than 1% drug in buffer stage 1, and batch B6 caused less than 80% drug release at buffer stage 2. Thus, the batches B1, B2 and B6 didn't meet USP compliance and kept out of further consideration. This might be for the reason that amount of Eudragit S12.5 deposited on the tablet surface of batches B1 and B2 was not sufficient to resist the release of drug in stage 1 phosphate buffer (pH 6.0). For batch B6 the amount deposited over tablet surface was highly dense which might have slowed down the penetration of water into core tablet resulting in lower release of drug at final stage (stage 2 phosphate buffer, pH 7.2). Among remaining batches, batch B4 was superior in terms of drug release than all others as well as than commercial product. Stability study of the tablets of batches B3, B4 and B5 found that the tablets were both physically and chemically stable during the storage period at accelerated storage condition (Table

10). The limitation of the study was lack of comparison of similarity and dissimilarity factors with the innovators' product due to its unavailability at local market. Due to constraints of facilities, *in vivo* behaviour of the tablet could not be characterized.

CONCLUSION

In this study a mesalazine 400 mg tablet was formulated successfully for colon-specific delivery through the quality by design (QbD) approach. In the optimized formulation of mesalazine core tablet, the percentages of SSG and PVP K-30 were 3.05% and 1.69%, respectively. The coated mesalazine tablets (batches B3-B5) obtained from optimized core complied with the specifications set for mesalamine delayed released tablet in the USP, thereby indicating their suitability to be used for colon specific drug delivery. Interestingly, coated mesalazine tablet of batch B4, which has a weight gain of 7.4% due to coating, exhibited the maximum release of drug (91.19 ± 0.11%) in buffer stage 2 following zero-order kinetics model. This formulation shows a highly promising drug product for the colon specific delivery of mesalazine and thus warrants *in vivo* studies, as well as evaluation of bioequivalence with the innovator's product.

ABBREVIATIONS

QbD: Quality by Design; SSG: Sodium starch glycolate; PVP K-30: Polyvinylpyrrolidone (PVP K-30); XRD: X-ray diffraction; SEM: Scanning electron microscope; TGA: Thermogravimetric analysis; FTIR: Fourier transform infrared spectroscopy; UC: Ulcerative colitis; IBD: Inflammatory bowel disease; DoE: Design of experiment; PB: Phosphate buffer; CP: Commercial product

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AUTHORS CONTRIBUTIONS

The conceptualization and supervision of the study was done by ASSR. The laboratory work and initial manuscript drafting was performed by DKS. The statistical analysis, laboratory supervision and manuscript writing & editing were done by UK, MAH and DNL. Final manuscript was checked and approved by all.

CONFLICT OF INTEREST

The authors declare 'no conflict of interest'.

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