Optimization of Budesonide pH Dependent Coated Pellets for Potential Colon Targeted Drug Delivery

¹Hemant H. Gangurde, ²Mayru A. Chordiya, ¹S. Tamizharasi and ¹T. Sivakumar

¹Department of Pharmaceutics, NANDHA College of Pharmacy, Perundurai Road, Erode, Tamil nadu, India

²Department of Pharmaceutics, C.L. Baidmehta College of Pharmacy, Chennai, Tamil nadu, India

ABSTRACT

Aim of study: Design and optimize budesonide loaded controlled release enteric coated pellets for colon targeted drug delivery. The need of this formulation is to release drug specifically and slowly in proximal colon without being released in upper gastrointestinal tract. **Methods:** Preliminary experimental batches was prepared and studied for micromeritic properties and in vitro drug release. Formulation that showed desirable lag time and dissolution profile were further optimized by applying 3² full factorial design to study the effect of extent of coating with Eudragit S100 and HPMC K100M (% w/w) over drug layered pellets were selected as independent variables X1 and X2 respectively and two responses Y1 and Y2 were studied as lag time of 5 h and 90% of drug release within 12 h after lag time as Q5 and Q90, respectively. The regression equation generated for Q5 = $+11.03-5.57*A-2.55*B$ and for Q90 = $+17.22+3.67*A+1.50*B-0.75*A*B$. **Results:** The drug release data of optimized formulation were close to that predicted by the model. The formulation containing 38.51%w/w Eudragit S100 and 10.40%w/w HPMC K100M was found to be optimum. The results of this study revealed that factorial design is a suitable tool for optimization of coating formulations to achieve colon delivery. **Conclusion:** The present study demonstrates that the budesonide enteric coated controlled release pellets could be successfully targeted for colonic delivery.

Key words: Colon delivery, statistical optimization, budesonide, eudragit S100, HPMC K100M, factorial design

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INTRODUCTION

From last few decades, colon targeted drug delivery systems (CTDDS) have achieved wide focus over the variety of formulations. To achieve an optimum therapeutic efficacy, the delivery system must convey the drug in adequate concentration and without significant loss before reaching to the proximal part of colon. Unfortunately, most of currently available therapeutic strategies lack this selectivity. ¹CTDDS has gained great significance not just for the delivery of drugs for topical treatment of local colon diseases like Inflammatory Bowel Diseases (IBD), inflammatory bowel syndrome (IBS) etc., for the delivery of proteins and peptides² but also for its potential delivery in diseases that have peak symptoms in early morning and exhibits circadian rhythm is evident like asthma, rheumatic disease etc.^{3,4}.

The pH of the gastrointestinal tract (GIT) gradually increases as one move down the tract from stomach (pH 1-3) to terminal ileum (pH 7-8). On entry to the colon, the pH drops to 5.5-7. Because the pH in the

terminal ileum and colon is higher than in any other region of the gastrointestinal tract, the dosage forms which disintegrate at suitably high pH levels have the potential for site-specific delivery into this region⁵.

IBD is an idiopathic inflammatory disorder involving the mucosa and sub-mucosa of the colon. In humans, the majority of IBD occurs in a variety of forms, the most mutual being Crohn's disease (CD) and ulcerative colitis (UC)⁶. In UC, inflammation is classically limited to the colon and is usually continuous, starting at the rectum. The endoscopic features of ulcerative colitis include ulcers, erythema and loss of vascular pattern, friability, spontaneous bleeding and pseudopolyps. Histologically, inflammation in ulcerative colitis is confined to the mucosa; other histological features include crypt distortion, crypt abscesses, goblet cell depletion and rarely mucin granulomas^{7,8}. In contrast to UC, CD affects any region of the gastrointestinal tract and is characteristically segmental with areas of sparing throughout the gastrointestinal tract. Compared to UC, inflammation in CD affects all layers of the bowel. Gross inspection of the bowel in well established CD demonstrates marked

Corresponding Author: Hemant H. Gangurde, Department of Pharmaceutics, NANDHA College of Pharmacy, Perundurai Road, Erode, Tamil nadu, India Tel: +91 9421601654

wall thickening from chronic transmural inflammation accompanied by luminal narrowing⁹.

Multiple approaches have been reported over the last few decades, in order to achieve specific colonic drug delivery. Most of the literatures on CTDDS have motivated on the development of a colonic delivery system based on pH dependent, time-dependent delivery or microflora activated systems¹⁰. Among all these approaches, the pH dependent systems is simple and suitable and have found practical application in the field of research¹¹. This delivery system, by means of controlled release mechanism, hardly releases drug in the upper part of the Gastrointestinal Tract (GIT) but releases drug in the colon following oral administration. In those studies immediate release and pH-dependant polymers have been applied as separate coating layers on top of each other¹².

Multiparticulate approach executed for colonic delivery includes formulations with smaller particle size compared to single unit dosage forms are capable of passing through the GIT easily, leading to low inter and intra subject variability and have potential benefits. Applying different coating materials onto multiparticulate, various desirable functional properties such as resistance to acid^{13,14}, sustained release and targeted release² may be obtained. The coating thickness is also one important factor for the coating systems. Therefore, evaluation and optimization of two factors is useful to achieve the best pH-dependent colonic drug delivery. Optimization with factorial designs is a powerful, efficient and systemic tool that shortens the time required for the development of pharmaceutical dosage forms and improves research and development work¹⁵. The response surface method has been applied to dosage form design for various kinds of drugs by many researchers¹⁶.

Budesonide (BSD) is a potent, synthetic non-halogenated corticosteroid with high topical anti-inflammatory effect and little systemic effects. BSD exhibits potent glucocorticoid activity and weak mineralocorticoid activity for the treatment of asthma, non-infectious rhinitis and for treatment and prevention of nasal polyposis. Additionally, BSD has low incidence of adverse effects and high topical effects and has important suggestions in the pharmacotherapy of IBD, both in the treatment of UC and CD. Different formulations of BSD in treatment of IBD are more effective with direct delivery of drugs to the affected area. It was found that less than 5% of the drug was available beyond the ileum and cecum and therefore, colonic delivery still needs to be optimized by a more reliable colon-specific system^{17,18,19}.

The current perspective of this study is to prolong the release of the corticosteroid anti-inflammatory BSD

Fig. 1: The basic structure of the optimized film coated pellets

to the colon using pH dependent polymeric system. This system is designed in such a way that it will stay intact and enables the drug to be delivered through out the proximal colon. The two coats which enclose middle layer of HPMC K100M and an outer most layer of Eudragit S100 shown in the Fig. 1. Eudragit to provide complete protection over the upper GIT including the small intestine and HPMC K100M release drug in delayed manner aimed to release most of drug to the colonic region in order to provide effective treatment for IBD. This approach can reduce side effects of the drug and increase its effectiveness in the site of action.

MATERIALS AND METHODS

Materials: Budesonide 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). HPMC K5M, HPMC K100M 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 Pharmacopeial grade.

Methods

Experimental design: To optimize the selected formulation of preliminary experimental batch, 3² full factorial design was executed. The independent variables were extent of Eudragit S100 coating (X1) and extent of coating of HPMC K100M (X2). The dependent variables (responses) Y1 = Q5 (lag time of 5 h) and Y2 = Q90 (90% of drug release within 12 h 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.

Preparation of drug-layered pellets: Drug loaded pellets were prepared by spraying drug solution over celpheres by fluidized bed coating technique.

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	40	50	Y1 = Q5 (lag time of 5 h)
X2 = Extent of HPMC K100M coating (% w/w)	8	10	12	Y2 = Q90 (90% of drug release within 12 h after lag time)

Table 2: Composition of experimental formulations (runs)

Batch No.	Extent of Eudragit S100 coating (%w/w)	Extent of HPMC K100M coating (%w/w)
D1	30	8
D2	40	8
D3	50	8
D4	30	10
D5	40	10
D6	50	10
D7	30	12
D8	40	12
D9	50	12

Budesonide was homogeneously dispersed in isopropyl alcohol containing polyvinyl pyrrolidone (PVP K30) as binder and talc as antiadhering agent stirring with a magnetic stirrer. The drug dispersion was passed through a 100 mesh sieve. The drug dispersion was then sprayed on celpheres using the fluidized bed coater, bottom spray (Miniglatt, Germany) with a 0.5 mm nozzle at a feed rate of 0.5-2 mL min⁻¹ 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 HPMC K100M and further with Eudragit S 100. The composition of drug loaded pellets and other processing parameters utilized for coating method are listed in Table 3 and 4, respectively.

HPMC K100M non aqueous coating over drug layered pellets:

In order to control the release rate of drug, a layer of release retardant polymer HPMC K100M high viscosity was applied over the drug layered pellets by fluidized bed coating technique. HPMC non aqueous coating solution was prepared by mixing required amount of HPMC were gradually added to the isopropyl alcohol with continuous agitation. A portion of Methylene chloride is added to solubilize the polymer and propylene glycol as plasticizer was then added stirring with a magnetic stirrer. After layering, the pellets were gently fluidized for 15 min and then kept in hot air oven for drying purpose for 30 min at 50°C. Coating composition and processing parameters are listed in Table 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

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

Ingredients	Quantities
Composition of drug layering solution	
Budesonide (g)	0.5
Polyvinyl pyrrolidone K30 (g)	3.5
Talc (g)	2
Isopropyl alcohol (mL)	100
Composition of HPMC K100M coating solution	
HPMC K100M (%)	1
Propylene glycol (%)	0.5
Isopropyl alcohol (%)	45
Methylene chloride (%)	q.s. 100
Composition of pH sensitive Eudragit S100 coating solution	
Eudragit S100 (g)	2.5
Triethyl citrate (g)	0.25
Acetone (g)	13.696
Isopropyl alcohol (g)	20.568
Purified water (g)	1.712

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

Process parameters	Drug layering	Release retardant coating	Enteric coating
Celpheres bed size (g)	50	50	50
Spray rate (g min ⁻¹)	1-2.5	1.0-1.5	0.6-2.5
Air flow (bar)	0.3-1.5	2-2.5	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)	50-60	40-50	35-45
Product temperature (°C)	35-40	35-40	30-35

and stirred the solution for few minutes²⁰. This solution was sprayed over the above processed drug layered HPMC 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 Table 3 and 4, respectively. In preliminary studies, it was identified two most important factors affecting budesonide release from pellets extent of coating with HPMC K100M (% w/w) and extent of Eudragit S100coating (% 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 ratio²¹ and the pellet circularity²² 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 9 mg of budesonide 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 $37 \pm 0.5^\circ\text{C}$ in media with 1.2 (HCl 0.1 N), 7.4 and 6.8 pH (phosphate buffer) for 2 and 3 h till the end of the test, respectively. About 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 budesonide by spectrophotometer (JASCO V630, Japan) at wavelength at 247 nm for acidic medium and at 245 nm for phosphate buffer²³. The dissolution data was analyzed to calculate % 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 $1000\times$ ²⁴.

Differential scanning calorimetry (DSC): The possibility of any interaction between budesonide, 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 $10^\circ\text{C min}^{-1}$ conducted over a range of 0–300°C under an inert atmosphere flushed with nitrogen at a rate of 20 mL min^{-1} .

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 K α radiation, voltage 40 kV and a current of 20 mA. The

scanning rate employed was 1°C min^{-1} over the 5° to 50° diffraction angle (2θ) range. The XRD patterns of drug powder, polymer and drug-loaded microspheres were recorded.

Fourier-transform infrared spectroscopy: Drug-polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug, polymer and drug-loaded microspheres using FTIR spectrophotometer (Jasco FTIR-410). Samples were prepared by KBr disk method and the scanned over the range of 400–4000 cm^{-1} and the resolution was 2 cm^{-1} .

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 8.0.7.1; 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.

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

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 90% drug release within 12 h after lag time. The fitted equations relating the response Y1 and Y2 to the transformed factor final equation in terms of coded factors are shown in Eq. 2 and 4, respectively and final equation in terms of actual factors are shown in Eq. 3 and 5, 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.

RESULTS AND DISCUSSION

Original structure/shape of the coated pellets: The drug layered and polymer coated budesonide pellets were effectively developed using fluidized bed bottom spray Glatt coating process. In drug loading step, the process had an efficiency of ~90% and ~85-90% 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 Fig. 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 1.08 and 0.94, respectively. The value of aspect ratio and pellet circularity closer to 1 indicates that the shape of Eudragit S100 coated pellets closer to spherical. 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.

The drug-loaded pellets were coated with successive layers of polymer HPMC K100M and Eudragit S100 respectively. In order to retard the release of drug, a layer of HPMC K100M was applied over the drug layered pellets, when it comes in the contact of aqueous medium will swell and form viscous layer by absorbing water, thus leads to slowdown the drug release. 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 HPMC from which it was confirmed that after addition of water the area of pellet was increased from. One can

also see that an opaque viscous 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 1.2, 7.4 and 6.8 pH were sequentially used referred to as sequential pH change method²⁵. 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:

Final equation in terms of coded factors:

$$Q5 = +11.03 - 5.57 * A - 2.55 * B \quad (2)$$

Final Equation in Terms of Actual Factors:

$$Q5 = +46.10944 - 0.55750 * \text{Eudragit} - 1.27750 * \text{HPMC} \quad (3)$$

Final Equation in Terms of Coded Factors:

$$Q90 = +17.22 + 3.67 * A + 1.50 * B - 0.75 * A * B \quad (4)$$

Table 5: Observed values for determination of shape of pellets

Parameters	Observed values
$d_{\max}(\mu\text{m})$	115.1
$d_{\min}(\mu\text{m})$	106.2
Projected area (m^2)	35082.92
Perimeter (μm)	683.89
Aspect ratio	1.08
Pellet circularity	0.94

Fig. 2(a-b): Microscopic swelling study of HPMC K100M coated pellet (a) Pellet before addition of water droplet and (b) Pellet after addition of water droplet Magnification 40X

Final equation in terms of actual factors:

$$Q90 = -19.944 + 0.74167 \cdot \text{Eudragit} + 2.2500 \cdot \text{HPMC} - 0.037500 \cdot \text{Eudragit} \cdot \text{HPMC} \quad (5)$$

The above equation 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 Fig. 3 and 4. Fig. 3a and b shows the effect of two formulation factors on lag time and indicates that increase in coating level of Eudragit S100 rises lag time

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	225.65	2	112.83	7.43	0.0238	Significant
A-Eudragit S100	186.48	1	186.48	12.29	0.0127	
B-HPMC K100M	39.17	1	39.17	2.58	0.1593	
Residual	91.07	6	15.18	-	-	
Cor total	316.72	8	-	-	-	
Analysis of Variance for Y2 (90% of drug release within 12h after lag time)						
Model	96.42	3	32.14	141.10	< 0.0001	Significant
A-Eudragit S100	80.67	1	80.67	354.15	< 0.0001	
B-HPMC K100M	13.50	1	13.50	59.27	0.0006	
AB	2.25	1	2.25	9.88	0.0256	
Residual	1.14	5	0.23	-	-	
Cor total	97.56	8	-	-	-

Fig. 3(a-b): (a) Q5 2-D contour plot and (b) Q5 3D surface response curve

Fig. 4(a-b): (a) Q90 2-D contour plot and (b) Q90 3D surface response curve

significantly. It was observed from the response curves and contour plots in Fig. 4 a and b for both responses that increasing level of coating of Eudragit S100 retard the water uptake and thus prolongs drug release time while increase in level of HPMC due to formation of swelled and thick viscous layer helps in releasing drug slowly and in controlled manner.

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 Q5 and Q90. The optimum formulation was selected based on the criteria of attaining the target value of Q5 and Q90. The optimized formulation was evaluated for lag time and percentage drug release within 12 h after lag time. The cumulative drug release summary of different enteric coated formulations was given in Fig. 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.

The linear correlation plots drawn between the predicted and actual (experimental) values for all the batches of the optimization formulation shown in Fig. 6a 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. Three solution formulations were found (Table 9), out of which the best conditions to optimize drug release corresponded to coating level 38.51% of Eudragit S100 and a coating level of 10.40% of HPMC K100M. By substituting X1 and X2 by the amounts of optimized formulation in Eq. 3 and 5, predicted responses were obtained. In order to check the validity of the optimization procedure, a new batch of pellets with the predicted levels was

Fig. 5: Drug release profile of optimization batches D1-D9

Fig. 6(a-b): (a) Predicted Vs Actual response for Q5 response and (b) Predicted Vs Actual responses for Q90 response

Table 7: Micromeritic properties of optimization batches D1-D9

Formulation code	Bulk density (g mL^{-1}) ^x	Tapped density (g mL^{-1}) ^x	Angle of repose ^x	Hausner ratio ^x
D1	0.871 ± 0.12	0.937 ± 0.16	28.01 ± 0.39	1.11 ± 0.20
D2	0.864 ± 0.15	0.941 ± 0.20	26.85 ± 0.42	1.12 ± 0.18
D3	0.794 ± 0.11	0.845 ± 0.14	25.38 ± 0.35	1.06 ± 0.18
D4	0.817 ± 0.14	0.857 ± 0.15	24.03 ± 0.91	1.04 ± 0.22
D5	0.814 ± 0.15	0.864 ± 0.18	25.94 ± 0.83	1.06 ± 0.31
D6	0.841 ± 0.18	0.894 ± 0.13	27.04 ± 0.81	1.06 ± 0.19
D7	0.868 ± 0.10	0.876 ± 0.15	24.38 ± 0.62	1.00 ± 0.25
D8	0.874 ± 0.17	0.885 ± 0.12	25.57 ± 0.92	1.01 ± 0.19
D9	0.812 ± 0.13	0.843 ± 0.10	26.49 ± 0.73	1.03 ± 0.34

^x Mean n=3

Table 8: Predicted and observed response variables of optimization formulations

Responses	Formulation	Actual value	Predicted value	Prediction Error* (%)
Q5	1	25.88	19.16	* 5.73
	2	8.80	13.59	-1.015
	3	7.87	8.01	-0.041
	4	14.90	16.61	-0.299
	5	8.14	11.03	-0.269
	6	6.50	5.46	0.179
	7	12.16	14.05	-0.553
	8	9.94	8.48	0.254
	9	5.12	2.90	0.656
Q90	1	11.00	11.31	-1.826
	2	16.00	15.72	0.399
	3	20.00	20.14	-0.730
	4	14.00	13.56	0.697
	5	17.00	17.22	-0.160
	6	21.00	20.89	0.153
	7	16.00	15.81	1.053
	8	18.00	18.72	-1.633
	9	22.00	21.64	* 2.33

Table 9: Optimized solution batches produced by Design expert software

Solutions No.	Eudragit S100 (%)	HPMC K100M (%)	Lag time (%)	90 % drug release (h)	Desirability	
1	38.51	10.40	11.3513	17	0.957	Selected
2	38.45	10.43	11.3514	17	0.957	
3	38.59	10.37	11.3515	17	0.957

Fig. 7: Cumulative % Drug Release profile of Budesonide optimized solution batch

prepared. The result shows that the observed responses were inside the constraints and close to predicted responses and, therefore, factorial design is valid for predicting the optimum formulation. The values of predicted and observed responses are shown in Table 8. The pellets were prepared according to optimum solution formulation batch and released negligible drug at pH 1.2 (0.1 N HCl) and pH 7.4 and showed controlled release at pH 6.8 (Fig. 7).

Scanning electron microscopy (SEM): The spherical nature of drug layered pellets also confirmed with the images taken by scanning electron microscopy Fig. 8 a, b, c and d. SEM study at X30, 30,500 and 1000 magnifications revealed that the drug layered pellets are dense with wrinkled, smooth circumference which is

due to gradual loss of water during drug layering process from the surface of pellets.

Differential scanning calorimetry (DSC): The DSC thermogram shows a sharp endothermic peak at 261°C for budesonide. While in final optimum formulation containing drug and polymer, the endothermic peak was observed at 262.45°C which was as same as close to pure drug (Fig. 9 B1 and B2). Evaluation and interpretation of the thermogram revealed no interaction between the drug and polymer in the optimized formulation.

X-ray diffractometry (XRD): Characteristic crystalline peak of budesonide (B1) was retained in the XRD of optimized formulation (B3) which suggested

Fig. 8(a-d): Scanning electron micrographs of Budesonide layered HPMC K100M and Eudragit S100 coated pellets (a): at X30, (b): at X30, (c): at X500 and (d): at X1000 magnification

Fig. 9(a-b): (a) DSC thermograph of budesonide (B1) and (b) Optimized formulation (B2)

Fig. 12: FT-IR peaks of compatibility study data

(2949), C-H Aromatic (3170) etc were seen in the subsequent spectra shown in Fig. 12.

CONCLUSION

The present study concludes that the budesonide pH dependant controlled release could be successful option for colon targeting by achieving the desired lag time. The optimization study using 3^2 full factorial design revealed that the extent of 38.51% of Eudragit S100 and a coating level of 10.40% of HPMC K100M over drug layered pellets had significant effect on lag time and pattern of release of drug from the multiparticulate dosage form. Lag time and targeted release was observed for optimized formulation. Acceptable results were found from evaluation of micromeritic parameters such as flow property, shape of pellets and surface topography. Thus, the designed formulation can be considered as one of the promising formulation technique for preparing a promising colon targeted drug delivery in management of IBD and other colon diseases.

REFERENCES

1. Lamprecht, A., 2003. Multiparticulate systems in the treatment of inflammatory bowel disease. *Curr. Drug Targets Inflamm. Allergy*, 2: 137-144.
2. Gupta, V.K., T.E. Beckert and J.C. Price, 2001. A novel pH-and time-based multi-unit potential colonic drug delivery system. I. Development. *Int. J. Pharm.*, 213: 83-91.
3. Halsas, M., J. Hietala, P. Veski, H. Jurjenson and M. Marvola, 1999. Morning versus evening dosing of ibuprofen using conventional and time controlled release formulations. *Int. J. Pharm.*, 189: 179-185.
4. Halsas, M., T. Penttinen, P. Veski, H. Jurjenson and M. Marvola, 2001. Time controlled release pseudoephedrine tablets: Bioavailability and *In vitro/In vivo* correlations. *Die Pharmazie*, 56: 718-723.
5. Evans, D.F., G. Pye, R. Bramley, A.G. Clark, T.J. Dyson and J.D. Hardcastle, 1988. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. *Gut*, 29: 1035-1041.

6. Klein, S., J. Stein and J. Dressman, 2005. Site-specific delivery of anti-inflammatory drugs in the gastrointestinal tract: An In-vitro release model. *J. Pharm. Pharmacol.*, 57: 709-719.
7. Kugathasan, S., R.H. Judd, R.G. Hoffmann, J. Heikenen and G. Telega *et al.*, 2003. Epidemiologic and clinical characteristics of children with newly diagnosed inflammatory bowel disease in Wisconsin: A statewide population-based study. *J. Pediatr.*, 143: 525-531.
8. Hildebrand, H., B. Fredrikzon, L. Holmquist, B. Kristiansson and B. Lindquist, 1991. Chronic inflammatory bowel disease in children and adolescents in Sweden. *J. Pediatr. Gastroenterol. Nutr.*, 13: 293-297.
9. Farmer, R.G., W.A. Hawk and R.B. Jr. Turnbull, 1975. Clinical patterns in Crohn's disease: A statistical study of 615 cases. *Gastroenterology*, 68: 627-635.
10. Gangurde, H.H., M.A. Chordiya, S. Tamizharasi and T. Sivakumar, 2012. Diseases, approaches and evaluation parameters for colon specific drug delivery: A review. *Int. J. Drug Res. Tech.*, 2: 239-262.
11. Ashford, M. and J.T. Fell, 1993. Colonic delivery of drugs: Current status on targeted drug delivery to gastrointestinal tract. Short Hills, Capsugel, Symposia Series, pp: 133-142.
12. Fukui, E., N. Miyamura, K. Uemura and M. Kobayashi, 2000. Preparation of enteric-coated timed release press-coated tablets and evaluation of their function by In vitro and In vivo tests for colon targeting. *Int. J. Pharm.*, 204: 7-15.
13. Rodriguez, M., J.L. Vila-Jato and D. Torres, 1998. Design of a new multiparticulate system for potential site specific and controlled drug delivery to the colonic region. *J. Control. Release*, 55: 67-77.
14. Akhgari, A., H.A. Garekani, F. Sadeghi and M. Azimaie, 2005. Statistical optimization of indomethacin pellets coated with pH-dependent methacrylic polymers for possible colonic drug delivery. *Int. J. Pharm.*, 305: 22-30.
15. Huyghebaert, N., A. Vermeire and J.P. Remon, 2005. In vitro evaluation of coating polymers for enteric coating and human ileal targeting. *Int. J. Pharm.*, 298: 26-37.
16. Gohel, M.C. and A.F. Amin, 1998. Formulation optimization of controlled release diclofenac sodium microspheres using factorial design. *J. Control. Release*, 51: 115-122.
17. Fedorak, R.N. and L. Bistriz, 2005. Targeted delivery, safety and efficacy of oral enteric-coated formulations of budesonide. *Adv. Drug Delivery Rev.*, 57: 303-316.
18. McKeage, K. and K.L. Goa, 2002. Budesonide (Entocort EC Capsules): A review of its therapeutic use in the management of active Crohn's disease in adults. *Drugs*, 62: 2263-2282.
19. Krishnamachari, Y., P. Madan and S. Lin, 2007. Development of pH and time dependent oral microparticles to optimize budesonide delivery to ileum and colon. *Int. J. Pharm.*, 338: 238-247.
20. Shivkumar, H.N., S. Suresh and B.G. Desai, 2006. Design and evaluation of pH sensitive multi-particulate systems for chronotherapeutic delivery of Diltiazem hydrochloride. *Ind. J. Pharm. Sci.*, 68: 781-787.
21. Krogars, K., J. Heinamaki, J. Vesalahti, M. Marvola, O. Antikainen and J. Yliruusi, 2000. Extrusion-spheronization of pH-sensitive polymeric matrix pellets for possible colonic drug delivery. *Int. J. Pharm.*, 199: 187-194.
22. Alvarez, L., A. Concheiro, J.L. Gomez-Amoza, C. Souto and R. Martinez-Pacheco, 2003. Powdered cellulose as excipient for extrusion-spheronization pellets of a cohesive hydrophobic drug. *Eur. J. Pharm. Biopharm.*, 55: 291-295.
23. Gang, C., F. An, M.J. Zou, J. Sun, X.H. Hao and Y.X. He, 2004. Time and pH-dependent colon-specific drug delivery for orally administered diclofenac sodium and 5-amino salicylic acid. *World J. Gastroenterol.*, 10: 1769-1774.
24. Jantzen, G.M. and J.R. Robinson, 1996. Sustained and Controlled-Release Drug Delivery Systems. In: *Modern Pharmaceutics*, Banker, G. and C. Rhodes (Eds.). 3rd Edn., Marcel Dekker, New York, pp: 575.
25. Rao, K.P. and C.C. Patil, 2007. Formulation and evaluation of colon targeted oral tablets of naproxen. *Ind. J. Pharm. Educ. Res.*, 41: 146-150.