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Research Article

Theme: Advanced Technologies for Oral Controlled Release
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Once Daily, High-Dose Mesalazine Controlled-Release Tablet for Colonic Delivery: Optimization of Formulation Variables Using Box–Behnken Design

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Abstract. The aim of this work was to statistically optimize a novel high-dose, mesalazine colonic delivery matrix system, potentially suitable for once daily administration, using simple wet granulation method. A hydrophobic–hydrophilic polymeric blend was used to manipulate drug release. A three-factor, three-level Box–Behnken design was used to construct polynomial models correlating the dependent and independent variables. Independent formulation variables were the percentages of the hydrophilic polymer Carbopol® 940, hydrophobic polymer Eudragit® RS, and the superdisintegrant croscarmellose sodium. The cumulative percentages of drug released at 6, 10, and 14 h were selected as dependent variables and restricted to 7.5–22.5% (Y_1), 42.5–57.5 % (Y_2), and 72.5–87.5% (Y_3), respectively. A second-order polynomial equation fitted to the data was used to optimize the independent formulation variables. Based on Box–Behnken experimental design, different mesalazine release profiles were obtained. The optimized formulation containing 5.72% Carbopol®, 9.77% Eudragit® RS, and 1.45% croscarmellose sodium was prepared according to the software determined levels. It provided a release profile which was very close to the targeted release profile, where the calculated values of f_1 and f_2 were 8.47 and 67.70, respectively, and followed zero-order release kinetics.

KEY WORDS: Box–Behnken; controlled release; Eudragit; mesalazine; optimization.

INTRODUCTION

The anti-inflammatory agent 5-aminosalicylic acid (5-ASA or mesalazine) is the recommended first-line therapy for the treatment of active symptoms, induction of remission, and maintenance of remission in patients with mild-to-moderate ulcerative colitis (1). Mesalazine acts topically on the colonic mucosa but when orally administered, it is extensively and rapidly absorbed in the small intestine, leading to little localization of mesalazine in the colon and hence, low efficiency with significant systemic side effects (2). Consequently, three methods have been commonly used for targeting of mesalazine to the colon: a pro-drug concept, enteric coating, and/or prolonged release of the drug through semipermeable membrane (3).

The recommended daily dose of mesalazine may reach 4.8 g in acute attack and 2.4 g in maintenance of remission in divided doses. Therefore, multiple daily dosing up to 12 tablets or capsules per day are required because of the low dosage strength of most currently commercially available mesalazine formulation (4). Reduced patient compliance and disease control are the results of these inconveniences of frequent daily dosing and the number of tablets or capsules required per day (5). Additionally, many traditional delayed-release formulations that lack any means for prolonging mesalazine release are characterized by the undesirable immediate release of mesalazine once they reach the colon. This leads to a relatively smaller amount of mesalazine delivered to the distal part of the colon, the area most commonly to be inflamed (6).

Lialda®, a delayed-release tablet (also known as Mezavant® XL in UK) with high-dose 1,200 mg mesalazine/tablet, was developed utilizing Multi-Matrix System (MMX) technology for the treatment of ulcerative colitis at a dosage of 2.4–4.8 g given only once daily with a view of improving patients compliance (7,8). The MMX technology involves incorporating mesalazine into a lipophilic matrix, which is itself dispersed as microparticles within a hydrophilic matrix. Then pH-dependent gastro-resistant film, designed to disintegrate when the pH is at least 7, was applied to delay the dissolution (5,9,10). The components of the MMX matrix are sodium-carmellose, sodium carboxymethyl-starch (type A), talc, stearic acid, and carnauba wax (11).

Factorial designs and analysis of the response surfaces are methods of experimental designs that could be used for the statistical optimization of pharmaceutical dosage forms (12). Box–Behnken statistical design is a type of response surface methodology that requires smaller number of experimental runs and is less time consuming than conventional formulation methods (13).

The current study is aimed at developing and optimizing a novel delayed-controlled zero-order release matrix tablet of mesalazine, suitable for once daily administration, employing a simpler method suitable for conventional tablets manufacture

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processes. The proposed method is based on a core matrix tablet, which mainly contains Eudragit® RS (hydrophobic polymer), Carbopol®940 (hydrophilic polymer), and croscarmellose sodium to manipulate drug release prepared by the traditional wet granulation technique followed by coating with pH-dependent polymer Eudragit® S.

In order to achieve this goal, computer-aided optimization techniques using three-factor, three-level Box–Behnken design was employed to investigate the effect of three formulation factors, namely, the contents of Carbopol®940, Eudragit® RS, and croscarmellose sodium on the cumulative percent of drug released and to statistically optimize the levels of these factors using mathematical equations and response surface plots in order to obtain the targeted dissolution profile for mesalazine.

MATERIALS AND METHODS

Materials

Mesalazine was kindly donated by Minapharm Pharmaceuticals (Cairo, Egypt); croscarmellose sodium by FMC BioPolymer (Brussels, Belgium); Carbopol® 940, Noveon Inc. (USA); polyvinylpyrrolidone K-30 (PVP), Fluka AG (Buchs, Switzerland); talc and magnesium stearate, Adwic, El-Nasr Pharmaceutical Chemicals Co. (Egypt); triethyl citrate, Alfa Aesar (Karlsruhe, Germany); and Eudragit® S 100 and Eudragit® RS PO, generously donated by Röhm Pharma, GmbH (Germany). All other chemicals and solvents were of analytical grade.

Methods

Compatibility of Mesalazine with Different Excipients

Differential Scanning Calorimetry. Thermal analysis by differential scanning calorimetry (DSC) was carried out using Shimadzu thermal analyzer (Shimadzu DSC 60, TA-60 WS, Japan) to investigate the compatibility between mesalazine and different excipients. The DSC thermograms of pure drug, individual excipients, and drug–excipient mixtures (1:1 *w*/*w*) were recorded. For each measurement a sample of approximately 6 mg was placed in an aluminum pan and scanned in the temperature range 30–350°C. A heating rate of 10°C/min was used, and the thermal analysis was performed under dynamic nitrogen atmosphere.

Fourier Transform–Infrared Spectroscopy. Fourier transform–infrared spectroscopy (FT-IR) spectra for the drug, the selected excipients and the drug-selected excipients powder mixtures (1:1 *w*/*w*) were recorded on FT-IR spectrophotometer (FTIR-8400S, Shimadzu, Japan) using the potassium bromide disk technique. The scanning range was 4,000 to 500 cm^{-1}.

Table I. Variables in Box–Behnken Design

Formulation variables	Level used (−1)	(0)	(+1)
X_1=Carbopol content (%)	0	4	8
X_2=Eudragit RS content (%)	1	5.5	10
X_3=Croscarmellose sodium content (%)	0	1	2
Responses variables	Constraints		
Y_1=release (%) after 6 h	$7.5\% \le Y_1 \le 22.5\%$		
Y_2=release (%) after 10 h	$42.5\% \le Y_2 \le 57.5\%$		
Y_3=release (%) after 14 h	$72.5\% \le Y_3 \le 87.5\%$		

Table II. The Composition and Observed Responses from Randomized Runs in Box–Behnken Design

	Factors			Responses		
Run	X_1	X_2	X_3	Y_1	Y_2	Y_3
1	0	5.5	2	6.810	32.717	53.345
2	4	5.5	1	9.642	71.949	96.120
3	0	5.5	0	3.014	11.340	20.540
4	8	10	1	2.015	13.599	44.660
5	4	1	2	17.210	65.420	95.134
6	8	5.5	0	4.520	20.240	49.210
7	4	5.5	1	10.500	66.392	90.320
8	4	1	0	12.100	63.227	93.497
9	4	5.5	1	9.012	69.170	92.650
10	8	1	1	7.896	39.029	83.505
11	4	10	2	11.020	44.660	84.296
12	4	10	0	2.010	6.558	15.604
13	0	10	1	3.510	12.210	18.260
14	8	5.5	2	4.320	46.886	92.098
15	0	1	1	16.010	60.120	92.227

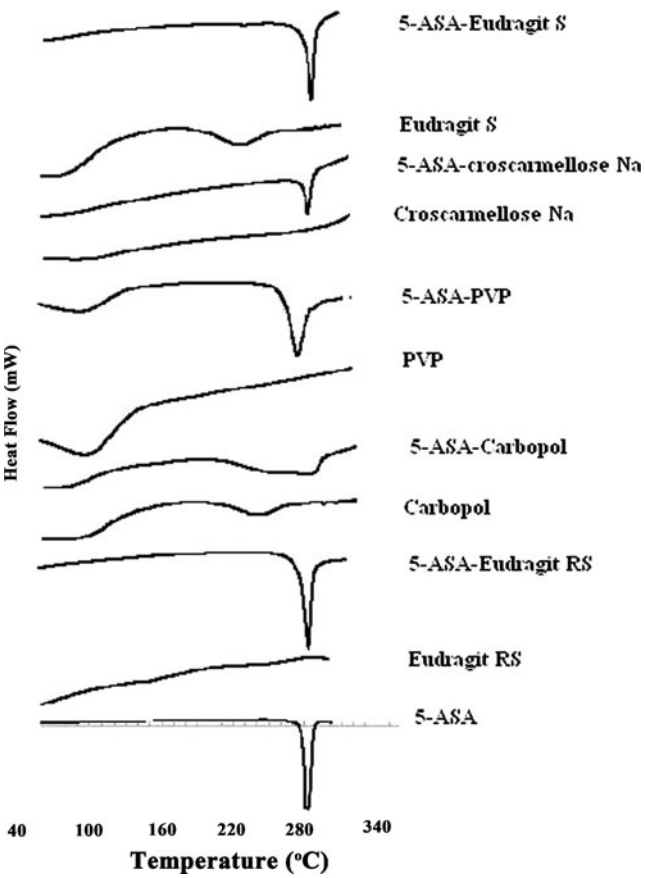

Fig. 1. DSC thermal scan of mesalazine, pharmaceutical excipients and their 1:1 *w*/*w* coground mixtures

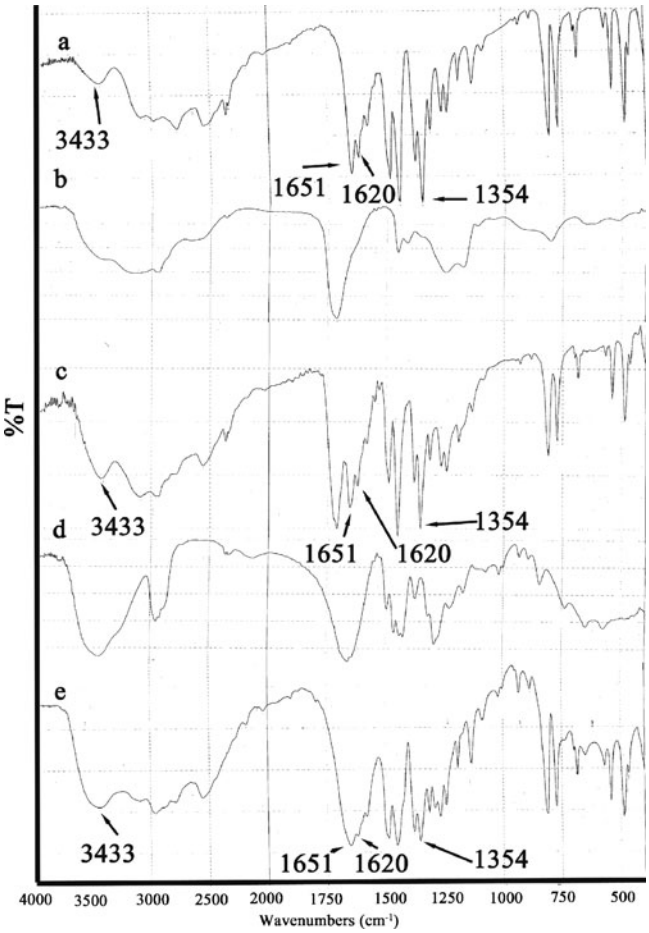

Fig. 2. FT-IR spectra of *a* mesalazine; *b* Carbopol®; *c* mesalazine–carbopol coground mixture (1: 1 *w*/*w*); *d* PVP; *e* mesalazine–PVP coground mixture (1: 1 *w*/*w*)

Experimental Design

A three-factor, three-level Box–Behnken design was employed for the optimization procedure using Design-Expert® 7.1.5 software (Stat-Ease, Inc., USA). The investigated factors (independent variables) were Carbopol® content (X_1), Eudragit® RS content (X_2), and croscarmellose sodium content (X_3). The levels for these three factors were determined from sufficient preliminary trials. The cumulative percentages of drug released at 6, 10, and 14 h (Y_1, Y_2, and Y_3, respectively) were selected as dependent variables as shown in Table I. A zero-order release profile of mesalazine over 16 h was suggested as a targeted release profile which was based on a theoretical release of about 8.3% of the drug per hour after a lag time of 4 h and was deduced from mesalazine release profile of the once daily marketed product.

Preparation of Mesalazine Core Tablets

Accurately weighed quantity of mesalazine, Carbopol®, and Eudragit® RS were mixed for 20 min using a glass mortar and pestle. The mixture was then granulated using a binder solution of PVP (5% *w*/*w*) in isopropyl alcohol. The wet mass was passed through 16# sieve and the resulted granules were dried in a tray drier for 30 min at 50°C. The dried granules were mixed with the required amounts of croscarmellose sodium, 2% *w*/*w* of talc, and 1% *w*/*w* magnesium stearate. Amounts of the resulting granules equivalent to 1,200 mg of mesalazine were compressed with a single-punch tablet press machine (Royal Artist, Bombay, India), using an oblong punch and die set (21×9 mm). Table II depicts the composition of the prepared tablets.

Coating of the Prepared Tablets

Twenty-five grams of Eudragit® S 100 was dissolved in 350 g of 95% ethanol under high-speed stirring until a clear solution was obtained. Two and half grams of triethyl citrate as a plasticizer and 1.25 g talc as a glidant were added (14,15). Then the mixture was stirred for 24 h to ensure sufficient plasticization of the polymer and to get homogeneous solution (16). Coating of tablets was performed by immersion (17) in the coating solution followed by solvent evaporation using hot air electric hand dryer (16). The process was repeated until the target weight gain of 5% (*w*/*w*) was achieved. This ratio was selected based on the results from the preliminary trials.

Characterization of Core Tablets

The prepared tablets were evaluated regarding hardness, friability, and drug content. The hardness of 10 tablets was measured using Monsanto (standard type) tablet hardness tester. Friability was determined by taking 10 tablets in digital tablet friability tester (Model DFI-1, Veego, Bombay, India) for 4 min at 25 rpm. For estimating drug content, 10 tablets were crushed and powdered. The aliquot of powder equivalent to 10 mg of drug was weighed and dissolved in 50 ml freshly prepared phosphate buffer (pH 7.4). The resultant solution was filtered and suitably diluted, then analyzed spectrophotometrically at predetermined λ_{max} of mesalazine (330 nm). From the absorbance value drug content was calculated on average weight basis.

In Vitro Release Studies

The release characteristics of mesalazine from the prepared formulations were determined according to the USP dissolution II paddle method using a dissolution tester (Vision® Classic 6^{TM} Dissolution Tester, Hanson Research Corporation, California, USA) at 37±0.5°C with a rotation speed of 50 rpm. The release profile was studied in a medium of changing pH. The initial condition was 350 ml of 0.1 N HCl (pH 1.2) for 0–2 h. At the end of second hour, 250 ml solution composed of 3.75 g of KH_2PO_4 and 1.2 g of NaOH was added to raise pH of dissolution medium to 4.5 and the total volume of the dissolution medium to 600 ml. At the end of fourth hour, 300 ml phosphate buffer concentrate (2.18 g of KH_2PO_4 and 1.46 g of NaOH in distilled water) was added to raise pH to 7.4. The study was then continued till the end in 900 ml volume (18). At predetermined time intervals, a 5 ml sample was withdrawn and replaced with fresh dissolution media. Collected samples were filtered through 0.45 μm Millipore filters. After appropriate dilutions, the concentration of mesalazine in samples was spectrophotometrically measured at predetermined $\lambda_{max(s)}$ using a UV spectrophotometer (Jenway UV/Vis. Spectrophotometer, Barloworld Scientific

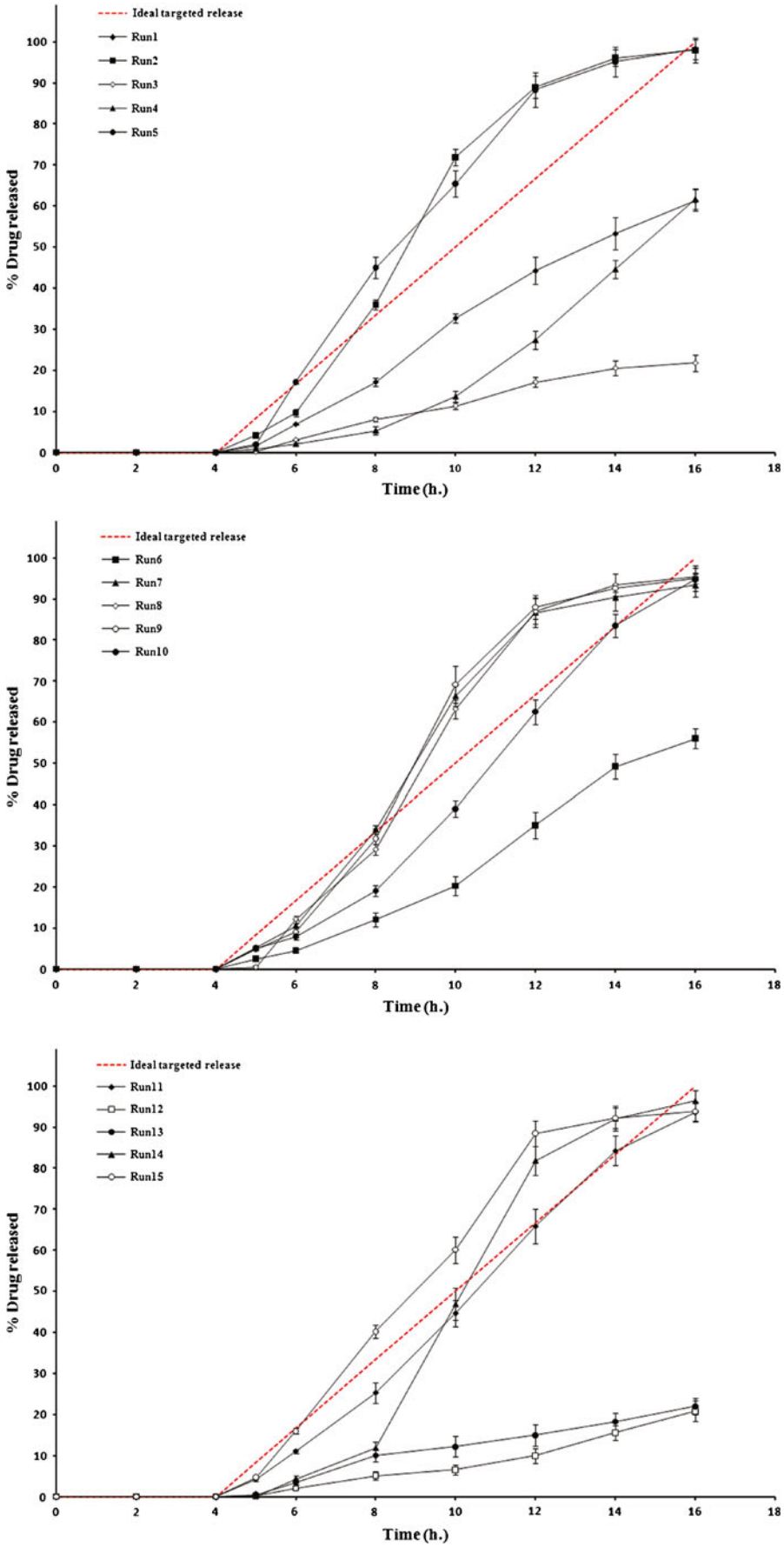

Fig. 3. *In vitro* release profiles of all formulations. Each data point is expressed as mean ± SD (n=3)

Table III. Summary of Results of: Model Analysis, Lack of Fit and R-square Analysis for Measured Responses

Source	Y_1		Y_2		Y_3	
	Sum of squares	P > F	Sum of squares	*P* > *F*	Sum of squares	*P* > *F*
Model analysis						
Mean *vs.* total	953.44		25,918.13		69,559.39	
Linear *vs.* mean	203.43	0.0115	3,817.85	0.0609	8,647.99	0.0087
2FI[a] *vs.* linear	18.75	0.7118	455.62	0.8094	1,457.86	0.3927
Quadratic *vs.* 2FI	81.69	0.0487	3,517.22	0.0023	3,032.47	0.0092
Cubic *vs.* quadratic	23.69	0.0667	238.23	0.0899	385.41	0.0628
Residual	1.12		15.44		17.04	
Total	1,282.11		33,962.49		83,100.16	
Lack of fit						
Linear	124.13	0.0395	4,211.07	0.0163	4,875.74	0.0156
2FI	105.38	0.0311	3,755.45	0.0122	3,417.88	0.0148
Quadratic	23.69	0.0667	238.23	0.0899	385.41	0.0628
Cubic	0.000		0.000		0.000	
Pure error	1.12		15.44		17.04	

	R^2	R_a^2	PRESS	R^2	R_a^2	PRESS	R^2	R_a^2	PRESS
R^2 analysis									
Linear	0.619	0.5150	258.03	0.475	0.3313	6,764.99	0.637	0.5401	8,608.39
2FI	0.676	0.4330	524.30	0.531	0.1797	10,468.81	0.746	0.5561	10,430.84
Quadratic	0.925	0.7886	381.63	0.968	0.9117	3,846.43	0.970	0.9168	6,204.88
Cubic	0.997	0.9762	ND[b]	0.998	0.9866	ND	0.999	0.9912	ND

[a] Two-factor interaction
[b] PRESS statistic not defined

Limited, Essex, UK). Cumulative percentage of drug released from the tablets were calculated and plotted as a function of time.

Kinetic Analysis of the Release Data

The mean *in vitro* drug release data were fitted to different kinetic models, namely zero-order kinetics (19), first order (20), Higuchi (21), and Korsmeyer-Peppas (22) using regression analysis to evaluate the kinetics of drug release from the prepared matrices. The model which shows the highest value of coefficient of determination (R^2) was selected as the best model that describes the kinetics of drug release.

Stability Study

According to the ICH guidelines the tablets of the optimized formula were exposed to 6 months accelerated stability study at 40°C/75% RH (23). At the end of 1, 3, and 6 months, the tablets were subjected to visual observation to detect any physical changes and evaluated regarding drug content and *in vitro* release.

Table IV. Standardized Main Effects of the Factors on the Responses

	Y_1			Y_2			Y_3		
	Coefficient estimate	*P* value	SME[a]	Coefficient estimate	*P* value	SME	Coefficient estimate	*P* value	SME
b_0	9.72	0.0240	7.56	69.17	0.0030	16.82	93.03	0.0026	17.96
b_1	−1.32	0.1535	−1.68	0.42	0.8738	0.17	10.64	0.0202	3.35
b_2	−4.33	0.0027	−5.50	−18.85	0.0007	−7.48	−25.19	0.0005	−7.94
b_3	2.21	0.0375	2.81	11.04	0.0071	4.38	18.25	0.0022	5.75
b_{12}	1.65	0.1975	1.49	5.62	0.1754	1.58	8.78	0.1076	1.96
b_{13}	−1.00	0.4109	−0.90	1.32	0.7266	0.37	2.52	0.5984	0.56
b_{23}	0.98	0.4214	0.88	8.98	0.0531	2.52	16.76	0.0135	3.74
b_{11}	−4.14	0.0160	−3.57	−27.55	0.0007	−7.43	−25.85	0.0026	−5.54
b_{22}	1.78	0.1854	1.53	−10.38	0.0380	−2.80	−7.52	0.1683	−1.61
b_{33}	−0.91	0.4669	−0.79	−13.82	0.0136	−3.73	−13.38	0.0352	−2.87

[a] Standardized main effects (SME) were calculated by dividing the main effect by the standard error of the main effect

RESULTS AND DISCUSSION

Compatibility of Mesalazine with Different Excipients

Figure 1 shows the DSC thermograms of mesalazine alone and as physical mixtures with different pharmaceutical excipients. The DSC thermogram of mesalazine exhibited a sharp endothermic peak at 281°C indicating the melting point of the drug (24). There is no observed change in the endothermic peak of mesalazine in cases of drug mixtures with Eudragit® RS, croscarmellose sodium, or Eudragit® S. This result could suggest the absence of interaction between the drug with all the aforementioned excipients.

In the DSC thermogram of the physical mixture of mesalazine with Carbopol®, a broadening and decreased intensity of the endothermic peak of mesalazine was observed. This result could suggest interaction between mesalazine and Carbopol® (25). This interaction could be attributed to hydrogen bond formation between mesalazine and Carbopol® (26,27). However, this interaction between the drug and Carbopol could contribute to reduction of the dissolution rate, which could be considered as an advantage in formulation of a controlled-release delivery system (28,29).

In the case of the physical binary mixture of mesalazine and PVP, the typical melting peak for mesalazine was observed, but broadening and shift of the endothermic peak temperature to a lower temperature (from 281°C to 277°C) were observed. Similar results have been reported between nateglinide (30), glipizide (31), ibuproxan (32), and ibuprofen (33) with PVP in a physical mixture. This shift could be attributed to some solid–solid interaction, although it does not necessarily indicate any incompatibility (33,34). It was reported that minor changes in the melting endotherm of drug could be due to mixing of drug and excipient, which lowers the purity of each component in the mixture and may not necessarily indicate potential incompatibility (30,31).

Accordingly, for a better understanding of the changes in the binary mixtures, physical mixtures of mesalazine with Carbopol® and PVP were subjected to FT-IR studies and their spectra were compared to FT-IR spectrum of mesalazine. Figure 2 shows the infrared spectra of mesalazine, the used excipients, and the drug-excipients physical mixtures. The infrared spectrum of pure mesalazine exhibited the characteristic bands corresponding to the functional groups of the drug at 3,433 cm^{-1} (due to the mutual overlapping of NH and OH stretching), 1,651 cm^{-1} (corresponds to the C=O stretch), 1,620 cm^{-1} (corresponds to NH bending), and 1,354 cm^{-1} (corresponds to CN stretching). The bands in a range of 2,000–3,000 cm^{-1} correspond to the stretching vibrations of the hydrogen bonds in the mesalazine molecule (35). It was remarked that all the spectra of the mixtures exhibited the characteristic bands of the drug. This indicates that there is no change in the drug structure and the absence of chemical interaction between mesalazine and these excipients. The compatibility between mesalazine and the selected excipients will be further investigated by carrying out stability studies on the optimized formulation.

Characterization of Core Tablets

All the prepared tablets were found to be of good quality with acceptable physical characteristics. The hardness was found to vary between 10 and 11 kg. Friability in all the formulations was less than 0.9%. Drug content varied with ±5% of the theoretical value (1,200 mg) for all formulations.

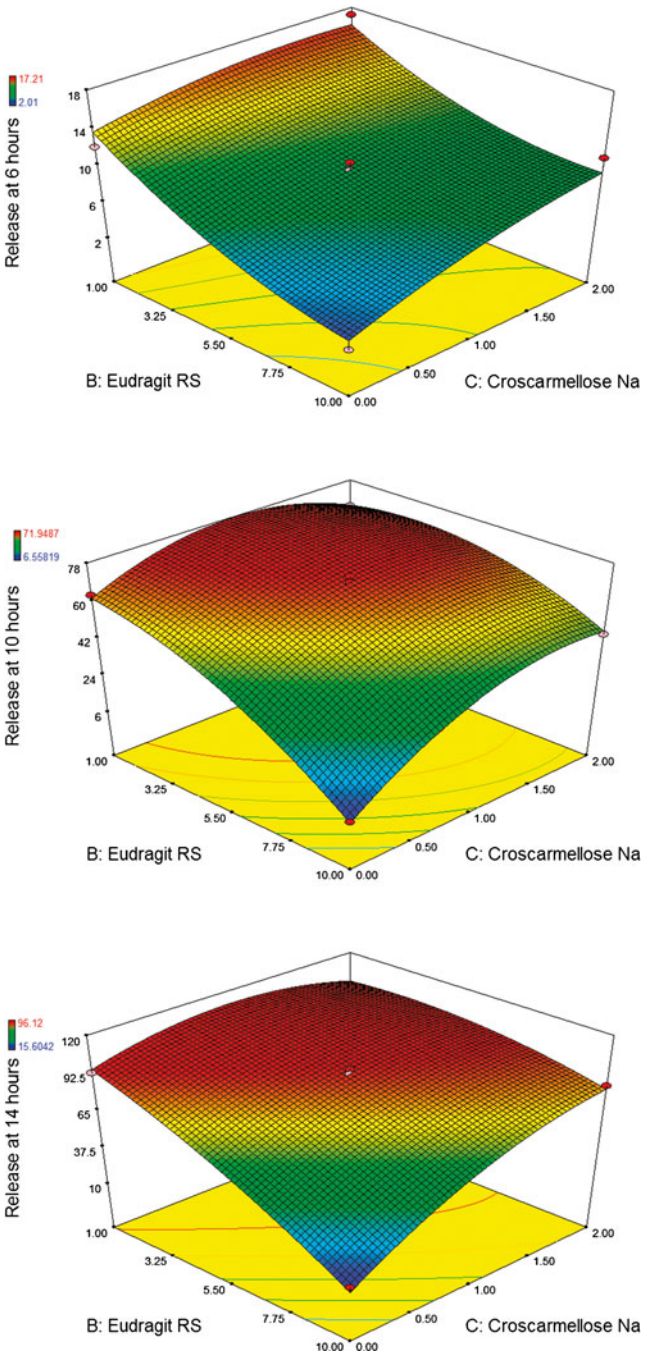

Fig. 4. Effect of the contents of Eudragit® RS (X_1) and croscarmellose Na (X_2) on responses using response surface and contour plots at 4% Carbopol® content

Preparation of Mesalazine Tablets

Because of the high loading of mesalazine (1,200 mg/tablet), we aimed to develop a formulation containing polymers and other excipients at amounts as little as possible, as well as releasing its content in a extended release profile over the specified length of time, and preferably with a zero-order kinetic. Hydrophobic insoluble polymer (36) is a good choice to address all of these requirements. Eudragit® RS is composed of poly (ethylacrylate-methylmethacrylate-trimethylammonioethyl methacrylate chloride) copolymers. Eudragit® RS is a hydrophobic, water-insoluble polymer, and pH-independent sustained drug release profile is exhibited by drug delivery systems prepared using it (37). It has been widely applied in modified release formulations (38–41). In the production of matrix tablets Eudragit® RS has the advantages of excellent compression properties, being suitable for producing tablets using all common process technologies, good binding properties, thermostability, thermoplastic properties, and plastic properties. The plastic properties of Eudragit® RS produce stable characteristics across a range of relevant production parameters such as compression force (42). Such property give rise to similar dissolution profiles for tablets produced at different compression forces.

Carbopol® is a hydrophilic polyacrylic acid polymer which has gel-forming and bioadhesive properties. Due to the chemical nature of Carbopol® polymers, swelling of the polymer occurs in the pH range 5–9, as a result of ionization of the carboxylic acid groups that lead to electronic repulsion of the polymer (43). Such pH-dependent swelling behavior of Carbopol® suggests that it is a good choice ingredient to be included in colon-targeting delivery systems.

A superdisintegrant, croscarmellose sodium, was incorporated extragranularly to assist the breakdown of tablets into smaller granules or fragments and thus, ensure more uniform distribution of mesalazine throughout the colon.

Wet granulation technique, although more time consuming than direct compression (44), was employed in this study because of the high load of the drug which has poor flowability and compressibility as observed in the preliminary trials.

Eudragit S is methylacrylic acid–methylmethacrylate copolymers, which tends to dissolve at pH higher than 7. This makes it a suitable coating material for the colonic drug delivery (45). After application of Eudragit® S coating, all the evaluated formulations released less than 1% of mesalazine in the first two stages of the release studies.

Determination of the Regression Model and Statistical Evaluation

Box–Behnken design is suitable for investigating the quadratic response surfaces and for constructing a second-order polynomial model. Consequently, statistical optimization of the pharmaceutical dosage form could be performed using a small number of experiments runs (15 runs) (46). The experiment runs with independent variables and the observed responses for the 15 formulations are shown in Fig. 3 and Table II. The selection of the best fitting mathematical model involving the individual main effects and interaction factors was based on the comparison of some statistical parameters including the multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (R_a^2), and the predicted residual sum of squares (PRESS), provided by the Design-Expert software. As shown in Table III, the quadratic model was chosen because it had the smallest value of PRESS. Predicted residual sum of squares indicates how well the model fits the data. The smaller the PRESS statistic is, the better the model fits to the data points (47). Additionally, the quadratic model showed a statistically insignificant lack of fit ($P>0.05$). Analysis of variance was applied to estimate the significance of the model at the 5% significance level. The nonlinear computer-generated quadratic model is given as Eq. 1:

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

Where Y is the measured response associated with each factor level combination; b_o is an intercept; b_1 to b_{33} are the estimated regression coefficients computed from the observed experimental values of Y; and X_1, X_2, and X_3 are the coded levels of independent variables. The terms X_iX_i and X_i^2 (i=1, 2, or 3) represent the interaction and quadratic terms, respectively (48).

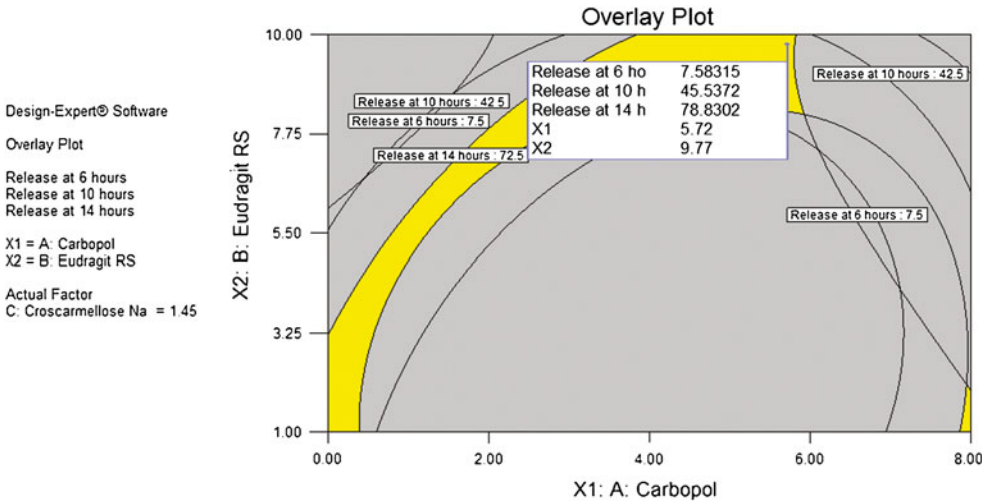

Fig. 5. Overlay plot for optimized variables

Table V. Comparative Levels of Predicted and Observed Responses for the Optimized Formulation

Responses (predicted, %)	Observed (%)	Predicted error[a] (%)
Y_1 (7.58)	8	5.54
Y_2 (45.54)	46.2	1.45
Y_3 (78.83)	82.3	4.40

[a] Predicted error (%)=(observed value−predicted value)/predicted value×100%

The coefficient estimate and standardized main effects (SME) values in the form of a polynomial equation for the responses are listed in Table IV. SME values were calculated by dividing the main effects by the standard error of the main effects (49).

From Table IV, it could be inferred that Eudragit® RS (X_2) and croscarmellose sodium (X_3) were significant in controlling the release of mesalazine throughout the dissolution time ($p \leq 0.05$). In addition, Eudragit® RS content (X_2) showed the largest SME (−5.50, −7.48, and −7.94 at Y_1, Y_2, and Y_3, respectively) indicating that Eudragit® RS content (X_2) was the main influential factor on drug release from the tested tablets in the whole stages of mesalazine *in vitro* release studies.

Figure 4 depicts the contour and three-dimensional response surface plots which show the effects of the independent variables on each response. Analysis of Fig. 4 shows that on increasing Eudragit® RS form 1% to 10% a decrease in drug release was observed at Y_1, Y_2, and Y_3. Such finding was as expected and is in agreement with the findings of many previous reports (41,50,51). These results stem from the fact that Eudragit® RS is insoluble in aqueous media and acts as a shield preventing the penetration of the dissolution medium into the tablets and mesalazine from dissolution (52).

As shown in Table IV, the effect of Carbopol® became only significant (P=0.02) at 14 h (Y_3). Also, the coefficient of b_1 is 10.64 (bearing positive sign) for Y_3 indicating that increasing Carbopol® content augments mesalazine release. This could be attributed to the fact that Carbopol® needs water to swell (43). Availability of water needed for Carbopol® to swell could be retarded by the coherent structure and hydrophobic nature of mesalazine–Eudragit® RS matrices (53).

Eudragit® RS matrix tablets could be thought as a coherent system in which the drug is dispersed. This structure is anticipated to be weakened by incorporating the water swellable polymer, Carbopol®, which swell in water up to 1,000 times its original volume (and 10 times its original diameter) to form a gel when exposed to a pH environment above 4.0 to 6.0 (54). Swelling is suggested to decrease the strength of the matrix and assist the drug leaking out. It is worthy to mention that in Carbopol®-containing tablets, a faster gradual detachment of smaller granules from the core was observed to take place with time during dissolution studies. A consequent increase in drug release is suspected due to the greater surface area available for the dissolution media. However, such effect was expected to be opposed by the formation of a viscous gel layer on the surface of the granules which is postulated to hinder drug release (55). The net effect of Carbopol® depends on which effect is predominant. At the low concentrations used in this study, Carbopol® enhanced mesalazine release from the detached granules. Such finding met that reported by Haney and Dash (56).

Although the correlation between tablet disintegration and drug dissolution is not always observable (57), analysis of Fig. 4 demonstrates that increasing croscarmellose sodium from 0% to 2% led to an increase in mesalazine release at Y_1, Y_2, and Y_3. This result could be attributed to the detachment of granules that was aided by the inclusion of the super-disintegrant croscarmellose sodium into the tablets; hence, the release rate was increased by increasing the surface exposed to the dissolution medium (57,58).

Optimization of Drug Release and Validation of Optimized Formulation

After generating the polynomial equations relating the dependent and independent variables, the release profile was optimized for the responses Y_1, Y_2, and Y_3. The desirable range of these responses was restricted to the values listed in Table I. The optimum values of the variables were obtained by graphical and numerical analyses using the Design-Expert software and based on the criterion of desirability (59,60). Figure 5 represents an overlay plot showing the optimized parameters suggested by the software to get the responses in the required range. The optimized formulation was achieved with 5.72% Carbopol®, 9.77% Eudragit® RS, and 1.45% croscarmellose sodium. To check the validity of the optimization procedure, a new batch of mesalazine tablets with the predicted levels of formulation factors was prepared. Table V illustrates the predicted and observed responses for the optimum formulation. The observed values of Y_1, Y_2, and Y_3 were in a very close agreement to the predicted ones. By this the validity of the optimization procedure was proven. Figure 6 demonstrated that the optimized formulation prepared according to computer-determined levels exhibited a release profile which was close to that of the ideal targeted release profile. Additionally, these dissolution profiles were compared using two fit factors, difference factor (f_1) and similarity factor (f_2). The calculated values of f_1 and f_2 were 8.47 and 67.70, respectively. Such values indicate that the release profiles of the optimized formulation and that of the ideal targeted release profile were similar.

Three kinetic models were applied to study the kinetics of mesalazine release from all the prepared formulations as well as from the optimized formula. Drug release kinetic parameters are presented in Table VI. As shown in Table VI, zero-order kinetic model gave the highest value of the coefficient of determination (R^2) for optimized formula (0.9974), indicating that zero-order kinetic model would be the most suitable model for describing the release of mesalazine.

The *in vitro* release profiles were further studied in terms of three time-based parameters; $t_{20\%}$, $t_{50\%}$, and $t_{80\%}$ values (time required for 20%, 50%, and 80% of drug release, respectively). It was suggested that a $t_{20\%}$ >6 h ensures that less than 20% of drug could be released during the initial gastrointestinal transit while $t_{50\%}$ of 10–11 h and $t_{80\%}$ <14 ensure that 50% of the drug could be released in the ascending and transverse colon and drug release could be completed in 14–16 h during the expected residence time of the dosage form in colon (61). The optimized formula exhibited $t_{20\%}$, $t_{50\%}$, and $t_{80\%}$ values of 7.12, 10.47, and

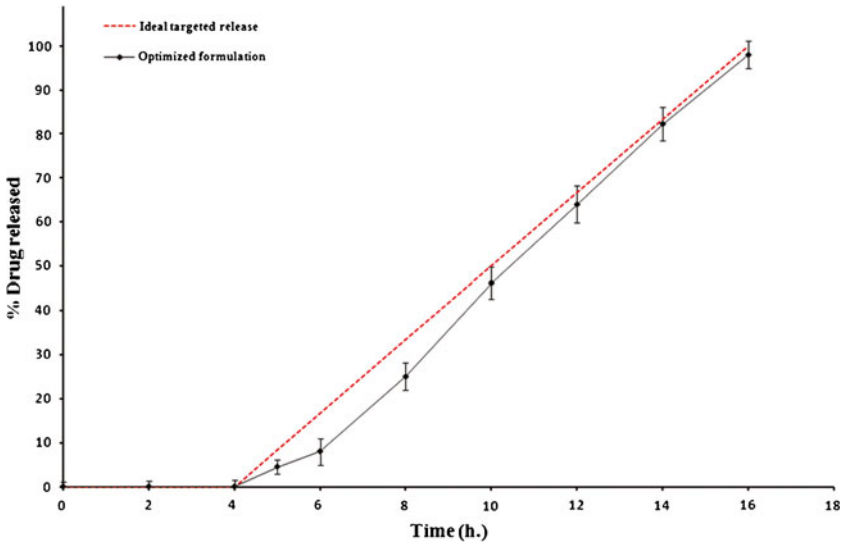

Fig. 6. *In vitro* release profiles of mesalazine for optimized formula and ideal targeted release profiles. Each data point is expressed as mean ± SD (n=3)

13.82 h, respectively, that were within the suggested range regarding these parameters (Table VI).

Stability Study

Neither physical changes nor significant changes in drug content of tablets from the optimized formula had been detected on storage and drug content of the tested tablets was found to be 98.77±0.46%. This result comes in agreement with previous report in which the decrease in the content of mesalazine did not exceed 1% in solid dosage forms even placed in stressed conditions for a period up to 2 years (62). The pair-wise procedures indicated statistically insignificant difference in the *in vitro* drug release profiles from the fresh and stored tablets of the optimized formula. Such results suggest the compatibility between mesalazine and the used excipients and the high stability of mesalazine in the optimized enteric coated tablets.

CONCLUSION

The optimized hydrophilic–hydrophobic, high-loading mesalazine matrix tablets demonstrated a controlled drug release manner potentially suitable for once daily administration. The optimized formulation, containing 5.72% Carbopol®, 9.77% Eudragit® RS, and 1.45% croscarmellose

Table VI. Release Rate Kinetics for the Box–Behnken and Optimized Formulations

Run	R^2				Mechanism	$t_{20\%}$[e]	$t_{50\%}$[e]	$t_{80\%}$[e]
	Zero order[a]	1st order[b]	Diffusion[c]	Peppas[d]				
Run1	0.9809	0.9992	0.9965	0.9743	1st order	8.02	13.23	23.38
Run2	0.9755	0.9187	0.9592	0.9932	Peppas	7.18	8.95	10.02
Run3	0.9746	0.9788	0.9853	0.9844	Diffusion	14.40	45.72	97.96
Run4	0.9974	0.9747	0.9900	0.9960	Zero order	9.74	15.16	20.57
Run5	0.9961	0.9390	0.9966	0.9910	Diffusion	5.99	8.51	12.05
Run6	0.9855	0.9834	0.9806	0.9894	Peppas	9.50	15.04	19.79
Run7	0.9928	0.9543	0.9861	0.9921	Zero order	6.22	9.56	12.91
Run8	0.9856	0.9229	0.9618	0.9921	Peppas	8.12	10.44	12.10
Run9	0.9845	0.9537	0.9776	0.9892	Peppas	6.99	9.97	12.51
Run10	0.9991	0.9499	0.9929	0.9965	Zero order	7.36	10.76	14.17
Run11	0.9961	0.9282	0.9715	0.9989	Peppas	7.30	10.76	13.77
Run12	0.9576	0.9485	0.9016	0.9813	Peppas	14.97	22.72	28.62
Run13	0.9878	0.9811	0.9923	0.9805	Diffusion	15.03	51.33	112.94
Run14	1.0000	0.9590	0.9977	0.9798	Zero order	7.42	10.38	13.34
Run15	0.9956	0.8970	0.9798	0.9970	Peppas	6.68	9.67	12.33
Optimized formulation	0.9974	0.9346	0.9917	0.9889	Zero order	7.12	10.47	13.82

M_t/M_∞ the fraction of drug released up to time t, k the kinetic constant, C constant
[a] Zero order: $M_t/M_\infty = kt + C$
[b] First order: $M_t/M_\infty = 1 - e^{-kt}$
[c] Higuchi: $M_t/M_\infty = k(t)^{0.5} + C$
[d] Korsmeyer-Peppas: $M_t/M_\infty = kt^n + C$
[e] $t_{20\%}$, $t_{50\%}$, and $t_{80\%}$ are the times required for 20%, 50%, and 80% of drug release, respectively

sodium in addition to other excipients, was fabricated utilizing the simple wet granulation technique and produced a zero-order drug release profile over a period of 16 h after a lag time of 4 h. This release profile was similar to that of the ideal target release model deduced from the dissolution profile of a marketed once daily tablet of mesalazine.

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