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Research Article

OPTIMIZATION AND *IN VIVO* EVALUATION OF MESALAMINE pH DEPENDENT COATED PELLETS FOR PROMISING ILEO-CECAL TARGETING

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ABSTRACT

The present research is a challenge to design, optimized and evaluates mesalamine loaded burst release pH dependent coated pellets for possible ileo-cecal targeting to treat effectively Crohn's disease. The novelty of this formulation is to release drug specifically and instantly in ileo-cecal region where the chances of Crohn's disease is more frequent, 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 of 5h 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. The regression equation generated for Q300 (lag time of 5h) = +5.72-31.97*A+0.82*B-0.49*A*B+26.36*A2-0.15*B2 and for Q390 (90% of drug release at pH7.2 within 90 minutes after lag time) = +84.63-40.09*A+4.62*B. The drug release data of optimized formulation were close to that predicted by the model. Various kinetic models were applied to the all optimized batches. *In vivo* evaluation of optimized formulations was performed to assess macroscopic, microscopic and biochemical parameters in rats and performed. The present study demonstrates that the mesalamine enteric coated pellets successfully targeted at ileo-cecal region.

Key words: Mesalamine, pulsatile, ileo-cecal targeting, celpheres, Croscarmellose sodium, eudragit S100

INTRODUCTION

Several 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 system (CTDDS) is considered to be beneficial in the local and systemic treatment of ileo-cecal and colon related diseases and disorders. In humans, the majority of IBD occurs in a variety of forms, the most common being Crohn's disease (CD) and ulcerative colitis (UC). CD affects any region of the gastrointestinal tract and is characteristically segmental with areas of sparing throughout the gastrointestinal tract. The endoscopic features of CD include linear ulcers, cobblestoning, and skip areas of normal mucosa and microscopic features include transmural inflammation, granulomas, and skip areas of normal uninflamed mucosa. (Cotran *et al.* 2000; Déo *et al.*, 2011) For effective treatment of this disease the drug must release at site of ileo-cecal region with immediate release. (Weathrall *et al.* 1996)

A variety 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 (Watts and Illum, 1997; Chourasia and Jain, 2003). Among these approaches, pH-dependant system is simple and suitable for CTDDS in different physiological and pathological conditions in GIT (Ashford *et al.*, 1993). The use of pH-dependant polymers as coating materials for colonic drug delivery has been reported previously. In this study immediate release and pH-dependant polymers have been applied as separate coating layers on top of each other (Fukui *et al.*, 2000). 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.

Mesalamine group of drugs are first line therapy treatment for patients with mild to moderate IBD, which include oral and rectal formulations of mesalamine and oral pro-drugs like sulfasalazine, olsalazine and balsalazide are the major drugs for maintaining remission. These are capable of preventing the production of prostaglandins and leukotrienes, thus preventing neutrophil chemotaxis. Mesalamine 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 (Cai *et al*., 2003; Qureshi & Cohen, 2005) for the long-term maintenance therapy to prevent relapses of CD and ulcerative colitis. Efficacy of mesalamine depends on achieving a high concentration at disease site. 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. (Klotz *et al*. 2000, Hanauer *et al*. 2004, Podolsky *et al*. 2005)

The aim and need of this work was to formulate, optimize and evaluate mesalamine pellets coated with croscarmellose sodium for immediate release and Eudragit S100, a pH dependant polymer to achieve successful ileo-cecal targeting. 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 (Anal, 2006, Mastiholimath *et al*. 2007). Here, we observed 5 hours of lag time in *in-vitro* release study and also optimized formulation was studied for microscopic, macroscopic and biochemical parameters in rats.

MATERIALS AND METHODS

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.

Experimental design

To optimize the selected formulation of preliminary experimental batch, the 3^2 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 I.

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 (Pam Miniglatt, Germany) with a 0.5mm nozzle at a feed rate of 0.5–2g/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 15min and were used for further coating with croscarmellose sodium and further with Eudragit S 100.

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.

Table I. 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 II. Process parameters of drug layering process and polymer coating

Process Parameters	Drug layering	Swellable coating	Enteric coating
Celpheres bed size (g)	50	50	50
Spray rate (g/min)	0.5-2	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 (°C)	60-70	60-70	30-38
Product temperature (°C)	35-40	35-40	25-30

Final solid content of Croscarmellose sodium layering solution was 3% w/w. After layering, the pellets were gently fluidized for 10min and then kept in hot air oven for drying purpose for 30min at 40°C.

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 (Shivkumar *et al.* 2006). 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 drug layering and coating processing parameters are listed below in tables II.

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.

Dissolution studies

Accurately weighed enteric-coated pellets equivalent to 250mg of mesalamine were transferred to the dissolution medium. The test was carried out by rotating basket method specified in the USP XXIII dissolution tester (Electrolab, TDT-08L, India) at a rotation speed of 100 rpm in 900mL dissolution medium at 37 ± 0.5°C in media with pH 1.2 (HCl 0.1 N), pH 7.4 and pH 6.8 (phosphate buffer) for 2h, 3h, and till the end of the test, respectively. 5mL 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 334nm for the first, second and third stages, respectively (Déo *et al.*, 2011). 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 20kV, chamber pressure of 0.6mm Hg, with original magnification up-to 500 (Gupta *et al.* 2001; Rao *et al.* 2007).

Drug release models

To describe the kinetics of the drug release from the controlled release microparticles, 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.

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

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 *Y*1 and *Y*2 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 Study

Animals

Albino Wistar rats (150 -200 g) of either sex and Albino male rabbits (1.5-2.0 Kg) were used in this study. On arrival rats were placed randomly in polypropylene cages (six per cage) with paddy husk as bedding and rabbits were housed in separate cages. The study protocol for the in vivo study was approved by the institutional animal ethics committee.

Induction of experimental IBD

For induction of IBD an 8mm soft pediatric catheter was advanced 6cm from anus under low dose ether anesthesia. Rats were hold in trendelenburg position during this process 1mL of 4% acetic acid or 0.9% normal saline solution was slowly administered intrarectally. The rats were maintained in head down position for 30sec. to prevent leakage and the rest of solution was aspirated. After this process, 2mL of phosphate buffer solution with pH 7 was administered intrarectally (Gorgulu and Yagci, 2006).

Administration of Mesalamine microspheres

Mesalamine microspheres dispersion for administration to animals were prepared using normal saline solution and administered using oral feeding tube. Twenty healthy wistar albino rats weighing 150-200gm were divided in to 4 groups with 5 animals in each group as follows:

Group A (Normal control) - received 2mL normal saline intrarectally; Group B (Control induced group) - received 2mL of normal saline intrarectally; Group C (Test) - received Mesalamine microspheres (100mg/kg/day/ p.o.); Group D (Standard) - received Mesalamine pure drug (100mg/kg/day/ p.o.)

The animals were pretreated with the respective drugs (volume of drugs was kept at constant 2mL/Kg) for 5days, along with the normal diet. On the 5th day animals were kept fasting for 12h (overnight) and IBD was induced next morning in the groups B, C, and D by administration of 1mL of 4% acetic acid solution intrarectally. Group A normal control animals received 0.9% normal saline intrarectally.

Macroscopic assessment of colonic injury and inflammation

All the animals were sacrificed after 48 h of IBD induction, by ether overdose. A midline incision was made in abdomen and 8-cm distal segment of colon was removed, opened, and washed in normal saline, mucosal injury was accessed macroscopically. The physical parameters like body weight, colon weight, colon length, and colon weight/length ratio were used to evaluate status in animals. (Biradar *et al.* 2011; El Medany *et al.* 2005) Severity of gross macroscopic injury was assessed using a scoring system reported previously with a slight modification as follows: 0, normal appearance; 1, erythema and inflammation without ulcer; 2, inflammation and ulcer; 3, ulcer with necrosis.

Assessment of histopathological examination

Sections of colon samples were fixed in phosphate-buffered formalin solution (10%), implanted in paraffin, stained with Haematoxylin and Eosin dye (H and E) and evaluated by light microscopy for morphological changes. Inflammation extent and severity, crypt damage and percent of involvement were considered to assess the colonic damage from the histopathological point of view (Williams *et al.* 2001). Total colitis was also calculated by summation the scores of inflammation severity, inflammation extent and crypt damage. The microscopic and histological scoring was carried out at pathology laboratory to normal control, control induced and drug treated groups.

Assessment of Biochemical parameters

Myloperoxidase Activity (MPO) activity

The measurement of MPO activity is the first quantitative assessment to quantify the severity of colitis. It is reliable index of inflammation caused via infiltration of activated neutrophils to the inflamed tissue. Activity was analyzed using standard method specified by Krawisz, *et al.* 1984.

Lipid peroxides (LPO) activity

Lipid peroxidation activity is an indicator of mucosal injury induced by reactive oxygen species was measured as thiobarbituric acid (TBA) reactive substance. The amount of colonic lipid peroxides was measured by the TBA assay as previously mentioned by Buege and Aust 1978.

Lactate dehydrogenase (LDH) activity

Lactate dehydrogenase (LDH) is an oxidoreductase which catalyzes the interconversion of lactate and pyruvate. When disease or injury affects tissues containing LDH, the cells release LDH into the bloodstream, where it is identified in higher than normal levels.

Reduced glutathione (GSH) activity

Colonic tissue specimens were homogenized in ice cold 10mmol/L Tris–HCL buffer of pH 7.2, and centrifuge at 2500rpm for 10min at 4°C. The supernatant was used for the measurement of GSH. Reduced GSH was determined using the method described by Owens and Belcher, 1965 which is based on the reaction of 5, 5-dithiobis-2-nitrobenzoic acid with GSH present in the tissue.

RESULT AND DISCUSSION

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.

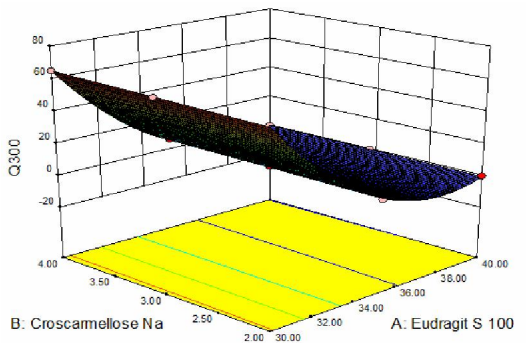

Figure 1(A). Q300 3D surface response curve

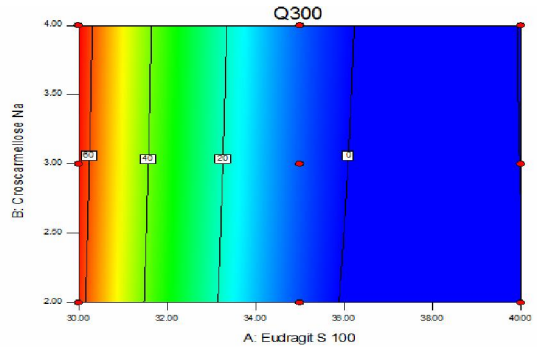

Figure 1(B). Q300 2D contour plot

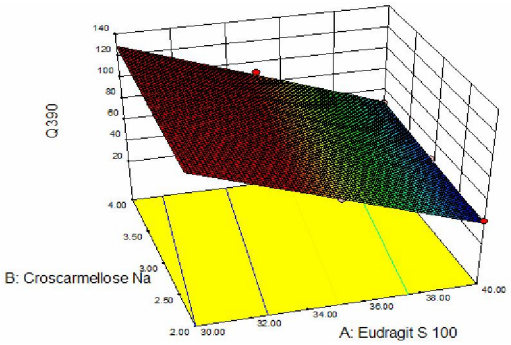

Figure 2(A). Q390 3D surface response curve

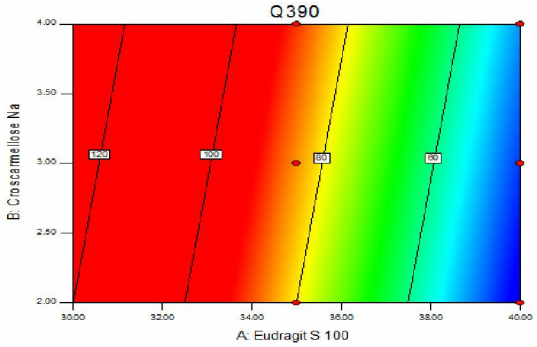

Figure 2(B). Q390 2D contour plot

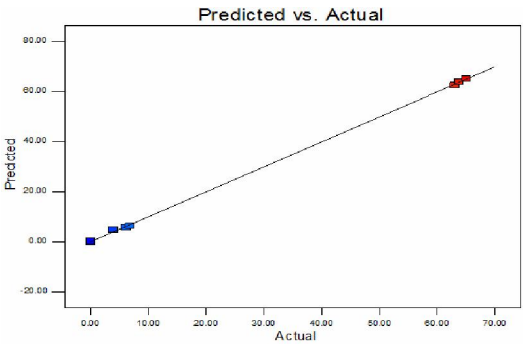

Figure 3(A). Predicted Vs Actual response for Q300 response

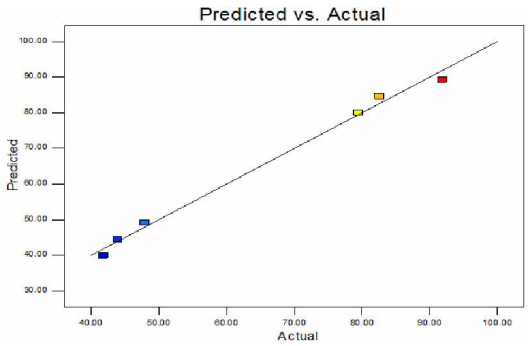

Figure 3(B). Predicted Vs Actual for Q390 response

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 (Akhgari *et al.*, 2005).

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.

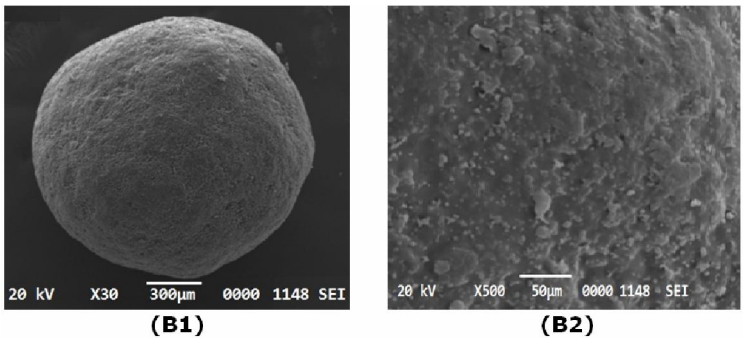

Figure 4. Scanning electron micrographs of mesalamine layered Eudragit S100 coated pellets B1: at x30 magnification, B2: at x500 magnification, B3: at x6000 magnification

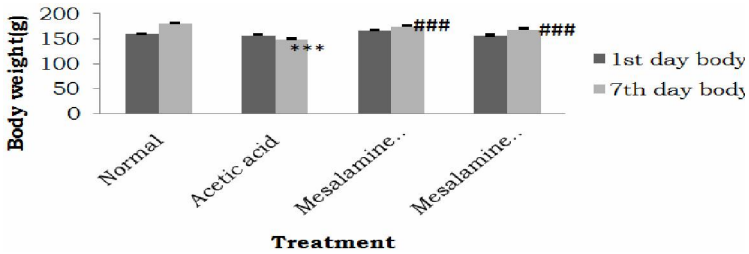

Figure 5. Effect of Mesalamine microspheres on physical parameters of body weight in acetic acid induced colitis

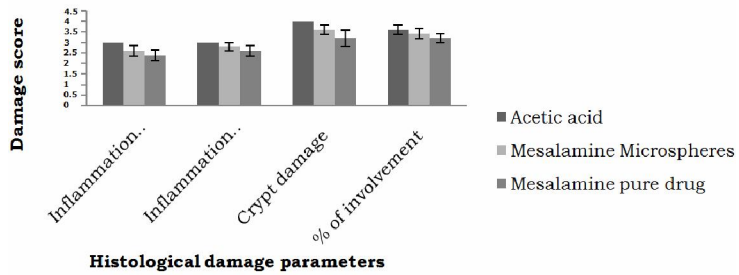

Figure 6. Histopathological examination scores of colitis in control and treatment groups

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 (Gang *et al.*, 2004; Kadam *et al.*, 2010). 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(lag time of 5h) = +5.72-31.97*X1+0.82* X2-0.49*X1*X2+26.36*X1²0.15*X2²**…….(2)**

Y2 (% of drug release within 90min. after lag time) = +84.63-40.09*X1+4.62*X2**.....(3)**

The above equation 2and 3 represents the quantitative effect of independent variables (*X*1 and *X*2) upon the responses (*Y*1 and *Y*2). Analysis of variance (ANOVA) (Table III)

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 1 and 2. Figure 1(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 2 (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 creats more pressure over outer Eudragit S100 coat 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 performed. 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 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 formulation shown in Figure 3 (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 with the predicted levels was prepared.

Scanning Electron Microscopy (SEM)

The spherical nature of drug layered pellets also confirmed with the images taken by scanning electron microscopy Figure 4 below (B1and B2). SEM study at x30 and x500 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.

***In Vivo* Study**

Effect of mesalamine microspheres on physical parameter

There will be significant decrease ($p<0.001$) in the body weight (which is expressed in percentage of original weight) of the acetic acid induced group compare with normal group (after induction). The drug treated groups mesalamine microspheres (100mg/kg/day/ p.o.) and the standard mesalamine pure drug (100mg/kg/day/ p.o.) group ($p<0.001$) showed retain in the total body weight compare with acetic acid induced group (Figure 5)

In control induced group, acetic acid induction showed significantly ($p<0.001$) increase in the colon weight compare to normal group. Whereas the drug treated mesalamine microspheres and mesalamine pure drug decreases the colon weight significantly ($p<0.001$) compared with the acetic acid induced group. The IBD induction by intra rectal administration of acetic acid cause shrinkage in the colon length in the control group significantly ($p<0.001$) compared with normal group. The drug treated mesalamine microspheres and mesalamine pure drug decreases the shrinkage of colon length significantly ($p<0.001$) compared to acetic acid induced group. The weight/length ratio (mg/cm) is increased significantly ($p<0.001$) in acetic acid induced group compared to normal group.

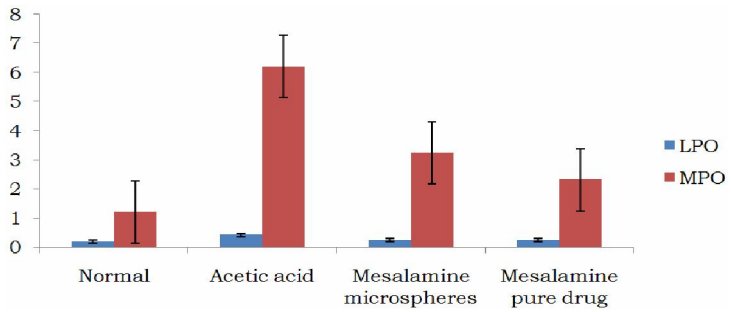

Figure 7. Effect of Mesalamine microspheres on MPO and LPO activity in acetic acid induced IBD in rats

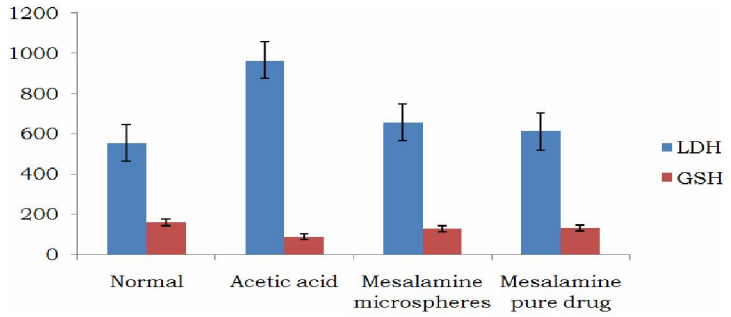

Figure 8. Effect of Mesalamine microspheres on LDH and GSH activity in acetic acid induced IBD in rats

Table III. 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 sodium	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 sodium	85.51	1	85.51	15.13	0.0301	
Residual	16.96	3	5.65	-	-	
Total	2512.83	5	-	-	-	

Drug treated mesalamine microspheres and mesalamine pure drug group significantly ($p<0.001$) decreases this ratio compared to acetic acid group.

Macroscopic assessment of colonic injury and inflammation

It was observed from the macroscopic tissue damage parameters visible severe inflammation; hemorrhage, ulcer, and necrosis as well as thickening of colon wall in control groups compared to normal groups in which no changes were observed. The treatment group Mesalamine microspheres and Mesalamine pure drug were effective to reduce macroscopic score , ulcer index and damage score in colon samples compared to control groups.

Assessment of histopathological examination

For scoring histological damage four parameters were selected as inflammation severity, inflammation extent, crypt damage, and percent of involvement. Total colitis was an particular parameter, which was the sum of inflammation severity, inflammation extent, and crypt damage. Treatment of rats with Mesalamine Microspheres and Mesalamine pure drug decreased all the histological scores of total colitis (Figure 6).

Evaluation of mesalamine microspheres on colonic mpo activity

Colitis induced by 4% acetic acid in rats resulted in significant ($P<0.001$) increase in colonic MPO content with respect to normal control rats. However, the former effect was reversed in the group of rats with colitis which was previously treated with Mesalamine microspheres (100mg/kg/day/ p.o.) and Mesalamine pure drug (100mg/kg/day/ p.o.) The greater inhibitory effect on MPO activity was achieved with the Mesalamine microspheres (100mg/kg/day/ p.o.), which indicates a reduction of neutrophil infiltration in colonic tissue. Mesalamine pure drug (100mg/kg/day/ p.o.) used as a drug reference, also substantially decreased MPO activity compared Mesalamine microspheres in rats with colitis (Figure 7).

Evaluation of mesalamine microspheres on colonic lpo activity

LPO assay showed significant ($p<0.001$) increase in LPO activity of acetic acid induced group compared to normal group. The drug treated Mesalamine microspheres (100mg/kg/day/p.o.) showed significant ($p<0.001$) and Mesalamine pure drug (100mg/kg/day/p.o.) treated group showed significant ($p<0.001$) reduction in LPO activity compared to the acetic acid induced group (Figure 7)

Evaluation of mesalamine microspheres on colonic ldh activity

The lactate dehydrogenase (LDH) assay showed significant ($P<0.001$) increase in LDH activity of acetic acid induced group (964.6 U/L) compared to normal (554.4 U/L). The drug treated Mesalamine microspheres (100mg/kg/day/p.o.) and Mesalamine pure drug (100mg/kg/day/p.o.) treated group showed significant ($p<0.001$) decrease in LDH compared to acetic acid induced group (Figure 8).

Evaluation of mesalamine microspheres on measurement of gsh activity

The reduced glutathione (GSH) concentration in the colon tissues was significantly decreased after induction of IBD as compared to normal control group ($p<0.001$). GSH levels were significantly decreased in colitis compared to control induced group. The depletion of GSH in colitis may be associated to inflammation. The Mesalamine microspheres and Mesalamine pure drug (100mg/kg/day/p.o.) significantly increased GSH content compared with control induced group (Figure 8).

CONCLUSION

The present study concludes that the mesalamine pH dependent 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* studies. Macroscopic, microscopic histopathological and biochemical results confirmed the optimized mesalamine pellets to reduce inflammation in IBD induced rat colon. Thus, the designed formulation could 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.

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