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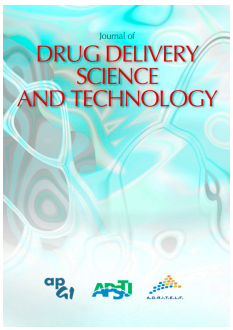


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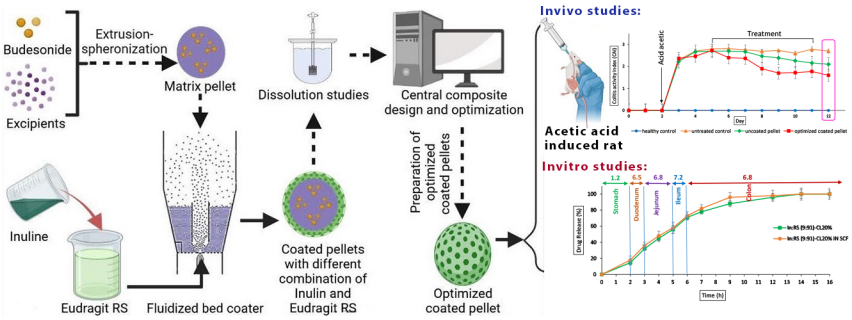


# Combination of time-dependent polymer and inulin as a coating for sustained delivery of budesonide pellets aimed for use in IBD treatment

**Fatemeh Soltani[1], Hossein Kamali[2], Abbas Akhgari[2,1], Mahboobeh Ghasemzadeh Rahbardar[3], Hadi Afrasiabi Garekani[3,1], Ali Nokhodchi[4,5,*] and Fatemeh Sadeghi[2,1,*]**

[1]Department of Pharmaceutics, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad 9177899191, Iran; [2]Targeted Drug Delivery Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad 9177899191, Iran; [3]Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad 9177899191, Iran; [4]Lupin Pharmaceutical Research Center, Coral Springs, FL 33065, USA; [5]School of Life Sciences, University of Sussex, Brighton BN1 9RH, UK

* Corresponding authors:

Fatemeh Sadeghi (sadeghif@mums.ac.ir)

Ali Nokhodchi (a.nokhodchi@sussex.ac.uk and AliNokhodchi@lupin.com)

**Abstract**

Crohn's disease and ulcerative colitis, both forms of inflammatory bowel disease (IBD), are prevalent conditions. Budesonide, a medication widely recommended as a first-line treatment for Crohn's disease, necessitates the development of formulations capable of delivering the drug to the intestinal region. Inulin, a highly effective polysaccharide and prebiotic in treating IBD, possesses inadequate film-forming abilities. However, harnessing the beneficial effects of inulin in IBD treatment, researchers designed a single-layer coating using a central composite design (CCD) that combined inulin and eudragit RS. This coating aimed to sustain the release of budesonide pellets, providing targeted therapy for Crohn's disease. The study focused on two independent variables: the percentage of inulin and the coating level. The responses evaluated were the release of the drug during a two-hour period at pH 1.2, as well as within three and ten hours at pH 6.8. The release profiles of the coated pellets were examined in media with varying pH levels to determine the optimal coating formulation. Subsequently, the optimized coated pellets underwent continuous mode dissolution testing to assess their release profile. Finally, the impact of the optimized formulation on reducing inflammation was evaluated in rats with experimentally induced colitis. The coating formulation composed of 9% inulin and 91% eudragit RS at a coating level of 20% displayed a complete and pH-independent release profile. Approximately 85% of the drug was gradually released throughout the small and large intestines. The presence of inulin in the coating formulation rendered it susceptible to microbial degradation, resulting in an increased drug release rate in the colonic medium containing rat cecal content. *In vivo* results demonstrated the favorable therapeutic effects of this delivery system in treating inflammation in rats. The controlled release of the drug throughout the gastrointestinal tract (GIT) contributed to its efficacy in mitigating inflammation.

***Keywords*:** Budesonide pellets, Target delivery, Inulin, Time-dependent, Crohn's disease

## 1. Introduction

Inflammatory bowel disease (IBD) is a chronic condition linked to the inflammation of the intestinal lining [1]. The two main types of IBD include ulcerative colitis and Crohn's disease [2]. In Crohn's disease, the inflammation can affect the entire gastrointestinal tract, from the mouth to the anus [3], while in ulcerative colitis, the inflammation is primarily limited to the colon and rectum [4]. The primary therapeutic goal in patients with IBD is to achieve mucosal healing [5], which can significantly improve their quality of life [6]. Although the medications currently used for IBD treatment have been effective [7], their potential side effects have prompted the search for new delivery systems that offer higher safety profiles [8, 9].

Due to its topical anti-inflammatory properties and minimal systemic absorption, budesonide has gained attention as an oral controlled-release formulation for the treatment of IBD [10]. It is currently recommended as a first-choice therapy for Crohn's disease [11]. While numerous budesonide dosage forms have been developed for IBD treatment [12-14] multi-particulate dosage forms, particularly pellets, are gaining prominence due to their potential advantages. These advantages include predictable movement within the gastrointestinal (GI) tract and reduced variations in gastric emptying compared to tablets [15, 16]. Thus, budesonide pellet formulations have been extensively developed to target IBD treatment [17-20].

Recent studies have revealed that natural products for instance prebiotics, probiotics, and synbiotics can be considered as alternative therapeutic options for IBD [21]. These natural products have shown potential in alleviating gastrointestinal symptoms by lowering the pH of the intestine and increasing the population of beneficial bacteria [22]. They can be used either as a standalone treatment or in combination with standard therapy for IBD [23, 24]. Several studies have confirmed that the concurrent administration of probiotics with standard medications is more effective than using chemical therapy alone [25, 26]. These findings

highlight the potential benefits of incorporating natural products, particularly probiotics, into the treatment regimen for IBD.

It is important to note that the use of probiotics is contraindicated in patients with compromised immune systems [27] due to the potential risks associated with the development of sepsis and bacteremia [28]. On the other hand, the use of commercial prebiotics is generally considered safe [29, 30]. Additionally, the production processes and storage requirements for prebiotics are typically easier compared to probiotics [31] making them more appealing for pharmaceutical industries. Due to their safety profile and ease of production, prebiotics hold a significant interest in the pharmaceutical industry.

Inulin is a widely used prebiotic in IBD patients [32, 33]. Oral administration of inulin can reduce inflammation in both IBD patients and colitis rat models [22, 34]. The modulation of the intestinal flora is the primary mechanism involved in the management of IBD with inulin [35]. Additionally, the metabolites produced through inulin fermentation can help regulate constipation by affecting intestinal peristalsis [36].

One notable characteristic of inulin is its ability to withstand the acidic environment of the stomach and only be hydrolyzed by specific intestinal microflora, not digestive enzymes [37]. Therefore, in addition to its pharmacological benefits, inulin holds promise as a vehicle for targeted drug delivery to the intestinal region [35, 38]. Some studies have explored the encapsulation of budesonide into inulin nanoparticles for targeted delivery to the colon [39, 40]. Furthermore, loading budesonide into inulin microparticles has shown significant effectiveness in targeting the colon [41, 42].

Studies have shown that loading polysaccharides within pellet matrices can lead to a decrease in drug release due to gel formation upon contact with a dissolution medium [43, 44]. Considering that budesonide is practically insoluble in water [45], incorporating inulin into the pellet structure and promoting gel formation may result in inadequate drug release at the

desired site of action [46]. Alternatively, using inulin as a coating material may lead to premature drug release in the upper parts of the gastrointestinal (GI) tract due to its high water solubility and poor film-forming ability [47]. To address these challenges, the combination of inulin with time-dependent polymethacrylates has been extensively studied as a system for targeted delivery to the intestinal region [4, 41, 43, 48]. Current evidence suggests that blending inulin with water-insoluble polymers can enhance film-forming ability and prevent early drug release in the upper segments of the GI tract [49]. This approach aims to optimize drug release profiles and ensure that the drug is delivered effectively to the intended site of action in the intestinal region.

In this study, the objective was to develop a sustained-release formulation of budesonide pellets for the treatment of Crohn's disease, while also leveraging the therapeutic advantages of inulin. To achieve this, an optimized single-layer coat was designed for the budesonide-loaded pellets using a blend of inulin and time-dependent polymers, specifically eudragit RS. The drug release profiles in these combined systems can be controlled by adjusting the concentration of inulin in the coating composition as well as the coating thickness [50, 51]. To optimize this system, a full factorial design was utilized, allowing for the investigation of the impact of varying inulin concentration and coating thickness on the drug release characteristics of the pellets. By systematically evaluating these factors, the researchers aimed to determine the optimal formulation parameters for achieving the desired sustained-release properties of budesonide.

## 2. Materials and methods

### *2.1. Materials*

Budesonide (Jaber Ebne Hayyan, Tehran, Iran), Avicel PH 102, Lactose monohydrate, triethyl citrate (TEC) and talc (Merck Company, Frankfurt, Germany), polyvinylpyrrolidone (PVP K30) (Rahavard Tamin, Tehran, Iran), inulin (high molecular weight) (Sigma-Aldrich, St.

Louis, USA) isopropyl alcohol (Dr. Mojallaly, Tehran, Iran), sodium lauryl sulfate (SLS) (Scharlau ,Barcelona, Spain) and eudragit RS 30D (Evonik Industries, AG Hanau, Germany) were utilized in this study. Other reagents and solvents utilized in this study were of analytical grade.

*2.2. Preparation of budesonide pellets*

Budesonide-loaded pellets were produced using the extrusion-spheronization technique, following a formulation developed previously for conventional budesonide pellets [46]. The selection of excipients and their concentrations aimed at achieving a high dissolution rate for the budesonide pellets. In summary, a blend containing budesonide (1.5% w/w), PVP K30 (5% w/w) serving as a binder, lactose monohydrate (25% w/w) as a soluble filler, and Avicel® PH 102 (68.5% w/w) as a pelletization aid was prepared through mixing (utilizing FUMA, Fu-1877 Hand Mixer, Japan) for a duration of 20 minutes. Distilled water was then slowly added to the powder blend until a wet mass with the desirable plasticity was achieved. This wet mass was subsequently extruded through an axial screw extruder (Dorsa Tech, EX-01, Iran) with a 1 mm screen. The spheronization of the extrudates was carried out (Dorsa Tech, EX-01, Iran) for 5 minutes at 1200 RPM. The resulting pellets were subjected to drying in an oven (40 °C) for 24 hours, then were sieved and the fraction of 850–1180 μm was used for further studies.

*2.3. Design of coating formulation*

The response surface method with a $3^2$ full factorial design was implemented to mode and optimize the coating formulation, by design expert software (Design-Expert software, Version 11, Stat-Ease, USA). The independent variables which were examined at three levels were the amount of inulin in the coating composition ($X_1$) as well as the coating level ($X_2$). The higher and lower levels of each independent variable were designated as +1 and -1, while the average

value was denoted as 0. The specific independent variables and their levels can be found in Table 1.

**Table 1**
Independent variables and their levels in design experiment.

| Independent variables | Levels used | | |
|---|---|---|---|
| | -1 | 0 | +1 |
| $X_1$ = amount of inulin (%) | 0 | 20 | 40 |
| $X_2$ = coating level (%) | 10 | 15 | 20 |

Considering the extension of inflammation in Crohn's disease from the stomach all the way down to the anus [3], therefore the coating formulations should be able to offer a sustained release of the drug from the stomach to the small and large intestines [4]. So the percentage of drug release during 2 hours at pH 1.2 ($Y_1$), the percentage of drug release within 3 hours at pH 6.8 ($Y_2$) (to avoid excessive release in the small intestine), as well as the percentage of drug release during 10 hours at pH 6.8 ($Y_3$), were chosen as response variables. Table 2 lists the suggested formulations suggested by the software.

**Table 2**
The runs and composition of experimental formulations.

| Run | Variable factors | |
|---|---|---|
| | $X_1$ (ratio of inulin to (inulin+RS)) | $X_2$ (coating level (%)) |
| **1** | 0 | 10 |
| **2** | 0 | 15 |
| **3** | 0 | 20 |
| **4** | 20 | 10 |
| **5** | 20 | 15 |
| **6** | 20 | 20 |
| **7** | 40 | 10 |
| **8** | 40 | 15 |
| **9** | 40 | 20 |

*2.4. Coating of pellets*

*2.4.1. Preparation of coating formulations*

First of all, a solution of inulin was prepared in hot distilled water (10% w/v) and then added to an appropriate amount of eudragit RS 30D under continuous stirring. As a plasticizer, triethyl citrate (TEC) was incorporated at a weight ratio of 10% to the eudragit RS and stirred for 1 hour. Following this, the resulting mixture was diluted with deionized water so that the final concentration of eudragit RS in the coating suspension was 10% (w/v). Finally, to prevent the adhesion of pellets during the coating procedure, talc was added to this dispersion at a weight ratio of 10% relative to the eudragit RS. The resulting suspension was stirred for 20 minutes prior to use.

*2.4.2. Coating procedures and conditions*

The obtained budesonide pellets (100 g) were placed in a fluidized bed coater (Wurster insert, Werner Glatt, Germany), and then different coating formulations were sprayed employing a peristaltic pump under specific coating conditions (inlet temperature 50-54 ℃, outlet temperature 44 to 48 ℃, nozzle diameter was 1 mm and atomization pressure set at 2 bar). Once the coating percentage reached the designed levels specified in Table 2, samples of coated pellets were collected and dried for 24 hours at a temperature of 45 °C in an oven.

*2.5. Dissolution studies*

The release profiles of coated pellets containing 9 mg of budesonide (n= 6) were tested using USP dissolution apparatus I (Pharmatest, PTWS 3E, Germany) with a 250 mL release medium containing 0.25% w/v SLS (as a help to provide sink condition). The dissolution studies were conducted at 37 ± 0.5 °C with a rotation speed of 75 RPM. To simulate various gastrointestinal conditions, the dissolution studies were performed for 2 hours in 0.1 N HCl at pH 1.2 (simulating the gastric region), and for 10 hours in phosphate buffers at pH 6.8 and 7.2 (simulating different parts of the small and large intestines). At specific time intervals, 5 mL

of release medium was manually withdrawn and filtered using a syringe filter (0.22 μm). The quantity of budesonide in the filtrate specimen was quantified using an HPLC (Shimadzu, Japan) with a Teknokroma column (BRISA LC2 C18 250 mm × 4.6 mm, 5 μm) at 240 nm. The injection volume was 20 μL and the mobile phase employed in this study was a blend of acetate buffer (pH 3.9) and acetonitrile (35:65), flowing at a rate of 1.5 mL/min.

A continuous mode of dissolution test was used to characterize the dissolution of pellets coated with optimum conditions. This test was performed using the method explained in our earlier study [20]. In brief, accurately weighed pellets (corresponding to 9 mg of budesonide) were tested at pH 1.2 (2 hours), pH 6.5 (1 hour), pH 6.8 (2 hours), pH 7.2 (1 hour) and pH 6.8 (10 hours) respectively. Following each designated resistance time, the pellets were immediately transferred to the next dissolution medium. At each sampling time, 5 mL of dissolution medium was withdrawn and subjected to HPLC analysis using the method mentioned above. To evaluate the effect of intestinal bacteria on the drug release rate, dissolution studies were also performed in the presence of rat cecal content (4% w/v) during the final part of the continuous dissolution test (pH 6.8 and 10 hours resistance time), with continuous $CO_2$ bubbling of the medium [4]. To analyze the drug release in media containing rat cecal content, 5 mL of the dissolution medium was centrifuged for 30 minutes at 4 °C with a rotation speed of 15,000 RPM, then, the resulting supernatant was sedimented through mixing with acetonitrile at a ratio of 1:3. The resulting suspension was filtered by a syringe filter (0.22 μm), and the quantity of budesonide in filtrate specimen was quantified using HPLC [43].

*2.6. Morphology of pellets*

A scanning electron microscope (SEM) (Leo, VP1450, Germany) was performed for the characterization of the surface morphology of optimized coated pellets as well as the thickness of the applied coat. Before imaging, specimens were sputter-coated with a thin layer of gold

by employing a Polaron, SC7620 sputter coater (England) for 180 seconds. Subsequently, a focused stream of electrons with a voltage of 20 kV interacted with them through an electron gun for the creation of high-resolution images.

*2.7. Animal treatment*

Acetic acid-induced colitis is a commonly utilized experimental model in IBD studies [52]. To assess the anti-inflammatory effect of the optimized coated pellets in comparison to uncoated budesonide pellets, an acetic acid-induced rat model of UC was utilized. Male Wistar rats, aged 10–12 weeks and weighing 250–300 g, were used in the research following ethical approval by the institutional ethics committee of Mashhad University of Medical Sciences (IR.MUMS.REC.1399.011). All the rats were housed in clean rooms at a temperature of 22 ± 3 °C, humidity of 40 ± 5%, and a 12-hour light–dark cycle. The rats were kept fasted with free access to water for 24 hours before colitis induction. To induce colitis, the rats were first anesthetized with an intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg) solution [53]. Then, 2 mL of acetic acid (3% v/v in normal saline) was rectally administered using a 2 mm diameter cannula [54]. In the healthy control group instead of acetic acid solution, 2 mL of normal saline was administered intrarectally. To prevent draining back of the fluid from the rectal region, rats were positioned upside-down for 1 minute before being returned to their cages. To allow for the development of colitis, they were left without treatment for the next 3 days [4]. The rats with induced colitis were grouped into 3 different sub-groups, each consisting of 6 rats. One group of rats was kept untreated and received 1 mL of normal saline (using an insulin syringe) during the treatment period, while two others were orally administrated an equivalent dose of budesonide (0.15 mg/day) from uncoated budesonide pellets as well as optimized coated pellets (using an insulin syringe with a cut-off head), for 7 consecutive days [55, 56]. During the study period, all animals were daily investigated for their

weight and stool consistency. 24 hours after the last dose consumption, the rats were sacrificed using $CO_2$ asphyxiation, and their entire colon was removed for additional investigations.

*2.8. Assessment of colitis treatment*

*2.8.1. Colitis activity index*

The severity of inflammation at different time points throughout the study was evaluated by calculating the colitis activity index (CAI). The CAI was determined based on several parameters, including weight loss, stool consistency, and anal bleeding [57]. The CAI scoring system provided a comprehensive assessment ranging from 0 (indicating a state of normal health) to 4 (representing the highest level of inflammation).

Weight loss was evaluated and assigned scores to reflect the degree of loss. Specifically, no weight loss was given a score of 0 points, while weight loss ranging from 1% to 5% was assigned a score of 1 point. For weight loss between 5% and 10%, a score of 2 points was given, and for weight loss ranging from 10% to 20% received a score of 3 points. Weight loss exceeding 20% was assigned the highest score of 4 points.

Stool consistency was assessed and scored accordingly. Well-formed pellets were assigned a score of 0 points, indicating normal consistency. On the other hand, pasty and semi-formed stools that did not stick to the anus received a score of 2 points, reflecting altered stool consistency. Lastly, liquid stools that stuck to the anus were given a score of 4 points, indicating the most severe alteration in stool consistency.

The presence and extent of anal bleeding were also considered in the CAI assessment. No evidence of blood in the stool resulted in a score of 0 points. Positive findings of blood in the stool received a score of 2 points, indicating the presence of bleeding. Visible anal bleeding, representing the most severe form of bleeding, was assigned a score of 4 points.

The overall CAI score, which provided a comprehensive measure of inflammation severity, was quantified by calculating the mean of the individual scores for weight loss, stool consistency, and anal bleeding.

*2.8.2. Colon/body weight ratio*

Prior to sacrifice, the rats were weighed to record their body mass. Following sacrifice, the entire colon tissues, extending from the colorectal junction to the anal verge, were carefully excised. The excised colon tissues were then horizontally cut and rinsed with a cooled phosphate buffer solution at a pH of 7.4. To assess the extent of inflammation, the ratio of the mass of the colon sample to the body mass of each rat was determined. This ratio served as an inflammation index, providing a quantitative measure of the inflammation present in the colon. [58].

*2.8.3. Weight/length ratio of colon*

The weight-to-length ratio of the washed colon tissue samples was used as a measure of the inflammation severity [59].

*2.8.4. Glutathione content of the colon tissue*

To determine the reduced glutathione (GSH) content in the tissue samples, a UV spectrophotometer was utilized by measuring the generation of a yellow color upon the addition of DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)), which reacts with sulfhydryl groups [60]. Here is a summary of the procedure:

Tissue samples were homogenized (10% w/v) in phosphate-buffered saline (0.1 M, pH 7.4).

To the 0.5 mL of the homogenized tissue, 0.5 mL of trichloroacetic acid (TCA) (10% w/v) was added. The mixture was then subjected to centrifugation at 25,000 g for 10 minutes at 4°C.

From the resulting supernatants, 0.5 mL was taken and combined with reaction mixtures containing 2.5 mL of phosphate buffer (pH 8) and 0.5 mL of DTNB. After 10 minutes, the absorbance of the resulting yellow solution was measured at 412 nm using a spectrophotometer (Jenway, 6105 UV/Vis, UK). The GSH content (nmol/g tissue) was quantified based on a standard curve constructed using the blank medium [12]. By measuring the yellow color developed through the reaction between DTNB and sulfhydryl groups, the GSH content in the tissue samples was determined using UV spectrophotometry.

*2.8.5. Malondialdehyde content of the colon tissue*

The concentration of malondialdehyde (MDA) in the colon tissue sample can serve as an indicator of the severity of inflammation [61]. The procedure for determining MDA is outlined as follows:

Homogenized tissue samples (10% w/v) in 1.15% w/v KCl were prepared.

To the homogenized samples, 3 mL of phosphoric acid (1% w/v) and 1 mL of thiobarbituric acid (TBA) (0.6% w/v) were included. The mixtures were then subjected to heating in a boiling water bath for 45 minutes. After the heating process, the mixtures were cooled, and 4 mL of n-butanol was added. The resulting mixture was vortex-mixed for 1 minute and then centrifuged at 3000 g for 10 minutes. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (Jenway 6105 UV/Vis, UK). The concentration of MDA (nmol/g tissue) was quantified by quantifying the absorbance based on a standard curve constructed using a blank medium [62]. By following this procedure, the concentration of MDA in the colon tissue sample, which serves as a biomarker of oxidative stress and inflammation, can be measured using spectrophotometry.

*2.8.6. Histological assessment of inflammation severity*

Colon tissue segments were first fixed in an aqueous solution of formalin (10% v/v) and then embedded in paraffin. The segments were stained with hematoxylin and eosin (H&E) following sectioning by microtome. The severity of inflammation in stained samples was graded on a scale ranging from 0 to 4 using an optical microscope. A score of 0 indicated no or mild inflammation, while a score of 1 was assigned when focal infiltration of inflammatory cells and a small affected area were observed. A score of 2 was given when inflammation involved a significant portion of the colon tissue with evidence of smooth muscle thickening. A score of 3 was assigned in cases where ulceration and infiltration of inflammatory cells were observed in the tissue sections. The highest score of 4 was given to cases showing extensive tissue damage characterized by necrosis and gangrene, indicating the most severe colitis [43, 63].

*2.9. Statistical analysis*

Statistical analysis of the collected data was done using Graph Pad Prism software (Graph Pad Prism, version 7, San Diego, CA). To compare the means and determine significant differences, ANOVA test was performed followed by Tukey–Kramer test (P values <0.05 indicates statistically significant).

## 3. Results and Discussion

*3.1. Dissolution studies*

Fig. 1 displays the release profiles of budesonide from all pellets coated under the experimental design conditions. As it could be seen, at various pH conditions the drug release rate from these formulations was almost the same. Considering the fact that the solubility of budesonide and all other materials that were applied in these formulations is not dependent on the pH of the dissolution medium [64], this pH-independency of release was predictable and such delivery

systems could be considered as a combination of time-dependent and bacterial degradation systems [65].

In all formulations, higher coating levels were accompanied by reduced drug release levels. So that formulations with a 20% coating level led to a slower release rate than the same formulation with a 10% coating level. Since diffusion has a significant role in drug release from time-dependent systems [66], such behaviors could be related to the longer pass for diffusion [67].

The formulation containing 20% inulin and 80% eudragit RS (ERS) with a 20% coating level released almost 15% of the drug after 2 hours and more than 60% during the first 5 hours of the release study (before reaching the terminal ileum), while the formulation containing 40% inulin and 60% ERS with the same amount of coating level released approximately 30% of the drug within 2 hours and more than 70% of the drug during the first 5 hours of the study, and those pellets with no inulin in their coating formulation, released less than 10% of their drug content within 2 hours and less than 50% during 5 hours. These results demonstrated that increasing the amount of inulin in the coating composition could increase the drug release rate. This can be ascribed to the solubility of inulin in the dissolution medium [47] and the development of pores within the coating layer [48].

Although it seems that formulations with no inulin in their coating compositions could be able to deliver a desired amount of drug to the colonic region, an incomplete drug release (almost 90% of drug content was released during 10 hours), could be a drawback of such formulations. High molecular weight inulin was used in this study due to the slower dissolution rate and hydrolysis in the colonic environment compared to low molecular weight inulin [47, 68]..

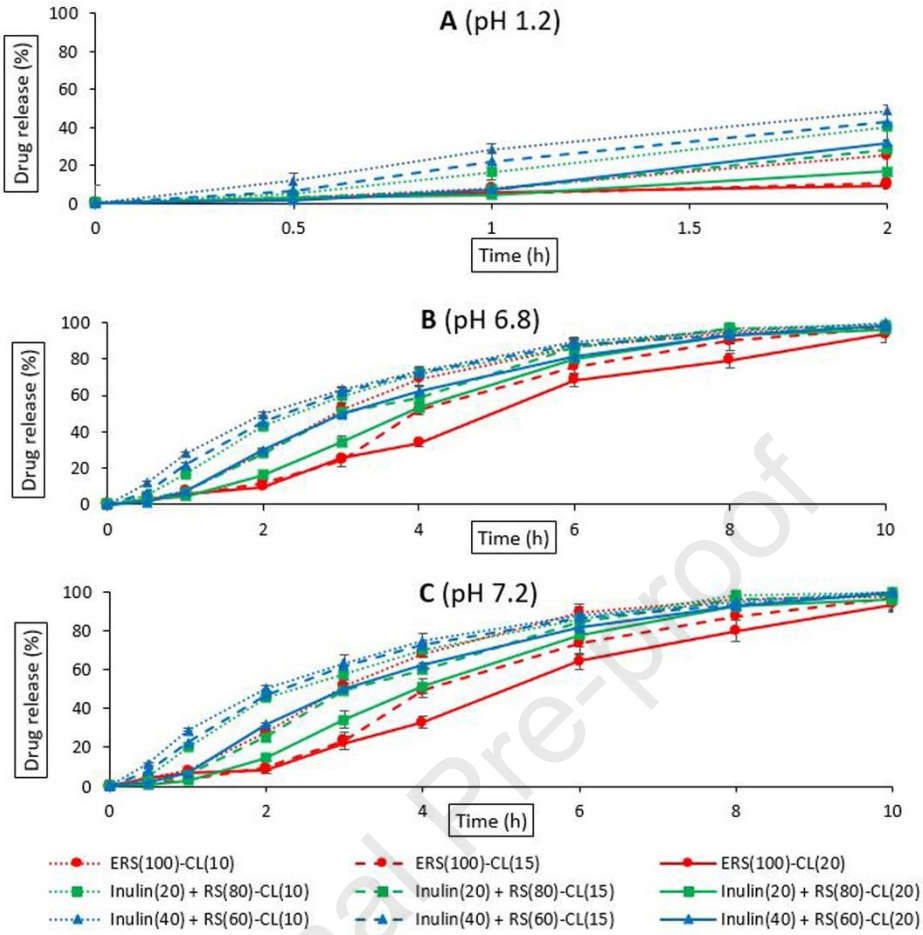



**Fig. 1.** The drug release profile from the pellets coated under the experimental design conditions at various pH conditions. CL: coating level.

*3.2. Determination of optimum coating composition and level*

Sequential p-values were computed using the design expert software, and a linear model was established to investigate the three responses. Upon conducting an analysis of variance (ANOVA) on the experimental data, it was observed that the model p-value for all responses was less than 0.0001. The model F-values corresponding to responses Y1, Y2, and Y3 were 205.68, 38.31, and 28.29 respectively. These values confirm the significance of the model for all three responses. Additionally, the R-squared (R2) values for Y1, Y2, and Y3 were determined as 0.9763, 0.8846, and 0.8500 respectively, indicating that the model offers a robust fit to the data [69].

Equations 1-3 represent the predicted mathematical relationship between independent variables ($X_s$) and responses ($Y_s$) in terms of coded factors:

$$Y_1 = + 28.04 + 12.66\ X_1 - 8.80\ X_2 \quad \text{(Equation 1)}$$

$$Y_2 = + 97.29 + 1.44\ X_1 - 1.28\ X_2 \quad \text{(Equation 2)}$$

$$Y_3 = + 48.01 + 12.07\ X_1 - 10.94\ X_2 \quad \text{(Equation 3)}$$

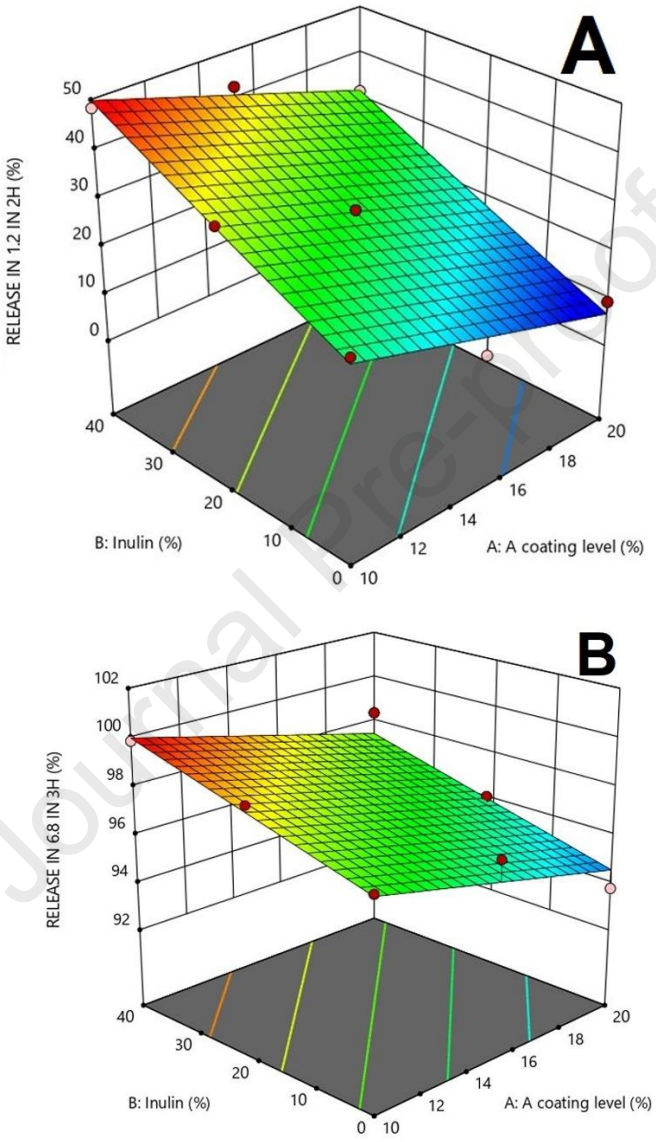
The positive coefficient of $X_1$ in all equations indicated a synergistic effect of this factor on all responses, while the negative value of $X_2$ demonstrated an opposing effect on them [19]. It could be concluded that an increase in the amount of inulin in the coating composition as well as a decrease in the coating level would result in more drug release.

Response surface plots (Fig. 2) were also presented in order to better illustrate the impact of independent variables on each response.

The optimization of the coating formulation and level was adjusted based on the residence time of the formulation in various regions of the GIT.

Since this designed delivery system was based on the time-dependent and bacterially degradable systems, to provide a suitable sustained release of the drug throughout the GIT, the optimal formulation should be able to stop excessive drug release in an acidic pH environment of the stomach within the first two hours ($Y_1$) and release the drug through the small and large intestines ($Y_3$) in a sustained manner [4]. As an attempt to prevent a high amount of drug release in the initial parts of the small intestine, the drug release at pH 6.8 within 3 hours was also considered as one of the responses ($Y_2$). So, desirable responses were considered drug release of less than 20% within two hours at pH 1.2, less than 30% within 3 hours at pH 6.8, while the release of 80 to 100% for the rest of 10 hours at pH 6.8.

Based on the obtained dissolution data, a coating composition consisting of 9% inulin and 91% ERS, at a coating level of 20% was predicted as the optimized coating formulation by the model. The optimized formulation was prepared and subjected to morphological analysis and dissolution tests.



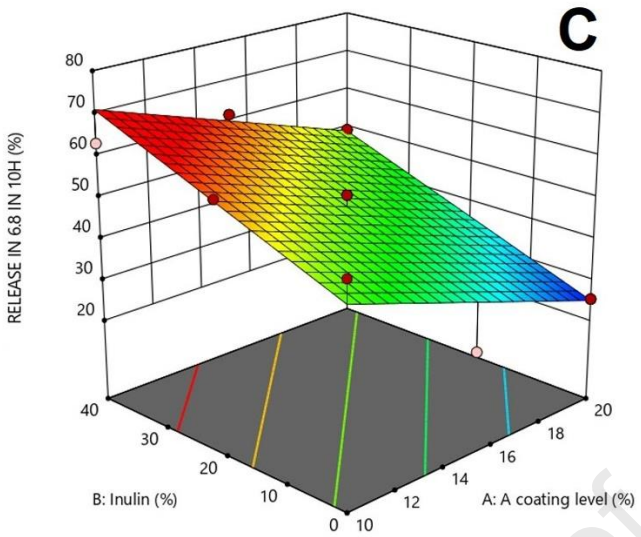



**Fig. 2.** The response surface plots for the responses predicted at different independent variables.

*3.3. Dissolution studies of budesonide pellets coated with optimized coating formulation*

Although based on the low standard deviation and predicted residual error sums of square values (PRESS) as well as high coefficient of determinations ($R^2$) and enough high F-value, it seems that the chosen predicting model (Linear) could represent high predictability [69], the measured response values were also compared with the anticipated values generated by the software to assess the validity of the optimization process. According to the results presented in Table 3, close agreement between these two values showed the validity of the optimization method.

**Table 3**
Observed and predicted values for the responses of optimum formulation.

| Responses | Observed | Predicted | Residual |
|---|---|---|---|
| **$Y_1$ (%)** | 12.60 | 12.46 | 0.14 |
| **$Y_2$ (%)** | 29.00 | 28.49 | 0.51 |
| **$Y_3$ (%)** | 98.84 | 95.51 | 3.33 |

The release profile of optimized coated pellets was studied in a continuous mode of dissolution test at different simulated GIT conditions. As mentioned earlier, budesonide is a practically-insoluble drug [64], and therefore incomplete drug release might be expected from its

diffusion-based sustained release formulations. It could be seen in Fig. 3 that, the optimized pellets released all of their drug content during the test period. So, it could be concluded that the problem of incomplete drug release, which is one of the drawbacks of some marketed brands of budesonide [70] and most of the time-dependent based colon drug delivery systems [65], could be addressed with this formulation. It has been shown that blending inulin with time-dependent polymers could improve the permeability of films and lead to more drug release [71].

Optimized pellets released only 15% of their cargo during the first 2 hours in the simulated gastric conditions (pH 1.2) and the remaining 85% of the drug was slowly released through other parts of GIT. The limited drug release during 2 hours in a simulated gastric environment could be attributed to the very low amount of inulin in the coating that could be dissolved in acidic media [72, 73] as well as the time required for ERS films to become permeable to the dissolution medium [74]. In the next 3 hours, following the dissolution of a higher amount of inulin, the polymeric network of the coating system became more permeable to the drug [44], so that almost 50% of the loaded drug was released prior to reaching the medium-simulating ileum and the remaining 35% of the loaded drug reached to the terminal ileum and colon. Therefore, it seems that optimized coated pellets could provide a favorable sustained release profile that enables effective drug delivery throughout the entire length of the small and large intestines.

The optimized formulation presented a slightly faster release of budesonide at pH 6.8 when rat cecal content (SCF) is present. This is attributed to the decomposition of inulin by bacterial enzymes [75]. Owing to this, pore formation within the coating matrix could be facilitated [44] and accelerate drug release.

Although due to the low concentration of cecum content (4% W/V) in the dissolution medium, the difference between drug release in the medium with and without cecal content was not

huge, this result could indicate enzymatic sensitivity of optimized formulation [76] and it is expected that the higher concentration of degrading enzymes in the human digestive tract could result to the marked difference in release between formulations without inulin and formulations containing inulin.

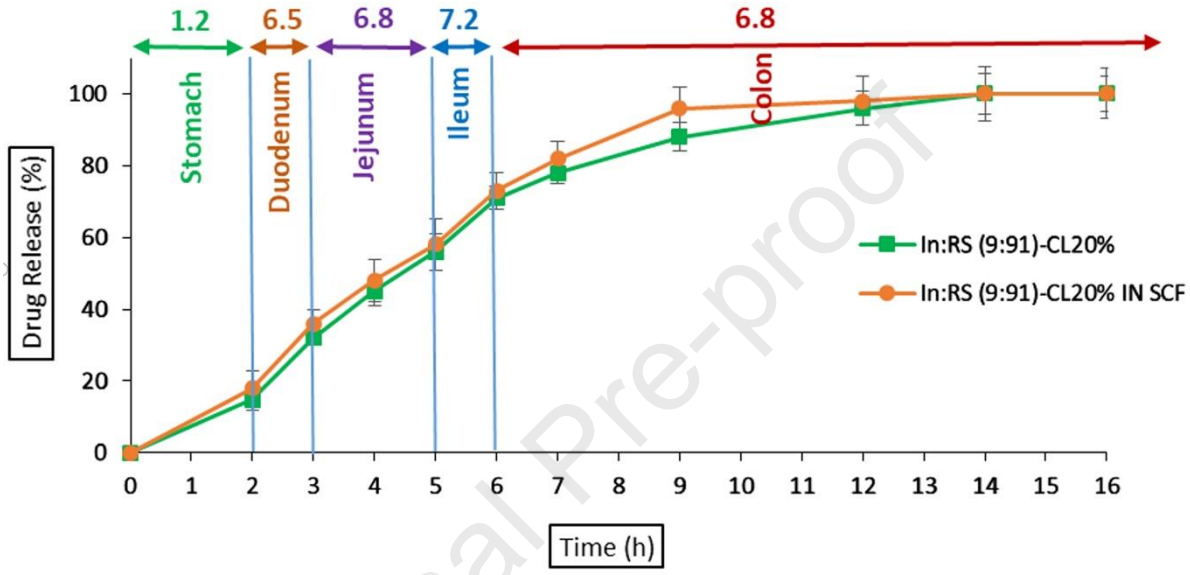
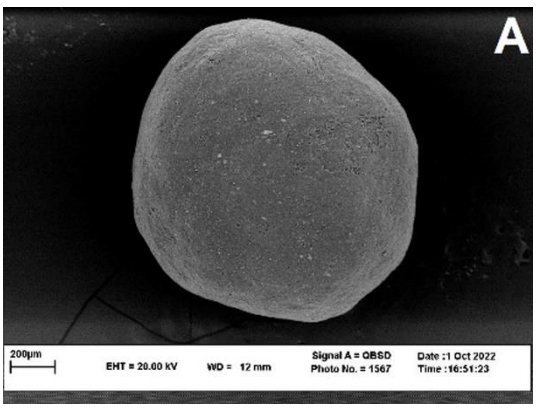



**Fig. 3.** The figure illustrates the release profile of budesonide from the optimized coated pellets in a continuous dissolution test, both with and without cecal content.

*3.4. Morphological characteristics*

SEM images of the optimized coated pellets illustrated the formation of a smooth and uniform coat with a thickness of about 70 µm around the core of the pellets (Fig. 4A & 4B).



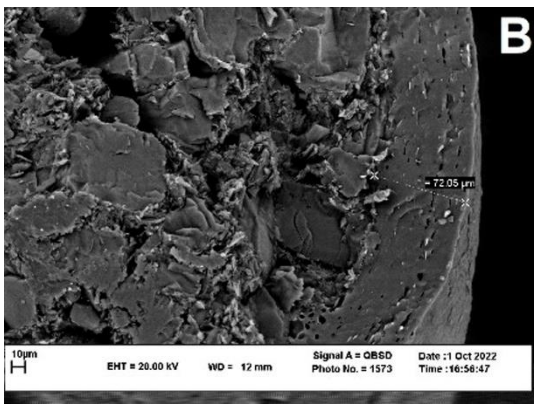



**Fig. 4.** Scanning electron microscopy of (A) the optimized coated pellets, and (B) the cross-sectional image of optimized coated pellets.

*3.5. In vivo therapeutic efficacy in rats*

The therapeutic efficacy of the optimized coated pellets was assessed and compared to uncoated pellets. To evaluate the severity of inflammation in the colon tissue, various parameters were examined, including the colitis activity index [15], colon/body weight, weight/length ratio of the colon, the level of malondialdehyde (MDA), the amount of reduced glutathione (GSH) in colon tissue [77], as well as histological studies.

Three days after rectal administration of acetic acid, every animal exhibited signs of weight loss and suffered from diarrhea. This observation as well as increasing the level of CAI (Fig. 5), demonstrated the development of experimental colitis in all animals [78]. During the treatment period, the level of CAI in treatment groups decreased consistently, so all rats that received treatment exhibited a significant reduction in CAI in comparison with untreated colitis rats on the day of sacrification ($p < 0.05$). Although these results demonstrated the reduction of inflammation in colonic tissue following the administration of the drug [79], it seems that optimized coated pellets are significantly more effective than uncoated pellets ($p < 0.05$).

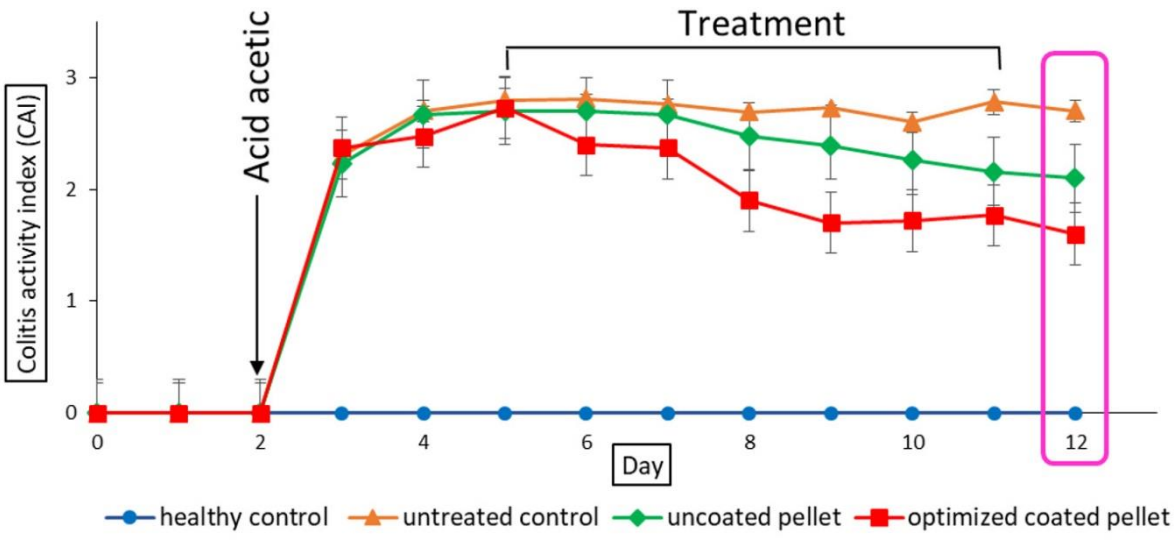


**Fig. 5.** Colitis activity index of the different rat groups. Data are presented as mean ± SD (n = 6 animals/group).

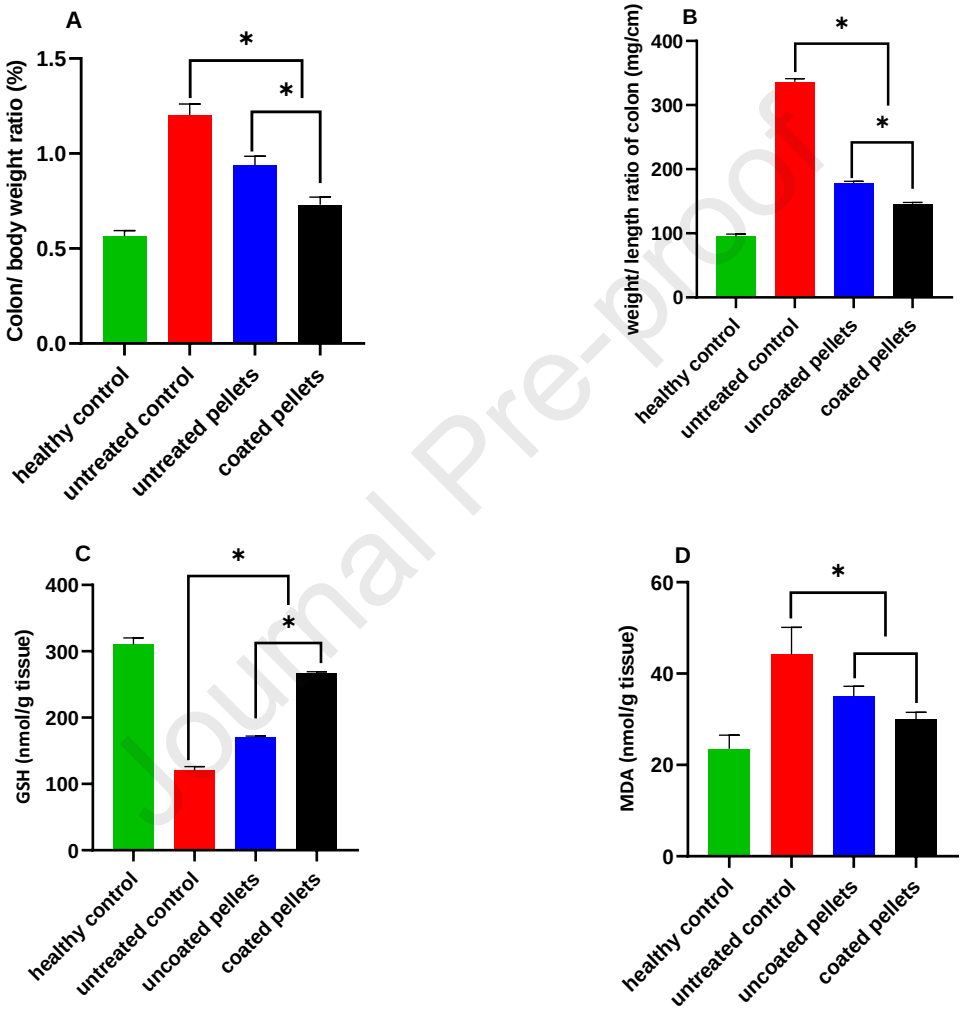
As it can be seen in Fig. 6A & 6B, in both treated groups, there was a remarkable decrease (p-<0.05) in both the colon/body weight ratio and the weight/length ratio in comparison to the untreated control group. This result could be related to the reduction of inflammation in colonic tissue [80]. Similarly to the CAI results, more therapeutic effects were seen by the administration of optimized coated pellets compared to uncoated pellets ( <0.05).

Fig. 6C & 6D illustrated that in treated rats the amount of GSH profoundly enhanced (p< 0.05), while the level of MDA exhibited a significant decrease (p<0.05) in comparison to the untreated control rats. Since MDA is a marker that increases tissue injuries and also GSH shows the anti-oxidative activity of cells following treatment [62], these results could demonstrate the reduction of inflammation upon the treatment.

The macroscopic observation of colon specimens (Fig. 6E) showed a decrease in colon length in untreated colitis rats when compared to the healthy controls (almost 8 cm). Furthermore, the colon tissue of colitis rats exhibited visible signs of thickened bowel wall and ulceration. Although the signs of inflammation appeared to be reduced in both treated groups, the rats treated with optimized coated pellets exhibited the greatest increase in colon length (almost 4 cm).

Fig. 6F represents the colitis damage score and Fig. 7 shows the histological sections of the colon tissues under a microscope. In Fig. 7A, normal colon histology is depicted, showing no signs of tissue abnormality or disruption. This image received a colitis score of zero. Conversely, in Fig. 7B, the colon tissue of untreated colitis rats exhibited tissue damage, goblet cell depletion and necrotic mucosal structure, warranting the highest score of four. Fig. 7C displayed some signs of smooth muscle thickening in the colon tissue of rats treated with uncoated pellets, resulting in a colitis score of two. Microscopic observation of the colon tissue

from rats that received optimized coated pellets showed trace regions of focal inflammatory cells infiltration, as shown in Fig. 7D, which earned a colitis score of one. Although both treatment groups exhibited some improvement in the microscopic characterization of the colonic tissues compared to the untreated group, the administration of optimized coated pellets demonstrated a condition that was much closer to the normal colon histology.



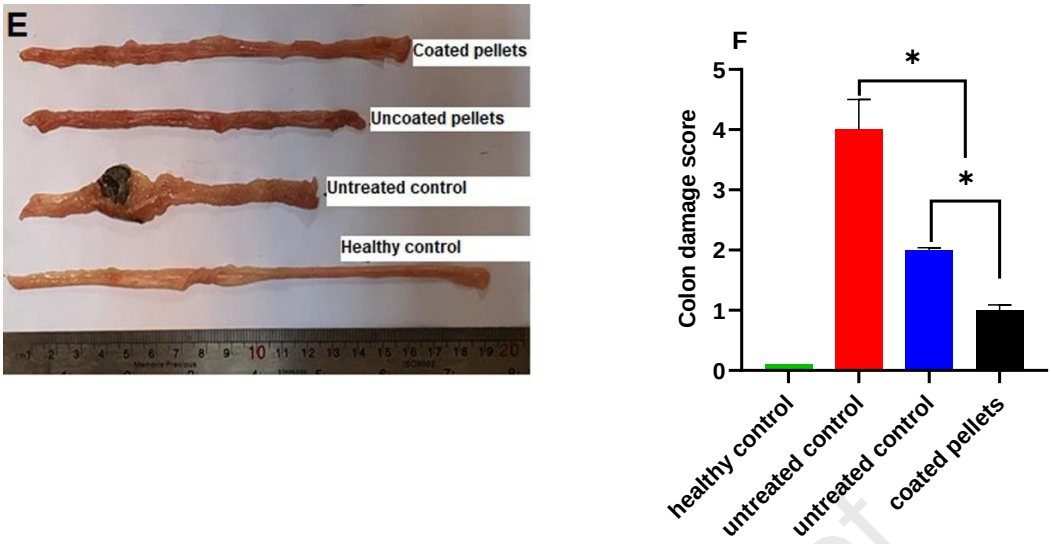



**Fig. 6.** The figure shows (A) Colon/body weight ratio, (B) Weight/length ratio of colon, (C) Amount of GSH, (D) Level of MDA, (E) Photographs for macroscopic examination, and (F) Colon damage score of the colon tissues. The data are presented as mean ± SD, with each group consisting of 6 animals. Significant differences between marked groups are denoted by an asterisk (*) with a significance level of $p < 0.05$.

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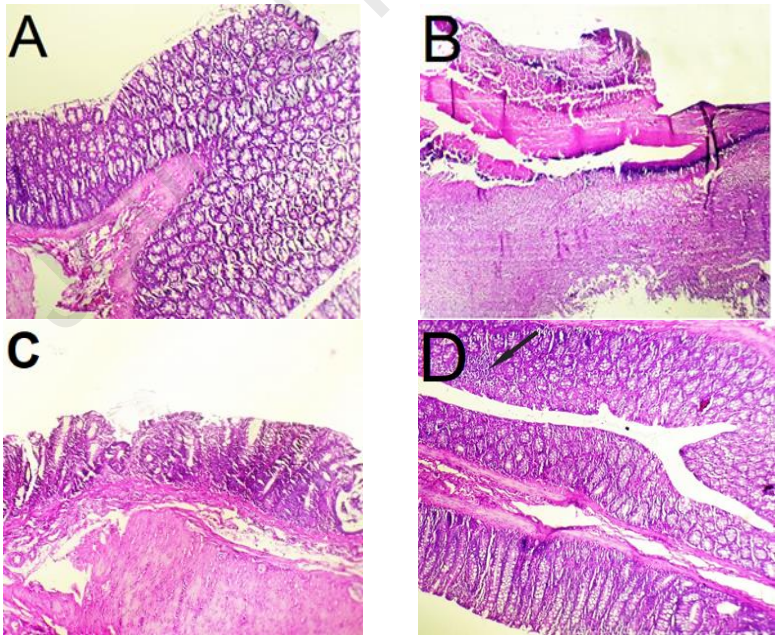


**Fig. 7**. The figure displays the histopathological characteristics of the colon tissues in rats subjected to different treatments. The subfigures show: (A) a rat with normal colonic tissues, (B) an untreated rat, (C) a rat treated with uncoated pellets, and (D) a rat treated with coated pellets.

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In summary, the findings from the *in vivo* studies indicated that the use of optimized coated pellets for the treatment of colitis, exhibited superior effectiveness compared to uncoated pellets. This higher efficacy demonstrated that designed coating systems successfully inhibited the rapid release of the drug in the stomach and delivered the drug in a sustained manner throughout the small intestine and colon and exhibited enzymatic sensitivity in the presence of cecal content in *in vitro* studies [81], indicating that the combined effects of budesonide and inulin might contribute to the effectiveness of this formulation in the treatment of colitis inflammation [34].

## 4. Conclusion

In this particular study, the researchers aimed to develop an optimized single-layer coating system for budesonide pellets by combining inulin and eudragit RS. The primary objective was to utilize a central composite design (CCD) to sustain the delivery of budesonide pellets while harnessing the therapeutic benefits of inulin in the treatment of inflammatory bowel disease (IBD). The optimized coating formulation comprised 9% inulin and 91% eudragit RS, with a coating level of 20% (w/w). This formulation effectively prevented premature drug release within the acidic environment of the stomach and facilitated complete and controlled drug release throughout the entire length of the intestine. By carefully tailoring the coating parameters, the researchers achieved the desired sustained release characteristics for budesonide. The efficacy of the optimized coated pellets was evaluated in comparison to uncoated pellets using a rat model of colitis. The results demonstrated that the optimized coated pellets exhibited superior therapeutic effects in treating colitis when compared to the uncoated pellets. This finding suggests that the optimized coating system effectively protected the drug from premature release, allowing for targeted drug delivery and improved treatment outcomes in the context of colitis.

Overall, this study highlights the successful design and optimization of a single-layer coating system combining inulin and eudragit RS for budesonide pellets. The optimized formulation demonstrated controlled drug release properties, which were advantageous for the treatment of IBD, particularly in the context of colitis.

## Declaration of Competing Interest

The authors declare no conflict of interest.

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**Author Contributions:**

**Fatemeh Soltani:** Investigation, methodology, data curation, formal analysis, writing original draft

**Hossein Kamali**: Software, methodology,

**Abbas Akhgari:** Conceptualization, data curation, supervision

**Mahboobeh Ghasemzadeh Rahbar**: Methodology,

**Hadi Afrasiabi Garekani**: Conceptualization, formal analysis, methodology, supervision

**Ali Nokhodchi:** Conceptualization, supervision, writing—review and editing

**Fatemeh Sadeghi:** Conceptualization, data curation, methodology, resources, Writing-review and editing, supervision

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**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: