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Statistical Optimization of Mesalamine Coated Pellets for Possible Ileo–cecal Targeting

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Abstract

The present study is an attempt to design and optimized mesalamine loaded pulsatile release pH sensitive coated pellets for ileo-cecal targeted pulsatile drug delivery. The novelty of this formulation is to release drug specifically and instantly in ileo-cecal region without being released in upper gastrointestinal tract. Preliminary experimental batches are studied for micromeritic properties and *in vitro* drug release. Formulation showed desirable lag time and dissolution profile were further optimized by applying 3^2 full factorial design to study the effect of extent of coating (% w/w) Eudragit S100 and Croscarmellose sodium over drug layered pellets selected as independent variables X1 and X2 respectively and two responses as lag time of 5h and 90% drug release within 90 minutes after lag time as Y1 and Y2 respectively. Various kinetic models such as Zero order, First order, Higuchi Matrix, Korsmeyer & Peppas were applied to the all optimized batches. The regression equation generated for Q300 = +5.72-31.97*A+0.82*B-0.49*A*B+26.36*A2-0.15*B2 and for Q390 = +84.63-40.09*A+4.62*B. The drug release data of optimized formulation were close to that predicted by the model. The formulation containing 35.22%w/w Eudragit S100 and 4.00%w/w Croscarmellose sodium was found to be optimum. Formulation of barium sulphate layered and optimized coated pellets studied for radio imaging study showed significant lag time of 5 hr and barium sulphate releases successfully in ileo-cecal region. The present study demonstrates that the mesalamine enteric coated pellets could be successfully targeted at ileo-cecal region.

Keyword: Mesalamine, Pulsatile, Ileo-cecal targeting, Celpheres, Croscarmellose sodium, Eudragit S100

INTRODUCTION

Many investigations have been carried out with the aim of discovering an ideal formulation for colon specific drug delivery to treat number of important implications in the field of pharmacotherapy. Colon targeted drug delivery systems (CTDDS) have been the focus of increasing interest for the last decade for local and systemic therapy. Crohn's disease (CD) is most commonly occurring inflammatory bowel disease (IBD) to any part of gastrointestinal tract (GIT) but the most susceptible part is Ileo-cecal valve region which has symptoms mostly similar as that of Ulcerative colitis[1,2]. CD affects all three layers/lining of the gut wall and the main pathological features of CD are shallow and deep ulcers, fistulae and strictures causing lumen obstruction. Inflammation of the lining of the gut causes symptoms of diarrhea which may be streaked with blood or mucus if the colon is involved. Episodes of cramps and intermittent abdominal pain are the most common symptom of Crohn's disease. The pain may evolve into a constant dull ache as the disease progresses. Diarrhea is present in 85% of patients; other symptoms include hematochezia, fever, weight loss, malaise, nausea, and arthralgias. For effective treatment of this disease the

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drug must release at site of ileo-cecal region with immediate release[3].

A range of approaches have been proposed and systems have been developed for targeting drug to the colon is mainly based on transit time dependant, pH dependant, pressure, and/or microflora degradable systems[4,5]. Among these approaches, pH-dependant system is simple and suitable for CTDDS in different physiological and pathological conditions in GIT[6]. The use of pH-dependant polymers as coating materials for colonic drug delivery has been reported previously. Therefore, the pH-dependant system was evaluated in order to achieve and ensure drug release at targeted site. In those studies immediate release and pH-dependant polymers have been applied as separate coating layers on top of each other[7]. There is no report on the use of these two kinds of polymers coating pattern for the development of a pulsatile multiparticulate colon targeted drug delivery system.

Multiparticulate approaches applied for colonic delivery includes formulations with smaller particle size compared to single unit dosage forms these systems are capable of passing through the GIT easily, leading to low inter and intra subject variability and have potential benefits like increased bioavailability, reduced risk of systemic toxicity, reduced risk of local irritation and predictable gastric emptying. Applying different coating materials onto multiparticulate, various desirable functional properties such as resistance to acid[8], sustained release and targeted release may be obtained[9].

Mesalamine or 5-aminosalicylic acid (5-ASA) has been used for several years as topical anti-inflammatory in the treatment of IBD due to its local effect on intestinal and colonic mucosa and to its few side effects for the long-term maintenance therapy to prevent relapses of CD and ulcerative colitis[10,11]. The mechanism of action of mesalamine in treatment of GIT disorders is not fully understood. It is contemplation to work as a topical anti-inflammatory agent through the inhibition of cyclooxygenase within the GI tract and the subsequent decrease in production of prostaglandins. The elimination half-life is 5 hours with a 40% of protein binding. Efficacy of mesalamine depends on achieving a high concentration at disease site. To be effective this drug must target the mucosa of the terminal ileum and colon for localized release of mesalamine. Release of drug in the stomach and upper small intestine is undesirable as this will lead to premature absorption and consequent drug wastage as well as possible systemic side effects[12-14].

The goal of this study was to optimize the formulation consisting of mesalamine layered nonpareil seeds coated with Croscarmellose sodium for immediate release and Eudragit S100, a pH dependant polymer to achieve successful ileo-cecal targeted drug delivery system. It is very important to correlate the *in vitro* performance of colon specific formulation with *in vivo* studies for ascertaining site specificity due to the varied conditions in GIT of the formulations targeted to the ileo-cecal region for its site specificity. In recent times the *in vivo* performance of colon specific drug delivery system was successfully accepted out by using X- ray imaging based on this studies *in vivo* X- ray imaging studies for optimized barium sulphate layered Eudragit S100 coated pellets was carried out[15]. We used New Zealand White strain rabbits since this strain has been used previously for an *in vivo* radio imaging study to assess the performance of the pH dependent pulsatile drug delivery system[16]. In the present investigation, *in vivo* radio imaging study conducted in rabbits. Here, we observed 5 hours of lag time in *in vitro* release which was then confirmed using a radio imaging technique for product performance in rabbits.

MATERIALS AND METHODS

Materials

Mesalamine was a kind gift from Ethypharma Pvt. Ltd. (Mumbai, India). Eudragit S100 was supplied as free gift sample from the Degussa India Pvt. Ltd. (Mumbai, India). Celpheres CP507 was purchased from Asahi Kasei Ltd. (Japan). Hypromellose (HPMC E5/AR), Croscarmellose sodium and Isopropyl alcohol (IPA) were purchased from Loba Chemicals (Mumbai, India). Other excipients used for coating were of standard pharmaceutical grade and were of standard Pharmacopoeial grade.

Experimental design

To optimize the selected formulation of preliminary experimental batch, the 32 full factorial design was executed. The independent variables were extent of Eudragit S100 coating (X1) and extent of layering of Croscarmellose sodium (X2). The dependent variables (responses) Y1 = Q300 (lag time of 5h) and Y2 = Q390 (% of drug release within 90 min after lag time). The independent and dependent variables and the used levels are summarized in Table 1 and the resulting formulations are listed in Table 2.

Table 1. Experimental design: Independent and dependent variables and the levels used for factorial design.

Factors (independent variables)	Levels used			Responses (dependent variables)
	-1	0	1	
X1=Extent of Eudragit S100 coating (% w/w)	30	35	40	Y1 = Q300 (lag time of 5h)
X2= Extent of Croscarmellose sodium Layering (% w/w)	2	3	4	Y2 = Q390 (% of drug release within 90 min. after lag time)

Table 2. Composition of experimental formulations (runs)

Batch No.	Extent of Eudragit S100 coating (%w/w)	Extent of Croscarmellose sodium layering (%w/w)
1	30	2
2	35	2
3	40	2
4	30	3
5	35	3
6	40	3
7	30	4
8	35	4
9	40	4

Preparation of drug-layered pellets

Drug loaded pellets were prepared by spraying drug solution over celpheres by fluidized bed coating technique. Mesalamine was homogeneously dispersed in an aqueous solution of hypromellose E5 and PEG 400 as plasticizer while stirring with a magnetic stirrer. The drug dispersion was passed through a 100 mesh sieve. The drug dispersion was then sprayed on celphere seeds using the fluidized bed coater, bottom spray (Miniglatt, Germany) with a 0.5 mm nozzle at a feed rate of 0.5–2 g/min using a peristaltic pump. The spraying process with the drug dispersion was continued to achieve the target drug loading level. The drug loaded pellets were finally dried at 45°C for 15 min and were used for further coating with Croscarmellose sodium and further with Eudragit S 100. The composition of drug loaded pellets and other processing parameters utilized for coating method are listed in Tables 3 and 4, respectively.

Table 3. Composition of drug-loaded celpheres pellets and experimental coating formulation

Ingredients	Quantities (g)
Composition of drug layering solution	
Mesalamine	12.5
Hypromellose E5	6.25
PEG 400	0.625
Talc	1.25
Purified water	46.75
Composition of Croscarmellose sodium layering solution	
Croscarmellose sodium	1.5
Hypromellose E5	0.75
PEG 400	0.075
Talc	0.15
Purified water	22.6
Composition of pH sensitive Eudragit S100 coating solution	
Eudragit S100	2.5
Triethyl citrate	0.25
Acetone	13.696
Isopropyl alcohol	20.568
Purified water	1.712

Table 4. Process parameters of drug layering process and polymer coating on Glatt coater.

Process Parameters	Drug layering	Swellable polymer coating	Enteric coating
Celpheres bed size (g)	50	50	50
Spray rate (g/min)	0.5-2.0	1.0-1.5	0.6-2.5
Air flow (bar)	0.3-1.5	1.2-1.5	0.8-1.5
Atomizing pressure (bar)	0.6-2.0	1.5-2.0	1.0-1.8
Nozzle diameter (mm)	0.5	0.5	0.5
Inlet temperature (^{o}C)	60-70	60-70	30-38
Product temperature (^{o}C)	35-40	35-40	25-30

Coating of Croscarmellose sodium over drug layered pellets

In order to bring the rupture of the outer functional coat, a layer of swelling agent Croscarmellose sodium was applied over the drug layered pellets by fluidized bed coating technique. Croscarmellose sodium coating solution was prepared by mixing required amount of hypromellose E5 and PEG 400 as plasticizer in aqueous medium, followed by adding Croscarmellose sodium stirring with a magnetic stirrer. Finally talc was dispersed uniformly into the prepared solution. Final solid content of Croscarmellose sodium layering solution was 3% w/w. After layering, the pellets were gently fluidized for 10 minutes and then kept in hot air oven for drying purpose for 30 min at 40°C. Coating composition and processing parameters are listed in Tables 3 and 4, respectively.

Application of outer enteric functional coat of Eudragit S100

Eudragit S100 coating solution preparation requires addition of Eudragit S100 to the mixture of solvents acetone, isopropyl alcohol and purified water which is mixed together properly stirring with a magnetic stirrer. This was followed by the addition of stated amount of triethyl citrate as plasticizer and stirred the solution for few minutes[17]. This solution was sprayed over the above processed drug layered Croscarmellose coated pellets in the fluidized bed coater with Wurster insert. Based on experimental design, the detailed composition of different batches processing parameters are listed below in Tables 3 and 4, respectively.

In preliminary studies, it was identified two most important factors affecting mesalamine release from pellets coated with Eudragit S100 (% w/w) weight gain and extent of Croscarmellose sodium layering (% w/w). The levels of these factors were selected on the basis of initial studies and observations. Pellets are evaluated for micromeritic properties such as bulk density, tapped density, angle of repose and hausner ratio. All the other formulation aspects and processing variables were kept invariant throughout the study period.

Pellet shape:

The shapes of the formulated pellets were investigated by optical microscopy. The image analyzer consisted of an optical microscope (magnification 10x) linked to a computer and a digital camera. The digitalized images were analyzed by image analyzing software. The maximum (d_{max}) and minimum (d_{min}) diameter, circumference and area were recorded for 10 pellets. The two parameters namely the aspect ratio[18] and the pellet circularity[19] were computed using the following formulae.

$$\text{Aspect ratio} = d_{max} / d_{min}$$

$$\text{Pellet circularity} = 4\pi A/P^2$$

Where A = the projected area, P = the perimeter of the pellet as seen through the microscope.

Dissolution studies

Accurately weighed enteric-coated pellets equivalent to 250 mg of mesalamine were transferred to the dissolution medium. The test was carried out in a USP dissolution type I assembly (Electrolab, TDT-08L, India) at a rotation speed of 100 rpm in 900 ml dissolution medium at 37 ± 0.5 °C in media with pH 1.2 (0.1 N HCl), pH 7.4 and pH 6.8 (phosphate buffer) for 2 h, 3 h, and till the end of the test, respectively. 5 ml aliquots of the dissolution fluid were removed at specified time intervals and replaced with fresh dissolution medium and assayed for the amount of mesalamine by spectrophotometer (JASCO V630, Japan) at wavelength 301, 330 and 334 nm for the first, second and third stages, respectively. The dissolution data was analyzed to calculate % drug released and % cumulative drug released at different time intervals.

Scanning electron microscopy (SEM)

SEM (Leica-Stereoscan-440) has been used to examine the surface morphology and texture of drug layered and polymer-coated pellets was observed under electron microanalyzer and photographs were taken using SM 4504 camera. A small amount of pellets was spread on glass stub. The stub containing the sample was placed in the SEM chamber. The scanning electron photomicrograph was taken at the acceleration voltage of 20 kV, chamber pressure of 0.6 mm Hg, with original magnification up-to 500[20, 21].

Differential scanning calorimetry (DSC)

The possibility of any interaction between mesalamine, polymers, and other excipients was assessed by DSC (Mettler Toledo Stare DSC 822c, Germany). The thermogram of the samples were obtained at a scanning rate of 10°C/min conducted over a range of 0–300°C under an inert atmosphere flushed with nitrogen at a rate of 20 ml/min.

X-ray diffractometry (XRD)

XRD was carried out to investigate the effect of microencapsulation process on crystallinity of drug. Powder XRD pattern was recorded on XRD (Philips-PW-1050) with filter Ni, Cu Kα radiation, voltage 40 kV, and a current of 20 mA. The scanning rate employed was 1°C/min over the 5° to 50° diffraction angle (2θ) range. The XRD patterns of drug powder, polymer, and drug-loaded microspheres were recorded.

Drug release models:

To describe the kinetics of the drug release from the controlled release pellets, the release data were evaluated with the help of mathematical models such as zero-order, first-order, Higuchi and Koresmeyer-Peppas model.

Statistical analysis of data

The effects of independent variables upon the responses were modeled using a second order polynomial equation. The mathematical model of the effects of independent variables upon the dependent variables was performed using Design Expert® software (Design Expert trial version 8.0.1; State-Ease Inc., Minneapolis, MN, USA) with a manual linear regression technique. A significant term ($p < 0.05$) was chosen for final equations. Finally, response surface plots resulting from equations were drawn.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \qquad (1)$$

In above equation, Y is the dependent variable; b_0 is the arithmetic average of all the quantitative outcomes of nine runs. b_1, b_2, b_{12}, b_{11}, b_{22} are the estimated coefficients computed from the observed experimental response values of Y and X_1 and X_2 are the coded levels of the independent variables. The interaction term (X_1X_2) shows how the response values change when two factors are simultaneously changed. The polynomial terms (X_1^2, X_2^2) are included to investigate nonlinearity.

All nine batches of design have shown wide variation in lag time and percentage of drug release within 90 min after lag time. The fitted equations relating the response Y1 and Y2 to the transformed factor are shown in equations 2 and 3, respectively.

Statistical validity of the polynomials was established on the basis of analysis of variance (ANOVA) provision in the software. Level of significance was considered at $p < 0.05$. The best-fitting mathematical model was selected based on the comparison of several statistical parameters, including the coefficient of variation (CV), the multiple correlation coefficient (R^2), the adjusted multiple correlation coefficient (adjusted R^2)

and the predicted residual sum of squares (PRESS) provided by the software. The 3-D response surface graphs and the 2-D contour plots were also generated by the software. These plots are very useful to see interaction effects of the factors on responses.

In vivo radio imaging study

The study protocol for the *in vivo* study was approved by the institutional animal ethics committee. To minimize the variation of gastric pH three adult male New Zealand white strain rabbits weighing approximately 2–2.5 kg were used for this study. The average pH in rabbit's stomach has been reported to be ~ 1.9[22], 6–8 in small intestine, and 7.2 in colon[23]. Rabbits were fasted overnight before start of the study. The optimized coating on barium sulphate layered pellets was administered through intubation tube followed by flushing of 25–30 ml of water. During the entire study, the rabbits had free access to water only. Photographs were taken at 0, 2, 5, and 6 h[24].

RESULT AND DISCUSSION

Original structure/shape of the coated pellets

The drug layered and polymer coated mesalamine pellets were effectively developed using fluidized bed bottom spray Glatt coating process. In drug loading step, the process had an efficiency of ~90% and ~80–85% in polymeric coating. The loss of coated product takes place due to the formation of some agglomerates and fines in the product bed, and the loss of coating solids to exhaust. The primary structure of the optimized film coated pellets has been schematically shown in Figure 1. The shape after Eudragit S100 coating pellets were observed and determined. The mean value of aspect ratio and pellet circularity was found to be 1.09 and 0.99 respectively. The value of aspect ratio and pellet circularity closer to 1 indicates that the shape of Eudragit S100 coated pellets closer to spherical as shown in Figure 2. The observed mean values of aspect ratio and pellet circularity are shown in Table 5. The release of drug layered pellets at pH 6.8 was more than 90% drug release in less than 5 min at pH 6.8. This exhibits that, despite poor water solubility, layering of the drug on the surface of pellets results in increased dissolution rate of drug. This is vital benefit of multiparticulate systems of poorly water soluble drugs contrast to single unit systems[25].

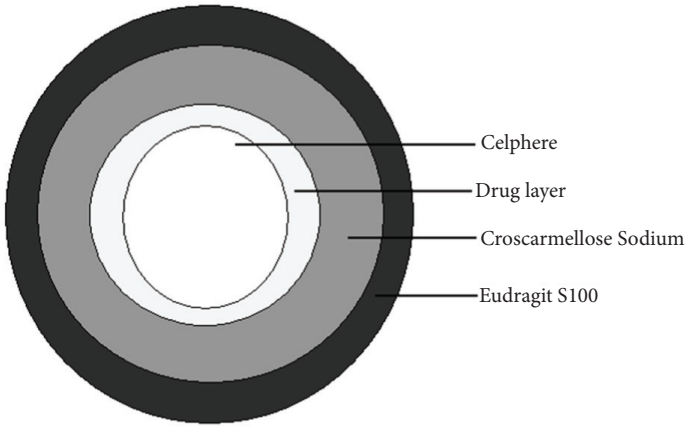

Figure 1. The basic structure of the optimized film coated pellets.

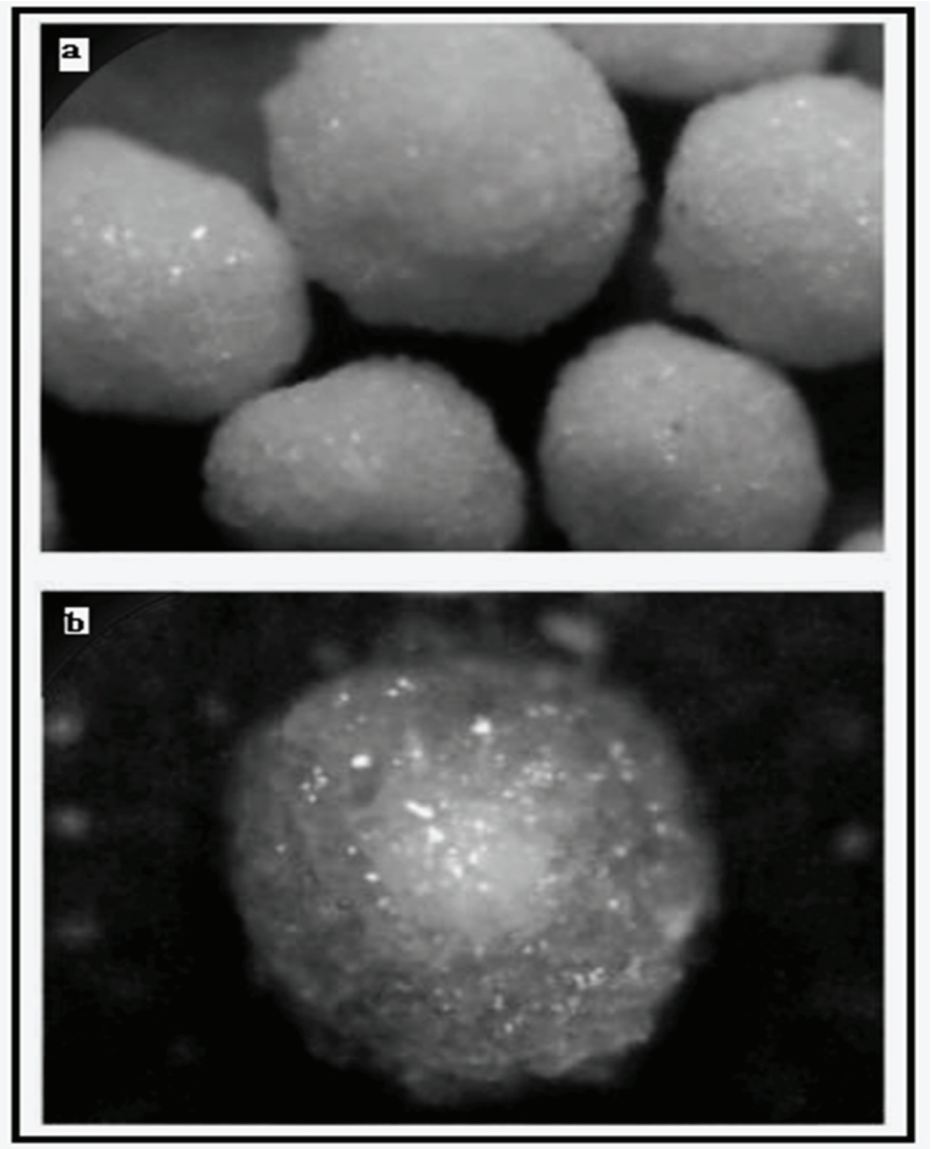

Figure 2. Microscopic swelling study of Croscarmellose sodium coated pellet (a): Pellet before addition of water droplet, (b): Pellet after addition of water droplet Magnification 40X.

Table 5. Observed values for determination of shape of pellets

Sr. No.	Parameters	Observed values
1	dmax (µm)	111.0
2	dmin (µm)	101.4
3	Projected area (m^2)	34908.84
4	Perimeter (µm)	662.98
5	Aspect ratio	1.09
6	Pellet circularity	0.99

The drug-loaded pellets were coated with successive layers of polymer Croscarmellose Sodium and Eudragit S100 respectively. In order to bring the rupture

of the outer enteric functional coat, a layer of Croscarmellose Sodium was applied over the drug layered pellets, when it comes in the contact of aqueous medium will swell by absorbing water, creates pressure thus leads to rupturing of outer membrane. Optical microscope was used for observing the increased size in the form of increased area of pellets at different time points. Figure 2 indicates the swelling behavior of Croscarmellose sodium from which it was confirmed that after addition of water the area of pellet was increased from 22584.9 m^2 to 52552.6 m^2 (raised more than 100%). One can also see that an opaque gel structure was formed around the surface of pellet. The enteric polymeric layer of Eudragit S100 is insoluble, thus for the purpose this layer may act as a barrier to any early drug release in upper GIT prior to reach to the targeted site and to provide an appropriate lag phase. In order to simulate the pH changes along the GI tract, three dissolution media with pH 1.2, 7.4, and 6.8 were sequentially used referred to as sequential pH change method[26, 27]. At pH 1.2 (simulating stomach) none of the formulations released their drug content up to 2 h. In order to determine the levels of factors which yield optimum dissolution responses, mathematical relationships were generated between the dependent and independent variables.

The equations of the responses are given below:

$$Y1 \text{ (lag time of 5h)} = +5.72-31.97*X1+0.82*X2-0.49*X1*X2+26.36*X1^2-0.15*X2^2 \quad (2)$$

$$Y2 \text{ (\% of drug release within 90 min after lag time)} = +84.63-40.09*X1+4.62*X2 \quad (3)$$

The above equation 2 and 3 represents the quantitative effect of independent variables (X1 and X2) upon the responses (Y1 and Y2). Analysis of variance (ANOVA) (Table 6) indicated the assumed regression models were significant and valid for each considered responses. The three-dimensional (3D) response surfaces and 2D contour plot were plotted to estimate the effect of independent variables on each response shown in Figures 3 and 4. Figure 3 (A&B) shows the effect of two formulation factors on lag time and indicates that increase in ratio of Eudragit S100 rises lag time significantly. It was observed from the response curves and contour plots in Figure 4 (A&B) for both the responses that increasing level of coating of Eudragit S100 retard the water uptake and thus prolongs the drug release time while increasing level of Croscarmellose sodium create more pressure over outer Eudragit S100 coat due to swelling and thus helps in releasing of drug by rupturing the outer membrane. A numerical optimization technique by the desirability approach was used to generate the optimum settings for the formulation. The process was optimized for the dependent (responses) variables Q300 and Q390. The optimum formulation was selected based on the criteria of attaining the maximum value of Q390 and minimum value of Q300. The optimized formulation was evaluated for lag time and percentage drug release within 90 min after lag time. The cumulative drug release summary of different enteric coated formulations was given in Figure 5. Micromeritic properties of all optimization formulations was found to be in the range which show that pellets exhibit good flow properties as shown in Table 7. Various kinetic models such as zero order, first order, Higuchi Matrix, Korsmeyer & Peppas were applied to the all optimization batches and values of coefficient of determination are shown in Table 8 which indicate that the release of drug from the formulated pelletized dosage forms follows zero order release kinetic model.

The linear correlation plots drawn between the predicted and actual (experimental) values for all the batches of the optimization

Table 6. Analysis of variance (ANOVA) of dependent variables.

Source	Sum of Squares	Degree of freedom	Mean Square	F Value	*p*-value	Prob>F
Analysis of Variance for Y1 (lag time of 5h)						
Model	7525.74	5	1505.15	2656.58	<0.0001	Significant
X1-Eudragit S100	6131.53	1	6131.53	10822.12	<0.0001	
X2-Croscarmellose Na	0.97	1	0.97	1.72	0.2813	
$X1^2$	1389.19	1	1389.19	2451.91	<0.0001	
$X2^2$	0.048	1	0.048	0.084	0.7907	
Residual	1.70	3	0.57	-	-	
Total	7527.44	8	-	-	-	
Analysis of Variance for Y2 (% of drug release within 90 min. after lag time)						
Model	2495.88	2	1247.94	220.80	0.0006	Significant
X1-Eudragit S100	2410.37	1	2410.37	426.47	0.0002	
X2-Croscarmellose Na	85.51	1	85.51	15.13	0.0301	
Residual	16.96	3	5.65	-	-	
Total	2512.83	5	-	-	-	

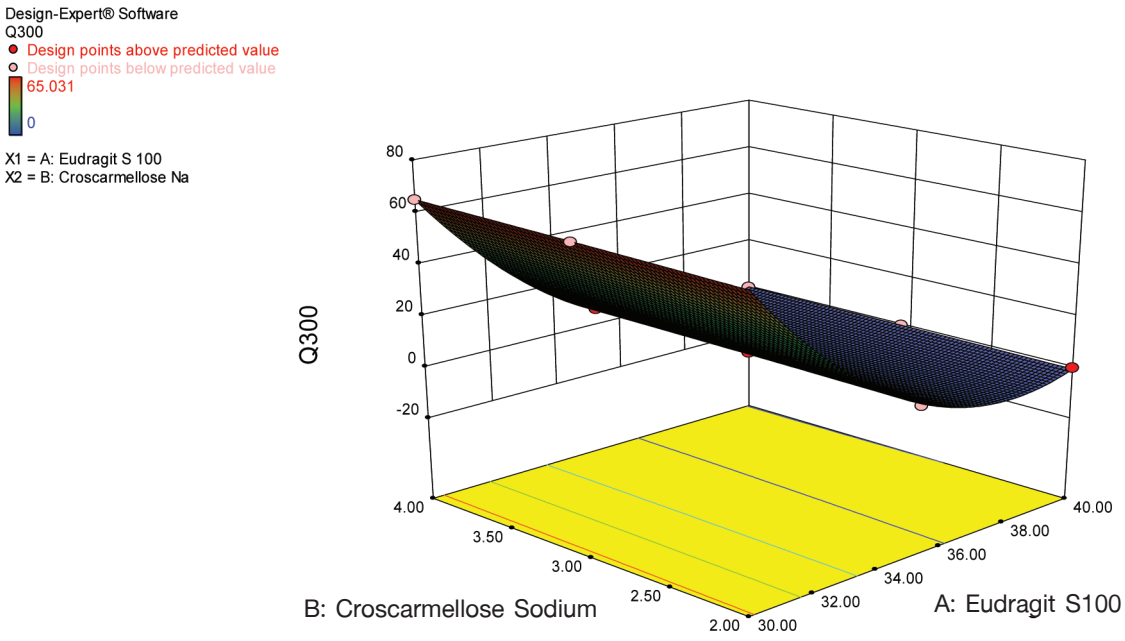

Figure 3(A). Q300 3D surface response curve

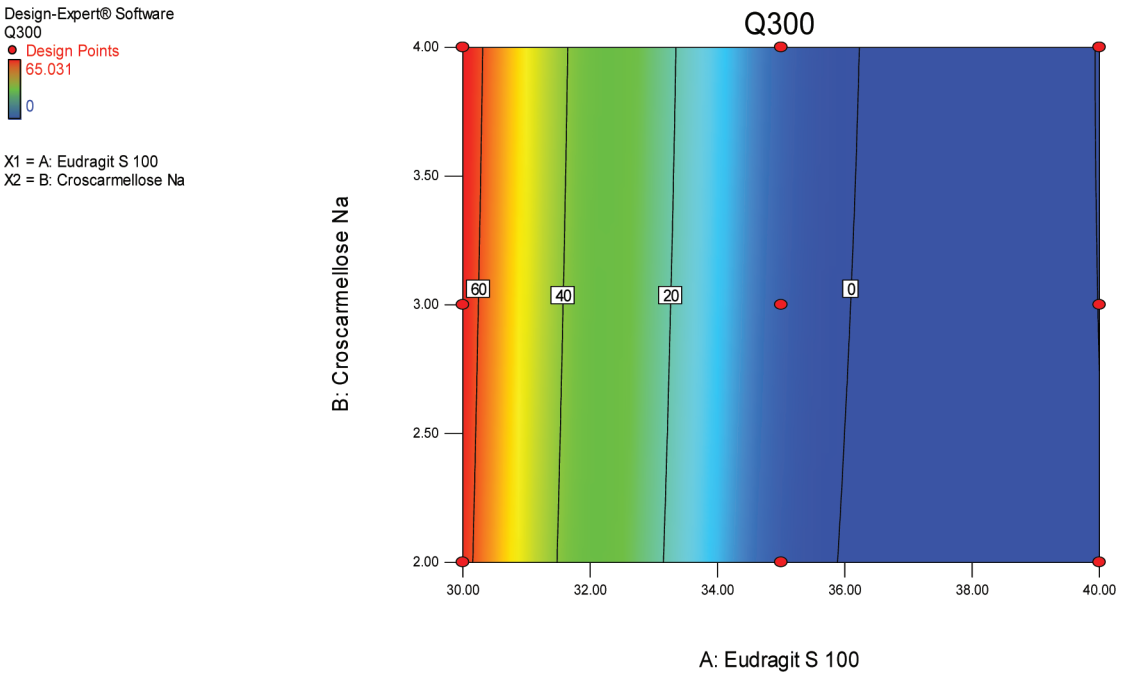

Figure 3(B). Q300 2D contour plot

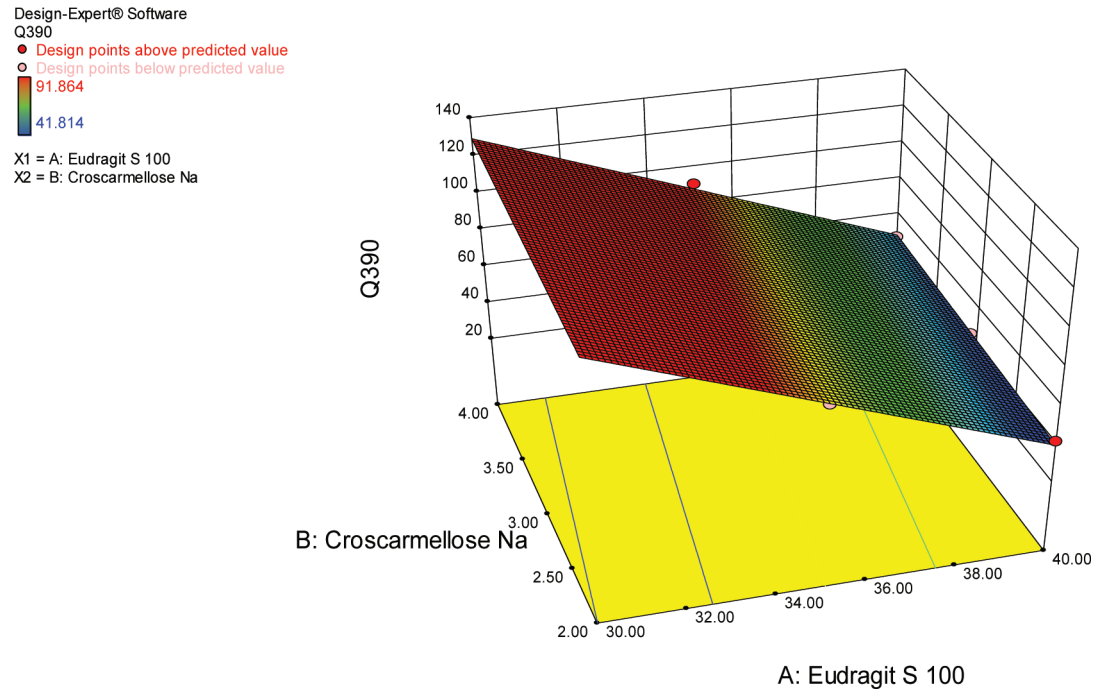

Figure 4(A). Q390 3D surface response curve

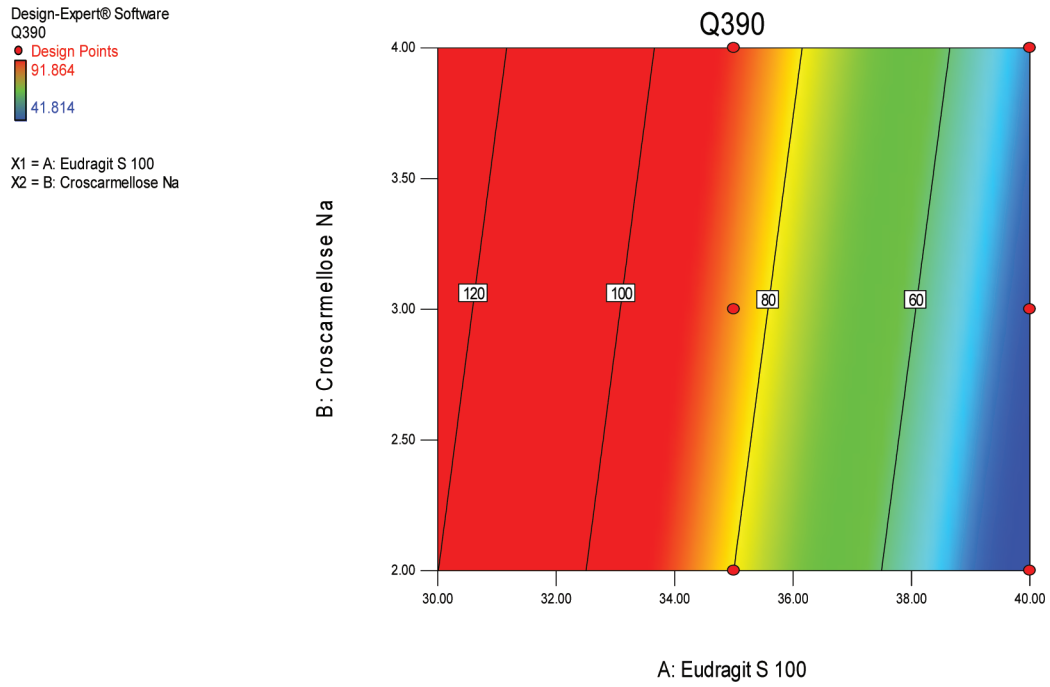

Figure 4(B). Q390 2D contour plot

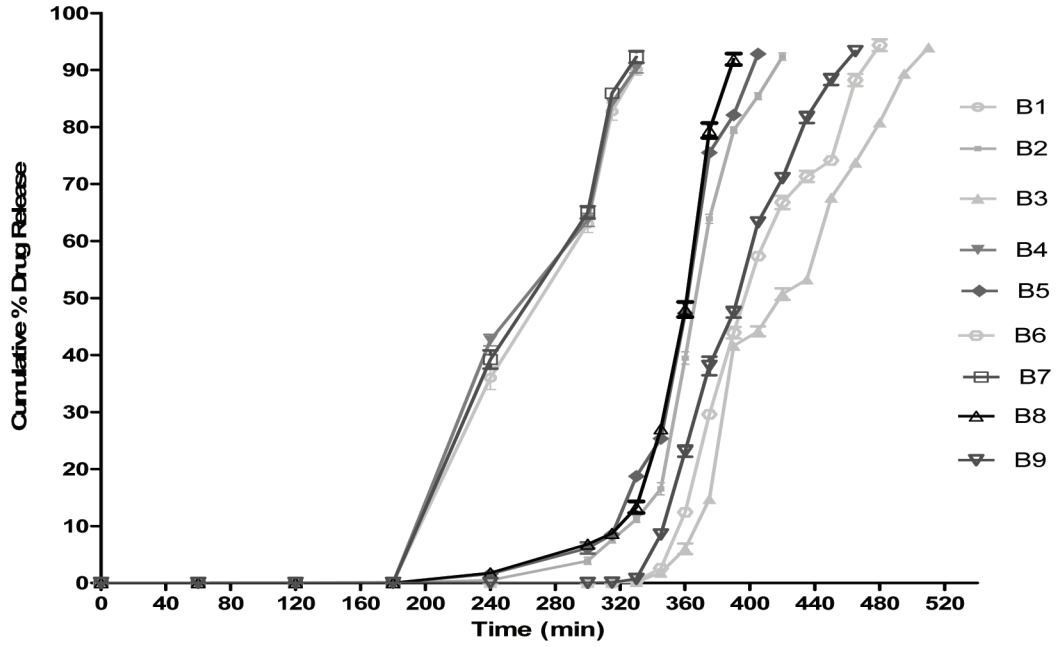

Figure 5. Drug release profile of optimization batches B1-B9

Table 7. Micromeritic properties of optimization batches B1-B9

Formulation Code	Bulk density (gm/ml)[x]	Tapped density (gm/ml)[x]	Angle of repose[x]	Hausner ratio[x]
B1	0.8273 ± 0.002	0.8537 ± 0.016	24.01 ± 0.296	1.03 ± 0.03
B2	0.8164 ± 0.005	0.8641 ± 0.020	24.85 ± 0.321	1.05 ± 0.028
B3	0.7954 ± 0.011	0.8453 ± 0.014	24.38 ± 0.135	1.06 ± 0.018
B4	0.8217 ± 0.004	0.8574 ± 0.015	25.03 ± 0.091	1.04 ± 0.022
B5	0.8124 ± 0.005	0.8642 ± 0.008	25.94 ± 0.283	1.06 ± 0.031
B6	0.8431 ± 0.008	0.8942 ± 0.13	26.04 ± 0.810	1.06 ± 0.019
B7	0.8268 ± 0.001	0.8763 ± 0.015	25.38 ± 0.312	1.07 ± 0.025
B8	0.8374 ± 0.002	0.8857 ± 0.012	26.57 ± 0.392	1.07 ± 0.029
B9	0.8012 ± 0.013	0.8439 ± 0.10	25.49 ± 0.573	1.05 ± 0.034

[x]Mean n = 3

Table 8. Kinetic modeling of Optimization Batches B1-B9

Formulations	R^2 (coefficient of determination) of various Kinetic Models			
	Zero order	First order	Higuchi release	Korsmeyer and Peppas release
B1	0.865	0.743	0.787	0.736
B2	0.570	0.464	0.477	0.642
B3	0.655	0.524	0.536	0.278
B4	0.873	0.757	0.799	0.734
B5	0.564	0.428	0.475	0.405
B6	0.623	0.492	0.512	0.431
B7	0.868	0.730	0.792	0.732
B8	0.487	0.324	0.408	0.412
B9	0.618	0.510	0.510	0.349

formulation shown in Figure 6 (A and B), which demonstrated high values of R^2 (0.999 and 0.993). Thus the low magnitudes of error as well as the values of R^2 in the present investigation prove the high prognostic ability of the optimization technique. According to the design the best area for formulation to obtain desired responses was found. The best conditions to optimize drug release corresponded to Croscarmellose Sodium with 4% coating level and of Eudragit S100 a coating level of 35.22%.In order to ensure the validity of the optimization procedure, a new batch of pellets with the predicted levels was prepared. The values of predicted and observed responses are shown in Table 9. The pellets were prepared according to optimum formulation and released negligible drug at pH 1.2 (0.1 N HCl) and pH 7.4, and showed burst release at pH 6.8 (Figure 7).

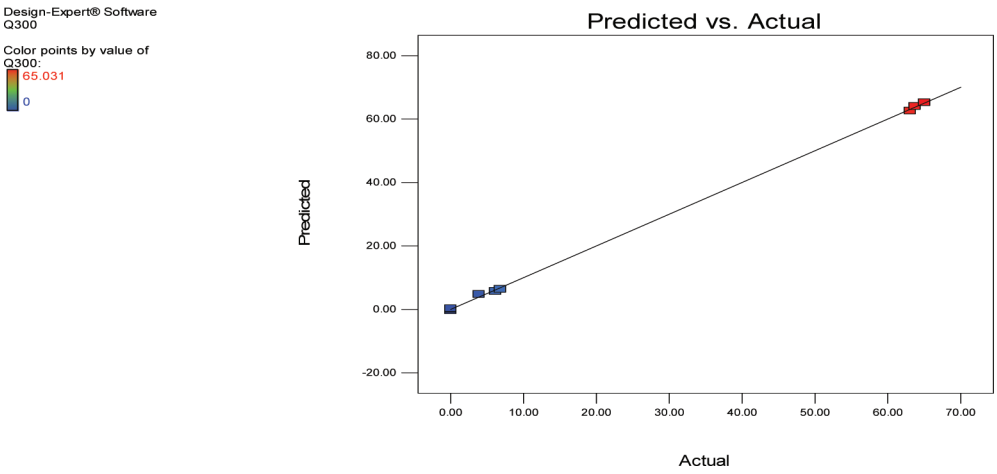

Figure 6(A). Predicted vs Actual response for Q300 response

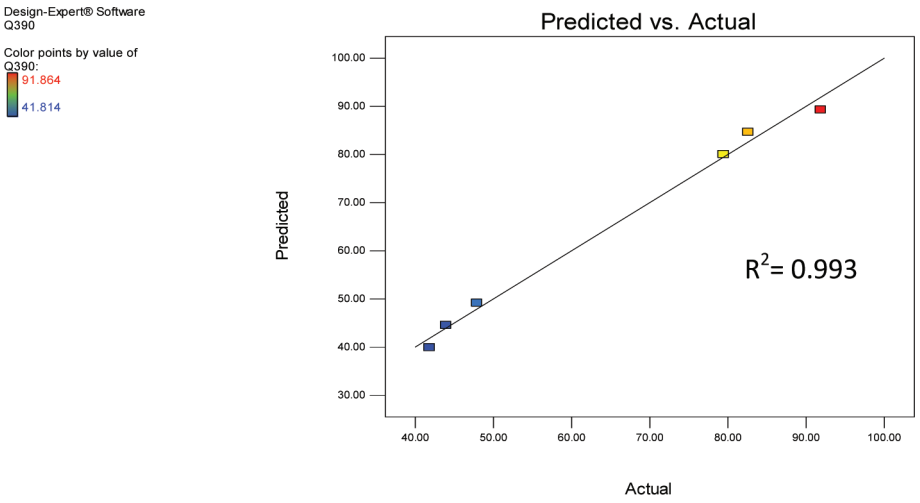

Figure 6(B). Predicted vs Actual for Q390 response

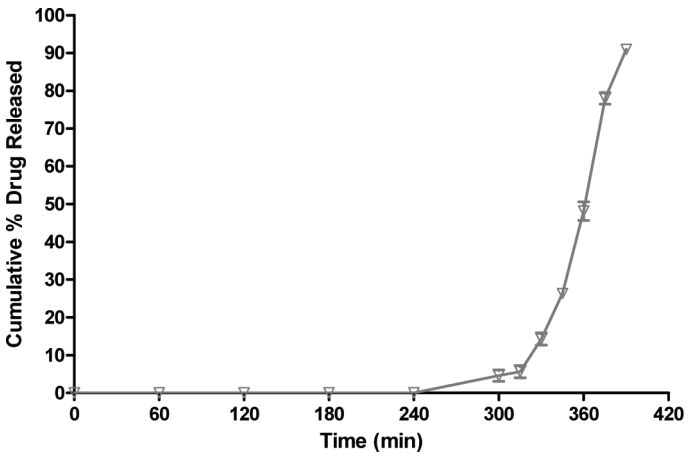

Figure 7. Cumulative % Drug Release profile of optimized batch

Scanning Electron Microscopy (SEM)

The spherical nature of drug layered pellets also confirmed with the images taken by scanning electron microscopy Figure 8 below (B1, B2 and B2). SEM study at x30, x500 as well as x6000 magnifications revealed that the drug layered pellets are dense with wrinkled, rough and porous circumference which is due to gradual loss of water during drug layering process from the surface of pellets. This rough and porous surface results in further increase in the solubility.

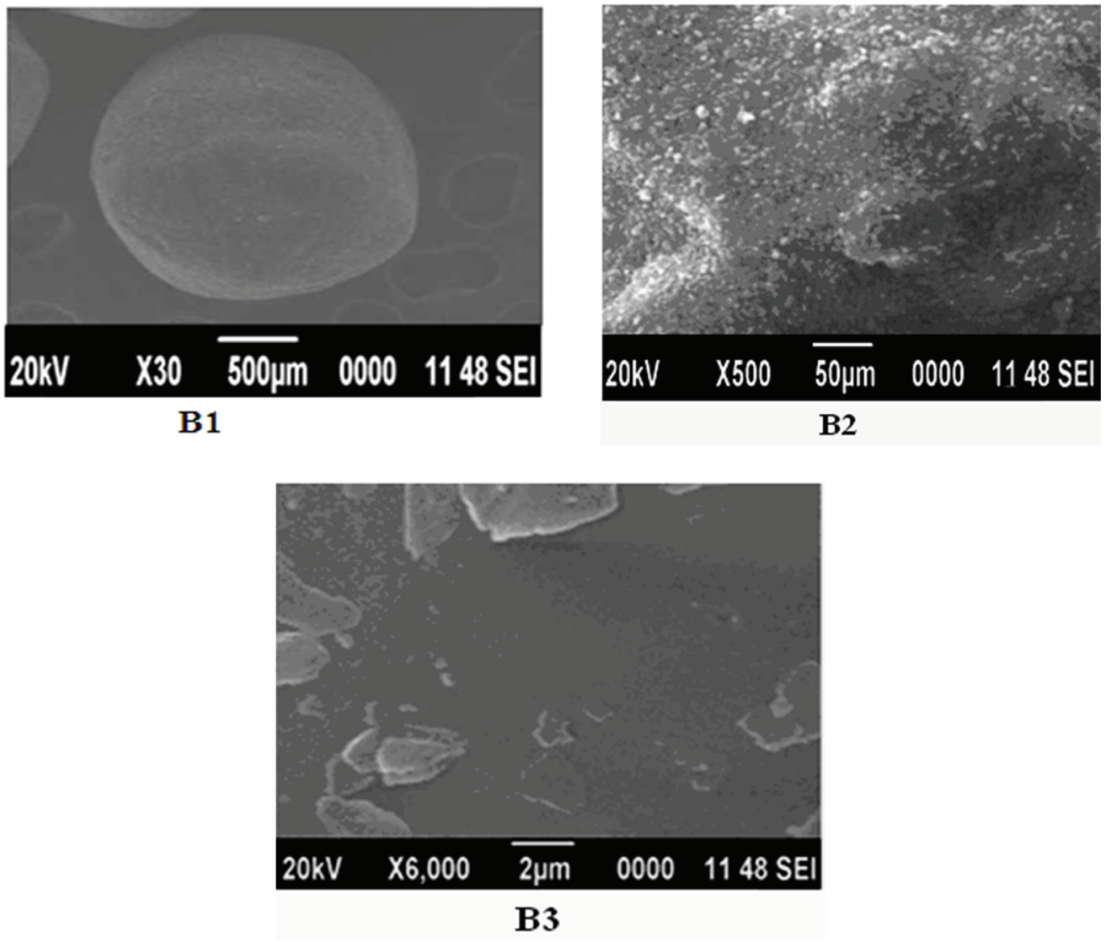

Figure 8. Scanning electron micrographs of Mesalamine layered Eudragit S100 coated pellets B1: at x30 magnification, B2: at x500 magnification, B3: at x6000 magnification

Differential scanning calorimetry (DSC)

The DSC thermogram shows a sharp endothermic peak at 285.16°C for mesalamine. While in final optimum formulation containing drug and polymer, the endothermic peak was observed at 262.45°C (Figure 9 A&B). Evaluation and interpretation of the thermogram revealed no interaction between the drug and polymer in the optimized formulation.

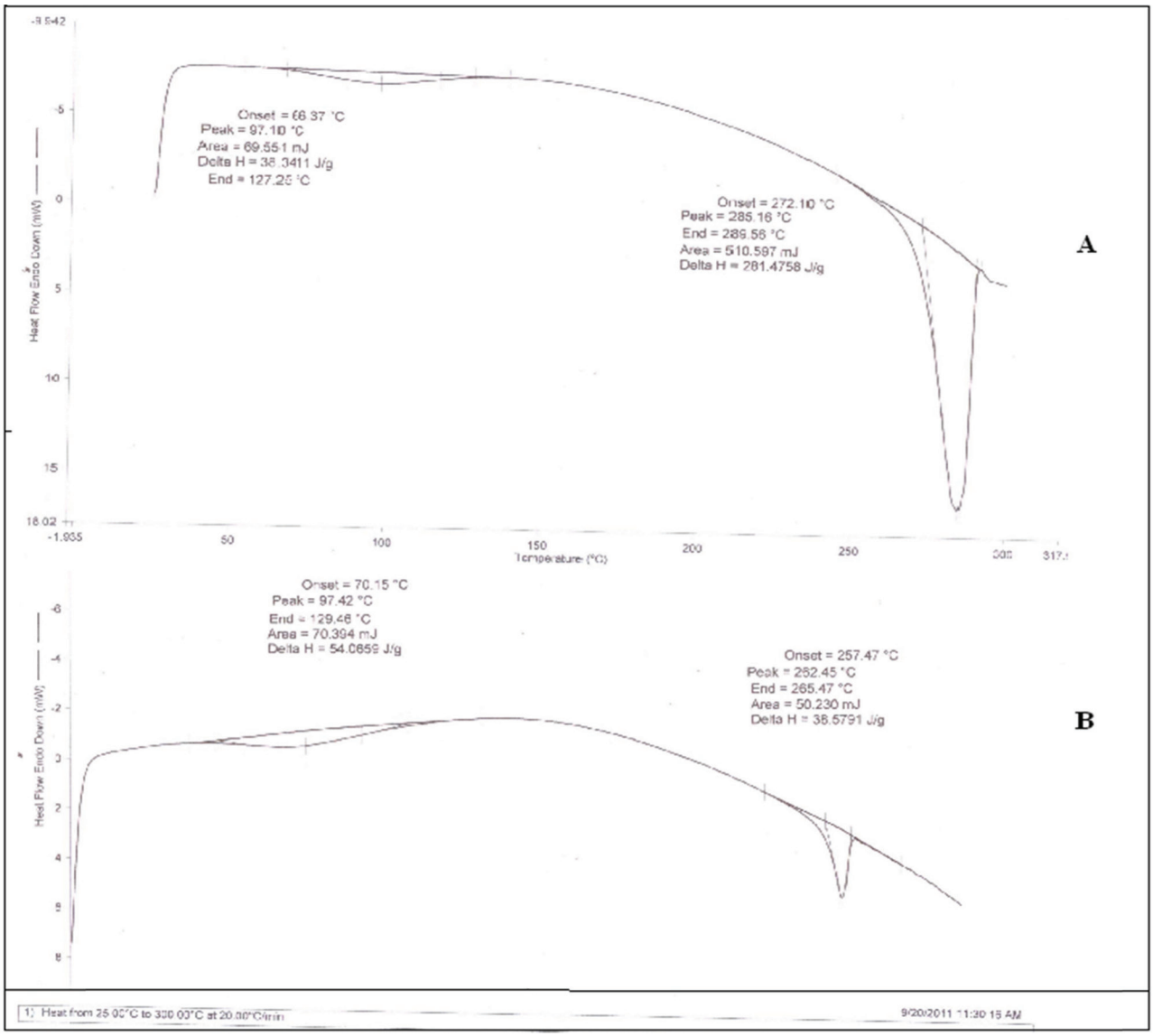

Figure 9. DSC thermograph of mesalamine (A) and optimized formulation (B)

X-ray diffractometry (XRD)

Characteristic crystalline a peak of mesalamine (M1) was retained in the XRD of optimized formulation (M2) which suggested that there no interaction between drug and polymers used for coating (Figure 10).

In vivo radio imaging study

From the radiographic images (Figure 11) it was proved that barium sulphate loaded Eudragit S 100 coated pellets remains intact in its structural integrity and shape in stomach and small intestine. At the end of 2 hrs shows fragmentation of capsule but no release of barium sulphate. At the end of 5 hrs barium sulphate was release from pellets and consider to release in ileo-cecal region.

CONCLUSION

The present study concludes that the mesalamine pH dependant pulsatile burst release could be successful option for ileo-cecal targeting by achieving the desired lag time. Lag time and quick release of drug after lag time was achieved with proper

selection of extent of Eudragit S100 coating and Croscarmellose layering over drug layered pellets. Lag time and target release was observed by good correlation between *in vitro* and *in vivo* by radio imaging studies. Thus, the designed formulation can be considered as one of the promising formulation technique for preparing a ileo-cecal targeted pulsatile drug delivery system in management of IBD and other diseases.

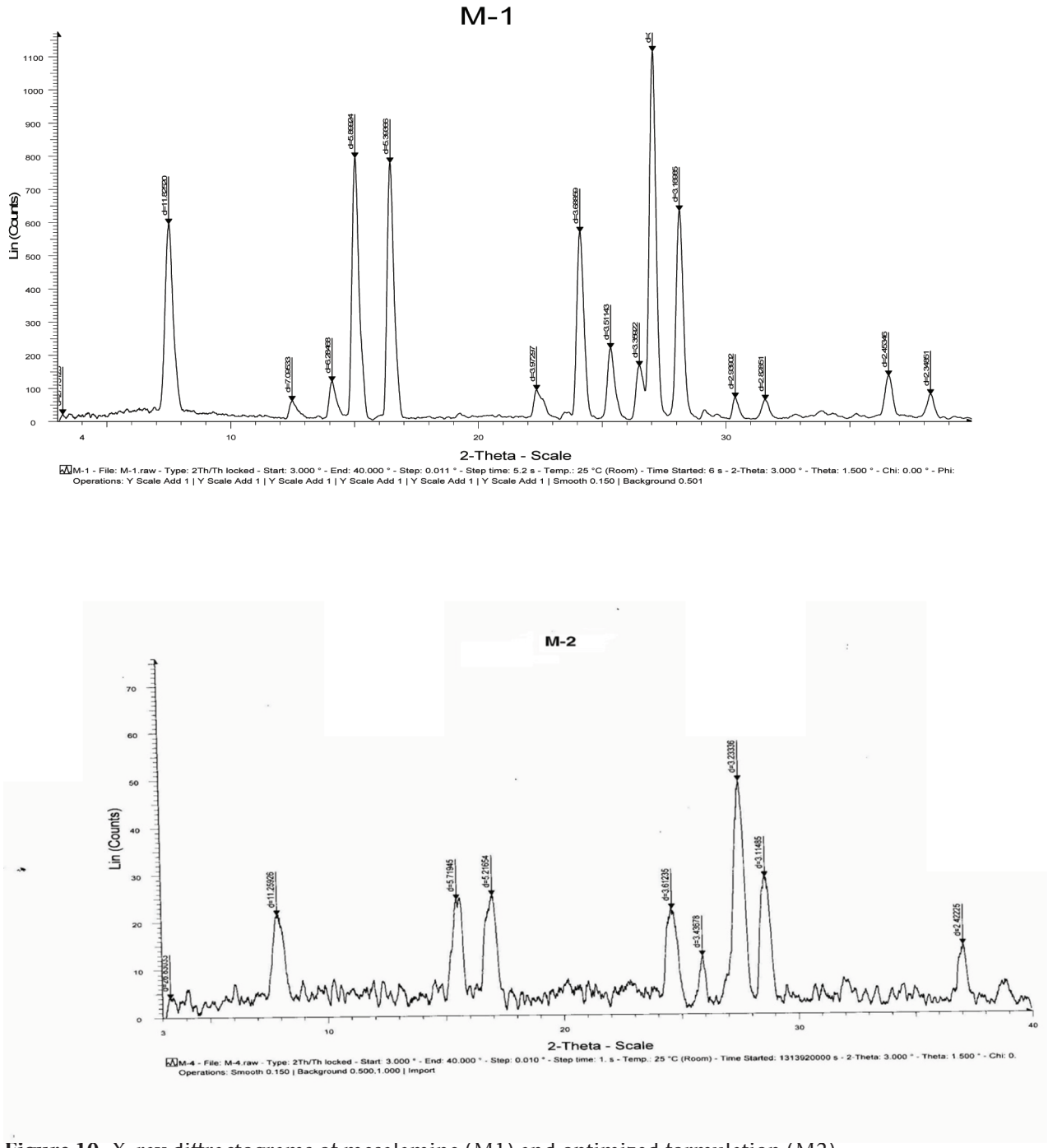

Figure 10. X-ray diffractograms of mesalamine (M1) and optimized formulation (M2)

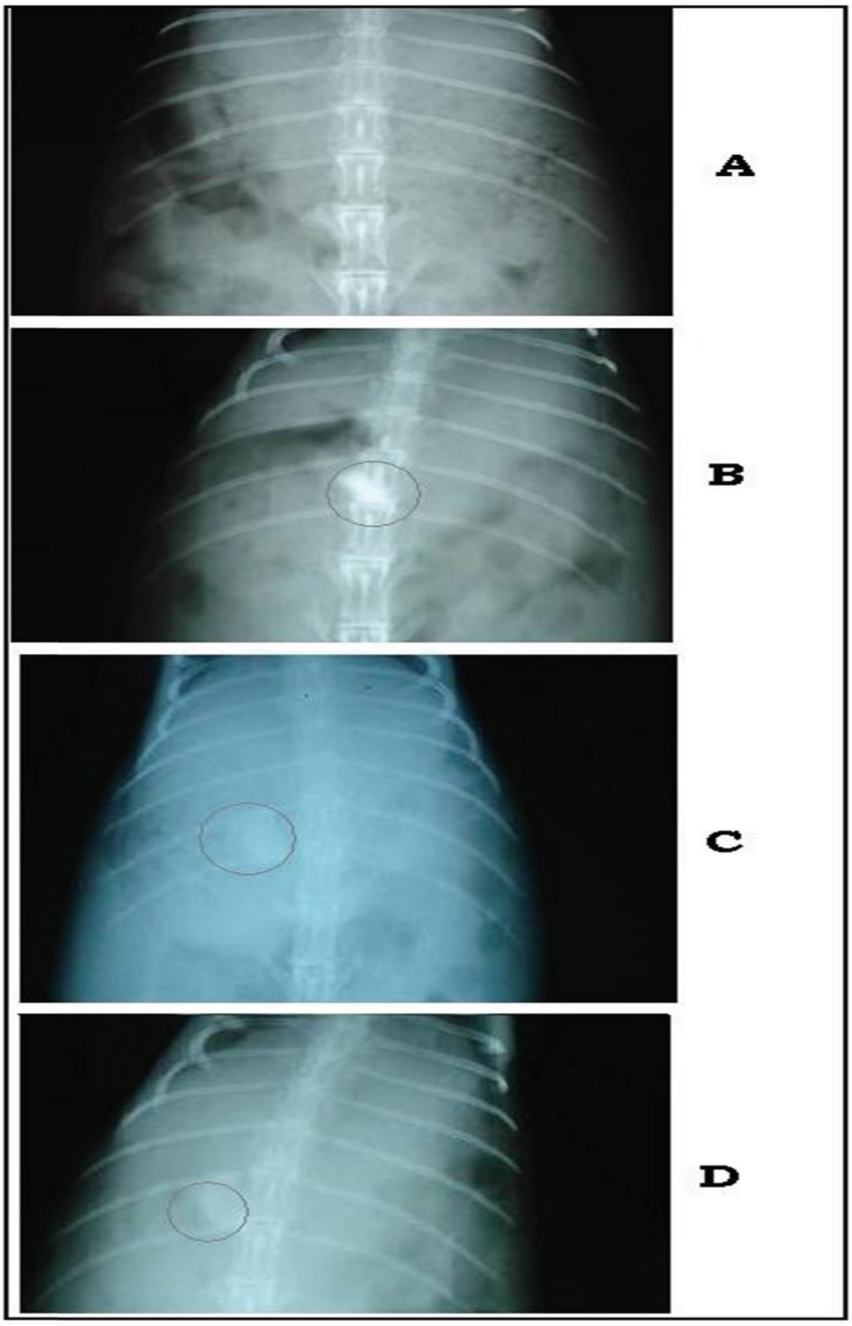

Figure 11. Radio images showing pellets filled in capsule at 0 h (A), 2 h (B) remain intact in stomach, pellets at 5 h (C) in ileo-cecal region, and after 6 h (D).

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