Formulation and Evaluation of Colon Targeted Oral Drug Delivery System using Natural Polymers and Methacrylic Acid Co-Polymers

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a. Title of the Thesis and abstract:

FORMULATION AND EVALUATION OF COLON TARGETED ORAL DRUG DELIVERY SYSTEM USING NATURAL POLYMERS AND METHACRYLIC ACID CO-POLYMERS

Abstract:

This research aimed to develop a colon-targeted Budesonide drug delivery system using microbial and pH triggers within the framework of Quality by Design (QbD). Natural gums were screened for their potential to create a colon-targeted drug delivery system based on viscosity analysis and enzyme sensitivity studies. Tamarind Gum was selected as the primary candidate based on its excellent viscosity characteristics. For tablet dosage forms, carboxymethyl (CM) Tamarind Gum was chosen, and these tablets were coated with Eudragit S 100 to delay drug release in the upper gastrointestinal tract. Pellet dosage forms were optimized using critical process parameters and formulation variables, super-coated with Eudragit S100 for enhanced colon targeting. Through the Box Behnken Design and Response Surface Optimization methods, the research identified the Design Space (DS) and optimal formulations meeting specific criteria. The results showed that these formulations released less than 10% of the drug in the first 5 hours and over 80% within 9 hours, aligning with colon-targeted drug delivery goals. Histopathology and biochemical analyses of IL-6 and TNF-α confirmed the efficiency of the optimized pellet formulation in treating induced ulcerative colitis in rats compared to standard Budesonide solution. Roentgenography demonstrated that the optimized formulation remained intact for 5 hours and fully disseminated after 7 hours. This research presents a systematic approach to developing colon-targeted Budesonide formulations with delay drug release characteristics, promising clinical applications for colon-related disorders.

b. Brief description on the state of the art of the research topic

Inflammatory Bowel Disease (IBD)/ Inflammatory Bowel Syndrome (IBS) is a worldwide prevalent health issue with a consistently rising occurrence.(1)

Despite the fact that 5-ASA (5-Amino Salicylic Acid) drugs have been the preferred treatment for CD (Crohn's disease) and UC (Ulcerative Colitis), there is contradictory information about their efficacy in individuals with Crohn's Disease, particularly as long - term treatment. Antibiotics have a minimal role in colonic CD therapy. Corticosteroids remain the preferred therapy for acute diseases unresponsive to more conservative treatment.(2)

Unfortunately, corticosteroids may result in undesirable side effects.(3) Budesonide is a relatively recent steroid drug that undergoes rapid hepatic metabolism, hence minimising corticosteroid-associated implications. Budesonide is much more effective than placebo or mesalamine in the treatment of severe ulcerative colitis.(4–6)

Budesonide is a corticosteroid possessing considerable anti-inflammatory effect at the site of application but limited systemic activity due to significant hepatic degradation.(3) Because of this, conventional oral formulations are less effective than prednisolone, a common corticosteroid with considerable side effects.(6) So, in the present investigation, budesonide was intended to target the colon via a colon-specific approach. Different approaches, including pH-Dependent(7,8), Time-Dependent(8,9), Pressure-dependent(10), and Microbial approaches (11,12), have been investigated by researchers in the last decade to target the drug to the colonic region.

c. Definition of the Problem

The development of an oral colonic drug delivery system is a complex undertaking that demands careful consideration of multiple factors. The ideal release profile for the colon should be determined by considering not only the composition and properties of the dosage form, but also the behaviour and environmental conditions that the dosage form experiences before reaching the colon.

While several strategies have been proposed to address these challenges, the systems that rely on the unique degrading ability of the colonic microbial flora to release the drug have emerged as a promising solution. Specifically, the use of polysaccharides, a class of compounds that resist degradation by the enzymes in the small intestine but are degraded by bacterial enzymes in the colon, has shown great potential as a carrier in the design of microbially controlled delivery systems for colon targeting.

Therefore, using the unique properties of polysaccharides for the purpose of achieving targeted drug release in the colon represents a potential direction for the development of efficient and secure oral drug delivery systems for the colon.

d. Objectives and Scope of work

1. To retard drug release in the upper part of GI tract and deliver drug in its intact form as close as possible to the target site.
2. To assess the strength and enzymatic sensitivity of various natural polymers like karaya gum, khaya gum, gum ghatti, xanthan gum, gellan gum, locust bean gum, tamarind gum for targeting to colon through viscosity parameter for preliminary Screening

3. To develop gum & pH dependent Polymer based colon targeted tablet-based DDS of Budesonide.
4. To develop gum & pH dependent Polymer based colon targeted pellet-based DDS of Budesonide.
5. To assess efficiency of optimized formulations in targeting of drug to the colon by roentgenography and histopathology studies.

e. Original contribution by the thesis

This study investigates the use of natural gums for colon-targeted drug delivery, using a microbial approach. Seven different natural gums were selected, and their potential to target the drug to the colonic region was assessed. To mimic the enzymatic environment of the colon, a novel dissolution biorelevant media was developed using a probiotic culture medium which was found to be more efficient than the traditional use of rat cecal content. The QbD approach was utilized to develop drug delivery systems in the form of tablets and pellets. The study demonstrates that natural gums have potential for colon-targeted drug delivery and offers a reliable method for predicting the quality of the product at every stage of the process, making it a significant contribution to the field of drug delivery.

f. Methodology of Research, Results / Comparisons

Pre-formulation studies

Identification of pure drug was done by melting point and UV scanning. drug polymers interaction was analysed by FTIR study

Analytical Study

Standard calibration curve of budesonide was carried out in 0.1 N HCl, Phosphate buffer (pH 7.4), and Phosphate buffer (pH 6.8)

Enzymatic susceptibility of Various Gums and Screening of Natural Gums by Viscometric procedure.

Viscosity profiles were used to screen natural gums for their potential to prolong drug release in the upper part of GI (gastrointestinal) tract, as this lag time is assessed by the viscosity profile. The viscosity of 1% solutions of various natural gums prepared in a phosphate buffer pH 6.8 was determined. Gellan Gum, Tamarind Gum, Locust bean Gum, and Karaya Gum's Viscosity profiles were found to be statistically significant in achieving the desired lag time. The viscometric approach was used to study the effect of degrading enzymes on polysaccharides (Natural gum) dispersion because it was simple and relevant approach. Comparing the effect of enzymes from Rat cecal content and probiotic media on the viscosity

of a Natural gum sample revealed that both enzyme systems were capable of degrading gum via randomized bond cleavage of the Polysaccharide. According to the findings, tamarind gum has a high viscosity for giving a substantial lag time and was more susceptible to enzymes produced by intestinal microflora than other selected gums.

Preparation of colon targeted tablet dosage form using tamarind gum

For preliminary assessment, the measured amount of budesonide and all other excipients were passed via sieve number 60. PVP K 30 was utilized as a binder for both direct compression and wet granulation methods. The drug and excipients were thoroughly blended with talc and magnesium stearate for lubrication and to improve the flow properties of the powder mass. Using an 8/32" flat punch, a weighed amount of powder mass was compressed using a rotating tablet compression machine (12 station D tooling, Model No- PR-TCM-007, Mfg.: Karnavati Engineering, Ahmedabad, India). Total 50 tablets were compressed per batch and the composition of the preliminary batches of tablets is reflected in table 1.

Table 1: Composition of Preliminary tablet formulations.

Ingredients	TD1 (mg)	TD2 (mg)	TD3 (mg)	TD4 (mg)	TW1 (mg)	TW2 (mg)	TW3 (mg)	TW4 (mg)
Budesonide	9	9	9	9	9	9	9	9
Tamarind gum	50	75	100	125	50	75	100	125
Lactose	125	100	75	50	125	100	75	50
PVP K30	10	10	10	10	10	10	10	10
Talc	4	4	4	4	4	4	4	4
Mg. Stearate	2	2	2	2	2	2	2	2
Total	**200**	**200**	**200**	**200**	**200**	**200**	**200**	**200**

* TD batches Prepared By Direct Compression

* TW Batches Prepared By wet Granulation (PVP K30 in IPA as binder)

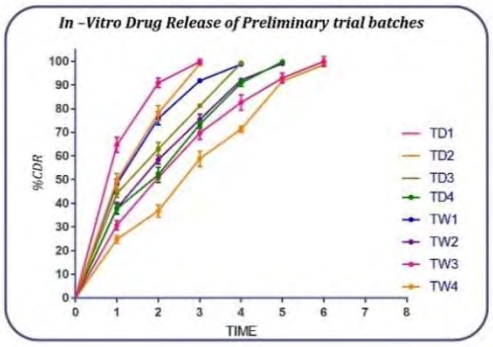

Figure 1: *In vitro* drug release of preliminary tablet formulations

From dissolution data of preliminary batches shown in Figure 1, it was observed that wet granulation provided better retardation compare to direct compression. But dissolution data of tablet formulation which were prepared by wet granulation, are not satisfactory or is too high than desire release limit. Based on data obtained from other preliminary batches, it was determined that the substitution of tamarind gum with carboxymethyl tamarind gum, and the

partial replacement of IPA (Isopropyl alcohol) with water, were recommended for the wet granulation process. Carboxymethyl tamarind gum, a grafted form with higher viscosity, was utilized to quickly form a mucilaginous mass, minimizing erosion and achieving the desired retardation. In the preparation of an optimized colon-targeted drug delivery system, a single microbial approach was deemed inadequate as per the dissolution data, necessitating a combination of pH and microbial approaches. Eudragit S 100, selected as the pH-dependent polymer, possesses the highest pH threshold among available pH-dependent (enteric) polymers.

Designing the Formulations by using Box-Behnken factorial design

Tablet dosage form for colonic delivery were optimized using Box-Behnken Design (Design Expert 11.0) containing the three-factor, three-level to define main, interaction and quadratic effects(13,14) of independent variables like amount of CM Tamarind Gum, % water proportion and % weight gain by Eudragit S100 on the selected responses (dependent variables) which were % drug release at 2 hours, % Drug Release at 5 hours and % drug release at 08 hours as shown in table 2 and batches designed by Box-Behnken design.

Table 2: Independent variables and Dependent variables of Box Behnken design for tablet dosage form

Independent variables	Levels			Dependent variables
	-1	0	+1	
X1=Amount of CM Tamarind Gum	75	100	125	Y_2 = % CDR at 2 Hours
X2= % Water Proportion	0	50	100	Y_5 = % CDR at 5 Hours
X3=% wt. Gain by Eudragit S100	2.5	5	7.5	Y_8 = % CDR at 8 Hours

Dissolution Method

In-Vitro drug release of Colon Specific Budesonide Tablet were conducted in USP Type II (Paddle) Apparatus at rotation speed of 50 rpm and at 37 ±0.5 ^{0}C. Initially test was done in 0.1 N HCl for 2 hrs. Then, test was performed in phosphate Buffer pH 7.4 for 3 hrs. The remaining study was carried out in biorelevant medium with a Phosphate buffer pH of 6.8 and CO_2 aeration to provide an environment that is favourable for anaerobic bacteria(15). *In vitro* release data of all formulation batches prepared as per the Box-Behnken design are shown in figure 2

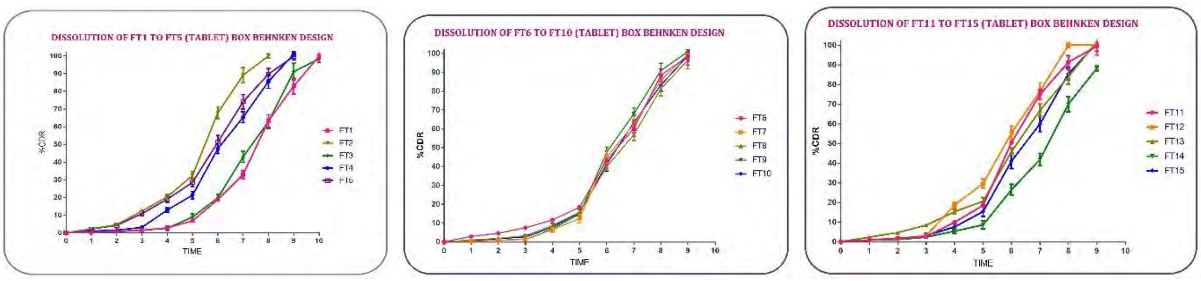

Figure 2: Dissolution profile of box Behnken batches (F1 to F15) for tablet dosage form.

Regression Analysis for generating the optimized design space

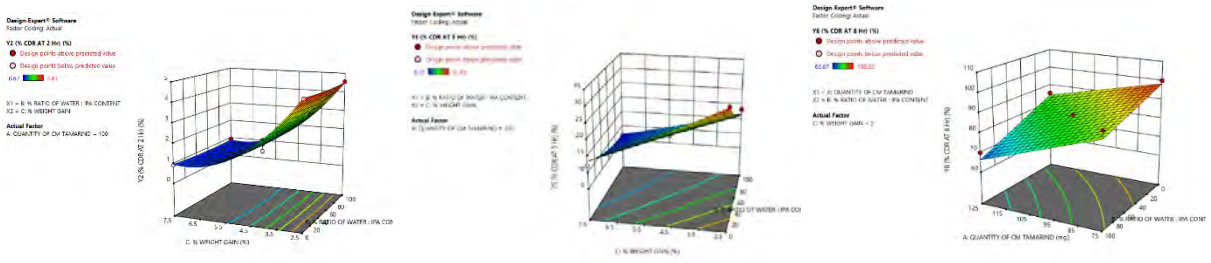

Figure 3: 3D Response Graph for % CDR at 2 hrs (a), % CDR at 5 hrs (b) and % CDR at 8 hrs (c)

Polynomial Equation
Y2 = +1.52-0.0925A-0.0400B-1.81C-0.1550AB-0.1750AC+ 0.0050BC - 0.0642A² + 0.0658B²+1.16 C²
Y5 = 15.50333 -3.99875 A -5.43375 B -7.1475 C -0.41 AB + 0.523 AC + 2.106 BC + 2.35083 A² +1.61583 B²+0.3733 C²
Y8 = + 84.118 - 8.7175 A -7.285 B - 7.4 C - 1.71 AB - 5.545 AC -2.73 BC

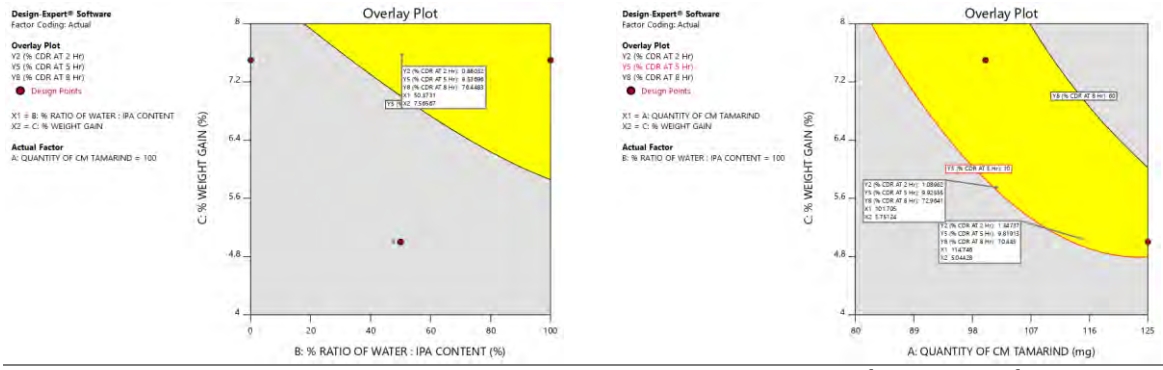

Figure 4: Design space (Overlay plot) prepared by keeping (a) X1 (2nd) (b) X2 (3rd) level constant

In this research, Design space (DS) was set up using RSM in conjunction with optimization. Design space (DS) were utilized to evaluate the impact of 2 factors on the dependent variables at a given time while a third variable was maintained constant. Figure 4a depicts the impact of X2 and X3 on Y2, Y5, and Y8 at 2 levels of X1. If the X3's % weight gain was between 5.8 % and 8.0 %, there was projected DS formation. Figure 4b depicts the impact of X1 and X3 on Y2, Y5, and Y9 at 3 levels of X2. If the X3's % weight gain was between 4.8 % and 8.0 %, there was projected DS formation. If all variables were within the DS, colon-targeted tablet would satisfy the compliance requirements.

For the robustness and accuracy of experimental design, five distinct check point batches were chosen within DS to compare predicted value to observed value. Based on the result data, it was determined that there is no substantial difference between the data, and it was therefore determined that selected design has excellent prediction power.

Pellet-based colon targeted dosage form:

Budesonide (0.2 g), Tamarind Gum, MCC, and lactose (q.s to create 10 g) were thoroughly combined to form a homogenous powder. The required quantity of water was added gently, and mixing was continued to achieve the desired consistency. The wet material was then extruded through a 1 mm-mesh screen using an extruder (Mfg.-Cronimach machinery, Ahmedabad) to generate extrudate (needle-shaped). The extruded material was spheronized at 1,000, 1,500, or 2,000 revolutions per minute using a spheronizer. The spheronization time ranged from 3 to 10 minutes. The investigation included the use of varying quantities of materials and process parameters in order to ascertain the elements that have an impact on the quality of pellets.(16)

Risk assessment study

The primary objective was to identify the CQAs for core pellets. On the basis of the literature, previous work experience, and preliminary batch data, the Fishbone diagram (figure 5) was generated and potential identified risk parameters were arranged in a hierarchical fashion.

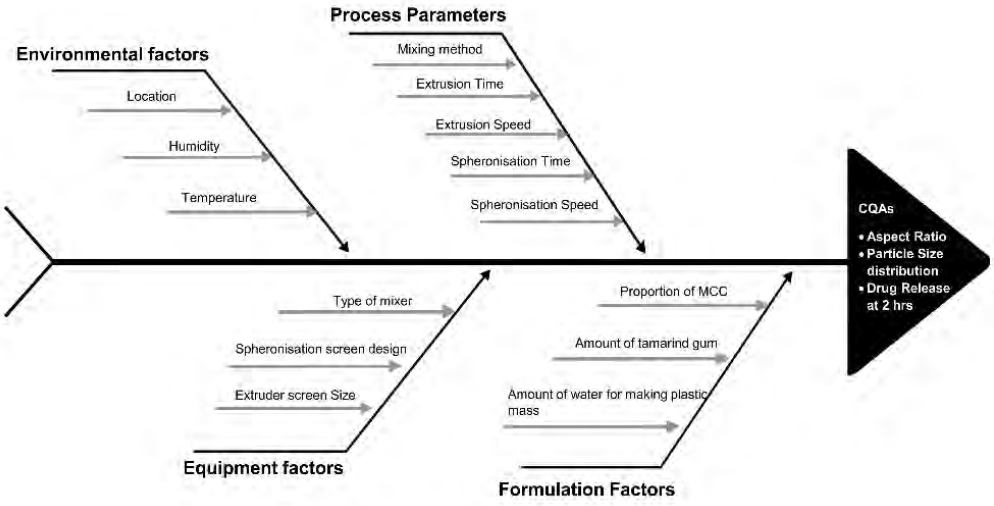

Figure. 5: A Fishbone diagram illustrating factors that may have impact on the critical quality attributes

FMEA (Failure Mode and Effects Analysis), a risk management tool, was utilised to determine the RPN (risk priority number) for all potential risks. Influence of environmental and equipment factors shown less significant because a research work was conducted at fixed laboratory setup. Multiplying S (severity), D (detectability), and P (probability) of risks yields the RPN. The maximum value (5) denotes a prominent influence, while the minimum value (1) denotes no effect of a specific risk on the selected CQA. A RPN value greater than 60 was used as a criterion for selecting factors for further research.

The RPN scores for all potential risk factors, which were calculated by multiplying the S, D, and P of individual risk factors, indicating that five risk factors, the amount of tamarind gum,

the ratio of MCC to lactose, the spheronizer speed, the spheronisation time, and the amount of water required to create plastic mass, have a significant impact on CQAs.

On the basis of preliminary batches, it was determined that 6 ml was sufficient for all formulations; consequently, it was chosen as a constant variable for further research.

Screening of Pellets Parameters (Formulation and Process) by using 2^4 full factorial design

In this study, Utilizing Design Expert 11.0 (StatEase, Minneapolis, MN) to build a four-factor, two-level full factorial design to assess the main, interaction, and quadratic effects of four components including amount of tamarind gum, proportion of MCC in relation to lactose, speed of spheronizer, and time of spheronization on selected responses, namely aspect ratio, particle size distribution, and % drug release at 2 hours(16), are outlined in Table 3

Table 3 Dependent variables and independent variables with levels for 2^4 full factorial design

Independent variables	levels		Dependent variables
	-1	+1	
X1= Amount of Tamarind Gum	1	5	R1 = Aspect Ratio
X2= Proportion of MCC (%)	25	75	R2 = Particle Size Distribution (D50)
X3=Speed of Spheronizer	1000	1500	R3 = % Drug Release at 2 Hours
X4 = Time for Spheronization	5	10	

In order to get the Design Space for attaining specified CQAs, a 2^4 Factorial design with 16 runs (factorial points) was used

From the data of the 2^4 full factorial design and design space, it can be determined that pellets with the desired characteristics may be produced by using Tamarind Gum in the range of 2gm to 3gm and MCC in the range of 30% to 40%. So, for the subsequent experimental design, the range of factors (A and B) has been reduced, while other factors of 2^4 factorial study were kept constant as per the data of factorial design and not included as independent variable in subsequent Box–Behnken experimental design.

Formulation optimization by using Box-Behnken Factorial Design

After Optimization of process parameters and formulation parameters for Pellets using 2^4 factorial design, Pellets for colonic delivery were optimized using Box-Behnken Design (Design Expert 11.0) containing the three-factor, three-level to define main, interaction and quadratic effects(13,14) of independent variables like amount of Tamarind Gum, MCC proportion with respect to Lactose (%) and % weight Gain by Eudragit S100 on the selected responses (dependent variables) which were % drug release at 2 hours, % drug release at 5 hours and % drug release at 9 hours as shown in table 4.

Table 4 Dependent variables and independent variables with levels for Box Behnken design

Independent variables	levels			Dependent variables
	-1	0	+1	
X1=Amount of Tamarind Gum	75	100	125	Y_2 = % CDR at 2 Hours
X2= MCC proportion (%)	0	50	100	Y_5 = % CDR at 5 Hours
X3=% wt. Gain by Eudragit S100	2.5	5	7.5	Y_9 = % CDR at 9 Hours

Regression Analysis for generating the optimized design space

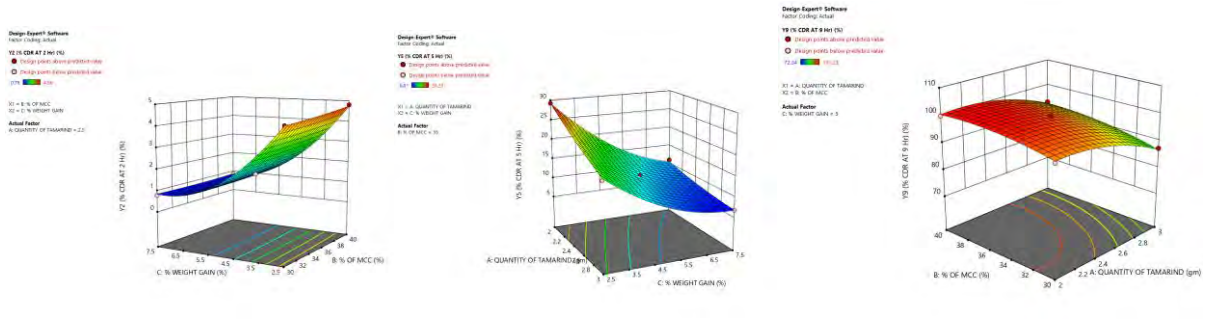

Figure 6: 3D Response Graph for % CDR at 2 hrs (a), % CDR at 5 hrs (b) and % CDR at 9 hrs (c)

Polynomial Equation

Y2 = 1.64333 + -0.24625 * A + 0.10375 * B + -1.845 * C + 0.02 * AB + 0.2425 * AC + -0.0975 * BC + -0.0441667 * A^2 + 0.0358333 * B^2 + 1.01833 * C^2

Y5 = 12.71 + -3.545 * A + 1.29625 * B + -7.65375 * C + -1.2475 * AB + 1.7125 * AC + 0.005 * BC + 0.4525 * A^2 + 1.085 * B^2 + 3.145 * C^2

Y9 = 99.0167 - 4.60875 * A + 2.26875 * B - 8.535 * C + 0.705 * AB - 4.5275 * AC + 1.6075 * BC - 3.12333 * A^2 -1.59333 * B^2 - 4.58083 * C^2

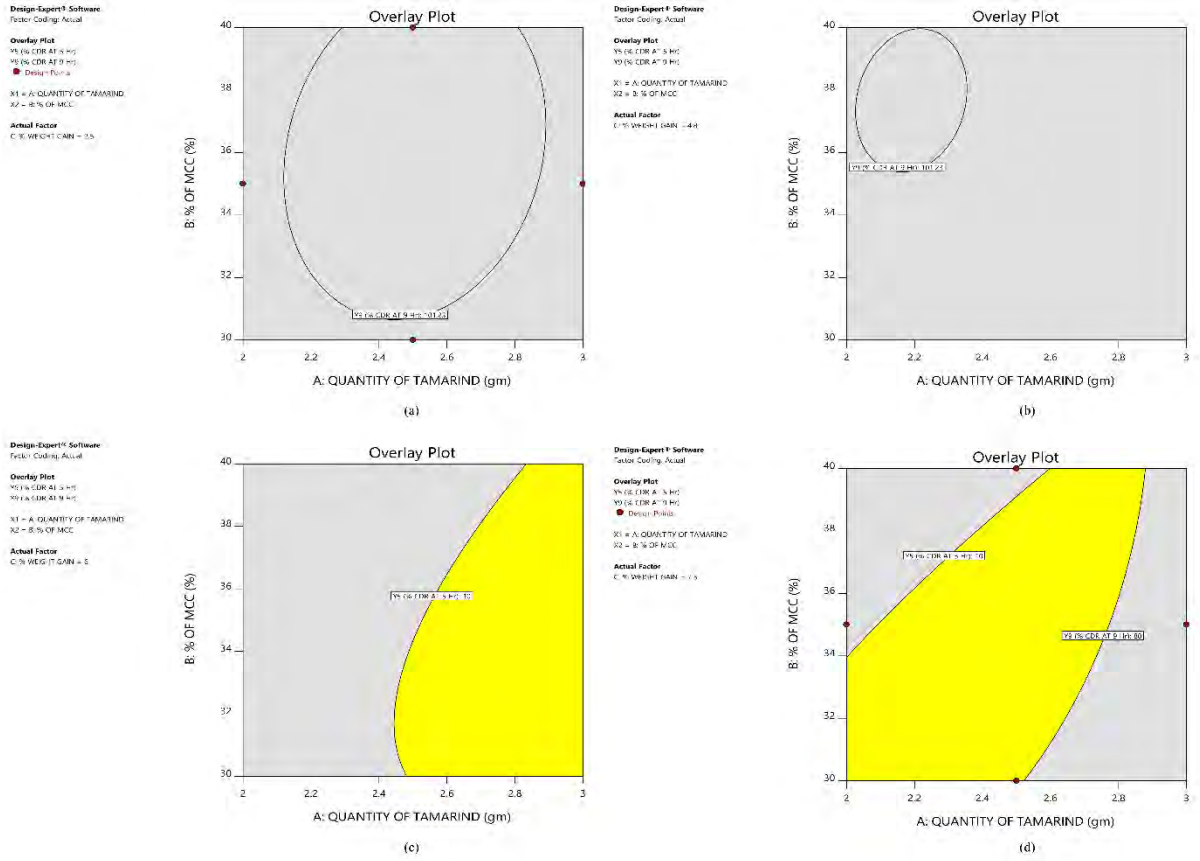

Figure *7*: Design space (Overlay plot) of prepared with Operating ranges of X3 (% weight gain) at (a) 2.5 % weight gain (b) % 4.8 weight gain (c) % 6 weight gain (d) 7.5 % weight gain

In this study, the Design space was established using Response Surface Methodology (RSM) in combination with optimization techniques. The study used design space methodology to assess the influence of two independent variables on the dependent variables at a certain moment, while keeping a third variable constant. Figure 7 illustrates the influence of variables X1 and X2 on the outcomes Y2, Y5, and Y9, specifically at the third levels of X3. If the X3 had a weight rise ranging from 2.5% to 4.8%, there was no projected start of DS as shown in Figure 7 a and b. The figure 7 (c and d) displays a graphical representation of the design space (DS), shown by the yellow region. This overlay plot illustrates the percentage weight gain achieved 6 % and 7.5 % by Eudragit S 100. The Design-Expert 11.0 software suggested that these levels to create an ideal dosage form that meets the specified parameters. If all variables were included within the data set, pellets designed to target the colon would meet the standards for compliance.

For the robustness and accuracy of experimental design, five distinct check point batches were chosen within DS to compare predicted value to observed value. Based on the data, it was determined that there is no substantial difference between the data, and it was therefore determined that Selected Design had excellent prediction power.

Comparison of Dissolution Profile between two formulations (Pellets and Tablet-based formulations)

The methods for the comparison of *in vitro* dissolution profiles can be classified into three groups: (1) model-dependent methods ((17–19), (2) model-independent methods ((18–20), and (3) the methods based on analysis of variance (ANOVA) ((18,21).

Model Dependent Methods:

Model-dependent approaches including zero order, first order, Hixson-Crowell, Higuchi, Korsmeyer Pappas and Weibull models were utilized for comparison between dissolution profile between pellet-based and tablet-based formulation in current study. Statistical parameter like R_2 Adjusted, n, AIC and MSC were assessed for comparison. As per the data it was concluded that there was similarity between dissolution profile of tablets and pellets optimised formulations. *In-vitro* dissolution profile comparison was also made using MSD (Multivariate Statistical distance) test and concluded that both formulations were similar.

Model Independent Methods:

Generally, Pair wise procedure was used for Model independent methods. These include difference factor $f1$ and similarity factor $f2$ and two indices of rescigno.

As per the data, $f1$ (5.9), $f2$ (72.87) and Rescigno index (i_1-0.033 & i_2-0.038), it was concluded that both formulations were similar.

One-way ANOVA was utilized to assessed the similarity between two formulations with respect to % dissolution efficiency (%DE) and mean dissolution time (MDT) for all batches. As per the statistical data, it was concluded that there is the similarity in dissolution profile between the formulations.

Consequently, it can be concluded that both formulations were capable of delivering the maximal amount of drug to the colon. However, from an economic standpoint, the tablet dosage form was produced using CM tamarind gum, whereas the pellets dosage form was created using Tamarind gum, indicating that pellets may contain significantly less expensive ingredients. According to the experimental design, the total quantity of CM Tamarind gum in tablet dosage form ranges from 37.5% to 62.5%, whereas the total quantity of Tamarind gum in pellet dosage form ranges from 20% to 30%. This information suggests that the total cost of pellet ingredients should be considerably lower, which may justify the pellets' high processing costs. In addition, pellets offer the advantage of a multi-particulate system. The pellet dosage form is therefore preferable to the tablet dosage form.

Stability Study

In the present study, the optimal batch F12 for pellets and F3 for tablet dosage form from the Box Behnken Design were chosen for the stability study, which was conducted in accordance with ICH (International Council on Harmonisation) guidelines by keeping the sample at 40±2 ^{0}C and 75±5 % RH for six months in a stability Chamber (Mfg.: Patel Instrument Pvt. Ltd., Ahmedabad, Gujarat, India)(22–24). A high-density Polyethylene bottle is the container closure system used in this study. The selected study intervals were the 1st, 3rd, and 6th months from the Initial time. The optimized tablet-based and Pellet-based formulation were examined for appearance (description), moisture content, drug content, % CDR (cumulative drug release) at 5 hours, friability, and microbial limit test(25). As per the data, it was concluded that the tablet and pellet dosage form was stable enough for 6 months under the accelerated conditions as per the ICH.

***In –Vivo* Study**

Induction of Ulcerative colitis and treatment with formulation in Rat

Male Wistar rats (200±20g) were housed in environmentally controlled conditions, with free access to water and standard chow pellet diet and will be fasted for 36 h before induction of colitis. Induction of colitis was performed according to the previously reported method (26,27). Under light ether anesthesia, a rubber canula (8-cm long) is inserted into the colon via the anus and 1 mL solution of 3% acetic acid was instilled into the lumen of the colon. The rats were divided into seven groups containing eight animals in each group as follows: Normal (Control Group), sham group (without induction of colitis) that received 2 ml normal saline intrarectally., untreated disease group, gum treated group (with induction of colitis) that received tamarind gum solution without drug orally., Test group I that received Budesonide Pellets (150 µg) orally after induction of colitis as the normal treatment of UC., Test group II that received budesonide pellets (300 µg) orally after induction of colitis as the normal treatment of UC., and Std group that received budesonide solution (300 µg) orally after induction of colitis as the normal treatment of UC.

Drug administration to animals was started 24h after induction of colitis and continued every 24h for 5 days. Twenty-four hours after administration of the last dose of formulations, rats were sacrificed with high dose of ether and a midline incision was made in abdomen.

The 8-cm distal segment of colon was removed, opened, and washed in normal saline. Histopathology and inflammatory Markers (TNF- α, IL-6) were be measured.

Histopathology Evaluation of colonic tissue

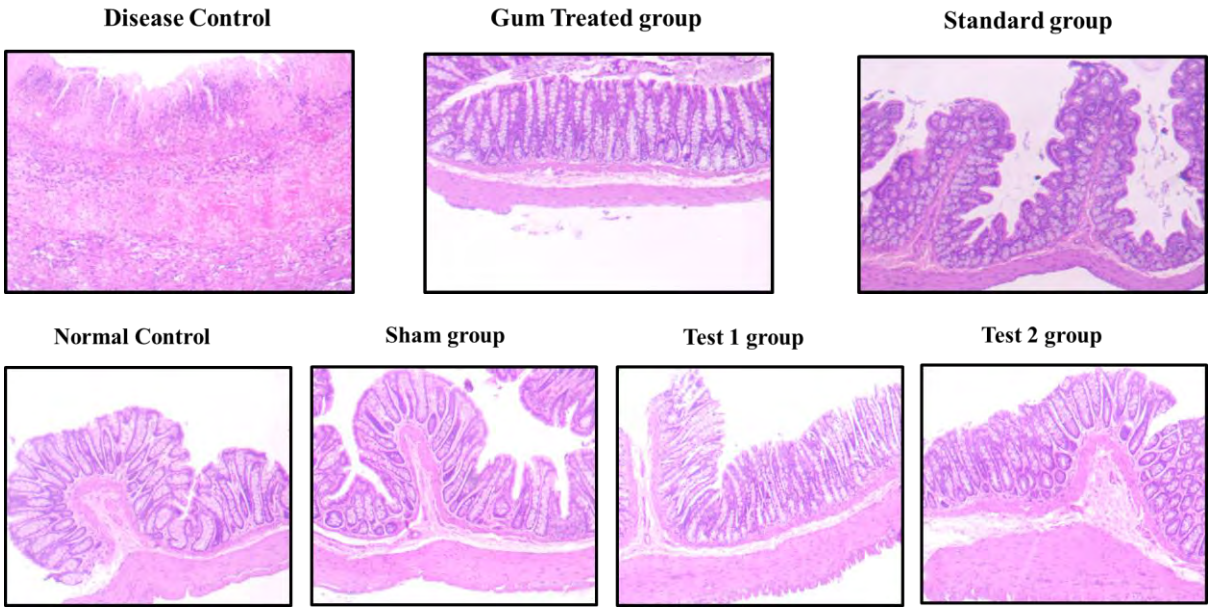

Figure 8: Histopathological analysis of the colonic tissue of rats of different groups

Inferences:

- **Disease Control animal** showing induction of sever ulceration and tissue necrosis associated with inflammatory infiltrate and goblet cell hyperplasia
- **Gum Treated animal** showing less goblet cells surrounded by transmucosal fewer lymphoplasmacytic infiltrate within stromal edema was seen.
- **Std treatment group** showing tall columnar epithelium, with superficial shredded epithelial cells, less eroded surface surrounded by few inflammatory edemas and less necrosis with colonic gland showed reparative epithelial changes
- All other animals' **Normal control and Sham group** looks normal
- **Test 1 and Test 2 groups** revealed intestinal section with better healed and improvement of intestinal mucosa compared to positive controlled sections with few mucosal lymphoplasmacytic infiltrate within stromal edema.

Biochemical (Interleukin 6 & TNF-α) Estimation

Inflammatory cytokines are known to play a crucial role in modulating mucosal immune system where the neutrophils and macrophages are responsible for disrupting epithelial integrity and causing colon injury. The pathogenesis of UC is characterized by migration of granulocytes and other leukocytes to the inflamed mucosa and superficial ulcers leading to increased levels of pro-inflammatory cytokines such as TNF-α and IL-6. In colon, TNF-α and IL-6 levels were assessed and quantified using enzyme-linked immunosorbent assay ELISA (Sandwich Method). The results were expressed as pg/mL

Biochemical (Interleukin 6 & TNF-α) Estimation using ELISA Test

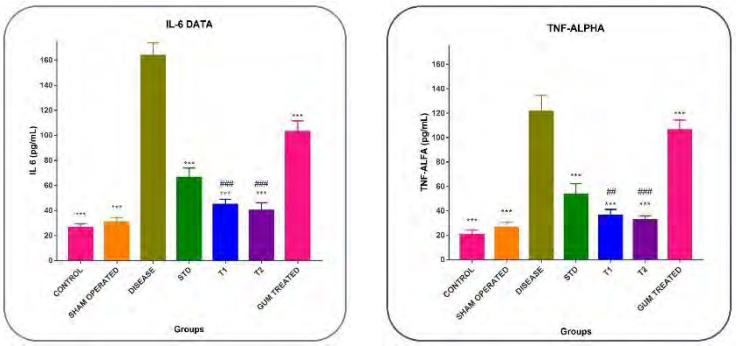

Figure 9: concentration of IL -6 (a) and TNF-Alpha (b) in colonic tissue homogenate of different groups

Inferences:

The disease group exhibited significantly elevated levels of TNF-α and IL-6 in the colon tissue compared to the control group, confirming the induction of inflammation as shown in figure 9. Histopathological analysis revealed evidence of epithelial cell necrosis, edema, and neutrophil infiltration, further supporting the inflammatory response in the disease group.

In contrast, the treatment group receiving budesonide colon targeted formulation (T1 & T2) showed a significant reduction in the levels of TNF-α and IL-6 compared to both the disease group and the group receiving budesonide solution in a 1% CMC solution.

***In vivo* Evaluation of Formulations in Rabbit**

In rabbits, the GI transit of a particular formulation was evaluated *in vivo*. White New Zealand Rabbits (1.5–2.5 kg) were chosen for the investigation. Before administering Pellets/Tablets, the animals were fasted overnight. The pellets/tablets were inserted into the animal's larynx using forceps, followed by the administration of 10-15 ml of water down the neck to aid their passage down the esophagus. Figure 10 (tablet dosage form) and Figure 11 (pellets dosage form) depict the results of X-ray examinations performed on the rabbit at regular intervals.

At Zero Hour **At 1st Hour** **At 3rd Hour** **At 5th Hour** **At 7th Hour**

Figure 10: X-Ray Examination of Rabbit after administration of optimized tablet dosage form

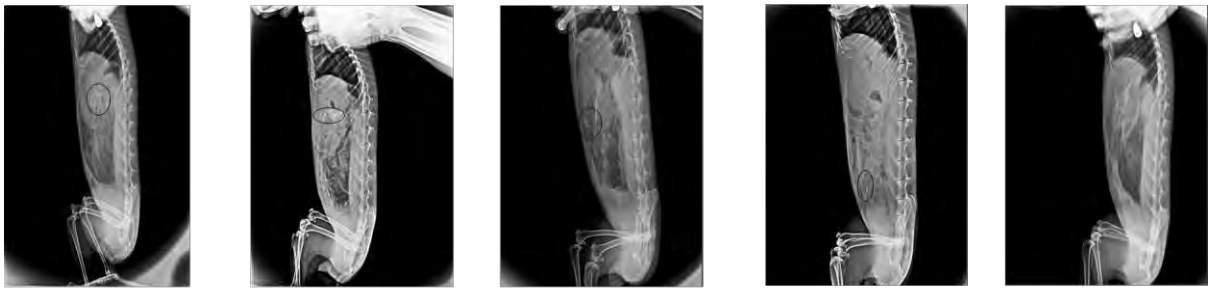

Figure 11: X-Ray Examination of Rabbit after administration of optimized pellet dosage form

g. Achievements with respect to objectives

The successful development of these colon-targeted dosage forms holds significant implications for the treatment of colonic diseases, such as inflammatory bowel disease (IBD). By delivering Budesonide directly to the colon, these formulations can improve therapeutic efficacy while reducing systemic side effects. This targeted drug delivery approach has the potential to revolutionize the treatment of colonic diseases, enhancing patient outcomes and quality of life. On the basis of viscometric analysis and an enzyme susceptibility study, it was concluded that tamarind gum was a potential candidate for a colon-targeted drug delivery system. Tablet-based and pellet-based colon targeted dosage forms with the use of carboxymethyl tamarind gum and tamarind gum, respectively, proved that they were excellent in delivering drugs directly to the colonic region.

The application of the Quality by Design (QbD) approach in this research ensured a systematic and scientific formulation development process. By carefully selecting critical parameters and optimizing formulation variables, formulations that met the desired selection criteria were achieved. Through histopathological, roentgenographic, and biochemical (IL-6 and TNF-α) estimation studies, it was determined that formulations were capable of delivering the drug to the colonic region.

h. Conclusion

In conclusion, this research successfully developed Microbial and pH-triggered Colon-targeted Budesonide Tablet and Pellet Dosage forms using the Quality by Design (QbD) approach. Through a viscosity-based methodology, the study identified tamarind gum and carboxymethyl (CM) tamarind gum as promising natural polymers for formulation development.

The tablet dosage form incorporated Carboxymethyl (CM) Tamarind Gum and was coated with Eudragit S 100 to retard the release of Budesonide in the upper gastrointestinal tract. The optimized formulations by Box-Behnken Design ensured targeted delivery of the drug to the

colon, maximizing its therapeutic effects while minimizing systemic exposure and potential side effects. For the pellet dosage form, critical process parameters (CPPs) and formulation variables were optimized using a 2^4-factorial design and Box-Behnken design. The formulations exhibited desirable drug release profiles, with less than 10% of the drug released in the initial 5 hours and more than 80% released within 9 hours.

Overall, the developed Microbial and pH-triggered Colon-targeted Budesonide Tablet and Pellet Dosage forms demonstrate the potential for effective site-specific drug delivery to the colon. Further studies and clinical trials are warranted to validate the effectiveness and safety of these dosage forms in real-world applications.

i. Copies of papers published/Accepted/communicated

Sr. No.	Details
Paper Published (Annexure – 1)	
1	Patel, J., Patel, K. & Shah, S. Fabrication of a Dual-Triggered Natural Gum–Based Multi-Particulate Colon-Targeted Drug Delivery System of Budesonide Using the QbD Approach. **J Pharm Innov (2023).** https://doi.org/10.1007/s12247-023-09764-z **(Impact Factor – 2.6 (Clarivate)**
Paper Accepted	
2	Title: Quality by Design Approach for Optimization of Microbial and pH-Triggered Colon-Targeted Tablet Formulation using Carboxymethyl Tamarind Gum Authors: Jaymin Patel, Ms. Kaushika Patel, Dr. Shreeraj Shah Journal: Assay and Drug development Technologies **(Impact Factor – 1.8 (Clarivate)**
3	Title: Methacrylic Acid Co-Polymers: Crucial agents for the Colon Targeted Oral Drug Delivery System Authors: Jaymin Patel, Ms. Kaushika Patel, Dr. Shreeraj Shah Journal: Research Journal of Pharmacy and Technology **(Scopus indexed (Q2))**

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ANNEXURE - 1

Journal of Pharmaceutical Innovation
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ORIGINAL ARTICLE

Fabrication of a Dual-Triggered Natural Gum–Based Multi-Particulate Colon-Targeted Drug Delivery System of Budesonide Using the QbD Approach

Jaymin Patel[1,2] · Kaushika Patel[1] · Shreeraj Shah[1]

Accepted: 29 July 2023

Abstract

Purpose To develop microbial and pH-triggered colon-targeted budesonide pellets utilising the Quality by Design (QbD) approach. Several polysaccharide-based natural gums selected using a retrospective research strategy were screened for their efficacy in developing a microbial degradation–based colon-targeted Drug Delivery System (DDS).

Methods The viscosity profiles were generated in the presence of a prebiotic culture medium that simulated the effect of 4% rat cecal content. Critical process parameters (CPPs) such as spheronization speed and time and formulation variables (CMAs) such as proportion of tamarind gum and microcrystalline cellulose (MCC):lactose were selected as independent variables for screening design 2^4 FFD (full factorial design) to sort out the crucial parameters for the further optimization of pellets. The significant dependent variables were aspect ratio, particle size distribution, and % CDR (cumulative drug release) at 2 h. Pellets were super coated with the enteric polymer Eudragit S100. The screened range was further revealed to Box Behnken Design (BBD) for response surface optimization.

Results On the basis of viscometric analysis, tamarind gum was selected for formulation development. Based on the screening design, considering the constraints of aspect ratio, particle size distribution, and % CDR at 2 h in the range of 1–1.2, 1–1.3, and < 25%, respectively, the independent variables selected for Box Behnken Design (BBD) were proportion of gum and % MCC in the ranges of 2–3 g and 30–40%, respectively. The optimization design space was generated based on the criteria of LT 10% of the drug in the first 5 h and MT 80% in the first 9 h to achieve colon targeting.

Conclusion Tamarind gum is efficient for colon targeting, and its proportion of 2.5–3 g along with an enteric coating of 6% leads to an optimised formulation.

Keywords Budesonide · Tamarind gum · pH and microbial approach · QbD · Probiotic culture medium · Colon-targeted pellets · Box-Behnken design

Introduction

Inflammatory bowel disease (IBD) or inflammatory bowel syndrome (IBS) is a prevalent worldwide health issue with a consistently rising occurrence [1].

Despite the fact that 5-ASA (5-amino salicylic acid) drugs have been the preferred treatment for CD (Crohn's disease) and UC (ulcerative colitis), there is contradictory information about their efficacy in individuals with Crohn's disease, particularly as long-term treatment. Antibiotics have a minimal role in colonic CD therapy [2]. Corticosteroids remain the preferred therapy for acute diseases unresponsive to more conservative treatment [3].

Unfortunately, corticosteroids may result in undesirable side effects [4]. Budesonide is a relatively recent steroid drug that undergoes rapid hepatic metabolism, thereby minimising corticosteroid-associated implications [5]. Budesonide is much more effective than placebo or mesalamine in the treatment of severe ulcerative colitis [6–8].

Budesonide is a corticosteroid with a considerable anti-inflammatory effect at the site of application but limited systemic activity due to significant hepatic degradation [4, 9]. Because of this, conventional oral formulations are

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ASSAY and Drug Development Technologies

Decision Letter (ADT-2023-066.R1)

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Subject: ASSAY and Drug Development Technologies - Decision on Manuscript ID ADT-2023-066.R1

Body: 05-Sep-2023

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Paper Title: Methacrylic Acid Co-Polymers: Crucial agents for the Colon Targeted Oral Drug Delivery System

Acceptance of Manuscript

With reference to your article titled **'Methacrylic Acid Co-Polymers: Crucial agents for the Colon Targeted Oral Drug Delivery System'** Author by **Jaymin Patel, Ms. Kaushika Patel, Dr. Shreeraj Shah**.

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