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Research Article

Formulation and Evaluation of Colon Targeted Rifaximin Drug Delivery System.

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ABSTRACT
The present work was aimed at developing a mucoadhesive colon targeted therapeutic drug delivery system for local efficacy of Rifaximin in multiparticulate oral dosage form by using different excipients. A 3^2 full factorial design with two different independent variables namely RPM of spheronizer and different quantity of Eudragit RLPO were used. The formulation was characterized on different evaluation parameters such as flow properties, friability, ex-vivo residence time, ex-vivo mucoadhesion study, % swelling index, in-vitro drug release and short-term accelerated study. There was no significant change in the pellets property after the stability period & pellets were found to be stable. Statistical analysis of percentage cumulative drug release irrespective of time at 7.4 pH in 0.1 M sodium phosphate buffer shows that, as the spheronizer speed and amount of Eudragit RLPO increases, % drug release decreases. From the results obtained, it can be concluded that 2095 RPM spheronizer and 0.7 gm Eudragit RLPO gives optimum values. Colon targeted drug delivery achieved with Eudragit S100 Coating.

KEYWORDS
Mucoadhesive, Extrusion spheronization, Rifaximin, Polymers

1. INTRODUCTION

Inflammatory bowel disease (IBD) is characterized by a spectrum of chronic inflammation and includes Ulcerative colitis (UC) and Crohn's disease (CD) [1].

There are reported links of Genetic polymorphisms related to Crohn's disease with abnormal bacterial composition (e.g. dysbiosis of the gut microbiota), thought to be through defects in innate immune responses and Paneth cell function [2–4]. In genetically susceptible individuals, dysbiosis of the gut microbiota and an abnormal immune response have been thought to play a role in the pathogenesis of Crohn's Disease [5–8]. These genetic factors associated with Crohn's disease also lead to abnormal responses of immune system and poor microbial clearance due to abnormal mucosal barrier function. Moreover, inappropriate containment of bacteria because of reduction in antimicrobial peptides and increased mucosal permeability adds to the pathophysiology of Crohn's Disease [9-10].

A targeted gut-specific antibiotic Rifaximin could provide an alternate to systemic delivery of antibiotics and immunosuppressive agents when suitable, since Rifaximin is known for its minimal systemic absorption, relatively favourable safety profile and superior preliminary efficacy data of delayed release 800mg with placebo which showed significant clinical remission [11-14].

In context of above results reported for Rifaximin and to explore this concept further, we have developed pellets of Rifaximin complexed with beta-cyclodextrin and using varying amount of Eudragit RLPO in a single dosage form wherein Eudragit RLPO is a sustained release polymer.

2. MATERIALS AND METHODS

2.1 Materials

Rifaximin, Eudragit RLPO and β-Cyclodextrin were received as a gift samples from Amneal Pharmaceuticals Ltd., Ahmedabad, India. Microcrystalline cellulose, polyvinyl pyrrolidone K-30 (PVP K-30), Carrageenan and magnesium stearate were generous gift samples from Shital Chemicals Ltd., India. All other chemical and reagent were of analytical grade and used as received.

2.2 Experimental Methodology

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of pure Drug and obtained by FTIR-8400S, CE (Shimadzu, Japan) spectrophotometer. The procedure consisted of dispersing samples with KBr and compressing into disc by applying a pressure for 5 min in a hydraulic press. The drug was placed in the light path and the scanning range used was 4000 to 400 cm-1 to obtain spectra.

2.3 Preparation of dosage form

2.3.1 Experimental Design

Formulation Optimization by using 3^2full factorial design.

A 3^2full factorial design was used to quantify the significant independent variables revealed from preliminary studies. In this design 2 factors were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations generated by Design Expert 11. As shown in Table no.17, in that two independent variables namely X1 (RPM of Spheronizer) & X2

(Different quantity of Eudragit RLPO). Y1 (Mucoadhesive Strength), Y2 (% Swelling Index), Y3 (% Drug release in 7.4 pH 0.1 M Sodium phosphate buffer at 6 hours), Y4 (%Drug release in 7.4 pH 0.1 M Sodium phosphate buffer at 30 mins), were selected as a dependent variable. On the bases of preliminary batches results, the low, medium and high values of independent variables were selected and the batches from F1to F9 were formulated.

Table 1. Composition Table of Factorial Batches.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
	Quantity (gm)								
Drug	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
MCC	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Carrageenan	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Eudragit RLPO	0.7	0.7	0.7	1.0	1.0	1.0	1.3	1.3	1.3
β-cd	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
10% PVP K30 IPA	3 ml	3 ml	3 ml	3 ml	3 ml	3 ml	3 ml	3 ml	3 ml
Glycerin	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
RPM	2000	2200	2400	2000	2200	2400	2000	2200	2400

Table 2. Coded and actual Value of Formulations.

Formulation Code	Coded Values		Actual Values		Dependent Variable			
	(RPM)	**X_2 (Eudragit RLPO conc.)**	**X_1 (RPM)**	**X_2 (Eudragit RLPO Quantity (gm))**	**Mucoadhesion Strength (Y_1)**	**% Swelling Index (Y_2)**	**% CDR at 6 hours in 7.4 pH 0.1 M Sodium Phosphate buffer (Y_3)**	**% CDR at 30 min in 7.4 pH 0.1 M Sodium Phosphate buffer (Y_4)**
F1	-1	-1	2000	0.7	0.137	50.5	91.10	38.82
F2	0	-1	2200	0.7	0.140	48.2	90.55	37.15
F3	+1	-1	2400	0.7	0.142	50.0	89.13	36.79
F4	-1	0	2000	1.0	0.119	43.4	86.49	35.92
F5	0	0	2200	1.0	0.115	44.3	85.23	35.13
F6	+1	0	2400	1.0	0.117	45.8	84.89	33.80
F7	-1	+1	2000	1.3	0.103	35.3	83.46	26.56
F8	0	+1	2200	1.3	0.104	36.2	79.72	26.38
F9	+1	+1	2400	1.3	0.103	37.8	78.14	26.29

% CDR: percentage cumulative drug release

Polynomial equation for 3^2 full factorial designs is:

$$Y= \beta_0+\beta_1 X_1+\beta_2X_2+\beta_{12}X_1X_2+\beta_{11}X_{12}+\beta_{22}X_{22}$$

Where, β_0=Intercept, β_1&β_2 = coefficient of X_1& X_2, β_{12} = coefficient of interaction, β_{11}&β_{22} = coefficient of quadratic terms &X_1& X_2 = Independent variable

2.3.2 Evaluation Parameters

Angle of repose

Angle of repose was determined by measuring the height, radius of the heap of the powder blend. A cut system funnel was fixed to stand and bottom of the funnel was fixed at a height off 2 cm from the plane. Powder blend was placed in funnel and allowed to flow freely and measured the height and radius of the heap [15].

$\tan_\theta$= h/r .... (1)

(Where, h= height of heap, r=radius of heap)

Bulk density

A bulk density is defined as the mass of powder divided by the volume. A bulk density is largely depending on the particle shape, as particles become more spherical in shape, bulk density is increase. In addition, as granules size increases, bulk density decreases. Powder weighing 10 g was placed into 100 ml measuring cylinder. Volume occupied by the powder was noted without disturbing the cylinder and bulk density was calculated in gm/ml by the following equation.

$$\boldsymbol{Bulk\ density} = \frac{\boldsymbol{weight\ of\ powder}}{\boldsymbol{volume\ of\ bulk\ powder\ in\ cylinder}} \quad \textbf{.... (2)}$$

Tapped density

Tapped density is achieved by mechanically tapping a measuring cylinder containing a powder sample. The mechanical tapping is achieved by raising the cylinder and allowing it to drop under own weight a specific distance. Device that rotates the cylinder during tapping may be preferred to minimize any possible separation of the mass during tapping down. Cylinder is tapped 20 times from 2 cm height. The final volume was recorded and the tap density was calculated by the following equation.

$$\boldsymbol{Tapped\ density} = \frac{\boldsymbol{weight\ of\ powder}}{\boldsymbol{volume\ of\ tapped\ powderin\ cylinder}} \quad \textbf{.... (3)}$$

Carr's Index

The percentage compressibility index will be calculated as 100 times the ratio of the difference between tapped density and bulk density to the tapped density. The sample will be graded as shown in table no. 21 below.

$$\boldsymbol{Carr's\ Index} = \frac{\boldsymbol{Tapped\ density - Bulk\ density}}{\boldsymbol{Tapped\ density}} * \textbf{10 .... (4)}$$

Hausner's Ratio

Hausner's ratio is the ratio of tapped density to bulk density. Lower the value of Hausner's ratio, better is the flow property and it will be calculated using the equation given below. Powders will be classified as shown in table no. 22.

$$\textbf{Hausner's ratio} = \frac{\textbf{Tapped density}}{\textbf{Bulk density}} \quad \textbf{.... (5)}$$

Ex-vivo mucoadhesion study

Mucoadhesion is essential for successful application of colonic drug delivery system in order to moderate the residence time at the site and hence to modify the drug release. The *ex-vivo* mucoadhesion strength was performed after application of the intestinal pellets on freshly cut goat mucosa. The fresh goat mucosa was tied on the glass slide, and a mucoadhesive pellets was wetted with phosphate buffer pH 7.4 and adhered to the mucosa by applying light force with a fingertip for 30 seconds. The modified physical balance was adjusted by keeping glass beaker on another side. Water was added by burette and weight of water needed to detach the pellet from goat mucosa was recorded for the measure of mucoadhesive strength in grams [16-17].

$$\textbf{Force of adhesion } (N) = \frac{\textbf{Mucoadhesive strength}}{1000} * 9.81 \textbf{ .... (6)}$$

Ex-vivo residence time

The *Ex-vivo* residence time was studied using a locally modified USP paddle apparatus. The dissolution medium was maintained at 37^{o}C. A segment of goat intestine was glued to the surface of a glass slab, vertically attached to the paddle. The mucoadhesive pellets was hydrated from one surface using phosphate buffer and then the hydrated surface was brought into contact with the mucosal membrane. The glass slide was vertically fixed to the paddle and allows rotating at 50 rpm. The time required for complete detachment of the pellets from the mucosal surface was recorded [18-20].

% Product yield

Yield of pellets, measured by weight of final product divided by initial total weight of powder blend.

Drug Loading

250 mg of pellets was weighed accurately and dissolved in 25 ml of 0.1 M Sodium phosphate buffer. Diluted suitably and drug loading was analyzed at 445.5 nm by UV spectrophotometer. The concentration was calculated using the standard calibration curve of Drug in 0.1 M Sodium phosphate buffer.

Mean particle size

Pellets were dispersed in liquid paraffin and mounted on clean glass slides and placed on the mechanical stage of the microscope (Aatur Instruments, Vadodara). An ocular micrometer was fitted with the microscope which was calibrated with the use of stage micrometer under 10×45 magnification. A particle size of 150 particles was measured using a calibrated stage micrometer and ocular micrometer. From the data, the average particle size was calculated.

Swelling Index (%)

The ability of each pellet to swell in pH 7.4phosphate buffer will determined by allowing them to swell up to their equilibrium. Weighed pellets (W_1) will placed in 25 ml beaker containing 5 ml of pH 7.4 0.1 M Sodium phosphate buffer solution medium of at 37 ± 0.5^{o}C. After 24h, swollen

pellets will withdraw from the medium, will blotted to remove surface water, and weighed (W_2) on a single pan balance.
The swelling index calculated as following equation:

$$\text{Swelling index} = \frac{W_2 - W_1}{W_1} \times 100 \ldots \textbf{(7)}$$

Where, W_1 is initial weight, W_2 is weight after swelling.

Shape
Sphericity or roughness of pellets is determined by using Optical Microscope.
Microstructure and surface
Pellet microstructure and surface morphology are assessed by scanning electron microscopy.
Friability
Pellets friability measured by Roche friabilator.200 mg of pellets will be placed in Roche friabilator for 25 rpm for 4 min.

$$\% \, Friability = \frac{w1-w2}{w1} * 100 \ldots \textbf{(8)}$$

Where, W_1 is initial weight, W_2 is weight retained after 100 revolutions.

Dissolution testing
The pellets were evaluated for the drug release by using standard USP Basket type I apparatus. Pellets containing 400 mg of drug immersed in the dissolution apparatus. First pellets were placed in basket and immersed in pH 7.4 0.1 M Sodium phosphate buffer solution maintained at 37 ± 5^{o}C and rotated at 100 rpm. Sample aliquots of 5 ml will be withdrawn every hour up to 12 h and the drug loading of withdrawn samples will be estimated spectrophotometrically at 445.50 nm. Drug release is assessed by USP-I basket Apparatus.
Short term Accelerated Stability Study
Stability is defined as the extent to which a product retains, within specified limits and throughout its period of storage and use (i.e. its shelf life), the same properties and characteristics that it possessed at the time of its manufacture. Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. Accelerated stability study was carried out for final formulation (n=3) at 40ºC±2 ºC. Samples were withdrawn after one month and analysed for visual appearance, drug release and drug loading.

3. RESULTS AND DISCUSSION

3.1 Drug-excipient interaction study: FTIR Spectral Analysis

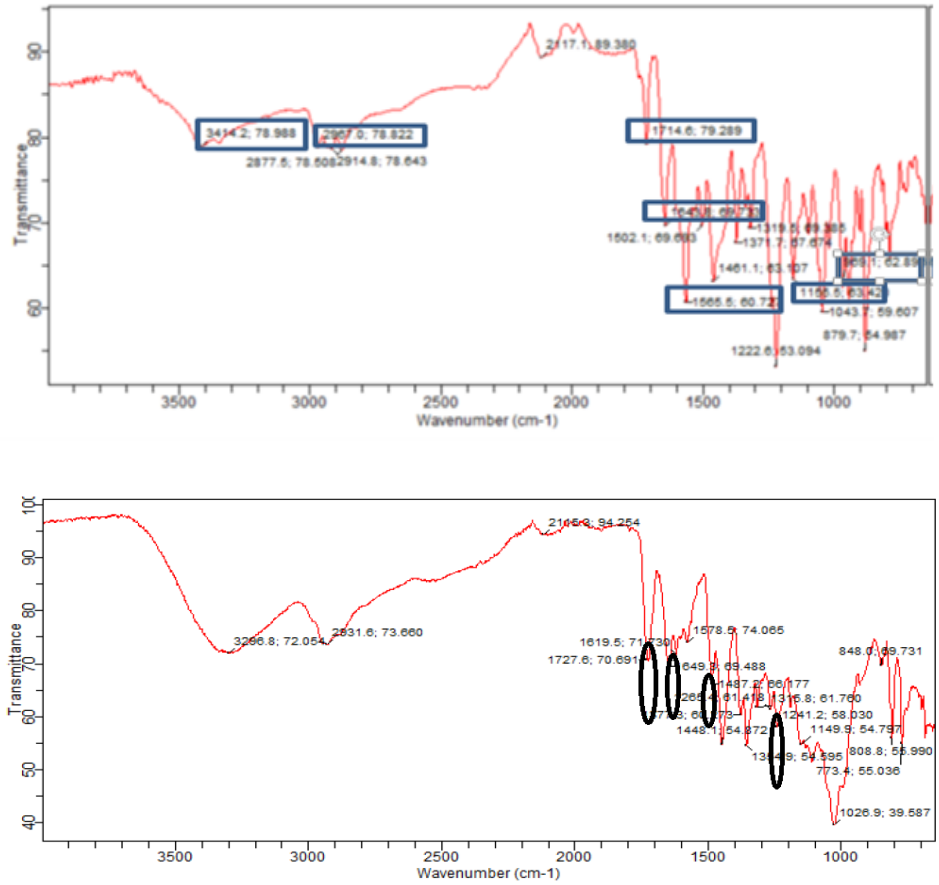

Figure 1: FTIR Spectra of Pure API Rifaximin [A] & Final Composition [B]

3.2 Drug Loading (%)

Drug - β-cyclodextrin Complex's % Drug Loading is about 80.26%.According to calculation of drug loading of β-cyclodextrin 3.72 gm complex contain 400 mg Rifaximin. This would be very high amount of by weight after pellets preparation. So, hereafter it is decided to use β-CD as excipient in pellet formulation directly and to explore its effect on dissolution enhancement.

3.3 Evaluation of Design Batches

Table 3. Post Formulation Parameters of Designed batches.

Batch Code	Bulk Density (gm/cm^3)	Tapped density (gm/cm^3)	% Carr's index	Hausner's ratio	% Friability
F1	0.64	0.71	9.85	1.10	0.98
F2	0.72	0.79	8.86	1.09	0.67
F3	0.70	0.77	9.09	1.10	0.90
F4	0.66	0.73	9.58	1.10	0.95
F5	0.74	0.81	8.64	1.09	0.80
F6	0.73	0.80	8.75	1.09	0.87
F7	0.76	0.84	9.52	1.10	0.92
F8	0.78	0.86	9.30	1.10	0.82
F9	0.75	0.83	9.63	1.10	0.89

From the above table Carr's index and Hausner's ratio were found to be excellent in pellets formulation which represents good flow property and % friability was found to be less than 1% as per IP standard.

3.4 Particle size determination using Microscope (10x)

Particle size determination by using 10x Microscope. Calibration factor was 13.5µm for 1 division.

Table 4. Particle size determination, % Yield&% Drug Loading.

Batches	Particle size range (µm)	% Yield	% Drug Loading
F1	607.5-324.0	83.11	93.00%
F2	580.5-297.0	93.50	94.46%
F3	594.0-310.5	90.90	93.07%
F4	702.0-378.0	82.50	91.66%
F5	675.0-351.0	90.00	92.33%
F6	729.0-405.0	85.00	90.89%
F7	837.0-486.0	87.95	89.07%
F8	810.0-459.0	93.97	89.38%
F9	877.5-526.5	90.36	88.86%

3.5 In-vitro Mucoadhesion study

Mucoadhesive Strength is important prerequisite requirement for colonic mucoadhesive pellets. As the amount of Eudragit RLPO increases mucoadhesive strength was decreased. Extent the drug release up to 12 hours by mucoadhesion.

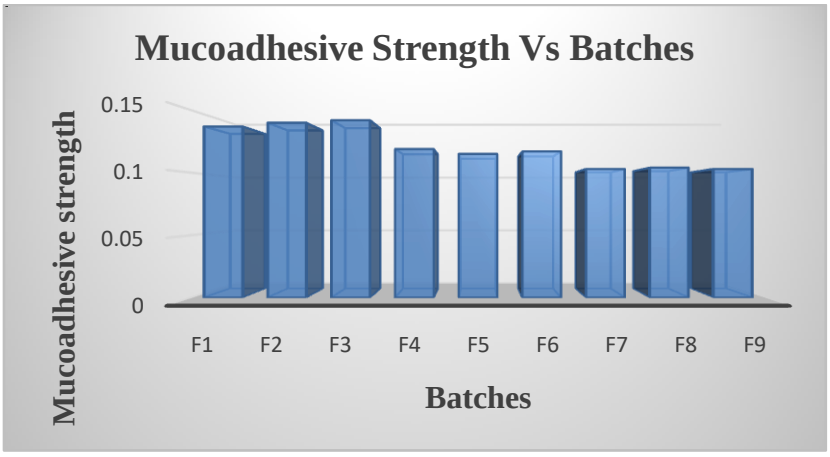

Figure 2. Mucoadhesive Strength for Designed Batches

Swollen mucoadhesive polymers attach to mucosal surfaces. Optimum hydration was required for swelling and swelling property is required for mucoadhesion measurement. Eudragit RLPO was water insoluble polymer. As the amount of Eudragit RLPO increases, swelling index decreases and mucoadhesion decreases. In F1-F3 Batches amount of Eudragit RLPO was 0.7 gm, In F4-F6 Batches amount of Eudragit RLPO was 1.0 gm, In F7-F9 Batches amount of Eudragit RLPO was 1.3. so, in batches F1-F3 optimum hydration was observed with maximum swelling index and maximum Mucoadhesive Strength compare to other batches. In batches F4-F6 moderate hydration was observed with moderate swelling index and moderate mucoadhesive strength. In batches F7-F9 minimum hydration was observed with minimum swelling index and minimum Mucoadhesive strength**.**

3.6Ex-vivoMucoadhesion time

Mucoadhesive residence time is important prerequisite requirement for colonic mucoadhesive pellets. As the amount of Eudragit RLPO increases mucoadhesive time was decreased. Mucoadhesive Residence time is 12 hrs for F1-F3, 11 hrs for F4-F6 & 10 hrs for F7-F9.

3.7 Swelling Index (%)

Swelling is required for the assessment of adhesion.

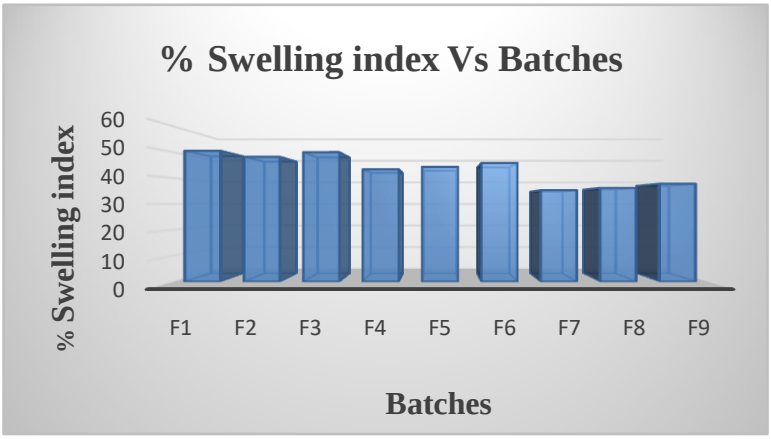

Figure 3. % Swelling Index for Designed Batches

The hydration ability of formulation is important because it influence pellets size, its residence time and its release kinetics. Initially rapid rise in swelling index due to the entry of 7.4 pH 0.1 M Sodium Phosphate Buffer via metastable pores. Eudragit RLPO was water insoluble polymer. As the amount of Eudragit RLPO increases swelling index decreases. Swelling depend on polymer concentration.

In F1-F3 Batches amount of Eudragit RLPO was 0.7 gm, In F4-F6 Batches amount of Eudragit RLPO was 1.0 gm, In F7-F9 Batches amount of Eudragit RLPO was 1.3 gm so, in batches F1-F3 optimum hydration was observed with maximum swelling index. In batches F4-F6 moderate hydration was observed with moderate swelling index. In batches F7-F9 minimum hydration was observed with minimum swelling index. Higher the hydration rate higher the mucoadhesion**,** where the system gets adhering to the mucus membrane of the colon, here the polymer swells and get adhere, adhesion involve formation of chemical or physical bonding between the polymer and surface of mucus membrane, improvement in both topical and systemic treatment in

colonic inflammatory disease is achieve by localized drug delivery there by improving drug resident time.

3.8 In-vitro dissolution study

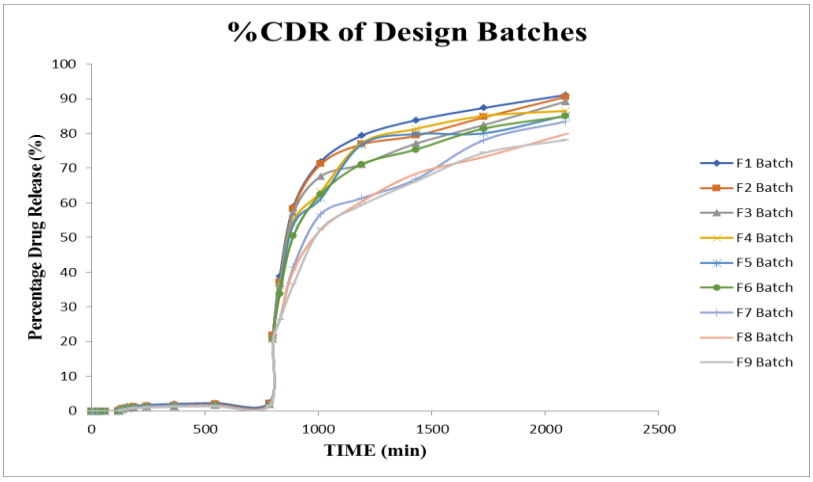

Figure 4. % CDR of Designed batches

Eudragit RLPO used for controlled drug delivery system. According to observation in F1-F3 Batches 0.7 gm Eudragit RLPO was used it gives maximum drug release. In F4-F6 batches 1.0 gm RLPO was used it give moderate drug release. In F7-F9 batches 1.3 gm Eudragit RLPO was used it gives minimum drug release. Eudragit RLPO was release retard polymer. As the % of Eudragit RLPO increase, Drug release decreases. Eudragit RLPO extends the drug release up to 12 Hours but, here as we added β-cd in pellets, drug release is higher. By keeping law concentration of the Eudragit RLPO one could achieve higher % CDR.As the spheronizer speed increases, drug release decreases.

Design Batches Coating done by Eudragit S100

Table 5. %Weight gain after Coating

Batches	Weight of pellets before coating (gm)	Weight of pellets after coating (gm)	% Weight gain
F1	6.4	7.04	10
F2	7..2	7.92	10
F3	7.0	7.70	10
F4	6.6	7.26	10
F5	7.2	7.92	10
F6	6.8	7.48	10
F7	7.3	8.03	10
F8	7.8	8.58	10
F9	7.5	8.25	10

Enteric coating achieved by Eudragit S100. Eudragit S100 was used for targeted drug delivery to the colon. It is insoluble in acidic media, dissolve above pH 7.0.

Eudragit S100 coating was done using Eudragit S100 as a coating polymer with the help of pan coater at 14 RPM.

3.9 Shape and Sphericity (Scanning Electron Microscopy)

Scanning Electron Microscopy was used to determine Sphericity and Shape of Pellets. Scanning electron microscopy was performed on pellets to assess the surface morphology like size and shape. Sample was fixed on an aluminum stub with conductive double sided adhesive tape and coated with gold in an argon atmosphere (50 Pa) at 50mA for 50s. The samples were scanned at a voltage of 5kV.SEM of photographs is taken before coating and after coating. The scanning electron microscopic (SEM) evaluation is important for determining the surface morphology, size, shape. Surface of pellets as shown in SEM photograph was smooth and sphericity was also good and which indicates pellets are spherical in shape. Result of pellets shape and sphericity was determined by Scanning Electron Microscopy which is shown in figure 23, figure 24, figure 25 and figure 26.

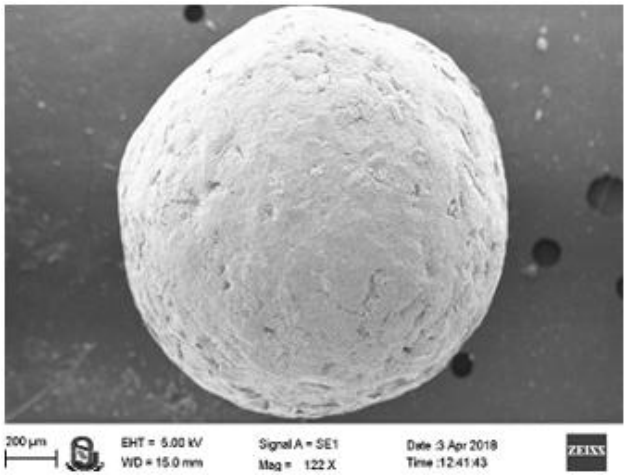

Figure 5. Pellets before Coating.

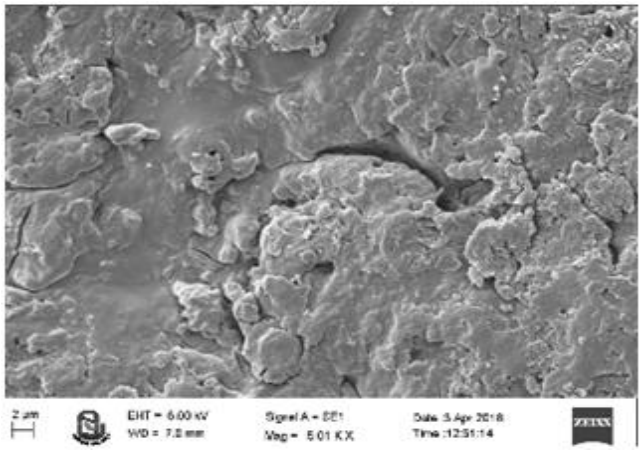

Figure 6. Surface morphology of Pellets (Before Coating)

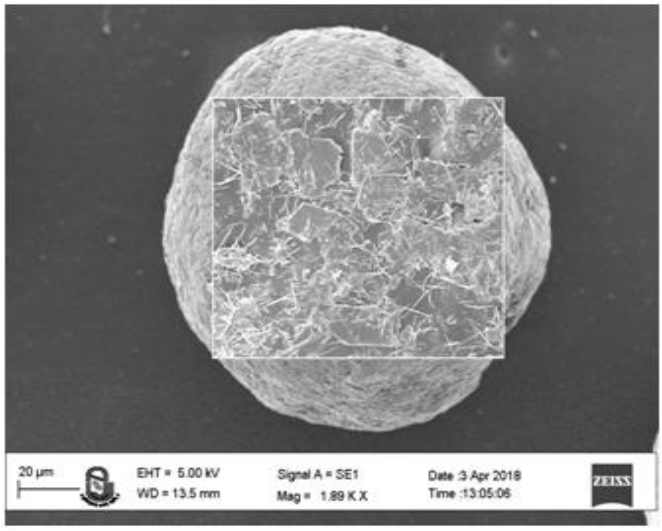

Figure 7. After Eudragit S100 Coating.

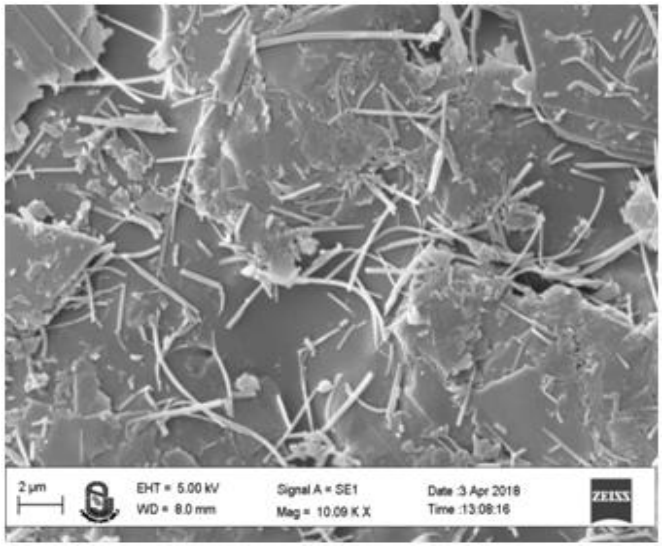

Figure 8. After Eudragit S100 Coating (Surface morphology).

1. Analysis of Experimental Data

The statistical analysis of the factorial design batches was performed by multiplelinear regression analysis. The Mucoadhesive Strength, % Swelling Index, % CDR at 6 hours in 7.4 pH 0.1 M Sodium phosphate buffer, % CDR at 30 min. in 7.4 pH Phosphate buffer was selected as dependent variables. The polynomial equations relating the responses, The Mucoadhesive Strength, % Swelling Index, % CDR at 6 hours in 7.4 pH 0.1 M Sodium phosphate buffer, % CDR at 30 min. in 7.4 pH 0.1 M Sodium Phosphate buffer to the transformed factor are described in table no. 1.

The data transformation simplifies the calculations for model development. The data generated by the experimental design was utilized for drawing contour plot, to obtain an optimized region

within the factorial space, and thereby produce an optimized formulation. Results of all the batches were subjected for regression analysis and the data is shown in tables no. 5, table no.6 and table no 7.

Table 6. Summary of Result of Full model.

	Y_1- Mucoadhesive Strength					
	β_0	**β_1**	**β_2**	**β_{12}**	**β_{11}**	**β_{22}**
Co-efficient	0.1167	0.0005	-0.0182	-0.0013	0.0005	0.0045
P-value	0.0017	0.5988	0.0002	0.3712	0.7572	0.0555
R^2	0.9936					
	Y_2 - % Swelling Index					
	β_0	**β_1**	**β_2**	**β_{12}**	**β_{11}**	**β_{22}**
Co-efficient	43.90	0.7333	-6.57	0.9000	-1.50	0.7500
P-value	0.0021	0.1138	0.0003	0.1618	0.2149	0.0795
R^2	0.9927					
	Y_3- % CDR at 6 hours in 7.4 pH 0.1 M Sodium Phosphate buffer					
	β_0	**β_1**	**β_2**	**β_{12}**	**β_{11}**	**β_{22}**
Co-efficient	85.41	-1.48	-4.91	0.8375	0.3683	-0.1867
P-value	0.0051	0.0236	0.0008	0.1434	0.5833	0.7765
R^2	0.9867					
	Y_4- % CDR at 30 mins in 7.4 pH 0.1 M Sodium Phosphate buffer					
	β_0	**β_1**	**β_2**	**β_{12}**	**β_{11}**	**β_{22}**
CO- efficient	34.85	-0.7367	-5.59	0.4400	0.1433	-2.95
P-value	0.0005	0.0279	< 0.0001	0.1457	0.6832	0.0027
R^2	0.9971					

Table 7. Summary of Result of Reduced Model.

	Y_1- Mucoadhesive Strength					
	β_0	**β_1**	**β_2**	**β_{12}**	**β_{11}**	**β_{22}**
Co-efficient	0.1170	0.0005	-0.0182	-	-	0.0045
P-value	<0.0001	0.5655	< 0.0001	-	-	0.0241
R^2	0.9903					
	Y_2 - % Swelling Index					
	β_0	**β_1**	**β_2**	**β_{12}**	**β_{11}**	**β_{22}**
Co-efficient	43.50	0.7333	-6.57	-	-	-
P-value	< 0.0001	0.2204	< 0.001	-	-	-
R^2	0.9620					
	Y_3- % CDR at 6 hours in 7.4 pH 0.1 M Sodium Phosphate buffer					
	β_0	**β_1**	**β_2**	**β_{12}**	**β_{11}**	**β_{22}**
Co-efficient	85.41	-1.48	-4.91	-	-	-
P-value	< 0.0001	0.0084	< 0.0001	-	-	-
R^2	0.9674					
	Y_4- % CDR at 30 mins in 7.4 pH 0.1 M Sodium Phosphate buffer					
	β_0	**β_1**	**β_2**	**β_{12}**	**β_{11}**	**β_{22}**
CO- efficient	34.95	-0.7367	-5.59	-	-	-2.95

P-value	< 0.0001	0.0196	< 0.0001	-	-	0.0005
R^2	0.9932					

Table 8. Result of ANOVA for reduced model of Dependent Variables.

Source of Variation	DF	SS	MS	F Calculated	F Significant	F Tabulated
	Y_1- Mucoadhesive Strength					
Regression	3	0.0020	0.0007	169.93	< 0.0001	5.40
Residual	5	6.03E-05	3.967E-06			
Total	8	0.0020				
	Y_2- % Swelling Index					
Regression	2	261.95	130.98	75.95	< 0.0001	5.14
Residual	6	10.35	1.72			
Total	8	272.30				
	Y_3- % CDR at 6 hours in 7.4 pH 0.1 M Sodium Phosphate buffer					
Regression	2	157.82	78.91	89.09	< 0.0001	5.14
Residual	6	5.31	0.8857			
Total	8	163.13				
	Y_4- % CDR at 30 mins in 7.4 pH 0.1 M Sodium Phosphate buffer					
Regression	3	208.06	69.35	243.51	< 0.0001	5.40
Residual	5	1.42	0.2848			
Total	8	209.48				

Statistical Analysis of Mucoadhesive Strength

The value obtained for Mucoadhesive Strength was ranging from 0.137 to 0.142 which indicates the highest Mucoadhesive Strength. A Quadratic model was applied and final polynomial equation was carried out from full model to the reduced model from regression analysis data and ANOVA test.

Mucoadhesive strength (Y_1) = 0.1167+ 0.0005 $\mathbf{X_1}$ - 0.01820 $\mathbf{X_2}$ + 0.0045 $\mathbf{X_{22}}$

From the polynomial equation it was observed that X_2 factor has the highest impact on the Mucoadhesive Strength.

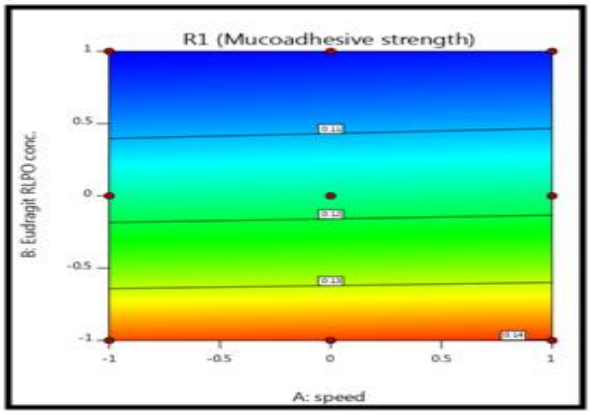

Figure 9. Contour Plot for Mucoadhesive Strength

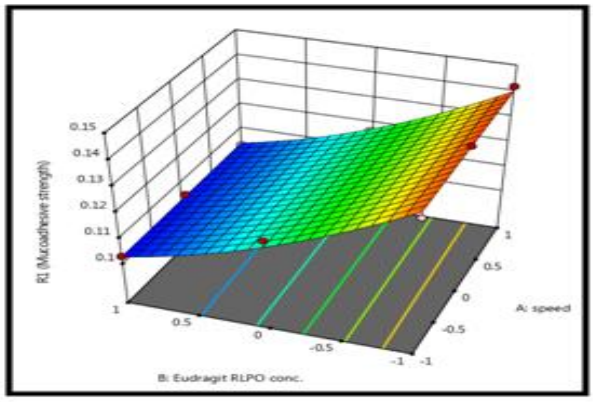

Figure 1. Response Surface plot for Mucoadhesive Strength

In the given contour and 3D surface plots, as the amount of Eudragit RLPO increased the Mucoadhesive Strength was decreased. So here the X_2 coefficient is inversely proportional with response.

Statistical Analysis of % Swelling Index

The value obtained for % Swelling Index was ranging from 48.2 to 50.5 which indicates that the highest % Swelling Index. A Quadratic model was applied and final polynomial equation was carried out from full model to the reduced model from regression analysis data and ANOVA test. Polynomial equation for the % Swelling Index was as following:

% Swelling Index (Y_2) = 43.50 + 0.7333$\mathbf{X_1}$ -6.75 $\mathbf{X_2}$

From the polynomial equation it was found that X2 factor has highest impact on the % Swelling Index. In the given contour and 3D surface plots, as the Eudragit RLPO decreases % Swelling Index increased. So here the X_2 coefficient is inversely proportional with response.

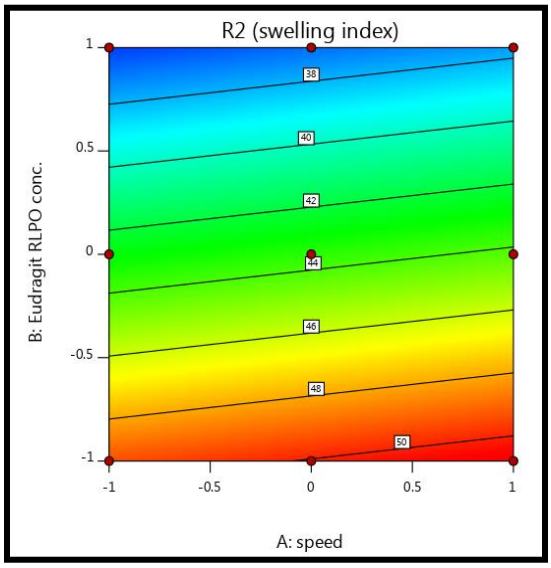

Figure 11. Response Surface plot for % swelling Index

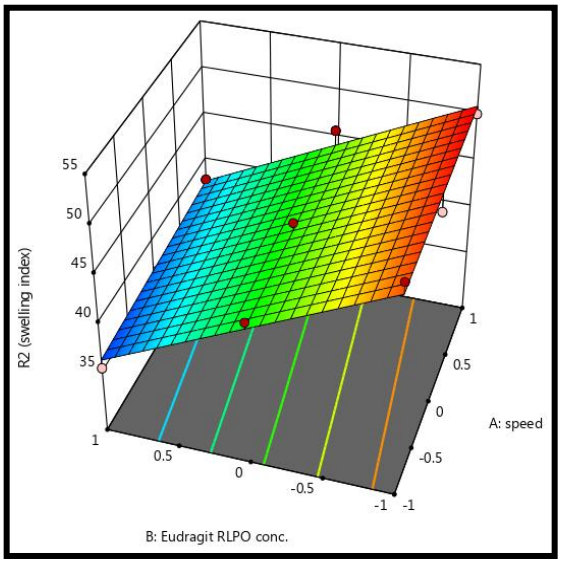

Figure 12. Contour plot for % Swelling Index

Statistical Analysis of % CDR at 6 hours in 7.4 pH 0.1 M Sodium phosphate buffer

The value obtained for % CDR at 6 hours in 7.4 pH 0.1 M Sodium phosphate buffer from 89.13 to 91.10 which indicates that the pellets formulation has better drug release. A Quadratic model was applied and final polynomial equation was carried out from full model to the reduced model from regression analysis data and ANOVA test. Polynomial equation for the % CDR at 6 hours in 7.4 pH 0.1 M Sodium Phosphate buffer was as following:

% CDR at 6 hours in 7.4 pH 0.1 M Sodium phosphate buffer (Y3) = 85.41 -1.48X1 - 4.91X2

From the polynomial equation it was found thatX2 factor has less impact Compare to X_1. X_1 has highest impact on responses.

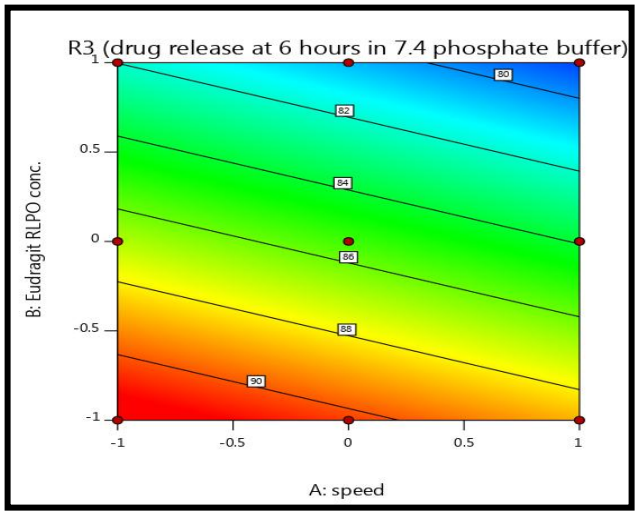

Figure 2. Contour plot for % CDR at 6 hours in 7.4 pH 0.1 M Sodium phosphate buffer

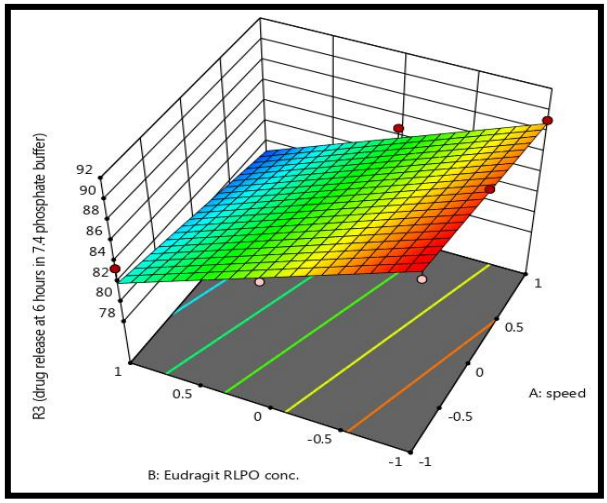

Figure 14. Response Surface plot for % CDR at 6 hours in 7.4 pH 0.1 M Sodium phosphate buffer

From the polynomial equation it was found that X_1 and X2 factor has highest impact on the % CDR at 30mins in 7.4 pH 0.1 M Sodium phosphate buffer.

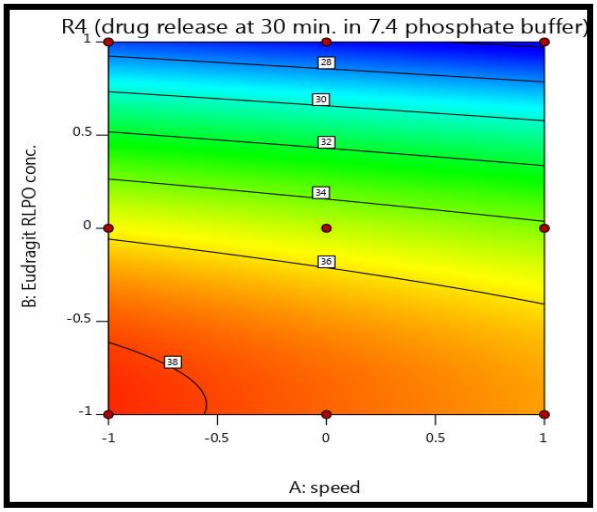

Figure 35. Contour plot for % CDR at 30 mins. in 7.4 pH 0.1 M Sodium phosphate buffer

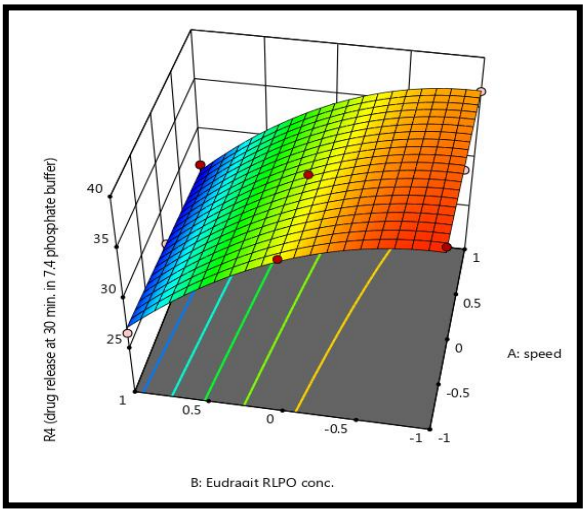

Figure 164. Response Surface plot for % CDR at 30 mins. In 7.4 pH

In the given contour and 3D surface plots, as the Spheronizer Speed and amount of Eudragit RLPO increases the % drug release Decreases. So here the X_1 and X_2 coefficients are inversely proportional with response.

Optimization by Overlay plot using Design Expert (11)

The aim of the optimization of pharmaceutical dosage form is to determine the levels of the variable from which a robust product with high quality characteristics may be produced. An Overlay plot relies on all the investigated formulation variables used to predict the ranges of

variables where the optimum formulation might occur. The measured responses were optimized using Design Expert 11.

Table 9. Constrain Value obtained from Designed Expert 11.

Response	Constrain		
	Minimum	**Maximum**	**Goal**
Y_1=Mucoadhesive Strength	0.103	0.142	Maximum
Y_2= % Swelling Index	35.3	50.5	Maximum
Y_3= % CDR at 6 hours in 7.4 pH 0.1 M Sodium Phosphate buffer	78.14	91.01	Maximum
Y_4= % CDR at 30 mins. in 7.4 pH 0.1 M Sodium Phosphate buffer	26.29	38.82	Maximum

Optimization of pellets for Colon targeted drug delivery system

As shown in the table no.52, 3 solutions were presented by Design Expert software from which F1 was selected for Optimisation cum check point preparation.

Table 10. Solutions Suggested by Design Expert 11 for Optimized cum check point Batch

No.	Spheronizer Speed	Eudragit RLPO conc.	Mucoadhesive Strength (N)	%Swelling Index	% CDR at 6 hours in 7.4 pH 0.1 M Sodium phosphate buffer	% CDR at 30 mins. in 7.4 pH 0.1 M Sodium phosphate buffer	Desirability
1.	0.525	-1.000	0.139	49.682	91.100	37.973	0.953
2.	-0.532	-1.000	0.139	49.676	91.111	37.979	0.953
3.	-0.540	-1.000	0.139	49.671	91.122	37.984	0.953

Stability study of optimized batch

Stability study of optimized batch was carried out for one month at 40˚C. At the end of30 days, the dissolution studies and of pellets was carried out. The profile was shown in table 64.For stability study pellets formulations are kept in dessicator over silica gel for 1 month. The dosage form did not show any significance difference as shown in Table no.64 and Figure no. 37.

Table 11. Stability Study Data

Parameters	Before Stability study	After Stability study	% Bias
% Drug loading	89.76%	89.33%	0.47
Mucoadhesive strength (N)	0.137	0.136	0.72

% Swelling Index	49.5%	49.2%	0.60
% CDR at 6 hours in 7.4 pH 0.1 M Phosphate buffer	90.02%	89.58%	0.68
% CDR at 30 mins in 7.4 pH 0.1 M Sodium phosphate buffer	37.04%	36.80%	0.64

From the above results, it was found that there was no significant change in the pellets property after the stability period & pellets were found to be stable. From the table it was concluded that the % Bias is <5 % which means that the selected design is valid for data obtain.

4. CONCLUSION

Rifaximin belongs to the family of amino salicylates and is currently used as first line therapy of IBD with efficacy based on localized delivery. Multiparticulate formulations have been reported to not only enable the drug to reach the colon quickly but also retain in the ascending colon for a relatively long period of time. Because of their smaller particle size as compared to single unit dosage forms these systems tend to be more uniformly dispersed in the GI tract and also ensure more uniform drug absorption.

Rifaximin was first complexed with beta cyclodextrin and the resultant complex requires 3.72 gm of complex in equivalence of 400 mg of drug which is much higher quantity as final pellet weight increases further with addition of other excipients. So, beta cyclodextrin was added as excipient along with other polymers after literature survey and the use of addition of beta cyclodextrin as pelletizer with drug was investigated for dissolution enhancement and study design from this point forward was designed accordingly.

A 3^2full factorial design was used to quantify the significant independent variables revealed from preliminary studies. In this design 3^2 factors were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations generated by Design Expert 11.

Statistical Analysis of % CDR at 6 hours in 7.4 pH 0.1 M Sodium phosphate buffer shows that contour and 3D surface plots, as the Spheronizer Speed and amount of Eudragit RLPO increases the % drug release decreases. So, here the X_1 and X_2 coefficients are inversely proportional with response.

Statistical Analysis of % CDR at 30 mins. in 7.4 pH 0.1 M Sodium phosphate buffer Shows that contour and 3D surface plots, as the Spheronizer Speed and amount of Eudragit RLPO increases the % drug release decreases. So, here the X_1 and X_2 coefficients are inversely proportional with response.

In all batches quadratic model was applied and final polynomial equation was carried out from full model to the reduced model from regression analysis data and ANOVA test. An Overlay plot relies on all the investigated formulation variables used to predict the ranges of variables where the optimum formulation might occur. The measured responses were optimized using Design Expert 11.According to designed batches observation desirability was found to be 0.953 and optimized batch was carried out with 2095 spheronizer RPM and 0.7 gm Eudragit RLPO. Result was found to be 0.137 Mucoadhesive Strength, 49.5% Swelling Index,90.02 % CDR at 6 hours in 7.4 pH 0.1 M Sodium phosphate buffer, and 37.04% CDR at 30 mins in 7.4 pH 0.1 M

Sodium phosphate buffer. From the results obtained, it can be concluded that 2095 RPM spheronizer and 0.7 gm Eudragit RLPO gives optimum values. Colon targeted drug delivery was achieved with Eudragit S100 Coating.

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