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A PRAGMATIC APPROACH ON COLONIC DELIVERY OF RIFAXIMIN USING POLYMER COATED MULTI-PARTICULATE SYSTEM

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Key words:

Colonic delivery, experimental design, statistical optimization, rifaximin, Eudragit L100, Eudragit S100.

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ABSTRACT: This study aimed to develop and optimize a multi-particulate formulation for colon targeted delivery of rifaximin by application of a functional coat of Eudragit L100 and Eudragit S100 mixture on the drug layered pellets. The rifaximin-excipients compatibility was confirmed with FTIR studies. Functional coat completely restricted drug release in pH 1.2 dissolution medium. Statistical optimization of the formulation variables, i.e., ratio of Eudragit L100 and S100, and % of coating, in respect to drug release at 2nd and 8th h in pH 6.8 dissolution medium was conducted with a central composite design and response surface methodology. Compared to % of coating, the impact of polymer ratio was more pronounced for modulating the drug release. Comparatively, higher influence of % coating was observed after 2 h of dissolution. As per graphical optimization, the minimum drug release at 2nd h and maximum drug release at 8th h could be achieved in formulation with 3.3 polymer ratio and 15.4 % coating where more than 80 % of drug should be delivered in colon. The high efficacy of the optimized formulation on colonic delivery of rifaximin was further confirmed with the animal study. Findings of this study indicate that application of Eudragit L100 and Eudragit S100 coating in an appropriate ratio on drug layered multi-particulates could be a promising approach for colonic delivery of rifaximin.

INTRODUCTION: Crohn's disease is a chronic inflammatory disease affecting any part of the gastrointestinal tract. The diagnostic algorithm identifies the patient's sign like diarrhoea, abdominal pain, and constipation.[1] Drugs that are found to be effective to decrease or control the inflammation on Crohn's disease, are cortisones,[2-4] sulfasalazine,[5] mesalazine,[6] immunosuppressant,[7, 8] antibiotics/ antibacterial [9, 10] and protein inhibitors of the actions of the Tumor Necrosis Factor (TNF).[11, 12]

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Patients with Crohn's disease could be benefited with antibiotic /antibacterial treatment as they reduce symptoms of the acute phase of the disease, e.g., diarrhoea, intestinal pain and meteorism; and prevent as well as cure septic complications, e.g., abscesses, fistulas and toxic state by decreasing growth of the luminal bacteria.[9, 10]

Rifaximin, a broad spectrum antibiotic, is active against many Gram-positive and Gram-negative bacteria, including aerobic and anaerobic bacteria.[13] A potential usefulness of rifaximin in the prevention of the relapse of Crohn's disease after endoscopic resection has been observed.[14] However, upon administration as conventional dosage forms, e.g. tablets, drug usually releases in stomach and disseminates over the stomach and the intestine. Thus, dose of the drug needs to increase for delivering the drug in a concentration that is

effective against local bacteria in the colon. Hence, the effective treatment of Crohn's disease has been required 600 mg /day of rifaximin for 16 weeks [15] which results in a poor patient compliance. Thus, there is a need in-the-art for a rifaximin pharmaceutical formulation to deliver it into the colon for the treatment of infections specifically located in the intestinal tract.

Among the different approaches, a combination of pH and time based formulation is found to more advantageous as it takes benefit both of the gastro-intestinal (GI) tract's pH variation, i.e., 1.2 in the stomach, 6.6 in the proximal small intestine and about 7.5 in the distal part of small intestine; as well as transit time of different regions of GI tract, i.e., around 2 h in stomach and 3h in the small intestine. Rifaximin crystal was coated with pH sensitive polymer and compressed into tablet with aid of other excipients for its delivery onto colon based on the combination of pH and time dependent approach.[16]

However, 5 to 75 % pH sensitive polymer, in respect to crystal weight, required to protect pellets during compression and to achieve desirable release profile which is not economical to industrial point of view. Attempt has been taken to deliver rifaximin into colon using tablet formulations.[17] Histo-pathological studies with Eudragit E100 and Eudragit RL100 coated tablet containing chitosan-chondroit inter-polymer complex could deliver rifaximin into colon with high efficacy for the treatment of inflammatory bowel diseases. However, a significant amount of drug release was observed in the proximal part of the small intestine as Eudragit E100 is soluble in GI tract fluid up to pH 5.0 and swellable as well as permeable above pH 5.0. Moreover, large discrepancy in the amount of drug reached to colon was observed with tablet formulation due to the high variation of gastric empting time.[18]

Compared to tablet, multi-particulate system should have more predictable gastric empting. Therefore, it is hypothesized that drug loaded multi-particulates coated with a combination of polymers which are soluble with in a pH range of 6.0 – 7.4, could provide better efficacy in delivery of rifaximin into colon.

Hence, the objective of this study was to develop and optimize a colon targeted formulation containing rifaximin for the treatment of Crohn's diseases. The formulations have been developed base on combination of pH uniqueness of different polymers, and transit time in the small intestine.

MATERIALS AND METHODS:

Materials:

Rifaximin purchased from Louhe nanjiecun pharma, Luohe, China. Sugar spheres from Paulaur Corporation, NJ, US, were used as drug carrier. Povidone (Kollidon K30, BASF India Ltd) and Hydroxypropyl methyl cellulose (HPMC E5, Colourcon India Pvt. Ltd.) were used as binder and sealing agent, respectively. Polyethylene glycol (PEG 6000, Qualigens fine chemicals, India) and Triethyl citrate (S. Zhaveri & Co, India) were plasticizers. Eudragits (L100 and S100, Evonik India Ltd, Mumbai, India) were employed as pH dependent polymer for functional coating of drug layered pellets. Red oxide of iron (Roha Dye Chem. Ltd India) was used as colouring agent. All other chemicals and solvents used in this study were of analytical grade.

Fourier Transform Infra-Red (FTIR) study:

FTIR spectra of rifaximin and its physical mixture with other excipients at range of 400 to 4000 cm^{-1} were obtained using a FTIR spectrophotometer (Perkin Elmer-Sprectrum 100, Japan).The drug and its formulation composite were mixed with potassium bromide and grounded into fine powder using mortar and pestle. The mixture was then compressed at 20 psi for 10 min to make it into a disc. The spectrum was obtained in a range of 4000-400 cm^{-1} and the characteristic peaks of FTIR transmission spectra of rifaximin and its formulation composite were recorded.

Experimental design:

A central composite design with two factors, i.e., ratio of Eudragit L100 and S100 and % of functional coat (B) was exercised using statistical software (design expert version 9, India) to explore the effect of the investigated factors on the drug release from coated pellets. Drug release at the end of 2^{nd} h dissolution in pH 6.8 (Y1) and at the end of 8^{th} hr in pH 6.8 were designated as response variables. Details of the design are summarized in

Table 1. The design had four axial points, four vertex points and five identical central points. Detail of different formulations in respect to investigated factors is presented in **Table 2**.

TABLE 1: THE FACTORS AND RESPONSES OF CENTRAL COMPOSIT DESIGN

Factors	Level			Response	
	-1	0	1	Type	Constrains
A= ratio of Eudragit L100:S100	1.0	3.5	6.0	Y_1= % drug release end of 2nd hr at pH 6.8	1 to 10 %
B= coating level (%)	12.00	16.00	20.00	Y_2= % drug release end of 8th hr at pH6.8	75 to 100%

TABLE 2: FACTORS, PHYSICAL CHARACTERISTICS AND OBSERVED RESPONSES

Formulation	Space type	Factors		Physical characteristics						Response	
		A	B	20-24# size fractions (%)	Bulk density (g/mL)	Tapped density (g/ml	Hausner's ratio	Carr's index	Drug Content (%)	Y1(%)	Y2(%)
F1	Centre	3.5	16.00	69.8	0.756	0.798	1.05	5.26	32.2	5.36	81.17
F2	Factorial	6.0	12.00	69.5	0.762	0.801	1.05	4.87	32.3	20.29	98.87
F3	Axial	3.5	10.34	70.8	0.767	0.798	1.04	3.98	32.4	8.34	82.33
F4	Axial	0.0	16.00	70.9	0.757	0.804	1.06	5.84	32.9	1.42	28.5
F5	Axial	7.0	16.00	69.9	0.748	0.798	1.06	6.26	32.4	25.33	98.47
F6	Factorial	6.0	20.00	70.5	0.760	0.788	1.03	3.55	32.1	17.9	90.5
F7	Factorial	1.0	20.00	70.3	0.746	0.796	1.06	6.28	32.3	3.08	40.37
F8	Centre	3.5	16.00	71.4	0.759	0.809	1.06	6.55	32.1	8.43	80.55
F9	Axial	3.5	21.66	69.8	0.763	0.81	1.06	5.8	32.4	4.45	69.15
F10	Centre	3.5	16.00	68.6	0.757	0.785	1.03	3.56	32.6	7.58	81.5
F11	Factorial	1.0	12.00	70.2	0.761	0.793	1.04	4.03	32.6	3.38	49.33
F12	Centre	3.5	16.00	69.4	0.765	0.795	1.03	3.8	32.4	5.37	79.65
F13	Centre	3.5	16.00	71.2	0.762	0.811	1.06	6	32.4	4.96	80.67

Preparation of rifaximin colon targeted delayed release pellets:

Preparation of multi-particulate formulation for colonic delivery of rifaximin involved three different steps.

Drug Layering:

Rifaximin pellets formulations were designed by powder layering to sugar spheres (30-35# fractions, 600 g) in a pan coating machine (Ideal Cure, Mumbai, India) rotating at 15-30 rpm. Povidon K30 (7.7 % of Non Pariel beeds), dissolved in isopropyl alcohol, was spread at the rate of 1-3 g/min using 1.0-2.5 bar atomizing pressure with continuous dusting of rifaximin powder blended with talc (4.8 % of rifaximin). The inlet and bed temperature were maintained at 50 – 60°C, and 40 – 50°C, respectively. This process was continued until desired drug loading was achieved.

Seal Coating:

The seal coating solution was prepared by dispersing HPMC E5 (8% w/w of total solution) and PEG 6000 (0.5% w/v) in purified water. The coating solution was then sprayed at the rate of 1-10 g/min on drug loaded pellets (500 g) using fluid bed processor (Wuster insert, Pam glatt GPCG 1.1 India) with inlet temperature 50 – 60°C. The process was continued until 2% weight build up was achieved.

Functional coating:

Functional coating was carried out in fluid bed processor (Wurster insert, Pam glatt GPCG 1.1, India) using solution containing Eudragit L100 and Eudragit S100 polymerin required proportion added with triethyl citrate (10% w/w of dry polymer), talc (5% w/w of dry polymer) and red iron oxide (0.25% of dry polymer).The coating suspension was sprayed at the rate of 1-10 g/min on the seal coated pellets. Once desired coating level was achieved, pellets were dried at 35-40°C.

Characterization of drug layered and functional coated pellets:

Drug content:

Dried pellets were triturated in a mortar and pestle. Accurately weighted powder (equivalent to 100 mg of rifaximin) was then transferred into a volumetric flask containing purified water. The content was sonicated (Power sonic 505, India), filtered and diluted. The drug content in the diluted solution was measured using a HPLC (Shimadzu

Corporation, Japan) fitted with Inertsil ODS 3V column (250mm x 4.6). Drug content was expressed as amount of drug per 100 mg of coated pellets.

Bulk and tapped Density:
Accurately weighted pellets samples were poured gently into a measuring cylinder and the volume of the pellets was recorded. The pellets were tapped using a tapping apparatus (Electro lab Model: ETD-1020) until no further change in volume was observed. Bulk (BD) and tapped (TD) densities were calculated from initial and final volumes. Carr's Index and Hausner ratio were derived using following equations.

$$Carr's\ Index = \frac{(TD-BD)}{TD} X100 \quad \text{(Eq.1)}$$

$$Hausner's\ Ratio = \frac{TD}{BD} \quad \text{(Eq.2)}$$

Size analysis:
Pellet size analysis by sieving was carried out on a sieve shaker (Retsch AS 200 digital Germany) at 1 mm amplitude for 10 min following the methods reported earlier.[19-21]A nest of sieves with mesh number on 16, 18, 20 and 24 was used. The fraction of pellets collected on each sieve was weighed and percentage retained was calculated.

Morphology of pellets by scanning electron microscopy (SEM):
The surface and cross-sectional morphologies of polymer coated drug layered pellets were examined upon gold coating using scanning electron microscope (Jeol JMS-840, Japan).[22] SEM photomicrographs on excitation voltage of 10 and 15 KV were captured at different magnifications.

***In-vitro* Dissolution Studies:**
In-vitro dissolution of drug layered and polymer coated pellets in 1000 mL of dissolution medium, maintained at 37±0.5°C, was performed using USP-1 dissolution apparatus at 75 rpm revolutions. Sodium Phosphate buffer (pH 7.4) containing 0.45% SLS was used as dissolution medium for drug layered pellets. Dissolution for functional coated pellets was performed in 0.1N HCl, pH 6.8 Phosphate buffer and pH 7.2 Phosphate buffer. Optimized formulation was assessed under continues dissolution method , i.e., 2 h at pH 1.2, followed by 1 h at pH 6.5 and 2 h at pH 6.8and rest at pH 7.2 media as reported in an earlier study.[23]

In each dissolution vessel, core or coated pellets, equivalent to 100 mg of rifaximin were placed. At predefined time intervals, a sample of 10 mL was withdrawn from each dissolution vessel, filtered through 0.45 µm filter, diluted and assessed with a UV spectrophotometer (Shimadzu UV-2101PC UV-VIS Scanning Spectrophotometer). Cumulative amount of drug release at each time point was calculated. The sample amount used for analysis was replaced by fresh dissolution medium.

Statistical analysis and optimization of investigated factors:
A non-linear quadratic model was applied by the design to fit the individual data set for the each of the responses using the second order polynomial equation (Eq. 3).

$$Y = b_0 + b_1A + b_2B + b_{12}AB + b_{11}A^2 + b_{22}B^2 \quad \text{(Eq.3)}$$

Where Y is the response, b_0 is the intercept; b, b_2, b_{12}, b_{11} and b_{22} are the regression coefficients for the factors. The significance of the model was evaluated by analysis of variance (ANOVA). The results were considered significant when the calculated p value was less than 0.05. The investigated factors were optimized by applying predefined constrains for the Y1 and Y2, i.e., 1% <Y1< 10% and 75% <Y2< 100%.

Animal Organ Distribution Examination:
The procedure involved in animal study was reviewed and approved by Institutional animal ethical committee (IAEC) and CPSEA members as per the rules and regulations (GPRCP/IAEC/02/14/8/PCE/AE - 2 - RATS - M/F-50). Healthy Wister rats weighing 250- 300 g were selected and set aside in well-spaced ventilated cages and preserved healthy with fixed diet (Bengal gram soaked in water). The animals were divided into standard and test groups. Each group contained 24 animals. The animals were kept on overnight fast (16-20 h). Pellets (equivalent to 100 mg rifaximin) from the optimized pellet formulation were administered orally via cannula to stomach by suspending the pellets in xanthan gum (0.2%) solution in water.

Two animals from each group were sacrificed after anesthesia at 2, 4, 6 and 8 h time intervals and the viscera was dissected to isolate portion starting from stomach to rectum. The position of pellets travelled in each time interval was noted and travelled distance was measured. The GI tract was then transversely dissected and content was transferred to 30 mL of pH 6.8 phosphate buffer mixed with 1 mL acetonitrile. After 1 h, the suspension was centrifuged. The samples from supernatant liquid were analyzed by UV method and drug content was calculated.

Stability study: The stability studies on the optimized formulation were conducted at 40°C/75% relative humidity for 3 months. The pellets at predefined interval were evaluated for appearance, assay and dissolution.

RESULTS AND DISCUSSION:

Pre-formulation study:

The FTIR spectra of rifaximin and its mixture with excipients are presented in **Fig.1**. The O-H, C=O stretching for rifaximin were observed at 3427 and 1716 cm^{-1}, respectively which remained intact in physical mixture of rifaximin and excipients. It indicated that there was no physical or chemical interaction between rifaximin and other excipients.

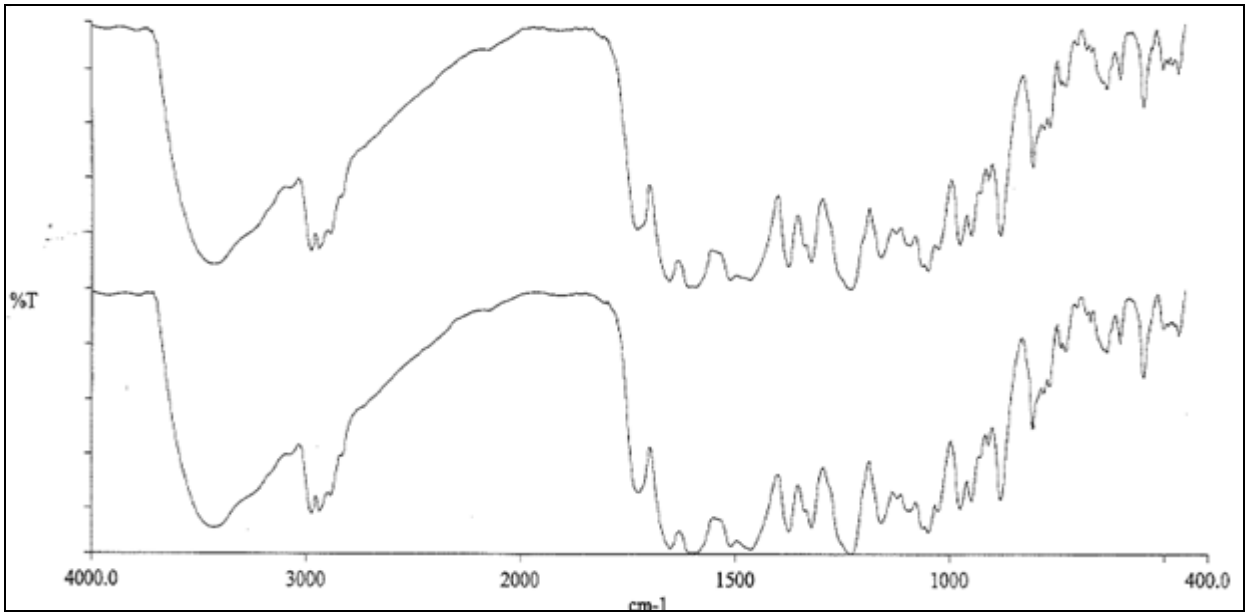

FIG.1: FTIR SPECTRUMS OF RIFAXIMIN AND MIXTURE OF RIFAXIMIN AND EXCIPIENTS

Characteristics of functional coated pellets:

Physical properties:

Different physical properties of the formulations are tabulated in **Table 2**. For all the investigated formulations more than 60 % pellets were in 20-24# size fraction. The smooth surface and spherical shape of the pellets, as per SEM photomicrograph (**Fig. 2a**), provided good flow ability to the pellets as indicated by their Hausner ratio values which were close to 1, and Carr's Index values which were less than 10 %.[24] Drug content of the formulation was ranging from 32.12 to 32.85 mg per 100 mg functional coated rifaximin pellets. Narrow size distribution, smooth surface area and uniform drug content indicated that the drug loading as well as coating processes were well controlled and all the pellets were coated uniformly with drug as well as coating polymer without forming significant amount of doublet and triplet formation. The uniformity of the drug content and coated layer was further supported by uniform coating thickness as shown in the SEM photomicrograph (**Fig.2b-2d**).

The pellet regularity at inner and outer layer can be seen in SEM photomicrographs of cross sections of functional coated pellets. It Thickness of functional coat was around 20 µm whereas the thickness of drug layer including the seal coat was around 184 µm. The weight gain of the pellets to achieve around 20 µm thickness of functional coating layer

was 15%. It was reported that the coating thickness of 18 to 22 µm is desirable for delayed release pellets.[25] This implied that the thickness of Eudragit coat applied in this study should be sufficient to provide delayed release formulation which is further proven by the dissolution study.

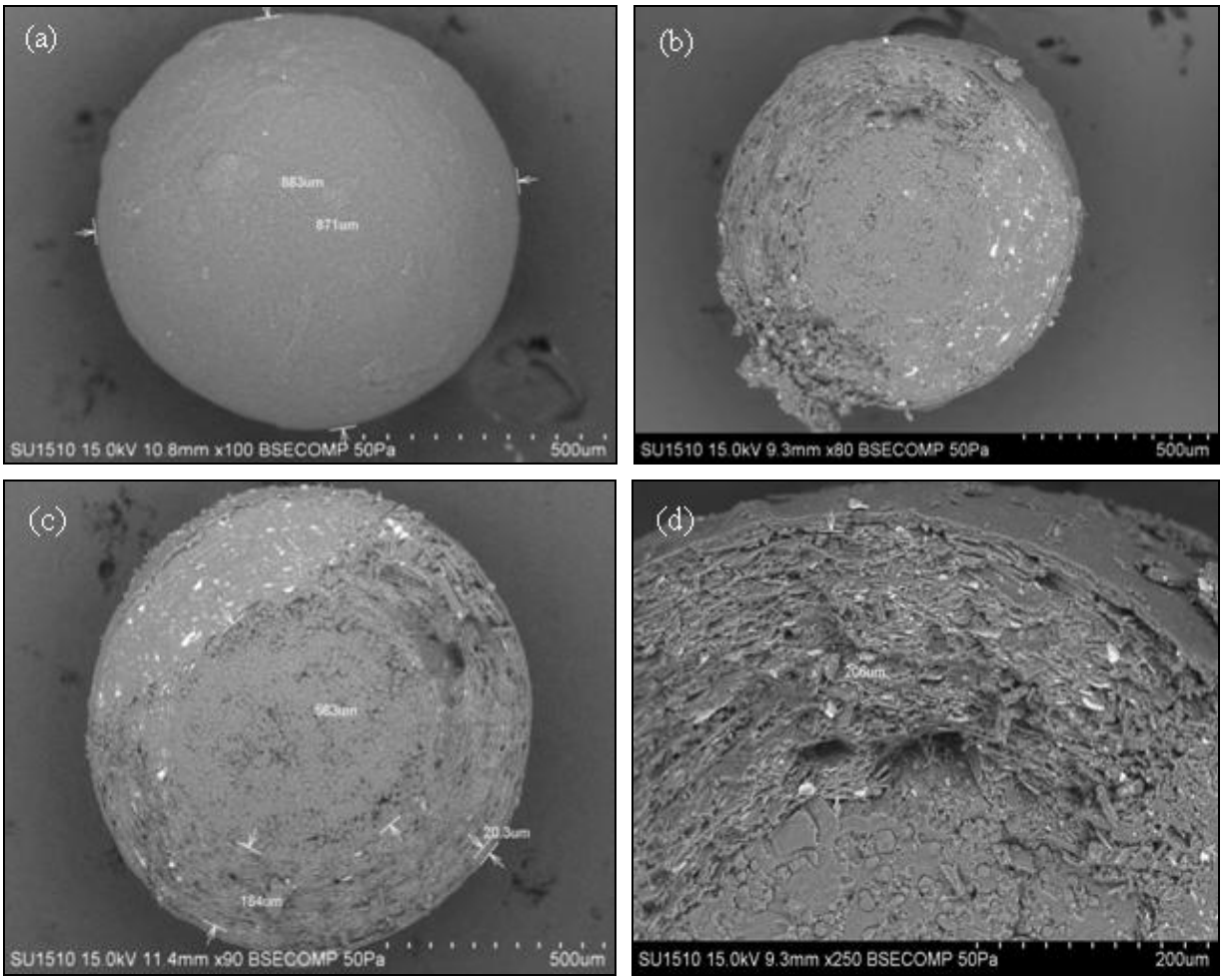

FIG.2: SEM PHOTOMICROGRAPH OF (a) RIFAXIMIN LOADED PELLETS AT 100 X MAGNIFICATION, (b) RIFAXIMIN PELLETS AFTER POLYMER COATING AT 80 X MAGNIFICATION, (c) CROSS SECTION OF COATED PELLETS AT 90 X MAGNIFICATION AND (d) CROSS SECTION OF COATED PELLETS AT 250 X MAGNIFICATION.

Drug release in different dissolution media:

Health human subject's GI tract has been measured by radio-telemetry to understand the pH variations in different regions. The pH of stomach stomach is 1.2. The pH progressively increase in duodenum reaching to 6.5. The pH from duodenum to end of ileum is progressively increased form 6.6 to 7.5. However, a decreased is observed in cecum (pH 6.4). A slow rise in pH is noted in colon reaching to a maximum pH of 7.0.[26] The solubility of rifaximin in 0.1 N HCl, pH 6.8 and pH 7.2 are 3.56, 3.22 and 3.12 mg/mL, respectively. Although drug does not have pH dependent solubility, the solubility of the polymers, i.e. Eudragit L100 and Eudragit S100 have pH dependent solubility. Eudragit S100 is soluble at pH 7 and above whereas Eudragit L100 is soluble at pH 6 and above. [27] Hence, dissolution of the formulation was conducted at pH 1.2, 6.8 and 7.2. The rate of solubility of uncoated drug pellets in all dissolution media was more than 80 % in 30 min and met the criteria for immediate release formulation.

Drug release of the functional coated pellets in all investigated dissolution media was reduced remarkably. Drug release at pH 6.8 (**Fig. 3**) and 7.2 (**Fig. 4**) was influenced by both polymer ratio and % of coating. At pH 6.8 the amount of drug release increased with increasing Eudragit L100 fraction in coating formulation and decreasing % of coating. Eudragit L 100 is soluble whereas Eudragit S100 is insoluble at pH 6.8. Therefore, higher amount of pore should formed in the coat containing higher fraction Eudragit L100 as it

dissolved in the dissolution media. Formation of higher amount of pore should provide more access of drug layer to the medium permitting dissolution of higher amount of drug. The drug release was further accelerated with decreasing the coating thickness which resulted with decreasing % of coating. As the Eudragit S100 is also soluble at pH 7.2, a similar trend but lower impact of Eudragit L100 and Eudragit S100 ratio and % of coating on drug release was noted at pH 7.2.

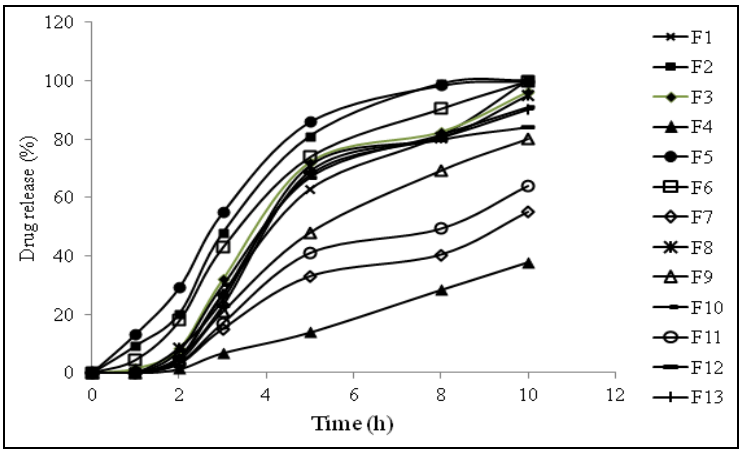

FIG.3: DISSOLUTION PROFILES OF FUNCTIONAL COATED PELLETS AT pH 6.8 SODIUM PHOSPHATE BUFFER

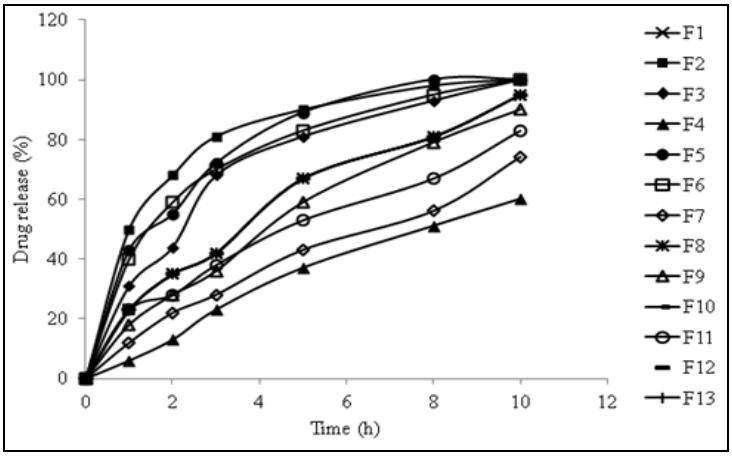

FIG.4: DISSOLUTION PROFILES OF FUNCTIONAL COATED PELLETS AT pH 7.2 PHOSPHATE BUFFER

Regression equation:

The investigational trials with the perceived responses are shown in **Table 2** and significance of surface reduced model for response tabulated in **Table 3**. In case of both responses, the p-values indicated that lack-of-fit test was not significant and the residuals did not reveal any major violations of the underlying assumptions. Furthermore, the p<0001 for model implied that the models were significant and fitted well. Significant model term in case of Y1 and Y2 were A, and A^2; and A, B, A^2 and B^2, respectively. The regression equations that were fitted to the data are given bellow.

$Y1= 6.34 + 8.19\ A - 1.02\ B - 0.52\ AB + 3.84\ A^2 + 0.35\ B^2$ (Eq. 4)

$Y2 = 80.71 + 24.83\ A - 4.50B + 0.15AB - 8.57A^2 - 2.45\ B^2$ (Eq. 5)

Factors with a positive coefficient implied that the response increased with increasing factor level whereas a negative coefficient denoted that the response increased as the factor level decreased. From above equations, it can be observed that factor A, i.e., ratio of Eudragit L100 and Eudragit S100 has a positive coefficient and exhibited positive effect on both Y1and Y2. The small negative coefficient for factor B indicated that % coating had very low negative but not significant effect on Y1. Compared to Y1, the impact of both factors, i.e. polymer ratio and % drug loading was relatively higher for Y2 as indicated by numerical higher values of their coefficient for Y2. The seal

coating layer present in between the drug layer and functional coat could take some time to be solvated with dissolution medium. Hence, solvation of the seal coating played a major role for the dug release in first 2 h. Once the seal coat layer solvated, the drug release was mainly controlled by the coating thickness and polymer ratio. As a result a higher impact of the polymer ratio and % of coating was noted for Y2.

TABLE 3: RESPONSE SURFACE REDUCED QUADRATIC MODEL FOR RESPONSES

Source	Y1					Y2				
	Sum of Squares	df	Mean Square	F Value	p-value	Sum of Squares	df	Mean Square	F Value	p-value
Model	649.0568	5	129.8114	62.64105	1.17E-05	5616.887	5	1123.377	3399.507	1.10E-11
A	536.9995	1	536.9995	259.1315	8.68E-07	4931.363	1	4931.363	14923.04	6.50E-13
B	8.387156	1	8.387156	4.04726	0.084145	161.7241	1	161.7241	489.4012	9.74E-08
AB	1.092025	1	1.092025	0.526962	0.491439	0.087025	1	0.087025	0.263351	0.623622
A^2	102.4112	1	102.4112	49.41898	0.000206	511.249	1	511.249	1547.115	1.79E-09
B^2	0.837024	1	0.837024	0.40391	0.545285	41.59477	1	41.59477	125.8719	9.97E-06
Residual	14.50613	7	2.072305	--	--	2.313171	7	0.330453	--	--
Lack of Fit	4.794734	3	1.598245	0.658296	0.619144	0.326691	3	0.108897	0.219277	0.878555
Pure Error	9.7114	4	2.42785			1.98648	4	0.49662		

In the equations, quadratic relationship or interaction terms are presented with coefficients with higher-order terms or two factors, respectively. Therefore, presence of higher-order terms or two factors in a response implied that that relations between the factors and the response are nor linear. Low interaction effects on the both responses were indicated by the very low coefficients of AB term. However, the interaction effects were not significant as implied by p> 0.05 for AB terms. Factor A showed a quadratic term for Y1 suggesting that factor A had a much greater effect on the response Y1. On the other hand, presence of quadratic term of both factors (A and B) in Y2, implied that both factors had much greater effect on the response.

Formulation optimization:

The process was optimized based on the three dimensional response surface **Fig. 5** and two dimensional contour **(Fig. 6)** plots generated by design expert. Three dimensional surface plots were drawn to approximate the properties of self-governing variables in each response. The counter plots exhibits the interaction effects of the design factors on the selected responses. It can be seen from the counter plot that the Y1 and Y2 predominantly influenced by the polymer ratio. In all the investigated coating levels, Y1<10 % was achieved when polymer ratio was below 4.0. In contrast, in all coating level Y2> 75 % was observed when polymer ratio was 2.0 and above.

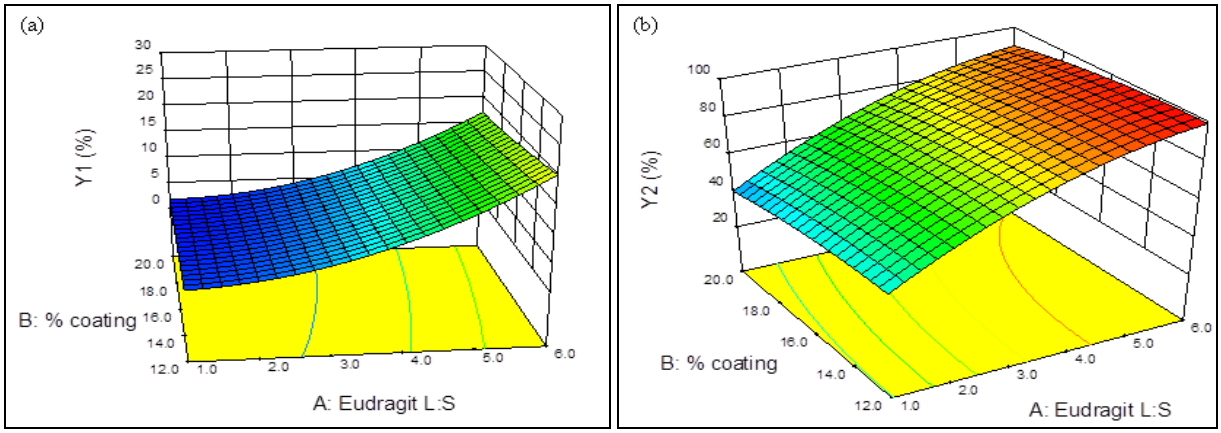

FIG.5: RESPONSE SURFACE PLOT FOR (a) Y1 AND (b) Y2

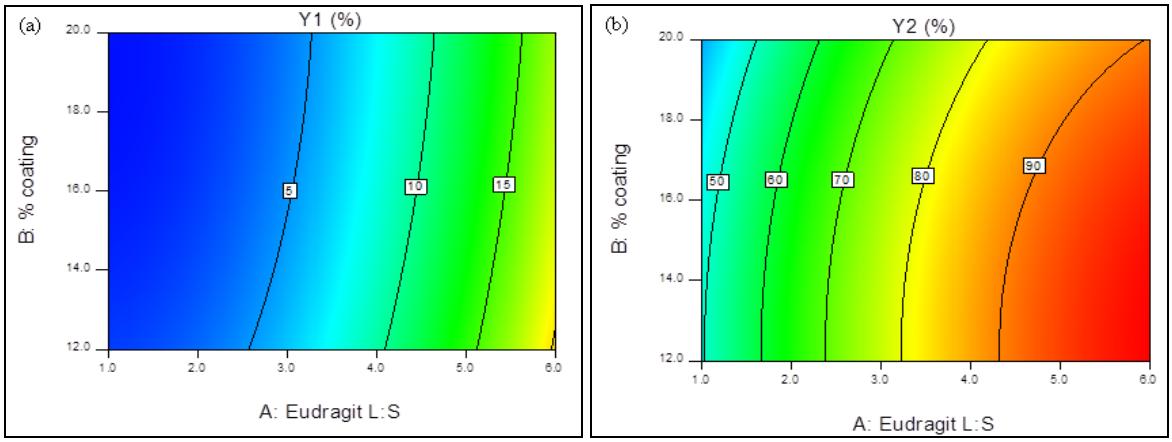

FIG. 6: COUNTER PLOT FOR (a) Y1 AND (b) Y2

With multiple responses regions, where the optimum parameters meet desired output or *sweet spot* were pursued. Applied Graphical optimization to get a sweet spot with following constrains applied to get response 1, 1% $<Y_1<$ 10% and response 2, 75% $<Y_2<$ 100% criteria. The factors A and B for the optimized formulation, as generated by design, should be 3.3 and 15.4% respectively to achieve both the responses most-favorable. With the optimized formulation, the predicted and actual values for Y1 were 5.9 and 5.0 respectively, whereas the predicted and actual values for Y2 were 79.4 % and 83.0 % respectively. The results demonstrated that actual values for both responses fitted very well with the predicted values.

Characterization of optimized formulation in continuous media:

The drug release of optimized formulation was conducted under continues dissolution in different media at pH 1.2 for 2 h, at pH 6.5 for 1 h, at pH 6.8 for 2 h and rest at pH 7.2. It was observed form the dissolution profile (**Fig. 7**) that the percentage drug release at the end of 6th h was observed to be less than 20 %. Hence, more than 80 % drug containing drug reached into colon. It is further confirmed by animal study where the drug content of the pellets recovered from the animal GI tract even after 6 h of administration was 83.5 ± 2.1 % (**Fig.8**) indicating that more than 80 % drug was able to deliver into the colon by the formulation.

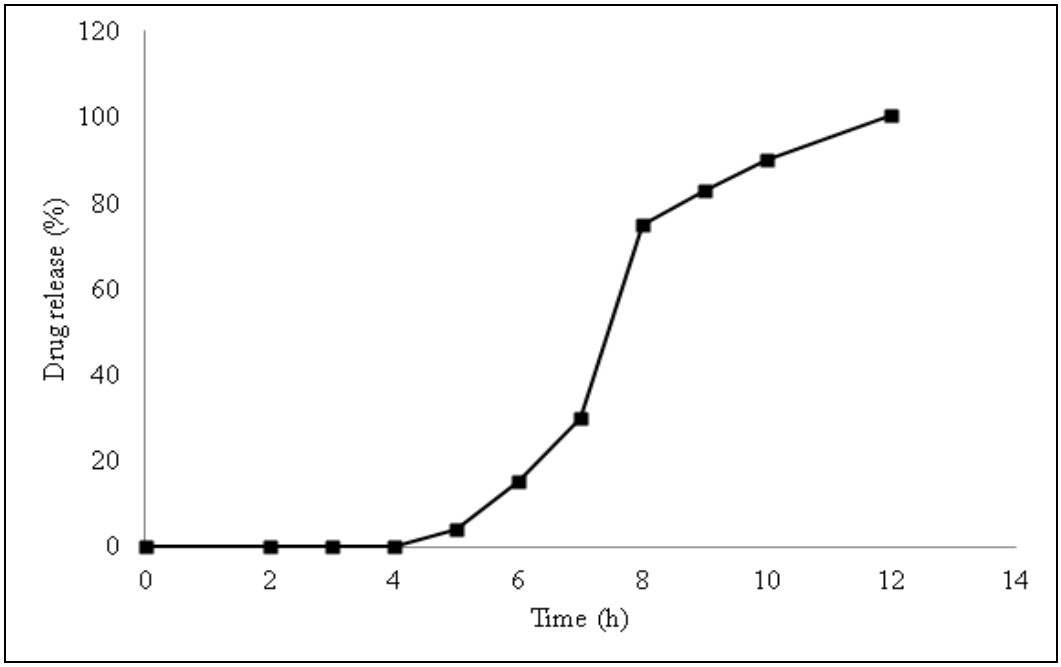

FIG.7: *IN-VITRO* DRUG RELEASE OF OPTIMIZED FORMULATION (A= 3.3, B= 15.4 %) IN CONTINUOUS DISSOLUTION METHOD (2 H AT pH 1.2, FOLLOWED BY 1 H AT pH 6.5 AND 2H pH 6.8 AND REST AT pH 7.2).

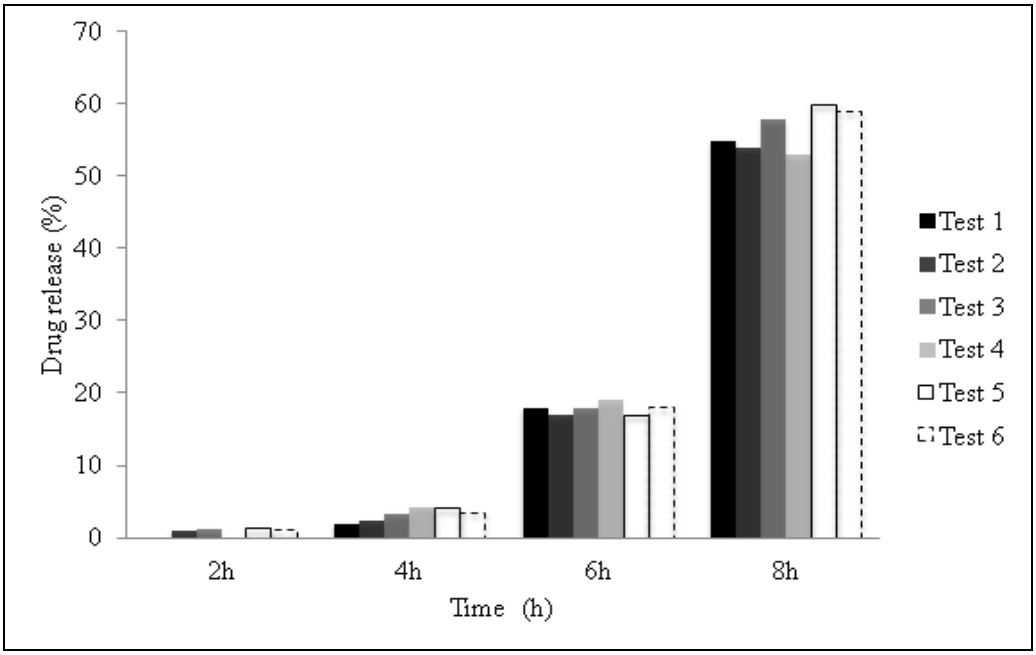

FIG. 8: AMOUNT OF DRUG RELEASE FROM THE FUNCTIONAL COATED PELLETS AT DIFFERENT TIME POINTS IN ANIMAL (WISTER RATS) GASTROINTESTINAL TRACT

The stability results of the optimized formulation are tabulated in **Table 4**. There was no remarkable changes was detected on the formulation in respect to drug content and dissolution profiles even after 3 months storage at 40°C and 75 % relative humidity. It indicates good stability profile of the formulation.

TABLE 4: STABILITY STUDY ON OPTIMIZED FORMULATION

Parameters	Time period			
	Initial	1 Month	2 Month	3 Month
Description	Complies	Complies	Complies	Complies
Drug content (%)	99.8	98.4	101.3	97.9
	At time h, cumulative % of drug release (%)			
0	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	4.21±1.28	4.5±.1.11	4.3±1.22	4.8±1.20
6	15.23±1.39	15.2±1.02	14.6±1.20	15.6±1.98
7	30.23±1.08	29.6±0.39	30.2±1.02	30.9±1.29
8	75.22±0.33	76.2±0.43	75.6±1.24	76.2±1.32
9	83.21±0.39	84.2±0.38	83.6±1.45	84.2±1.35
10	90.19±0.49	91.2±0.29	90.6±0.93	91.3±1.25
12	100.3±0.39	99.5±0.33	99.8±0.49	100±1.54

CONCLUSIONS: The rifaximin pellets coated with a blend of Eudragit L100 and S100 exhibited a promising *in-vitro* dissolution profile for the therapy of Crohn's disease. The compatibility of rifaximin with excipients was evident from FTIR studies. Drug release was predominantly influenced by the Eudragit L100 to Eudragit S100 ratio in the coating formulation which could modify drug release after a suitable lag time in different media. Compared to the drug released in first 2 h, release after 2 h was impacted more by the coating percentage. Based on the design, it was observed that formulation containing 15.4 % drug load and coated with polymer having Eudragit L100: Eudragit S100 at 3.3 could delivered maximum amount of drug into colon region. The optimized formulation could able to deliver more than 80 % drug into colon. Therefore, it can be a good candidate for delivery of rifaximin to the colon at different physiological pH conditions.

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