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Design and optimization of rifaximin macroparticles for colon targeting

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ABSTRACT: This study is focused to design a colon specific pellet formulation of rifaximin based on the combination of time-dependent and pH sensitive delivery system using Quality-by-Design (QbD) approach for better and promising treatment of inflammatory bowel disease (IBD). An extrusion/spheronization process was utilized for the preparation of core macroparticles using ethyl cellulose (EC) as matrix former and microcrystalline cellulose (MCC) as a spheronizing aid. Two critical process parameters (CPPs) i.e., spheronization time and spheronizer speed were taken as independent factors while aspect ratio, sphericity, carr's index, and particle size were taken as dependent responses to optimize the composition of the core macroparticles. To regulate the drug release, core macroparticles were coated with Eudragit NE40D and Eudragit FS30D to impart time-dependent and pH sensitive release of drug. The optimized coated macroparticles were characterized for drug content and in vitrodrug release in different pH media of stomach and intestine. The coating levels of the inner and outer polymers were further optimized for the time required for 10 %, 50 % and 90 % drug release. The result showed that the 90% of drug of P3 and P4 formulation were found to be released in 10.87 and 13.19hrs respectively. When exposed to Scanning Electron Microscopy the images of coated macroparticles suggested a uniform and smooth coat of polymers over the surface of macroparticles. This formulation reduces the dose and the side effects due to its specific targeting at the site of inflammation makes it a better choice over the tablets. The result indicates that the developed formulation may possibly reduce the dosing frequency and side effects associated with the conventional tablet formulation for the site-specific targeting at inflammation site.

KEYWORDS: Macroparticles; colon targeted; rifaximin; Eudragit; MCC; Optimization.

1. INTRODUCTION

The majority of medications require repeated daily doses to achieve the optimum blood concentration and produce therapeutic effect. It is extremely challenging to administer medications correctly for the treatment of inflammatory bowel diseases, specifically for local action, since the release of the medication must be blocked in the upper GIT [1]. One major difficulty with this drug delivery strategy is keeping the formulation intact as it passes through the stomach [2]. In these circumstances, the development of delivery systems that can reach the precise site of pharmacological activity is necessary. Additionally, it gives a considerable cost and manufacturing ease advantage.

Therapeutic bioavailability at the target location would increase with administration of pharmaceuticals orally in the form of a specific drug delivery at the colonic region, which would also result in a lower the dose of drug and less systemic side effects. Nevertheless, Common oral dosage forms are insufficient to deliver drugs to the colon because they are absorbed or broken down in the upper GIT region. Numerous methods have been used to target medications for the colon specifically, includes pH-sensitive coating polymer, time-dependent dosage forms, microflora triggered drug delivery systems, pressure-dependent systems, and prodrugs [3]. Eudragit® FS 30D is a methyl acrylate, methyl methacrylate, and methacrylic acid anionic copolymer. In case of colonic administration, This polymeric material has been used as a pH-sensitive material [4].

Combining pH-dependent and time-dependent systems to assure medication release under various physiological situations could overcome this issue [5].

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Due to their constant transit duration in the GIT and easy passing via the ileo-caecal valve, macroparticles—spherical multiparticulate dose forms have grown in popularity as colon-specific drug delivery devices. Macroparticles can be compacted into tablets or hard gelatin capsules with a coating and filling [6].

Rifaximin (RFX) inhibits bacterial RNA synthesis by acting on the beta subunit of the DNA (Deoxyribonucleic Acid) dependent RNA (Ribonucleic Acid) polymerase enzyme. RFX is utilized for the treatment of local problems in the GIT because it is not absorbed when taken orally [7]. In this research work, macroparticles of rifaximin was formulated using spheronizing process to deliver the drug at the colon for local action.

Rifaximin has been studied for its effectiveness in treating various gastrointestinal conditions, including those affecting the colon. The rationale for developing rifaximin for colon targeting lies in its unique properties and potential therapeutic benefits. The key reasons include poor absorption in the small intestine, selective action in the colon, reduction of gut microbial load and minimization of systemic effects [8].

Colon-targeted formulations of rifaximin may enhance patient compliance by delivering the drug directly to the site of action. This can be particularly beneficial in chronic conditions requiring long-term therapy.

2. RESULTS

To develop macroparticles of rifaximin for colon specific delivery MCC and ethyl cellulose were selected. Both of these polymers were taken in ratio ranges from 1:9 to 9:1. Ethyl cellulose was selected to produce the macroparticles with desired firmness and release characteristics.MCC found as spheronizing aid and helps in ease of the process of pellet production. All the trial batches were given in Table 1.The combination was analyzed for the pellet formation and the sphericity of macroparticles. Out of all the trial batches, randomly 2:8 ratio (ethyl cellulose: MCC) was selected for the further optimization of some more parameters. This is based on the formation of macroparticles and their sphericity.

Table 1. Trial batches for selection of polymer ratio

Formulations	F1	F2	F3	F4	F5	F6	F7	F8	F9
EC:MCC	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1
Macroparticles formation	Y	Y	Y	Y	Y	N	N	N	N
Sphericity	Y	Y	Y	Y	N	N	N	N	N

Y represents: Yes, N represents: No

Water was chosen as the granulating fluid due to its effective binding; when extruded, the extrudates formed correctly after the water molecules were well adsorbed onto the surface. Additionally, water's non-volatile nature aids in maintaining sphericity after spheronization.

2.1 Optimization of the process parameters [8-10]

The process variables were optimized by taking the F2 batch with 2:8 ratios of EC and MCC. Spheronization time and spheronization speed were selected as independent process parameters for optimization purpose with the help of 3^2 full factorial design. The parameters related to the extrusion process named extrusion speed and extrusion time were kept fixed in the optimization. At different level of Spheronization time and spheronization speed, the macroparticles were optimized for sphericity, aspect ratio, and pellet size.

2.2 Evaluation parameters of the rifaximin core macroparticles [11, 12]

The flow characteristics of macroparticles are the one of the significant considerable parameter for filling of rifaximin macroparticles into hard gelatin capsule shells. The value of angle of repose ranges from 24.20 ± 0.37^o to 35.64 ± 2.01^o for the entire factorial batches. The values less or equal to 30^o indicate excellent flow characteristics. The value above 40^o, nevertheless, the powder flows with difficulty. The values of various flow characteristics are given in Table 2.

The value of bulk density and tapped density was found from 0.65 ± 0.11gm/cm3 to 0.86 ± 0.09gm/cm3 and 0.82 ± 0.01 gm/cm3 to 0.97±0.08, respectively. The value of Carr's index below 15 % excellent flow characteristics of macroparticles, whereas value above 25 % indicates poor flowability.

Hausner's Ratio (H) is an indirect index of ease of powder flow. The batches R6 and R9 were found to have excellent flow efficiency.

Aspect ratio and sphericity are essential parameters for the macroparticles characterization, given in Table 3. Aspect ratio closer to 1 and sphericity nearer to 100 % indicates the macroparticles of spherical shape. All the batches of macroparticles were also evaluated for the morphological characteristics with the help of photomicrograph (Optical microscope, Olympus CX 31).

Table2. Flow property of macroparticles

Formulation code	Angle of repose (°)	Bulk density (gm/cm3)	Tapped density (gm/cm3)	Hausner's ratio	Carr's index (%)
R1	35.64 ± 2.01	0.65 ± 0.11	0.82 ± 0.01	1.23 ± 0.003	18.42 ± 0.009
R2	31.94 ± 0.51	0.73 ± 0.07	0.87 ± 0.06	1.17 ± 0.005	14.67 ± 0.192
R3	31.33 ± 1.32	0.74 ± 0.03	0.85 ± 0.08	1.12 ± 0.004	10.61 ± 0.091
R4	27.93 ± 1.46	0.78 ± 0.04	0.85 ± 0.03	1.12 ± 0.008	10.57 ± 0.113
R5	24.47 ± 1.08	0.85 ± 0.06	0.94 ± 0.09	1.10 ± 0.004	9.47 ± 0.171
R6	24.20 ± 0.37	0.78 ± 0.04	0.82 ± 0.05	1.05 ± 0.002	4.82 ± 0.103
R7	33.99 ± 1.59	0.86 ± 0.09	0.97 ± 0.08	1.14 ± 0.007	13.27 ± 0.165
R8	32.07 ± 2.11	0.75 ± 0.06	0.84 ± 0.06	1.15 ± 0.004	11.78 ± 0.201
R9	25.73 ± 1.65	0.80 ± 0.04	0.85 ± 0.05	1.04 ± 0.003	4.72 ± 0.459

Table3. Morphological characteristics of macroparticles

Batches	Shape	Aspect ratio	Sphericity (%)	Pellet size (mm)
R1	Cylinder shaped	2.39 - 5.30	38.41 - 42.31	1.58 - 3.11
R2	Cylindrical /Rod shaped	1.22 - 1.48	62.52 - 73.62	5.97 - 6.52
R3	Cylindrical + Dumb-bell	1.10 - 1.16	76.31 - 78.71	1.42 - 1.56
R4	Dumbbell + egg-shaped	1.04 - 1.20	81.63 - 85.12	0.76 - 0.78
R5	Ellipsoidal + Spherical	1.03 - 1.10	87.13 - 93.15	0.94 - 1.21
R6	Sphere	1.0 - 1.05	99.13 – 100.0	0.78–0.99
R7	Dumbbell + Ellipsoid	1.69 - 1.73	41.667 - 48.07	0.11 - 0.18
R8	Ellipsoid + Ova shaped	1.20 - 1.28	63.76 - 67.43	0.15 - 0.69
R9	Oval + Spherical	1.0 - 1.06	97.07 - 99.80	0.11 - 0.234

Out of all the factorial batches, R6 represents the values of aspect ratio and sphericity 1.0 to 1.051 and 99.103 % to 100.0 % respectively and batch R9 found 1.0 to 1.068 and 97.072 to 99.807 in that order. Although the pellet size of batch R9 represented very fine, i.e. 0.112 mm to 0.239 mm in contrast to batch R6, i.e. 0.879 mm to 0.993 mm. These coarser size macroparticles are advantageous taking into account the flow and coating.

The photo micrographic study of the macroparticles reveals that batch R6 had more spherical and uniform macroparticles with a even surface compared to other batches. Therefore, Batch R6 was identified as a final optimized batch and used for further study. Optimized value of two process parameters for the extrusion spheronization is spheronization time and spheronization speed with value of 800rpm and 20min respectively.

2.3 Coating of rifaximin macroparticles [13-15]

The main goal of present work is to examine the effect of inner as well as outer coating level, in combination, on the characteristics of release of drug to achieve the colon specificity. For achieving efficient colon targeted drug delivery system, time-dependent and pH sensitive polymers in combination was used to reduce the drug release in gastric pH (for the first 2 h) and should be evidence for maximum release at the colonic pH for the rest of the period. The main factors to target the drug at colon site are the residence time and pH at different regions of the GI tract.

While Eudragit NE30D is a pH-independent, insoluble polymer whose permeability increases over time and is an appropriate option for the development of oral sustained release dosage forms, Eudragit FS30D exhibits a peculiar pH-sensitivity and also helps in protecting the macroparticles in the gastric environment. Therefore, in the combination of pH and time dependent systems, time dependent polymer (Eudragit NE30D) contributes for controlling the release if pH-sensitive polymer (Eudragit FS30D) could not achieve release in colon due to pH variability. The coated pellet's size and surface morphology were measured using a SEM analysis given in Figure 1.

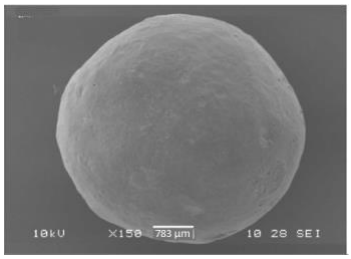

Figure 1.SEM image of coated rifaximin pellet

2.4 Optimization of coating levels using 3^2 factorial design [16-18]

The 3^2 factorial design was utilized for optimization of coating level of the two different grades of eudragit. The coating levels were optimized by selecting composition and manufacturing process of uncoated macroparticles to achieve inner and outer coat for various gain in weight for both Eudragit grades. The coated macroparticles were tested for the content of drug and drug release profile up to 24 h.

2.5 Evaluation of coated macroparticles

Drug content represents the uniform distribution of drug in the dosage form which confirms the safety, efficacy and quality of product. The drug loaded macroparticles of rifaximin manufactured with optimized polymeric composition showed drug content in the range of 97.2-99.8 % as given in Table4. The foremost factors to target the drug at the site of colon include pH and residence time at various parts of GI tract. The pH-sensitivity of Eudragit FS30D is accountable to protect the macroparticles in gastric surroundings, while pH independent permeability of water-insoluble film of Eudragit NE40D increases with time, and thus found as suitable choice to formulate oral sustained release dosage forms. The invitro drug release pattern of all the batches confirms that the controlled drug release can be effectively achieved by using both pH and time dependent polymers.

2.6 Statistical analysis of drug release from coated macroparticles

A 3^2 experimental design was used for the statistical optimization of both the polymer coating levels in macroparticles. Moreover, it studies the impact of independent variables i.e. inner coating level of Eudragit NE40D (X_{C1} in %) and outer coating level of Eudragit FS30D (X_{C2} in %) on the desired responses. Nine formulations of were prepared using different coating levels of polymers and characterized for the three responses named $t_{10\%}$, $t_{50\%}$ and $t_{90\%}$ which signifies the time required to get 10 %, 50 % and 90 % drug release as shown in Table 5. The time for drug release from the macroparticles reflects the efficiency influence of both inner coating level of coating of polymers on the selected responses.

For the concentrations of Eudragit NE40D (XC1) and Eudragit FS30D (XC2) in the matrix of coated macroparticles, mathematical correlations were established and coefficients of a second order polynomial equation produced. Factors and observed responses for coated macroparticles based on 3^2 design matrix are given in table 7.

$$Y_C = \beta_0 + \beta_1 X_{C1} + \beta_2 X_{C2} + \beta_3 X_{C1} X_{C2} + \beta_4 (X_{C1})^2 + \beta 5 (X_{C2})^2$$

Where, Y_C is the response of the dependent variables; β_0–β_5 are the regression coefficients; and X_{C1}, X_{C2} are independent variables.

Table 4. Cumulative drug release (%) of all formulation batches

Time [h]	P1	P2	P3	P4	P5	P6	P7	P8	P9
1	9.13±0.91	4.83±0.75	2.61±0.19	6.26±0.54	2.73±0.28	1.72±0.47	5.16±0.56	2.31±0.16	1.38±0.44
2	18.08±0.48	8.96±0.63	6.38±0.31	10.00±0.80	4.43±0.24	3.31±0.22	9.21±0.43	3.68±0.15	2.81±0.35
3	29.13±0.73	20.41±0.67	15.81±0.46	16.80±0.96	11.69±0.68	7.43±0.89	14.79±0.91	7.72±0.21	5.51±0.51
4	37.39±1.68	27.34±0.40	23.68±0.71	22.17±1.89	16.32±1.09	14.68±0.74	19.51±0.83	12.31±0.26	8.45±1.12
5	49.65±1.89	36.92±0.98	30.21±0.39	30.64±1.12	23.12±0.87	21.51±1.09	24.69±0.90	14.69±0.43	11.73±0.26
6	60.09±0.63	47.09±2.11	36.76±1.51	37.03±0.99	27.73±0.36	26.73±1.03	29.79±0.68	19.82±1.13	14.91±0.68
7	72.32±1.13	59.41±0.78	47.39±1.07	43.02±1.11	35.56±0.57	35.09±0.87	34.91±1.02	24.31±0.59	18.31±0.38
8	86.03±2.68	75.19±1.09	56.19±0.18	49.99±1.68	41.68±1.43	41.58±1.28	39.53±1.46	26.68±0.54	22.13±0.51
9	99.03±0.29	87.03±1.03	64.55±1.43	57.96±1.05	50.91±1.09	48.19±2.53	47.66±1.17	30.41±0.23	24.91±1.43
10	-	98.68±0.53	74.69±1.83	67.18±0.55	57.63±1.51	55.43±1.81	52.53±1.23	35.13±0.69	29.62±0.73
11	-	-	87.51±0.11	76.70±1.26	66.72±1.18	62.28±2.02	60.12±1.41	40.74±0.85	33.81±1.52
12	-	-	98.37±0.08	83.05±1.28	74.29±1.12	69.43±1.36	63.32±2.03	46.91±1.87	37.92±1.83
18	-	-	-	99.31±0.47	88.33±0.62	79.42±1.31	78.49±1.07	63.23±0.13	55.59±1.31
24	-	-	-	-	98.41±1.09	91.91±0.67	89.31±1.13	78.36±0.37	73.39±0.68

The data is presented as mean ± SD, n = 3

Table 5. Factors and observed responses for coated macroparticles based on 3^2 design matri

Trials Actual Name	Factors (independent variables) Inner coating level	Outer coating level	Response (dependent variables) $t_{10\%}$	$t_{50\%}$	$t_{90\%}$
Formulation code	X_{C1} (%)	X_{C2} (%)	Y_{C1} (h)	Y_{C2} (h)	Y_{C3} (h)
P1	4	4	1.21	4.93	6.37
P2	4	8	2.37	6.83	9.41
P3	4	12	4.18	8.48	10.87
P4	6	4	2.16	8.09	13.19
P5	6	8	2.58	9.02	19.27
P6	6	12	2.69	9.47	24.03
P7	8	4	2.07	9.86	24.96
P8	8	8	3.41	13.07	27.83
P9	8	12	4.34	16.53	29.61

For the estimation of the levels of factors which will yield optimum response of dissolution, mathematical relationships were developed between the dependent and independent variables using the experimental design software Design-Expert 11.0. The resulting equations in terms of coded factors for all the responses are given as follows:

$t_{10\%} = 3.0+0.31A+1.23B-0.17AB-0.026A^2-0.083B^2$

$t_{50\%} = 9.02+2.73A+1.52B+0.78AB+0.61A^2+0.026B^2$

$t_{90\%} = 18.40+8.73A+3.09B+0.64AB+0.11A^2 - 0.16\ B^2$

(A-Inner coating, B-Outer coating)

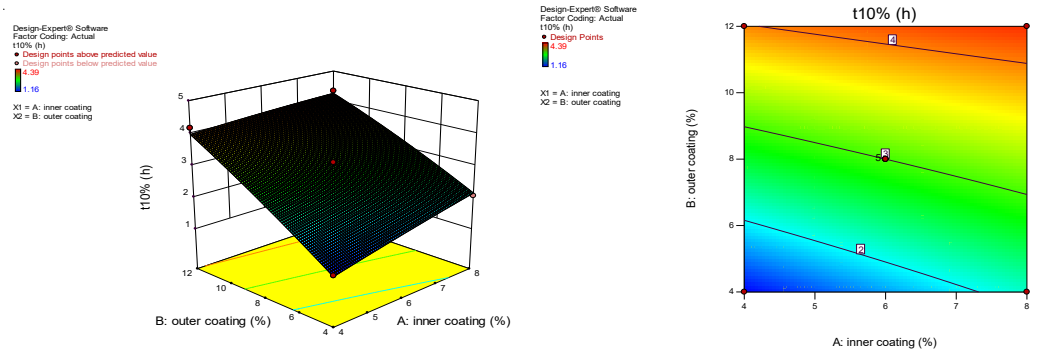

Figure 2. Surface response plot and contour plots for Response t10% of coated macroparticles

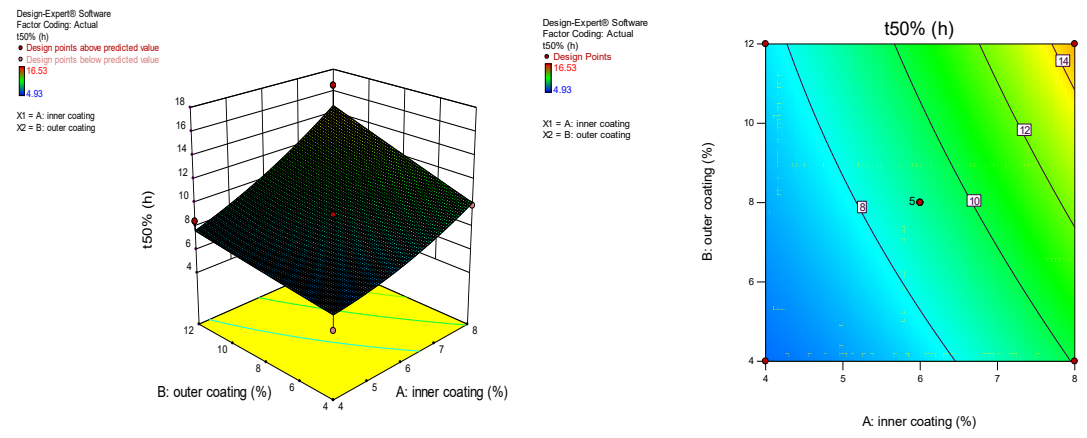

Figure 3. Surface response plot and contour plots for Response t50% of coated macroparticles

2.7 Kinetic modeling of drug release

Two basic parameters for presenting the release kinetics data are the release constant (k) and the coefficient of determination (R^2), both are given in Table 8. The value of release constant for the batch P4 was found to be 4.89 h^{-1}, 39.03 h^{-1}, 39.01 $h^{-1/2}$ and 1.613$^{1/3h-1}$. The data found more fitted to the Hixson-Crowell cube root model with the R^2 value of 0.988 as shown in table 6.The value The experimental data confirms the release of drug from the macroparticles was supported by Hixson-Crowell cube root model This ensures the release of drug followed dissolution mechanism and the release will be affected by variation in surface area as well as in diffusion path length throughout the process of dissolution.

Table 6. Release kinetic values of optimized rifaximin coated macroparticles

Zero Order		First order		Higuchi		Hixson-Crowell	
R^2	K_0	R^2	K_1	R^2	K_H	R^2	K_{HC}
0.9711	4.8932	0.9618	39.0883	0.9539	39.01	0.988	1.613

3. CONCLUSION

The main conclusions of the study should be presented in a short Conclusions section, which stands alone. You should explain whether your findings supported your hypothesis in this section. Avoid using references in conclusion section. The Extrusion-spheronization method was used for the development of macroparticles of rifaximin in this research work. The core macroparticles were prepared using MCC (spheronization aid), Ethyl Cellulose (release retardant) and water as a granulating agent. Out of various trial batches, ethyl cellulose: MCC ratio of 2:8 was selected for the formulation of macroparticles of rifaximin. The macroparticles were further coated by Eudragit NE40D and Eudragit FS30D to achieve site specific drug release at the colon. The ration of both of the eudragit grades were optimized with the help of design expert 11.0 software. The independent and dependent variables were correlated by getting polynomial equations after the statistical interpretations. The% in vitrodrug release profile was estimated at 1.2 pH and pH 6.8 phosphate buffers wherein formulation exhibited sustained release effect. Furthermore, all the outcome getting while formulation of the macroparticles were as per the preferred set of the objectives. Therefore, the coated pellet formulations can be a good choiceto target the release of rifaximin at the colon specifically and thereby reducing the irritation in stomach, decreasing dose frequency and by improving patient compliance for the managment of inflammatory bowel disease and ulcerative colitis conditions.

4. MATERIALS AND METHODS

Rifaximin was procured from Ankur Drugs and Pharma Ltd., Baddi, Himachal Pradesh. Eudragit NE40D and Eudragit FS30D were procured as a gift sample from Evonik Degussa Pvt. Ltd., Mumbai. EC was purchased from LobaChem laboratories, Nasik, India. The additional chemicals and reagents employed were all of analytical grade.

4.1 Preparation of Rifaximin Core Macroparticles

Rifaximin core macroparticles were prepared by the pelletization procedure using extrusion/spheronization [8]. Before the process of pelletization, the drug and other excipients were passed through sieve No. 40 and then combined evenly. Water was added, as a granulating fluid, drop wise to the mixture to obtain damp mass. This prepared semisolid powder mixture was extruded using a piston extruder (1mm orifice). The extrudates were instantaneously spheronized with varying time period and rotation speed. The developed macroparticles were dried. For the preparation of macroparticles different ratio of MCC and EC ranges from 1:9 to 9:1 were used. The selection of the polymer ratio for core macroparticles was done on the results of formation of macroparticles, physical appearance and sphericity.

Figure 4. Prepared macroparticles

4.2 Optimization of extrusion spheronization process

The extrusion-spheronization process was used for development of macroparticles of rifaximin. Spheronizer-250 (Anish Pharma) with 4.2mm of size of plate and Extruder-20 (Anish Pharma) with the 1 mm of sieve size were both employed in the pelletization process. The process of macroparticles development was optimized by changing the spheronization speed and spheronization time for formulating the macroparticles with preferred characteristics likewise aspect ratio, sphericity, Carr's index and pellet size. The experimental grid was given in table 7.

Table 7. Experimental design grid of physical units for Extrusion spheronization

Coded Values	**Actual Values (%)**		**Response**			
	X1 Spheronization speed (rpm)	**X2 Spheronization time (min)**	Y1	Y2	Y3	Y4
-1	600	10	Aspect	Sphericity	Carr's	Pellet
0	800	15	ratio		index	size
+1	1000	20				

4.3 Evaluation of core macroparticles of rifaximin [19-23]

The developed drug macroparticles were screened for micromeretics parameters, and invitro drug release. The macroparticles were evaluated for the flow properties by the determination of angle of repose, Hausner ratio and Carr's index. The roundness, aspect ratio and size of the macroparticles were estimated from the image analyser having a computer system linked to a video camera (Olympus SP-350, Tokyo, Japan) and optical microscope (Olympus CX31, Tokyo, Japan). Analysis of output digitalized image done with the help of Magnus Pro Version 3.0 Software.

4.4 Development of coated macroparticles of rifaximin [24-26]

The fabricated core macroparticles of rifaximin were coated in a fluidized bed coater. Two polymers were utilized for the coating of macroparticles, for inner coating time dependent polymer Eudragit NE40D was utilized and for outer coating a sensitive polymer Eudragit FS30D was employed. Eudragit NE40D was dissolved in 5% w/w glyceryl monostearate (GMS) and Polysorbate-80. Eudragit FS30D was dissolved in triethyl citrate (5%w/w) and GMS. 20 g of macroparticles were kept into the compartment outfitted with a spray nozzle of 0.8 mm diameter and situated in the bottom of fluidized bed. The spray rate of the polymeric dispersion was 1.0 g/min with the atomization pressure of 0.1 MPa, the inlet and outlet air temperature were kept 40^{o}C and 38^{o}C respectively. The coated macroparticles were further dried at 40^{o}Cfor 2 h in an oven for the proper film formation of the polymeric dispersion.

4.5 Optimization of enteric coated rifaximin Macroparticles [26-30]

The below mentioned polymers were considered for two independent variables namely,
Eudragit NE40D: inner coating level (X_1) = 4 %, 6 %, 8 %
Eudragit FS30D: outer coating level (X_2) = 4 %, 8 %, 12 %

The estimated levels of these independent variables (coating polymers) were selected from release profile of drug from the macroparticles tested from preliminary batches. The time to release the 10%,50% and 90% drug was studied to optimize the coating level. The variables were presented in table 8.

Table 8. Study variables and polymeric concentrations

Levels	**Factors**		**Response**		
	Eudragit NE40D Concentration (Xc_1)	**Eudragit FS30D Concentration (Xc_2)**	**Y1**	**Y2**	**Y3**
-1	4	4	t10%	t50%	t90%
0	6	8			
+1	8	12			

By using optimized concentrations of Validation of experimental design was done by calculating relative error using the following Eq. (1).
Relative error %= (Predicted values _ Practical values) / Predicted value -----(1)

4.6 Evaluation of coated macroparticles

The evaluation of coated macroparticles were performed for the drug content and in vitro release of drug. The coated macroparticles of rifaximin were evaluated for the drug kinetics for the estimation of mechanism and rate of release of drug was studied by fitting the data of dissolution into several mathematical models, including zero-order, first-order, Higuchi and Hixson- Crowell equations.

4.6.1 Determination of drug content

Using a UV-1800 Spectrophotometer set to 247 nm, the drug content of the produced pellets was measured spectrophotometrically. Rifaximin-loaded pellets were broken up in a mortar and pestle, and the resulting mixture of 9 mg of the medication was added to a 100 ml volumetric flask filled with methanol. Phosphate buffer (pH 7.4) was added to further dilute it, bringing the level up to 100 ml. After the proper dilution, the solution was filtered, and the drug's quantity was determined spectrophotometrically at 247 nm.

4.6.2 In vitro dissolution study

USP dissolving equipment I (Basket type) was used to measure the release at 50 rpm. A simulated stomach fluid (pH 1.2) in 250 ml was used for the test, which was conducted at 37 ± 0.5 °C. After precisely weighing 9 mg of the produced material, it was put in a basket and submerged in the dissolution flask at 50 rpm for the first two hours. Phosphate buffer 7.4 was then added to the dissolution medium for the final four hours. In the end, the pellets were dissolved in phosphate buffer with a pH of 6.8 for six hours. At a predetermined interval of one hour, five milliliter aliquots were removed and replaced with an equal volume of brand-new dissolving medium. Using spectrophotometric analysis at 247 nm, the sample's cumulative percentage drug release was determined. The depiction of the in vitro dissolution profiles was investigated by utilizing the subsequent kinetic models as given in Table 9.

Table 9. Kinetic models

Model	Equation	Abbreviations
Zero order	Q = Q0 - K0t	Q is the amount of drug released at time t Q0 is the amount of drug remaining in the formulation K0 is zero-order release rate constant K1 is first-order rate constant K2 is Higuchi rate constant Q/Q0 is fraction of drug release at time, t K is a constant and n is diffusion exponent indicating the mechanism of drug release.
First order	In Q = In Q0 - K1 t	
Higuchi model	Q = K2t1/2	
Peppas model	Q/Q0 = K tn	

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