Journal Pre-proofs

Comparison of 5-ASA layered or matrix pellets coated with a combination of ethylcellulose and Eudragits L and S in the treatment of ulcerative colitis in rats

Hossein Shahdadi Sardou, Fatemeh Sadeghi, Hadi Afrasiabi Garekani, Abbas Akhgari, Amir Hossein Jafarian, Mohammadreza Abbaspour, Ali Nokhodchi

PII: S0378-5173(23)00401-5
DOI: https://doi.org/10.1016/j.ijpharm.2023.122981
Reference: IJP 122981

To appear in: *International Journal of Pharmaceutics*

Received Date: 10 January 2023
Revised Date: 7 April 2023
Accepted Date: 20 April 2023

Please cite this article as: H. Shahdadi Sardou, F. Sadeghi, H. Afrasiabi Garekani, A. Akhgari, A. Hossein Jafarian, M. Abbaspour, A. Nokhodchi, Comparison of 5-ASA layered or matrix pellets coated with a combination of ethylcellulose and Eudragits L and S in the treatment of ulcerative colitis in rats, *International Journal of Pharmaceutics* (2023), doi: https://doi.org/10.1016/j.ijpharm.2023.122981

Comparison of 5-ASA layered or matrix pellets coated with a combination of ethylcellulose and Eudragits L and S in the treatment of ulcerative colitis in rats

Hossein Shahdadi Sardou[a,b], Fatemeh Sadeghi[a,b], Hadi Afrasiabi Garekani[c,b], Abbas Akhgari[a,b], Amir Hossein Jafarian[d], Mohammadreza Abbaspour*[a,b], Ali Nokhodchi*[e]

[a]Targeted Drug Delivery Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran; [b]Department of Pharmaceutics, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran; [c]Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran; [d]Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; [e]Lupin Research Inc, Coral Springs, Florida, USA, [f]School of Life Sciences, University of Sussex, Brighton, UK

*Corresponding authors:

Ali Nokhodchi (a.nokhodchi@sussex.ac.uk)

Mohammadreza Abbaspour (abbaspourmr@mums.ac.ir)

Abstract

The aim of this study was to evaluate and optimize the combination of time and pH-dependent polymers as a single coating for the design of the colon-specific drug delivery system of 5-aminosalicylic acid (5-ASA) pellets. 5-ASA matrix pellets with a 70% drug load were prepared by the extrusion-spheronization method. The optimal coating formula which included Eudragit S (ES)+Eudragit L (EL)+Ethylcellulose (EC) was predicted for the targeted drug delivery to the colonic area by a 3^2 factorial design. The ratio of ES:EL:EC and coating level were considered as independent variables while the responses were the release of less than 10% of the drug within 2 h (Y_1), the release of 60-70% within 10 h at pH 6.8 (Y_2) and lag time of less than 1 h at pH 7.2 (Y_3). Also, 5-ASA layered pellets were prepared by the powder layering of 5-ASA on nonpareils (0.4–0.6 mm) in a fluidized bed coater and then coated with the same optimum coating composition. The coated 5-ASA layered or matrix pellets were tested in a rat model of ulcerative colitis (UC) and compared with the commercial form of 5-ASA pellets (Pentasa®). The ratio of ES:EL:EC of 33:52:15 w/w at a coating level of 7% was discovered as the optimum coating for the delivery of 5-ASA matrix pellets to the colon. The coated 5-ASA pellets were spherical with uniform coating as shown by SEM and met all of our release criteria as predicted. *In-vivo* studies demonstrated that the optimum 5-ASA layered or matrix pellets had superior anti-inflammatory activities than Pentasa® in terms of colitis activity index (CAI), colon damage score (CDS), colon/body weight ratio and colon's tissue enzymes of glutathione (GSH) and malondialdehyde (MDA). The optimum coating formulation showed a high potential for colonic delivery of 5-ASA layered or matrix pellets and triggered drug release based on pH and time.

Keywords: 5-ASA; pellets; Ethylcellulose; Eudragit ulcerative colitis; colon-specific delivery

1. Introduction

Ulcerative colitis (UC) is a long-term inflammatory condition of the colon and rectum which has been shown to respond to oral doses of anti-inflammatory drugs such as 5-aminosalicylic acid (5-

ASA) (Kobayashi et al., 2020; Shahdadi Sardou et al., 2019b). 5-ASA, which is used as the first line of treatment for UC needs to be absorbed locally in the colonic area to reduce the inflammatory factors (Catenacci et al., 2020). However, if 5-ASA is taken orally, most of the drug is absorbed in the upper parts of the gastrointestinal tract (GIT) and as such a small amount of the drug reaches the colon (Murray et al., 2020). To overcome this issue, various drug delivery systems have been employed to deliver 5-ASA to the colon (Lee et al., 2020). The most important of these systems are pH-dependent, time-dependent and microbially-degradative systems (Shahdadi Sardou et al., 2019a; Veloso et al., 2021). Figure 1 shows the key physiological factors in the GIT that ought to be considered in formulation design for colonic delivery.

The combination of several drug delivery systems has been trialed for the design of new colonic delivery systems. The main advantage of combined delivery systems is that they can be less sensitive to the changes in the physiological conditions of the GIT such as pH and the duration of dosage form residence time in each part of the GIT (Moghimipour et al., 2018). Consequently, the drug release from the dosage form will be more predictable under different GI conditions, which provides an opportunity to prevent the release of the drug in the upper parts of GIT thereby achieving the gradual release of the drug at the target site (colon) (Varum et al., 2020b).

Some synthetic polymers have been evaluated for targeted drug delivery to the colon (S. Thakral et al., 2013). Among these polymers, Eudragit S (ES) and ethylcellulose (EC) are used in Asacol® and Pentasa® brands, respectively (Goyanes et al., 2015). ES is a pH-dependent polymer that can disintegrate and release the drug at pH ≥7 (Franco and Marco, 2020). The use of this polymer in the coating formulation of the Asacol® brand may prevent drug release in patients with gastrointestinal pH>7 (H. and A., 2020). To overcome this challenge, Eudragit L (EL) which is soluble in the range of pH 6, can be used in combination with ES to manipulate or adjust the drug release in the range of pH 6 to 7 (Elbaz et al., 2020). Therefore, the concomitant use of ES & EL in the polymeric coat of dosage forms is necessary for ensuring drug release at pH < 7 (Elbaz et al., 2020; Kotagale et al., 2010). The use of EC as a time-dependent polymer along with ES & EL can also prevent the sudden release of the drug from the dosage form at pH < 7, hence leading to a gradual release of the drug after a lag time (Abinusawa and Tenjarla, 2015; Parmar et al., 2018). Various studies have shown that Pentasa® releases more than half of the drug content in the upper GIT, while a small amount of the drug reaches the end parts of the GIT which may be the reason for the lack of optimal treatment for UC with this brand (Abinusawa and Tenjarla, 2015; Van Camp et al., 2022). The combination of specific amounts of ES, EL and EC can therefore be used to achieve a sustainable polymer coat with pH and time-dependent characteristics for colonic delivery of 5-ASA pellets.

The location and rate of drug release are very important elements that need to be considered in the design of drug formulations for the treatment of UC (Arévalo-Pérez et al., 2020; Shahdadi Sardou et al., 2023). In other words, the ideal formulation of 5-ASA should deliver specifically the drug to the target site of the colon and have the ability to gradually release the drug in the site of action along with the prevention of drug release in the upper parts of the GIT (Teruel et al., 2020; Varum et al., 2020a). For this purpose, the residence time of the dosage form and pH of each part of the GIT should be considered in the design of drug formulation for drug delivery to the colon (Wang et al., 2021). Therefore, to design an ideal formulation, it is necessary to use the combination of two drug delivery systems based on pH and time-dependent polymers to achieve the optimal release profile in the colon region.

After oral administration of dosage forms (tablet or pellets), it takes about 1.5 h to pass through the stomach with an average pH of 1.2 This then enters the initial part of the small intestine (duodenum residence time of 1 h) with an average pH of 6.5. After passing through the jejunum area (residence time of 2 h with pH about 6.8), it then enters the terminal ileum area (residence time of 1 h) with an average pH of 7.2 (Maurer et al., 2015; Zeeshan et al., 2019). In general, it takes about 5-6 h for oral dosage forms to reach the initial part of the colon and at least about 10 h to remain in the colonic area with a pH of about 6.8 (Maurer et al., 2015; Zeeshan et al., 2019). The reason for the decreasing pH in the colon is as the result of the activity of microorganisms in this area which leads to its acidification by bacterial fermentation of the contents of the colon (Hua, 2020). An ideal formulation should therefore be one that prevents drug release at the upper GIT fluids with the initial drug release happening in the terminal ileum fluid. The majority of the drug content then ought to gradually release in the colonic fluid.

Since UC is a chronic disease and the patient needs to take high doses of 5-ASA daily, the dose of the drug should be high to reduce the number of dosage forms (tablets or pellets) taken by the patient (Naganuma, 2020). On the other hand, to prevent the release of the drug in the upper GIT and the gradual release of the drug in the colonic area, a thin coating layer must be used on the tablet or pellets with pH and time-dependent properties. In our previous study, matrix pellets with a dose of 60% (w/w) 5-ASA and other excipients (Avicel®, lactose, and polyvinylpyrrolidone (PVP)) were prepared using the extrusion spheronization method and an optimal coating layer with a thickness of about 30 µm including Eudragit RS (ERS) as a time-dependent and ES and EL as pH-dependent polymers (Shahdadi Sardou et al., 2022). As the increase in the dose of the drug in the dosage form to reduce the frequency of capsules (contains pellets) administration was challenging in that study, in order to increase the dose to 70%, it was prudent in this current study to remove lactose from the formulation. This was to also reduce the dissolution in the upper part of the GIT. Furthermore, there is an endeavour to reduce the thickness of the coating layer to about 10-15 µm, as reducing the coating layer could allow for higher loading of the drug in the capsule body. Studies have shown that dosage forms covered with the EC polymer have more resistance in simulated GIT media compared to the dosage forms covered with ERS (Akhgari and Tavakol, 2016; Niranjan, 2017). Therefore, due to the lower permeability of the EC polymeric film compared to the ERS film, better control of drug release could be achieved with the replacement of ERS with EC.

This study aims at determining the best possible combination of ES+EL and EC as a thin coating layer in achieving a colonic delivery system for 5-ASA matrix pellets. The optimal coating formulation obtained from the 5-ASA matrix pellets was also applied on 5-ASA layered pellets. These were then evaluated for their release profile against two commercial products (Asacol® and Pentasa® brands). As the gavage of Asacol® tablets in rats was not possible, only the Pentasa® brand was used in animal studies for comparative purposes with the optimally coated 5-ASA layered or matrix pellets in the treatment of UC.

2. Materials and methods

2.1. Materials

5-ASA was purchased from Cosar Pharmaceutical Company (Tehran, Iran). Eudragit S and L were kind donations from Evonik (Germany). Ethylcellulose was purchased from ICN (USA). Avicel® PH-102 and polyvinylpyrrolidone (PVP K90) were purchased from Akbarieh (Tehran, Iran).

Monobasic potassium phosphate, sodium hydroxide, butanol, methanol, acetonitrile, formalin, isopropyl alcohol, triethyl citrate, thiobarbituric acid, DTNB or Ellman′s reagent, orthophosphoric acid and trichloroacetic acid (TCA) were all purchased from Merck (Germany).

2.2. Preparation of matrix pellets

5-ASA matrix pellets containing 5-ASA (70% w/w), Avicel® (27%) and PVP K90 (3%) were prepared by an extrusion spheronization method (Shahdadi Sardou et al., 2021). Briefly, the excipients and 5-ASA were mixed using a kitchen mixer for 15 min. The obtained powder was then mixed with 43 ml of distilled water to prepare a dough. This dough was then pushed through an axial screw extruder at 100 rpm. The resulting fiber-like extrudates were then transferred onto a spheronizer with a cross-hatched friction plate at 700 rpm rotation speed for 1.5 min. The obtained pellets were dried in the oven at 50 °C for 24 h and then sifted through a sieve to separate the pellets within the range of 800–1200 μm.

2.3. Preparation of nonpareil and 5-ASA layering

The nonpareil core formulation contained Avicel® (97%) and PVP K90 (3%) and was prepared by an extrusion spheronization method. The suspension containing 30% w/v 5-ASA (<100 μm) was prepared by dispersing the drug in 3% (w/v) PVP K90 solution in water. The drug was then loaded onto the nonpareils (400-600 μm) using a fluidized bed coater (Wurster, Germany) under the following coating conditions: inlet air temperature (55–60 °C), outlet air temperature (51–55 °C), atomization pressure (2 bar) and spray flow rate (1.2 g/min) (Akhgari et al., 2005). The 5-ASA layering process was carried out to produce pellets with a dose of 70% (w/w) drug.

2.2.1. Experimental design

In order to predict the optimum ratio of ES to EL and also the coating level, a 3^2 full factorial design (Design-Expert software, Version 13, Stat-Ease, USA) was used. The studied factors (independent variables) were the ratio of ES to EL using the constant amount of EC (15%) in the coating composition (X_1 with the levels of 0:85, 25:60, and 50:35) and the coating level (X_2 with the levels of 2.5, 5 and 7.5% of the total pellet weight). The responses or dependent variables were the drug release of less than 10% of the drug within 2 h (Y_1), release of 60-70% within 10 h at pH 6.8 (Y_2), and lag time of less than 1 h at pH 7.2 (Y_3) (Table 1).

2.2.2. Coating of 5-ASA matrix pellets

5-ASA matrix pellets were coated in the fluid bed coater with solutions containing specific amounts of polymers (Table 2). For this purpose, ES, EL, and EC were dissolved in a beaker containing ethanol as a solvent under agitation for 30 min. Also, talc (0.5%, w/v) and triethyl citrate (17%, w/w based on total polymer weight) were added to the solutions as anti-adhering and plasticizer agents, respectively. After preparing the coating solutions, the 5-ASA pellets were coated at the temperature of 27-33 ^{o}C, a pressure of 1.7 bar and a spray flow rate of 0.44 g/min until the coating level of 2.5, 5 and 7.5% (w/w) were reached (Akhgari et al., 2005).

2.2.3. Preparation of different dissolution media

All of the GIT-simulated media were prepared according to USP 39. For the simulated gastric medium, hydrochloric acid 0.1 N (pH 1.2) was used. In the case of the preparation of the dissolution media for other regions of GIT, phosphate buffer was used to generate and simulate pHs 6.5 (duodenum), 6.8 (jejunum), 7.2 (terminal ileum) and 6.8 (colon).

2.2.4. Dissolution studies

The above-mentioned dissolution media were poured into the glass flasks of an automatic dissolution system and the release profile of 5-ASA from the pellets was examined according to the USP XXIII dissolution apparatus I (Pharmatest, PTWS, Germany). To perform the dissolution test, the pellets containing 250 mg of 5-ASA were placed in the basket and immersed in the dissolution medium with a volume of 900 mL. The rotation speed of the baskets was set to 100 rpm and the concentration of 5-ASA was measured at different time intervals by a UV spectrophotometer (Shimadzu, UV-1204, Japan) at 302 and 330 nm for the simulated gastric medium and other dissolution media (pHs 6.5, 6.8 and 7.2), respectively (Nguyen et al., 2012). The continuous dissolution test was performed for the optimal formulations by considering the residence time of the pellets in different parts of the GIT. For this purpose, the dissolution pattern of the 5-ASA in the flasks containing gastric-simulated medium was monitored up to 2 h. For the small intestine-simulated media, the 5-ASA release was followed up to 1, 2, and 1 h, where the dissolution media with pH 6.5, 6.8, and 7.2, were used, respectively. For the colon-simulated media, the 5-ASA dissolution profile was observed up to 10 h at pH 6.8.

2.2.5. Morphology of 5-ASA layered or matrix pellets

To evaluate the morphology of the pellets, 20 pellets were randomly selected, glued on a black background paper and examined by a stereomicroscope (Olympus, DP25, Okura, Japan) with magnification (×8) equipped with a digital camera (Canon, Japan). The resulting images were analyzed with Image analyzing software (ImageJ 1.52v) and the parameters of sphericity and the aspect ratio were calculated as follows:

$$\text{Sphericity} = 4\pi A/P_m^2 \quad \text{(eq. 1)}$$

$$\text{Aspect ratio} = d_{max}/d_{min} \quad \text{(eq. 2)}$$

Where A is area, Pm is perimeter, d_{max} and d_{min} are longest and shortest Feret's diameters.

2.2.5.1. Scanning electron microscope analysis

The TESCAN MIRA3 SEM (Czech Republic) was used to observe the surface and cross-sectional characteristics of the 5-ASA layered or matrix pellets. Briefly, a thin layer of gold was sputter coated on the pellets to allow visualization on the SEM. To observe the cross-section of the coated pellets, several pellets were divided/cut into half using a surgical knife and then also sputter coated with a thin layer of gold. Images were taken by applying an electron beam acceleration voltage of 10 kV.

2.3. Rat model of colitis and treatment groups

Male Wistar rats with an average weight of 250 g (range, 230–250 g), 12 weeks; n = 4/group were used in the experiment. After emptying the colon from feces, the rats were deprived of food for 48 h before the start of the experiment but had access to water. In order to induce colitis, 2 mL acetic acid (2% v/v in normal saline) was injected into the rat's colon using a flexible plastic tube and after 30 s, the acidic solution was drained (Ansari et al., 2021). In the control group, rats received only 2 mL of normal saline by intra-rectal injection. The colitis-induced rats were then randomly divided into the untreated group, the vehicle-treated group (1% w/v Na-CMC) and the treatment groups (uncoated 5-ASA layered or matrix pellets, coated 5-ASA layered or matrix pellets and Pentasa®). These rats had access to water and food until the end of the experiment. The pellets were gavaged as a dose of 120 mg/Kg/day of 5-ASA to these groups for ten consecutive days (Kaffash et al., 2019). The *in-vivo* study was performed under ethical standards and the international regulations of the usage and welfare of laboratory animals and was approved by the clinical ethics committee of Mashhad University of Medical Sciences with the ethical code: IR.MUMS.AEC.1401.061.

2.3.1. Evaluation of colitis treatment

During the treatment process, the health status, stool consistency, rectal bleeding and weight of the rats were evaluated and recorded daily. After the end of the treatment period (15th day), the rats were sacrificed by CO_2 gas.

2.3.2. Colitis activity index

The colitis activity index (CAI) was calculated by evaluating and scoring the average indicators of weight loss, stool consistency, and rectal bleeding. In this system, the score is classified from 0 (healthy) to 4 (maximum colitis activity). For the weight loss factor: no weight loss (score 0), weight loss 1 to 5% (score 1), weight loss 5 to 10% (score 2), weight loss 10 to 20% (score 3),

weight loss greater than 20 % (score 4). For stool consistency factor: normal and ball-like stools (score 0), pasty and semi-formed stools that do not stick to the anus (score 2) and watery (liquid) stools that stick to the anus (score 4). For the rectal bleeding factor: no bleeding (score 0), observation of blood in the stool (score 2) and severe bleeding (score 4) were utilized (Tan et al., 2022).

2.3.3. Colon/body weight ratio

After the rats were sacrificed via CO_2 asphyxiation, their abdomen was opened with surgical scissors and the colon was retrieved. The colon was cut longitudinally and its internal contents were washed with normal saline. The excess water on the colon was removed with tissue paper and the weight of the colon was measured with a 3-digit analytical scale. The ratio of the weight of the colon to the weight of the same rat before sacrificing was calculated (Mura et al., 2011).

2.3.4. Length/weight ratio of colon

To calculate this parameter, the rat's colon was cut from the sigmoid region (first part) and the colo-cecal junction (end part). The colon was washed with normal saline and the excess water was removed with tissue paper. The length and weight of the colon were then measured (Varshosaz et al., 2010).

2.3.5. Assessment of colitis severity

The histopathological features of the colon tissue were evaluated in order to assess the effectiveness of the pellets. To perform this test, after the rat's colon tissue was accessed and washed with normal saline, the beginning, middle and end parts of the colon were cut and immersed in formalin solution (pH 7.4). Subsequently, the colon tissue was embedded in paraffin and cut with a microtome machine. The slices were placed on a microscope slide and stained with hematoxylin-eosin (H&E) and analysed under a light microscope equipped with a digital camera. For the assessed severity of colitis, scores from 0 to 4 were used. In this regard, the score 0 was considered for mild inflammation, a score of 1 for crypt abscess formation and acute focal inflammation, a score of 2 for smooth muscle thickening, a score of 3 for the presence of ulceration and cellular infiltration, with a score of 4 was considered for mucosal tissue necrosis and gangrene (Choi et al., 2016).

2.3.6. Glutathione (GSH) and malondialdehyde (MDA) content of the colon tissue

As markers of lipid peroxidation, GSH and MDA levels were measured using a spectrophotometric method at 412 and 532 nm, respectively. These two parameters were measured according to a previous study (Shahdadi Sardou et al., 2021).

2.4. Statistical analysis

Statistical analysis was carried out using GraphPad Prism software (version 8.4.0). Multiple comparisons between groups were assessed by one-way ANOVA with the Tukey–Kramer test as a multiple comparison post-ANOVA test at the significance level of 0.05.

3. Results and discussion

3.1. Dissolution studies

Figure 2A depicts the drug release profile from the uncoated 5-ASA layered or matrix pellets in acidic and phosphate buffer media with pH 1.2 and 6.8 respectively. As can be observed, the 5-ASA layered pellets released their drug content quickly in less than 30 min in both media, while the 5-ASA matrix pellets displayed a slower release rate. The results show that the time to release 100% of the drug from the matrix pellets in the acidic and phosphate buffer media was around 2.5 and 5 h, respectively. Since in the 5-ASA layered pellets the drug is spray-coated on the surface of the nonpareil core, when immersed in the dissolution medium, the drug is exposed to the dissolution medium and thus quickly dissolves. In the matrix pellets, the drug is distributed throughout the structure of the pellet, consequently, the drug is released at a slower rate (Afrasiabi Garekani et al., 2013). It is important to note that the use of Avicel® as an excipient inside the matrix pellet proved to be a powerful factor in reducing the rate of drug release. In this regard, various studies have shown that increasing the amount of this excipient in the structure of matrix pellets decreases the rate of drug release (Bautzová et al., 2012; Choi et al., 2016).

According to Table 2, the matrix pellets were coated with different coating formulations at three coating levels and their release profile was investigated in different dissolution media (Figures 2B to 2E). Generally, all formulations showed almost no drug release in the simulated gastric fluid for the 2 h period (Figure 2B). The use of the pH-dependent polymers in the coating formulation of the pellets thus proved useful in preventing the release of the 5-ASA in the simulated gastric medium (Veloso et al., 2021).

The comparison of drug release at pH 6.5 and 6.8 shows that the rate of drug release increases for all the formulations with the increase in the pH, the decrease in the coating level and also the decrease in the ratio of ES to EL in the coating formulation. Several studies have reported that pH-dependent polymers are ionized faster by increasing the pH of the dissolution media (Dos Santos et al., 2021; Lei et al., 2016). Depending on the type of polymer, the disintegration of these polymers is different (Seema Thakral et al., 2013). For example, EL, Eudragit FS (FS), and ES are FDA-approved polymers that are widely used in colon-targeted drug delivery systems and can be dissolved in pH about 6, 6.8, and 7 respectively (Cai et al., 2021; Patole and Pandit, 2017; S. Thakral et al., 2013). For this reason, by increasing the ratio of EL to ES in the coating formulation, faster release of the drug at both pH 6.5 and 6.8 is observed. Another observation from the drug

release profile at these two pHs is the inverse effect of the coating level on the drug release rate; the rate of drug release decreases with an increase in the coating level (Figures 2C to 2D). The cause of this phenomenon is the increase in the length of the passage of the drug (potential diffusion) in entering the dissolution medium (i.e. the coating thickness delays the imbibement of dissolution media into the pellets). There are some studies that have demonstrated that the rate of drug release decreases with the increase in the coating level (Chen et al., 2018; Kaffash et al., 2019; Maderuelo et al., 2019).

In general, with the increase in pH from 6.5 to 6.8 and then 7.2 (Figure 2E), an acceleration in the release of all coated formulations can be observed. This denotes the sensitivity of the coating formulations to the change in pH. On the other hand, evaluating the drug release profile from coated formulations at pH 7.2 can show the role of a time-dependent polymer in combination with ES & EL. According to Figure 2, a gradual release is observed in all coated formulations, which is due to the use of EC in the coating formulation. Generally, the combination of time-dependent polymers in combination with pH-dependent polymers prevents the sudden release of the drug at high pH and provides a controlled drug release (Akhgari et al., 2006).

By comparing the drug release from different coating formulations in the simulated medium of the colon at pH 6.8, it can be seen that the formulation containing ES 50 compared to the ES 25 and ES 0 significantly reduced the drug release within 10 h, which is due to the increase of ES in the coating formulation. Also, the coating level had a significant effect on the drug release from all the coating formulations. In other words, drug release was significantly decreased with the increase in coating level from 2.5 to 7.5%.

3.2. Optimization process

To find the optimal combination of coating materials and coating level, A 3^2 full factorial design was used (Table 1-2 and Fig. 2 F-H). For this purpose, the following equations characterized the relationship between the dependent and independent variables.

$$Y_1 = + 63.35057 - 0.70575\ X_1 - 8.74728\ X_2 + 0.03600\ X_1 X_2 + 2.48276\ X_1^2 + 0.42483\ X_2^2 \quad \text{(eq. 3)}$$

$$Y_2 = + 113.41379 + 0.88874\ X_1 - 7.89195\ X_2 - 0.11200\ X_1 X_2 - 0.029241\ X_1^2 + 0.51586\ X_2^2 \quad \text{(eq. 4)}$$

$$Y_3 = - 7.24359 + 0.23333\ X_1 + 3.66667\ X_2 \quad \text{(eq. 5)}$$

Where X_1 (ratio of ES to EL) and X_2 (coating level) are the independent variables and Y_1 and Y_2 as dependent variables represent the release of less than 10% of the drug within 2 and the release of 60-70% within 10 h at pH 6.8, respectively, while Y_3 is the lag time before drug release at pH 7.2.

Table 2 shows variable factors, predicted and experimental responses. The predicted and experimental responses were very close to each other. For example, Y equations were obtained from the experimental and predicted data for Y_1 (43 and 44 %), Y_2 (98 and 97 %), and Y_3 (5 and 4 min), which all indicate no significant differences between the predicted and experimental responses. Also, for all Y_s, the residual sum of squares (RSS) is much less than the total sum of

squares (TSS), which indicates the accurate fit of the model with the data. These two parameters are used as optimization criteria in parameter and model selection. In addition, the coefficient of determination (R^2) is close to 1, and also a very large F-value indicates that these models can all explain the variances in the dependent (response) values by the independent factors.

Figures 2F to 2G demonstrate that the drug release at pH 6.8 decreases by increasing the amount of ES and also the coating level from 2.5 to 7.5%. On the other hand, the reduction of these factors leads to a decrease in the lag time in drug release (Figure 2H). According to the 3D diagrams obtained from the experimental results (Figures 2F to 2H) and equations 3 to 5, coating of pellets by a formulation containing 33% ES, 52% EL, and 15% EC at a coating level of 7% can have access to the desired answers or target points (Y_1, Y_2, and Y_3). It can therefore be concluded that coating the 5-ASA matrix pellets with the optimal coating formulation can prevent the release of the drug in the upper parts of GIT and gradually release the drug in the colon area.

3.3. Morphological observation

The sphericity and aspect ratio of 5-ASA matrix pellets were 0.84 ± 0.08 and 1.19 ± 0.13 respectively while the corresponding values for 5-ASA layered pellets were 0.97 ± 0.04 and 1.02 ± 0.05. In the pellet production process, sphericity higher than 0.8 and aspect ratio less than 1.2 were considered ideal (Chopra et al., 2002). In our study, both series of prepared pellets had the ideal and suitable criteria. Fig. 3, shows 5-ASA layered or matrix pellets with and without optimal coating along with the cross-section of optimum coated pellets, which were acquired on the SEM. Figures 3A to 3C display the nonpareil core produced by the extrusion-spheronization method, drug was loaded on them and finally coated with the optimal coating formula. Also, the cross-section of the optimum coated pellets shows a uniform thickness of about 15 μm for the coating layer (Figures 3D and 3H). The comparison between 5-ASA layered and matrix pellets demonstrated that drug loading on the nonpareil core leads to the formation of more spherical pellets than the 5-ASA matrix pellets, which could be as a result of the uniform coating of pellets with the drug. In order to produce 5-ASA matrix pellets, it should be noted that an increase in the drug dosage in the pellets is accompanied by a decrease in the amount of pelletization aid excipients such as Avicel® which could be responsible for the failure to obtain completely spherical pellets (Desai and Momin, 2020; Dukić-Ott et al., 2009). For this reason, 5-ASA matrix pellets (Figure 3E) have lower sphericity than 5-ASA layered pellets (Figure 3B). Generally, spherical pellets can be easily distributed throughout the intestine and passed at high speed through the upper parts of the GIT (Muley et al., 2016). On the other hand, the covering of these pellets with coating materials causes the same thickness of the coating layer to be placed on the pellets. Therefore, the sphericity of the pellets is an important factor for targeted drug delivery to the end regions of the GIT (Muley et al., 2016).

3.4. Dissolution studies of the optimum coated pellets

Figure 4 shows the drug release profile from coated 5-ASA layered or matrix pellets with the optimal coating formulation in different simulated GIT media. Evaluating the drug release profile from the pellets coated with ES33+EL52+EC15 with a coating level of 7% in the simulated dissolution medium of the gastric at pH 1.2 shows a release of about 5% of the 5-ASA layered

pellets, while the 5-ASA matrix pellets kept their drug content completely (Figure 4A). The comparison of drug release at pH 6.5 and 6.8 shows that with an increase in pH, the rate of drug release increases significantly. The 5-ASA layered pellets also showed a higher drug release rate than the 5-ASA matrix pellets at these two pH levels (Figures 4B to 4C). The 5-ASA matrix pellets and 5-ASA layered pellets released about 8 to 10% within 2 h and 65 to 75% of their drug content within 10 h at pH 6.8, respectively (Figure 4 C). With the increase of pH from 6.8 to 7.2, the high speed of drug release of 5-ASA layered or matrix pellets could be observed. Similarly, the rate of drug release was higher from the 5-ASA layered pellets compared to 5-ASA matrix pellets at pH 7.2. In other words, it took about 4 and 6 h that around 90% of the drug content of 5-ASA layered or matrix pellets to be released at pH 7.2 respectively (Figure 4 D).

According to Figure 4E, both 5-ASA layered and matrix pellets showed a delayed release of about 5 h. After the lag time, the formulations started to release the drug in the simulated medium of the colon (pH 6.8) within 10 h. The drug release at pH 6.8 in the case of matrix pellets was slower than layered pellets. These results confirm the suitability of developed coating for a variety of drug-loaded cores. SEM images of 5-ASA layered or matrix pellets after the continuous dissolution test showed that their surface was wrinkled and also that the size of these pellets became smaller (Figure 5). The drug release profile of the Asacol® brand shows the prevention of drug release for about 5 h in the upper GIT, while drug release was complete in the medium with pH 7.2 within 1 h. In other words, an explosive release occurs in the simulated medium of the terminal ileum. This fast release is due to the dissolution of ES that was employed on the surface of the Asacol® tablet as the coating layer (Kotla et al., 2018). The release of the entire drug content at the end of the small intestine causes most of the drug to be absorbed in the initial areas of the colon, and therefore as a result, a small amount of it reaches the terminal parts of the colon such as the descending colon and/or sigmoid region. On the other hand, the drug release profile of the Pentasa® brand shows that most of the drug is released in the upper GIT, in such a way that, about 60% and 40% of the drug is released in the simulated area of the gastric and the other part of GIT media, respectively. The reason for this type of release may have to do with the relatively lower thickness of the coating layer of EC used for coating the Pentasa® pellets. The highlight of this study is therefore the use of combined different drug delivery systems in a single coating for the purpose of targeted drug delivery of 5-ASA to the colon. In other words, applying ES and EC, which are used in the coatings of Asacol® and Pentasa® brands, respectively, in a single coating layer can overcome the disadvantages of these two formulations. Due to the variability in physiological conditions of the GIT and the failure to reach pH above 7 to dissolve the tablet coat, some patients report the excretion of Asacol® in the intact form (H. and A., 2020). It seems that this challenge can be mitigated by using EL in combination with ES.

In several studies, applying pH and time-dependent polymers has been used for better efficiency and more assurance of drug delivery to the colon (Fude et al., 2007; Gupta et al., 2001; Xu et al., 2014). Gupta et al. designed pellets containing 5-ASA with two drug delivery systems, so that in one step, the pellets were coated with a mixture of Eudragit RS and Eudragit RL (as time-dependent polymers) and in the next step, these pellets were coated with a pH-dependent polymer (FS) (Gupta et al., 2001). They showed that when these pellets were exposed to the dissolution medium with a pH above 7, the outer coating layer (FS) dissolved completely and the inner coating layer controlled the drug release rate. In other words, the outer layer played a protective role in the passage of pellets through the upper GIT media (Gupta et al., 2001). In a similar study, Xu et al. obtained similar results. The authors coated 5-ASA pellets with two separate coating layers of an

aqueous dispersion of EC and ES. The results showed using ES as an external coating layer prevented drug release in the gastric simulated medium while 5-ASA pellets coated with EC alone released about 20% of the drug content in the same medium (Xu et al., 2014). In another study, 5-ASA pellets were coated with three separate coating layers including a mixture of pectin and HPMC as an internal coating, EC as a middle coating, and Eudragit L30D-55 as an external coating. The release study of the 5-ASA showed that the pellets maintained their drug content in the simulated gastric medium and released all of the drug content within 2 h when placed in a buffered medium at pH 6.8 (Fude et al., 2007).

It, therefore, seems that the application of separate coating layers on the pellets, the need for multiple coating processes and also considering a specific pH for the entirety of the small intestine and colon are the disadvantages of these studies. Due to the similarity and proximity of pH between the small intestine and the colon, in pH-dependent systems, the location of drug release can be unpredictable (Amidon et al., 2015). This challenge can be partially mitigated by combining the pH-dependent system and the time-dependent system in a single coating in order to ensure drug release at different physiological conditions (Akhgari et al., 2006). One-step coating saves cost and time in the production process. More importantly, the coating layer placed on the pellet will thus simultaneously represent both pH and time-dependent properties. This advantage prevents the sudden release of the drug when the pellets reach the target location and provide the gradual release of the drug in the colonic area (Shahdadi Sardou et al., 2022).

3.5. Preclinical efficacy of 5-ASA layered or matrix pellets

The effectiveness of the optimum 5-ASA layered or matrix pellets was observed in the treatment process of rats with UC (Figures 6 and 7). Daily use of the drug with the dose of 120 mg/kg during the treatment period decreased CIA, colon/body weight ratio, colon damage score, MDA, and increased length/weight ratio and GSH. In all the investigated factors, optimum 5-ASA layered or matrix pellets showed higher efficiency compared with other treatment groups during the treatment period.

After three days of induced colitis in rats, the CAI increased from zero to about 3.5, then treatment continued for 10 consecutive days. The rats that were treated with the optimum 5-ASA layered or matrix pellets were able to reduce CAI to the range of 1.5, while the untreated and vehicle groups didn't show any changes. Also, uncoated 5-ASA layered or matrix pellets and Pentasa® showed almost the same efficiency and reduced this factor to the range of 2.5 (Figure 6 A).

The evaluation of the ratio of colon weight to rat body weight showed that this ratio was higher for the groups without treatment and vehicle. On the other hand, the lowest ratio was for the control group and the groups treated with 5-ASA layered or matrix pellets respectively (Figure 6B).

The length/weight ratio of the colon (Figure 6C) showed an inverse relationship with the colon/body weight ratio test (Figure 6B). For example, the control group, which had the lowest colon/body weight ratio, showed the highest length/weight ratio. In this experiment, after the control group, the groups receiving the optimal 5-ASA layered or matrix pellets had the highest length/weight ratio of the colon. Also, the uncoated 5-ASA layered or matrix pellets and the Pentasa® did not show therapeutic effects similar to that of the optimum 5-ASA layered or matrix pellets (Figures 6B and 6C).

The colon damage score demonstrated that the group without treatment or the vehicle group had the highest score. Tissue necrosis, ulcer and gangrene were observed in these groups which indicated the high intensity of inflammation. On the other hand, the groups treated with the optimum 5-ASA layered or matrix pellets were able to significantly reduce this score, which indicates the excellent efficiency of these pellets in the treatment of colitis. However, the Pentasa® showed performance similar to uncoated pellets (Figure 6D).

Due to the importance of the oxidative stress pathway in colitis and the fact that in this disease, the amount of MDA and GSH in the colon tissue increases and decreases respectively, therefore, the compounds that are able to control the disease could lead to an increase and decrease in the level of GSH and MDA respectively (Chen et al., 2021; Ito et al., 2019). These markers of lipid peroxidation showed the effectiveness of optimum 5-ASA layered or matrix pellets in the treatment of colitis as compared to the Pentasa® and led to decreased and increased MDA and GSH, respectively (Figure 6E and 6F).

An evaluation and comparison of histology of colon tissues confirmed the results of CAI, colon/body weight ratio, length/weight ratio of the colon, colon damage score and lipid peroxidation markers such as MDA and GSH (Figure 7). In general, the administration of optimum 5-ASA layered or matrix pellets caused the disappearance of inflammatory markers including tissue necrosis, crypt tissues, muscle thickening, ulcer, and neutrophils in the colon tissue. In contrast to the groups that were not treated, inflammatory markers were clearly observed in them. However, muscle thickening was observed in the groups that were treated with the Pentasa® and uncoated 5-ASA layered or matrix pellets. In general, during the ten-day treatment period, optimum 5-ASA layered or matrix pellets compared to the Pentasa® showed excellent therapeutic performance and were able to return the colon tissue to an almost normal shape, which indicates the targeted delivery of 5-ASA to the colon area. The use of two drug delivery systems in the coat of optimal 5-ASA layered or matrix pellets may have led to providing a better performance therapy compared to the Pentasa® which had been designed based on one drug delivery system. Moreover, in the *in-vivo* studies, no significant difference was observed between optimum 5-ASA layered or matrix pellets which are probably related to the similar drug release of these pellets which were also shown in the continuous dissolution test (Figure 4E).

4. Conclusion

This study showed that uncoated 5-ASA layered or matrix pellets showed a rapid release of the drug in simulated gastric and intestinal media. This indicated the necessity of applying a coating layer on the 5-ASA pellets in controlling the rate and location of drug release. To control the rate of drug release in the initial parts of the GIT as well as accurate drug delivery to the target site or colon, the integration of two drug delivery systems (pH and time-dependent systems) was evaluated. The ratio of ES33+EL52+EC15 w/w at a coating level of 7% was found to be the optimum coating layer for this purpose. The drug release from this optimal 5-ASA layered or matrix pellets compared to Asacol® and Pentasa® provided a gradual release of the drug in the colon area, while Pentasa® released most of the drug content in the upper GIT with Asacol® showing a burst release in the terminal ileum's simulated medium. It seems that the use of two drug delivery systems in one coating layer prevented the release of the drug in the upper GIT and

delivered the drug to the colon specifically. Also, the optimum 5-ASA layered or matrix pellets showed excellent therapeutic results compared to the Pentasa® brand in animal studies.

Acknowledgments

This study was related to the research project (Grant: 981370, 4010687) supported by the Vice-Chancellor for Research and Technology of Mashhad University of Medical Sciences, Mashhad, Iran (MUMS).

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abinusawa, A., Tenjarla, S., 2015. Release of 5-Aminosalicylic Acid (5-ASA) from Mesalamine Formulations at Various pH Levels. Adv. Ther. 32, 477–484. https://doi.org/10.1007/s12325-015-0206-4

Afrasiabi Garekani, H., Nokhodchi, A., Rayeni, M.A., Sadeghi, F., 2013. Preparation and characterization and release properties of Eudragit RS based ibuprofen pellets prepared by extrusion spheronization: effect of binder type and concentration. Drug Dev. Ind. Pharm. 39, 1238–1246. https://doi.org/10.3109/03639045.2012.707207

Akhgari, A., Garekani, H.A., Sadeghi, F., Azimaie, M., 2005. Statistical optimization of indomethacin pellets coated with pH-dependent methacrylic polymers for possible colonic drug delivery. Int. J. Pharm. 305, 22–30. https://doi.org/10.1016/j.ijpharm.2005.08.025

Akhgari, A., Sadeghi, F., Garekani, H.A., 2006. Combination of time-dependent and pH-dependent polymethacrylates as a single coating formulation for colonic delivery of indomethacin pellets. Int. J. Pharm. 320, 137–142. https://doi.org/10.1016/j.ijpharm.2006.05.011

Akhgari, A., Tavakol, A., 2016. Prediction of Optimum Combination of Eudragit RS/Eudragit RL/Ethyl Cellulose Polymeric Free Films Based on Experimental Design for Using as a Coating System for Sustained Release Theophylline Pellets. Adv. Pharm. Bull. 6, 219–225. https://doi.org/10.15171/apb.2016.030

Amidon, S., Brown, J.E., Dave, V.S., 2015. Colon-targeted oral drug delivery systems: design trends and approaches. AAPS PharmSciTech 16, 731–741. https://doi.org/10.1208/s12249-

015-0350-9

Ansari, M.N., Rehman, N.U., Karim, A., Soliman, G.A., Ganaie, M.A., Raish, M., Hamad, A.M., 2021. Role of oxidative stress and inflammatory cytokines (Tnf-α and il-6) in acetic acid-induced ulcerative colitis in rats: Ameliorated by otostegia fruticosa. Life 11, 1–17. https://doi.org/10.3390/life11030195

Arévalo-Pérez, R., Maderuelo, C., Lanao, J.M., 2020. Recent advances in colon drug delivery systems. J. Control. Release 327, 703–724. https://doi.org/10.1016/j.jconrel.2020.09.026

Bautzová, T., Rabišková, M., Béduneau, A., Pellequer, Y., Lamprecht, A., 2012. Bioadhesive pellets increase local 5-aminosalicylic acid concentration in experimental colitis. Eur. J. Pharm. Biopharm. 81, 379–385. https://doi.org/10.1016/j.ejpb.2012.02.011

Cai, X., Wang, X., He, M., Wang, Y., Lan, M., Zhao, Y., Gao, F., 2021. Colon-targeted delivery of tacrolimus using pH-responsive polymeric nanoparticles for murine colitis therapy. Int. J. Pharm. 606, 120836. https://doi.org/10.1016/j.ijpharm.2021.120836

Catenacci, L., Sorrenti, M., Perteghella, S., Mandracchia, D., Torre, M.L., Trapani, A., Milanese, C., Tripodo, G., 2020. Combination of inulin and β-cyclodextrin properties for colon delivery of hydrophobic drugs. Int. J. Pharm. 589, 119861. https://doi.org/10.1016/j.ijpharm.2020.119861

Chen, J., Li, X., Chen, L., Xie, F., 2018. Starch film-coated microparticles for oral colon-specific drug delivery. Carbohydr. Polym. 191, 242–254. https://doi.org/10.1016/j.carbpol.2018.03.025

Chen, S., Chen, Z., Wang, Yi, Hao, W., Yuan, Q., Zhou, H., Gao, C., Wang, Yitao, Wu, X., Wang, S., 2021. Targeted delivery of Chinese herb pair-based berberine/tannin acid self-assemblies for the treatment of ulcerative colitis. J. Adv. Res. 40, 263–276. https://doi.org/10.1016/j.jare.2021.11.017

Choi, K., Cho, S., Kook, S., Chun, S., Bhattarai, G., Bahadur, S., Kim, M., Lee, K., Lee, J., 2016. Intestinal anti-in fl ammatory activity of the seeds of Raphanus sativus L . in experimental ulcerative colitis models. J. Ethnopharmacol. 179, 55–65. https://doi.org/10.1016/j.jep.2015.12.045

Chopra, R., Podczeck, F., Newton, J.M., Alderborn, G., 2002. The influence of pellet shape and film coating on the filling of pellets into hard shell capsules. Eur. J. Pharm. Biopharm. 53, 327–333. https://doi.org/10.1016/S0939-6411(02)00015-2

Desai, N., Momin, M., 2020. Colon targeted bioadhesive pellets of curcumin and cyclosporine for improved management of inflammatory bowel disease. Drug Deliv. Transl. Res. 10, 1288–1301. https://doi.org/10.1007/s13346-020-00756-x

Dos Santos, J., da Silva, G.S., Velho, M.C., Beck, R.C.R., 2021. Eudragit®: A versatile family of polymers for hot melt extrusion and 3D printing processes in pharmaceutics. Pharmaceutics. https://doi.org/10.3390/pharmaceutics13091424

Dukić-Ott, A., Thommes, M., Remon, J.P., Kleinebudde, P., Vervaet, C., 2009. Production of pellets via extrusion-spheronisation without the incorporation of microcrystalline cellulose: A critical review. Eur. J. Pharm. Biopharm. 71, 38–46. https://doi.org/10.1016/j.ejpb.2008.08.005

Elbaz, N.M., Owen, A., Rannard, S., McDonald, T.O., 2020. Controlled synthesis of calcium carbonate nanoparticles and stimuli-responsive multi-layered nanocapsules for oral drug delivery. Int. J. Pharm. 574, 118866. https://doi.org/10.1016/j.ijpharm.2019.118866

Franco, P., Marco, I. De, 2020. Eudragit : A Novel Carrier for Controlled Drug Delivery in Supercritical Antisolvent Coprecipitation. Polymers (Basel). 12, 234. https://doi.org/10.3390/polym12010234

Fude, C., Lei, Y., Jie, J., Hongze, P., Wenhui, L., Dongmei, C., 2007. Preparation and in vitro evaluation of pH, time-based and enzyme-degradable pellets for colonic drug delivery. Drug Dev. Ind. Pharm. 33, 999–1007. https://doi.org/10.1080/03639040601150393

Goyanes, A., Hatton, G.B., Merchant, H.A., Basit, A.W., 2015. Gastrointestinal release behaviour of modified-release drug products: Dynamic dissolution testing of mesalazine formulations. Int. J. Pharm. 484, 103–108. https://doi.org/10.1016/j.ijpharm.2015.02.051

Gupta, V.K., Assmus, M.W., Beckert, T.E., Price, J.C., 2001. A novel pH- and time-based multi-unit potential colonic drug delivery system. II. Optimization of multiple response variables 213, 93–102. https://doi.org/10.1016/S0378-5173(00)00650-5

H., B., A., B., 2020. Protective effect of asacol in combination with pantoprazole in ulcerative colitis patients who defecate asacol tablets intactly: A clinical trial study. Clin. Exp. Gastroenterol. 13, 47–51.

Hua, S., 2020. Advances in oral drug delivery for regional targeting in the gastrointestinal tract - influence of physiological, pathophysiological and pharmaceutical Factors. Front. Pharmacol. 11, 1–22. https://doi.org/10.3389/fphar.2020.00524

Ito, F., Sono, Y., Ito, T., 2019. Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: Oxidative stress in diabetes, atherosclerosis, and chronic inflammation. Antioxidants 8, 325–331. https://doi.org/10.3390/antiox8030072

Kaffash, E., Saremnejad, F., Abbaspour, M., Ahmad, S., Afrasiabi, H., Hossein, A., Shahdadi, H., Akhgari, A., Nokhodchi, A., 2019. Statistical optimization of alginate-based oral dosage form of 5-aminosalicylic acid aimed to colonic delivery : In vitro and in vivo evaluation. J. Drug Deliv. Sci. Technol. 52, 177–188. https://doi.org/10.1016/j.jddst.2019.04.006

Kobayashi, T., Siegmund, B., Le Berre, C., Wei, S.C., Ferrante, M., Shen, B., Bernstein, C.N., Danese, S., Peyrin-Biroulet, L., Hibi, T., 2020. Ulcerative colitis. Nat. Rev. Dis. Prim. 6, 2–20. https://doi.org/10.1038/s41572-020-0205-x

Kotagale, N., Maniyar, M., Somvanshi, S., Umekar, M., Patel, C.J., 2010. Eudragit-S, Eudragit-L and cellulose acetate phthalate coated polysaccharide tablets for colonic targeted delivery of

azathioprine. Pharm. Dev. Technol. 15, 431–437. https://doi.org/10.3109/10837450903264401

Kotla, N.G., Rana, S., Sivaraman, G., Sunnapu, O., Vemula, P.K., Pandit, A., Rochev, Y., 2018. Bioresponsive drug delivery systems in intestinal in flammation : State-of-the-art and future perspectives. Adv. Drug Deliv. Rev. 146, 248–266. https://doi.org/10.1016/j.addr.2018.06.021

Lee, S.H., Bajracharya, R., Min, J.Y., Han, J.W., Park, B.J., Han, H.K., 2020. Strategic approaches for colon targeted drug delivery: An overview of recent advancements. Pharmaceutics 12, 68. https://doi.org/10.3390/pharmaceutics12010068

Lei, J., Yi-cun, D., Yu, Z., 2016. A novel pH – enzyme-dependent mesalamine colon-specific delivery system. Drug Des. Devel. Ther. 10, 2021–2028. https://doi.org/10.2147/DDDT.S107283

Maderuelo, C., Lanao, J.M., Zarzuelo, A., 2019. Enteric coating of oral solid dosage forms as a tool to improve drug bioavailability. Eur. J. Pharm. Sci. 138, 105019. https://doi.org/10.1016/j.ejps.2019.105019

Maurer, J.M., Schellekens, R.C.A., Van Rieke, H.M., Wanke, C., Iordanov, V., Stellaard, F., Wutzke, K.D., Dijkstra, G., Van Der Zee, M., Woerdenbag, H.J., Frijlink, H.W., Kosterink, J.G.W., 2015. Gastrointestinal pH and transit time profiling in healthy volunteers using the IntelliCap system confirms ileo-colonic release of ColoPulse tablets. PLoS One 10, 1–17. https://doi.org/10.1371/journal.pone.0129076

Moghimipour, E., Dorkoosh, F.A., Rezaei, M., Kouchak, M., Fatahiasl, J., Angali, K.A., Ramezani, Z., Amini, M., Handali, S., 2018. In vivo evaluation of pH and time-dependent polymers as coating agent for colonic delivery using central composite design. J. Drug Deliv. Sci. Technol. 43, 50–56. https://doi.org/10.1016/j.jddst.2017.09.010

Muley, S., Nandgude, T., Poddar, S., 2016. Extrusion–spheronization a promising pelletization technique: In-depth review. Asian J. Pharm. Sci. 11, 684–699. https://doi.org/10.1016/j.ajps.2016.08.001

Mura, C., Nácher, A., Merino, V., Merino-Sanjuan, M., Carda, C., Ruiz, A., Manconi, M., Loy, G., Fadda, A.M., Diez-Sales, O., 2011. N-Succinyl-chitosan systems for 5-aminosalicylic acid colon delivery: In vivo study with TNBS-induced colitis model in rats. Int. J. Pharm. 416, 145–154. https://doi.org/10.1016/j.ijpharm.2011.06.025

Murray, A., Nguyen, T.M., Parker, C.E., Feagan, B.G., MacDonald, J.K., 2020. Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. Cochrane Database Syst. Rev. 8, 114. https://doi.org/10.1002/14651858.CD000543.pub5

Naganuma, M., 2020. Solving the questions regarding 5-aminosalitylate formulation in the treatment of ulcerative colitis. J. Gastroenterol. 55, 1013–1022. https://doi.org/10.1007/s00535-020-01713-8

Nguyen, C., Christensen, J.M., Ayres, J.W., 2012. Novel mesalamine-loaded beads in tablets for delayed release of drug to the colon. Pharm. Dev. Technol. 17, 73–83. https://doi.org/10.3109/10837450.2010.516436

Niranjan, P.C., 2017. Pharmaceutical significance of Eudragit: A review. Futur. J. Pharm. Sci. 3, 33–45.

Parmar, C., Parikh, K., Mundada, P., Bhavsar, D., Sawant, K., 2018. Formulation and optimization of enteric coated bilayer tablets of mesalamine by RSM: In vitro – In vivo investigations and roentogenographic study. J. Drug Deliv. Sci. Technol. 44, 388–398. https://doi.org/10.1016/j.jddst.2018.01.008

Patole, V.C., Pandit, A.P., 2017. Mesalamine-loaded alginate microspheres filled in enteric coated HPMC capsules for local treatment of ulcerative colitis: in vitro and in vivo characterization. J. Pharm. Investig. 48, 257–267. https://doi.org/10.1007/s40005-017-0304-1

Shahdadi Sardou, H., Akhgari, A., Afrasiabi Garekani, H., Sadeghi, F., 2019a. Screening of different polysaccharides in a composite film based on Eudragit RS for subsequent use as a coating for delivery of 5-ASA to colon. Int. J. Pharm. 568, 118527. https://doi.org/10.1016/j.ijpharm.2019.118527

Shahdadi Sardou, H., Akhgari, A., Hooshang Mohammadpour, A., Kamali, H., Hossein Jafarian, A., Afrasiabi Garekani, H., Sadeghi, F., 2022. Optimization study of combined enteric and time-dependent polymethacrylates as a coating for colon targeted delivery of 5-ASA pellets in rats with ulcerative colitis. Eur. J. Pharm. Sci. 168, 106072. https://doi.org/10.1016/j.ejps.2021.106072

Shahdadi Sardou, H., Akhgari, A., Hooshang Mohammadpour, A., Kamali, H., Hossein Jafarian, A., Afrasiabi Garekani, H., Sadeghi, F., 2021. Application of inulin/Eudragit RS in 5-ASA pellet coating with tuned, sustained-release feature in an animal model of ulcerative colitis. Int. J. Pharm. 597, 120347. https://doi.org/10.1016/j.ijpharm.2021.120347

Shahdadi Sardou, H., Saremnejad, F., Bagheri, S., Akhgari, A., Afrasiabi Garekanid, H., Sadeghi, F., 2019b. A review on 5-aminosalicylic acid colon-targeted oral drug delivery systems. Int. J. Pharm. 558, 367–379. https://doi.org/10.1016/j.ijpharm.2019.01.022

Shahdadi Sardou, H., Vosough, P.R., Abbaspour, M., Akhgari, A., Sathyapalan, T., Sahebkar, A., 2023. A review on curcumin colon-targeted oral drug delivery systems for the treatment of inflammatory bowel disease. Inflammopharmacology. https://doi.org/10.1007/s10787-023-01140-0

Tan, C., Fan, H., Ding, J., Han, C., Guan, Y., Zhu, F., Wu, H., Liu, Y., Zhang, W., Hou, X., Tan, S., Tang, Q., 2022. ROS-responsive nanoparticles for oral delivery of luteolin and targeted therapy of ulcerative colitis by regulating pathological microenvironment. Mater. Today Bio 14, 100246. https://doi.org/10.1016/j.mtbio.2022.100246

Teruel, A.H., Gonzalez-alvarez, I., Bermejo, M., Merino, V., Marcos, M.D., Sancenon, F., Gonzalez-alvarez, M., Martinez-mañez, R., 2020. New insights of oral colonic drug delivery

systems for inflammatory bowel disease therapy. Int. J. Mol. Sci. 21, 6502. https://doi.org/10.3390/ijms21186502

Thakral, S., Thakral, N., Majumdar, D., 2013. Eudragit®: a technology evaluation. Expert Opin. Drug Deliv. 10, 131–149. https://doi.org/10.1517/17425247.2013.736962

Thakral, Seema, Thakral, N.K., Majumdar, D.K., 2013. Eudragit®: a technology evaluation. Expert Opin. Drug Deliv. 10, 131–149. https://doi.org/10.1517/17425247.2013.736962

Van Camp, A., Vanuytsel, T., Brouwers, J., Augustijns, P., 2022. The effect of esomeprazole on the upper GI tract release and systemic absorption of mesalazine from colon targeted formulations. Int. J. Pharm. 619, 121701. https://doi.org/10.1016/j.ijpharm.2022.121701

Varshosaz, J., Ahmadi, F., Emami, J., Tavakoli, N., Minaiyan, M., Mahzouni, P., Dorkoosh, F., 2010. Colon delivery of budesonide using solid dispersion in dextran for the treatment and secondary prevention of ulcerative colitis in rat. Int. J. Prev. Med. 1, 115–23.

Varum, F., Freire, A.C., Bravo, R., Basit, A.W., 2020a. OPTICORE™, an innovative and accurate colonic targeting technology. Int. J. Pharm. 583, 119372. https://doi.org/10.1016/j.ijpharm.2020.119372

Varum, F., Freire, A.C., Fadda, H.M., Bravo, R., Basit, A.W., 2020b. A dual pH and microbiota-triggered coating (Phloral™) for fail-safe colonic drug release. Int. J. Pharm. 583, 119379. https://doi.org/10.1016/j.ijpharm.2020.119379

Veloso, P.M., Machado, R., Nobre, C., 2021. Mesalazine and inflammatory bowel disease – From well-established therapies to progress beyond the state of the art. Eur. J. Pharm. Biopharm. 167, 89–103. https://doi.org/10.1016/j.ejpb.2021.07.014

Wang, Y., Li, Y., He, L., Mao, B., Chen, S., Martinez, V., Guo, X., Shen, X., Liu, B., Li, C., 2021. Commensal flora triggered target anti-inflammation of alginate-curcumin micelle for ulcerative colitis treatment. Colloids Surfaces B Biointerfaces 203, 111756. https://doi.org/10.1016/j.colsurfb.2021.111756

Xu, M., Sun, M., Qiao, H., Ping, Q., Elamin, E.S., 2014. Preparation and evaluation of colon adhesive pellets of 5-aminosalicylic acid. Int. J. Pharm. 468, 165–171. https://doi.org/10.1016/j.ijpharm.2014.04.040

Zeeshan, M., Ali, H., Khan, S., Ahmad, S., Weigmann, B., 2019. Advances in orally-delivered pH-sensitive nanocarrier systems; an optimistic approach for the treatment of inflammatory bowel disease. Int. J. Pharm. 558, 201–214. https://doi.org/10.1016/j.ijpharm.2018.12.074

Table 1. Experimental design: factors and responses

Factors (independent variables)	Levels used			Responses (dependent variables)
	−1	0	+1	
X_1	0:85:15	25:60:15	50:35:15	Y_1 = percent drug release within 2h at pH 6.8
X_2	2.5	5	7.5	Y_2 = percent drug release within 10h at pH 6.8
				Y_3= lag time at pH 7.2 (min)

X_1 = ratio of ES to EL (by considering the constant amount of EC)

X_2 = coating level (%)

Table 2. Variable factors, experimental and predicted responses as well as the analysis of variances for each response.

Run	Variable factors		Responses (Exp. /Pred.)			Y_1 stat.	Y_2 stat.	Y_3 stat.
	X_1 (ES:EL:EC)	X_2 %CL	Y_1 (% of release)	Y_2 (% of release)	Y_3 (min)	TSS	TSS	TSS
1	0:85:15	2.5	43/44	98/97	5/4	1527.23	7171.69	876.92
2	0:85:15	5	31/29	88/85	15/13	**RSS**	**RSS**	**RSS**
3	0:85:15	7.5	22/22	81/80	20/20	8.95	43.22	168.59
4	25:60:15	2.5	31/28	90/88	10/10	$\mathbf{R^2}$	$\mathbf{R^2}$	$\mathbf{R^2}$
5	25:60:15	5	17 /17	77/76	15/14	0.994	0.994	0.807

6	25:60:15	7.5	10/11	69/68	20/18	***F*-value**	***F*-value**	***F*-value**
7	50:35:15	2.5	13/12	57/55	10/9	237.62	230.92	21.01
8	50:35:15	5	5/5	28/26	25/23	***P*-value**	***P*-value**	***P*-value**
9	50:35:15	7.5	1/1	11/12	40/36	0.0001	0.0001	0.0003

TSS: the total sum of squares.

RSS: residual sum of squares.

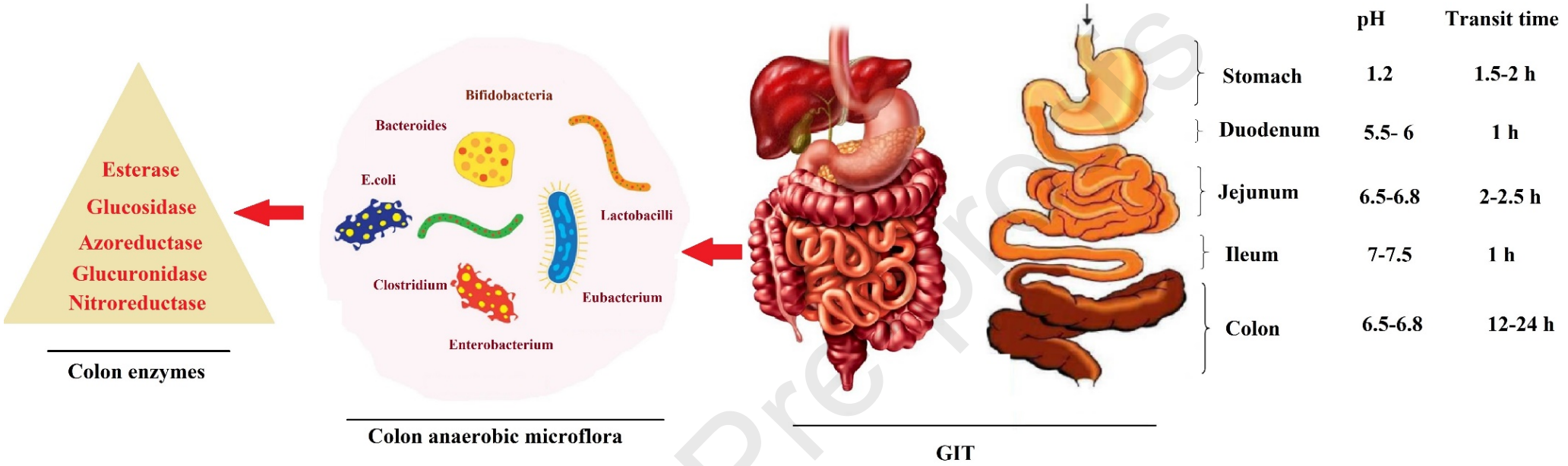


Figure 1. Key physiological factors in the GIT for designing drug formulation.

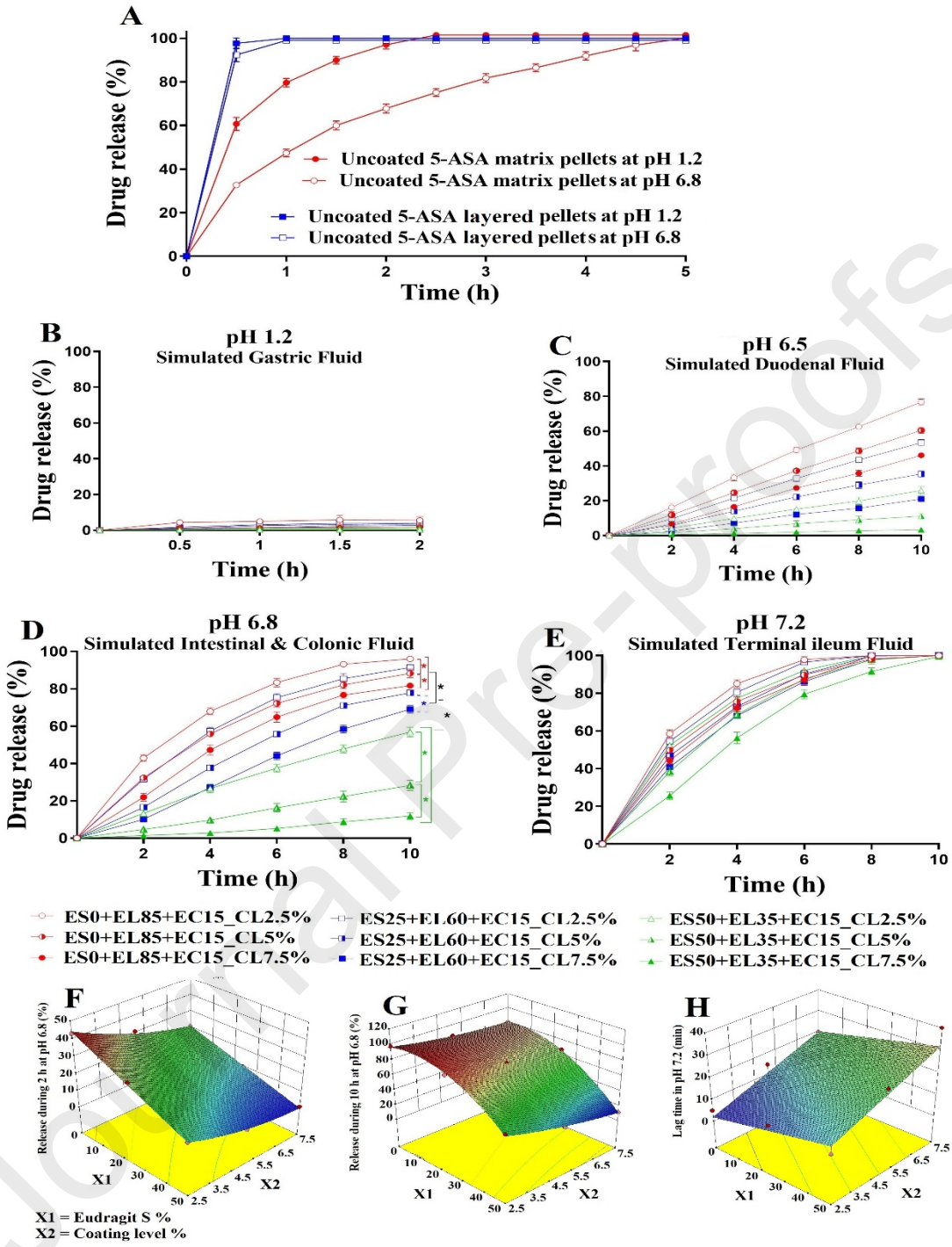
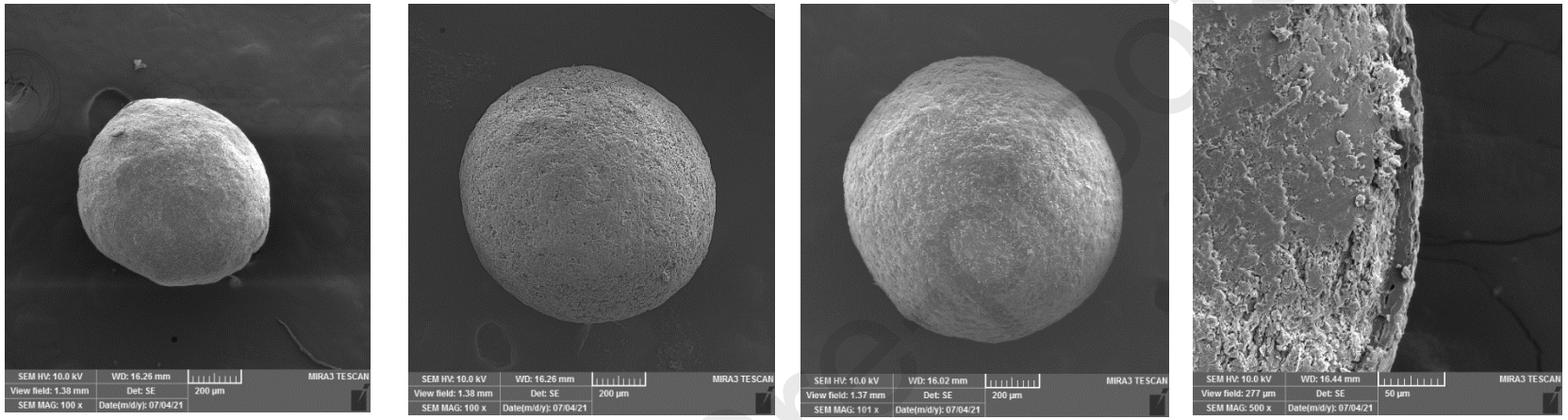


Figure 2. The release profile of drug from uncoated 5-ASA layered or matrix pellets at pH 1.2 and 6.8 (A) and 5-ASA matrix pellet coated with different combinations of ES+EL+EC at varying coating levels as suggested by experimental design. The release of drug from the pellets coated under the experimental design condition at varied pH conditions (B-E). F, G, and H show respectively the surface plot related to the percent drug released within 2h at pH 6.8, percent drug released within 10h at pH 6.8 and lag time before drug release at pH 7.2. Data are shown as mean ± standard deviation (n = 3). * points out the statistical differences between the groups ($p < 0.05$). CL: coating level.

A **B** **C** **D**



E **F** **G** **H**

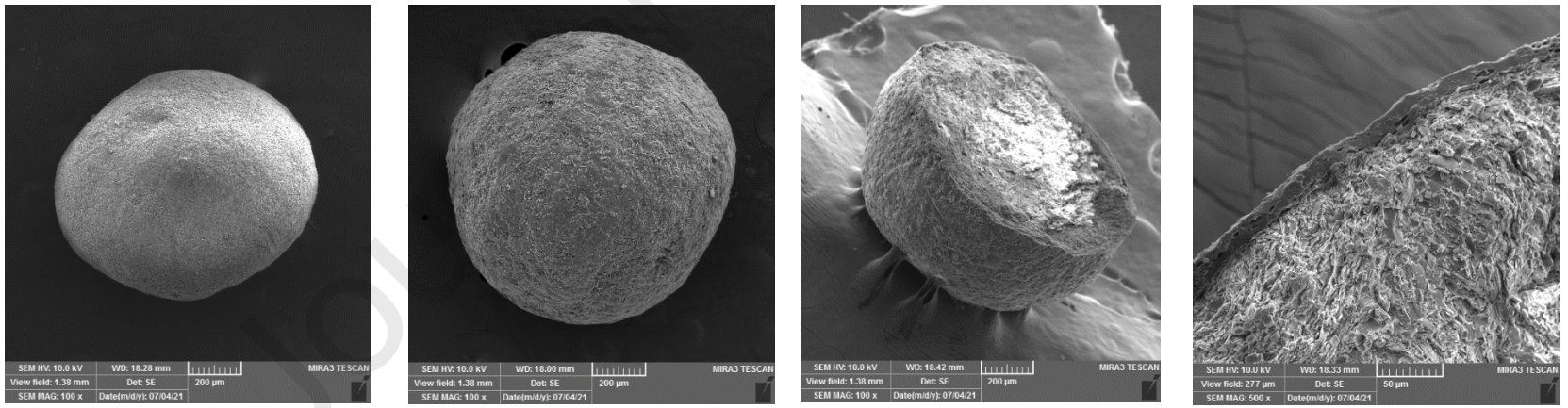


Figure 3. Scanning electron microscopy of the (A) Nonpareil core, (B) 5-ASA layered pellet, (C) 5-ASA layered pellet coated with optimum coating, (D) Cross-sectional image of the 5-ASA layered pellet coated with optimum coating, (E) 5-ASA matrix pellet, (F) 5-ASA matrix pellet coated with optimum coating, (G) and (H) show respectively the cross-sectional image of the optimum coated 5-ASA matrix pellet. The cross-sectional image of the optimum 5-ASA layered or matrix pellets indicates the 15 μm thickness of the coating at 7 % coating level. Scale on (D & H) is 50 μm and all others is 200 μm.

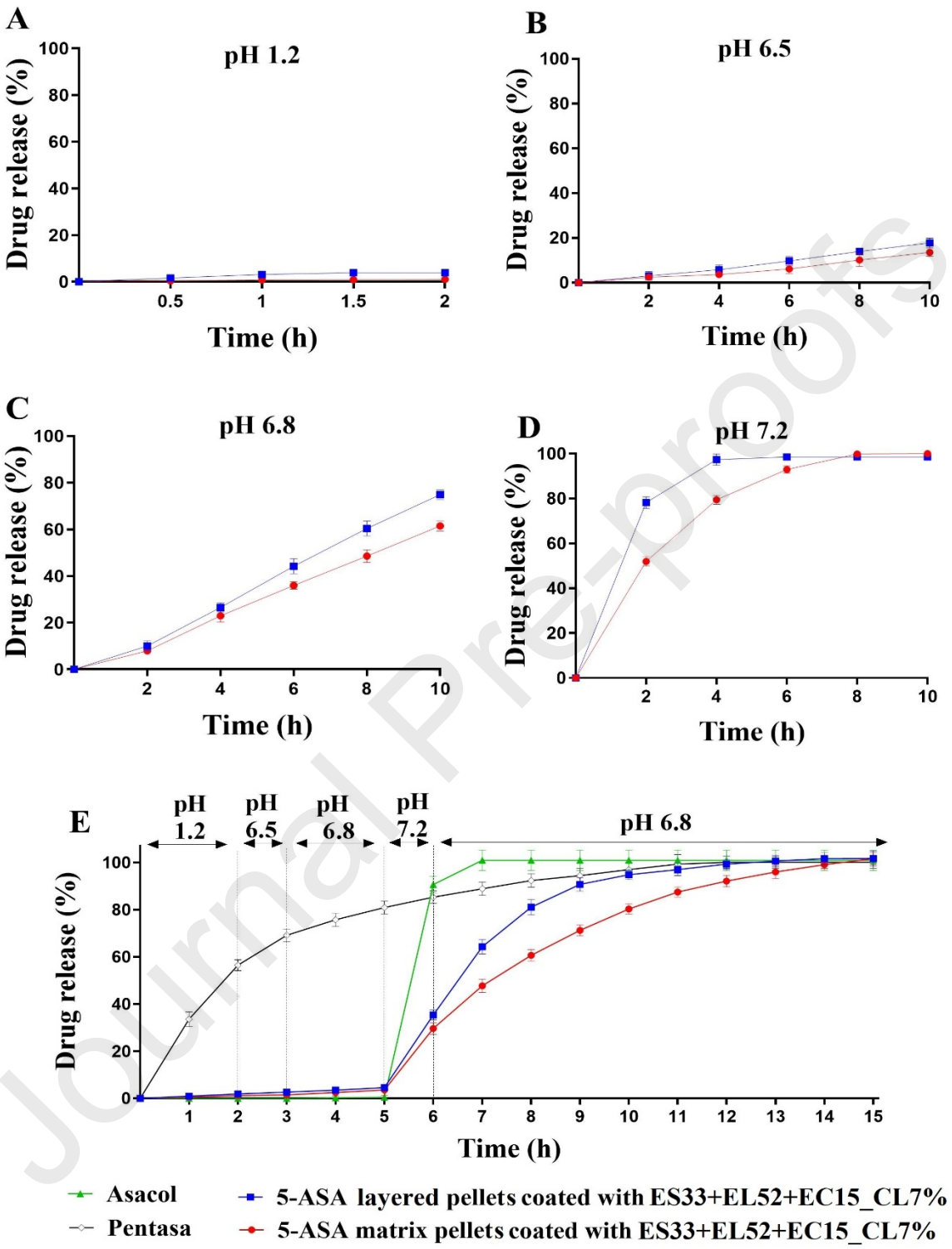


Figure 4. The release profile of 5-ASA layered or matrix pellets coated with optimum coating conditions suggested by the experimental design. A-D shows the drug release profile of the optimum 5-ASA layered or matrix pellets at different pHs. (E) exhibits the release profile of the drug from Asacol®, Pentasa® and optimum 5-ASA layered or matrix pellets in the continuous dissolution test. Data are shown as mean ± standard deviation (n = 3).

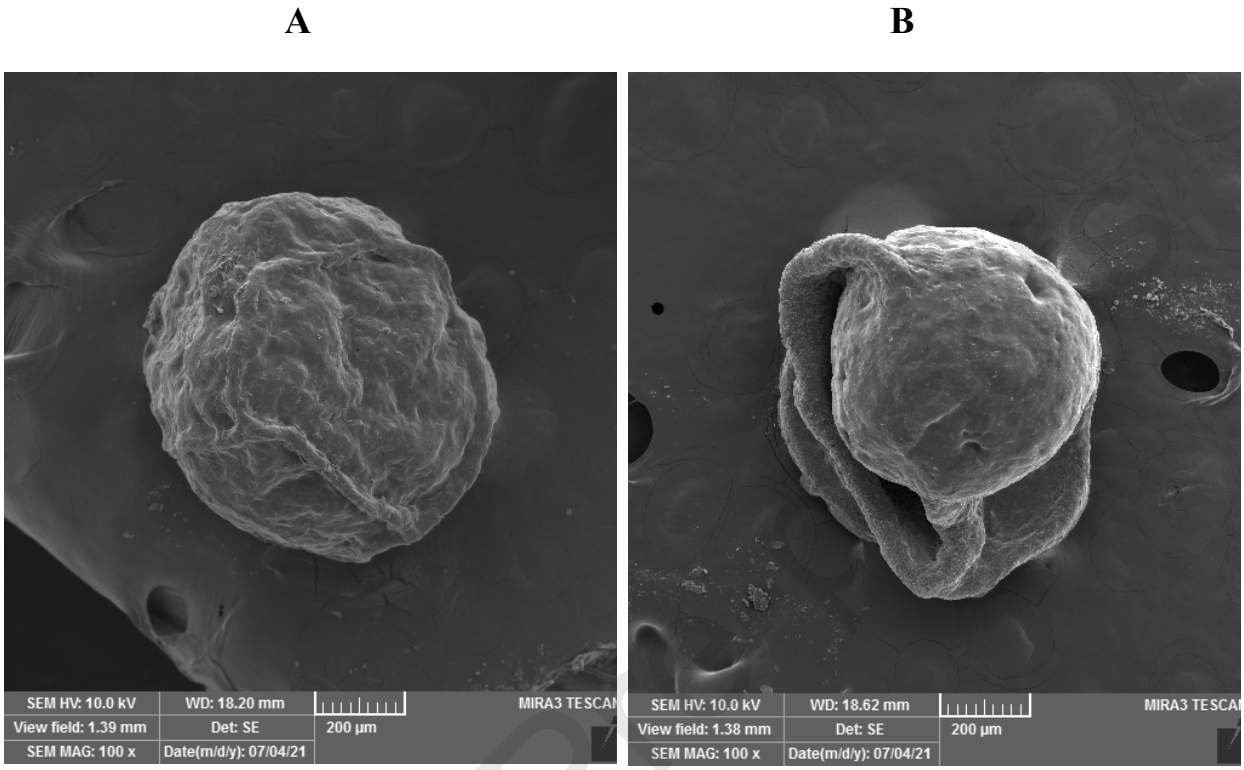


Figure 5. Scanning electron microscopy of the coated pellets after continuous dissolution test shows the smaller size and greater shrinkage of 5-ASA layered pellets (B) compared to the 5-ASA matrix pellets (A).

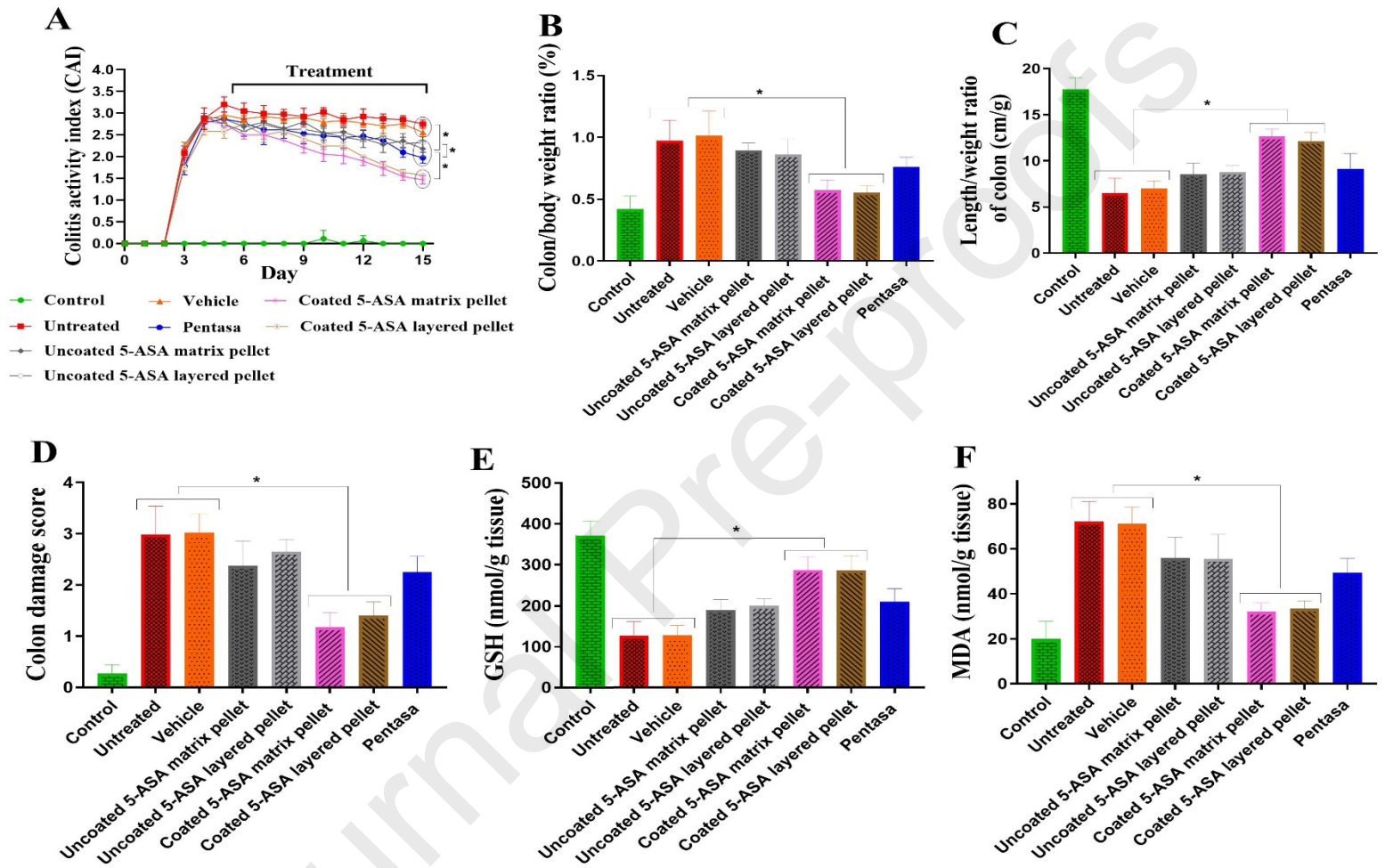


Figure 6. Therapeutic properties of 5-ASA layered or matrix pellets and Pentasa® in UC-rat model. (A) shows the profile of colitis activity index (CAI) in the rats treated with the 5-ASA layered or matrix pellets daily and in those left untreated. Colon/body weight ratio (B), length/weight ratio of colon (C), colon damage score (D), glutathione (GSH; E) and the level of malondialdehyde (MDA; F), all denote the higher therapeutic efficacy of the optimum 5-ASA layered or matrix pellets than Pentasa®. Data are shown as mean ± standard deviation (n = 4). * indicates significant differences between marked groups ($p < 0.05$).

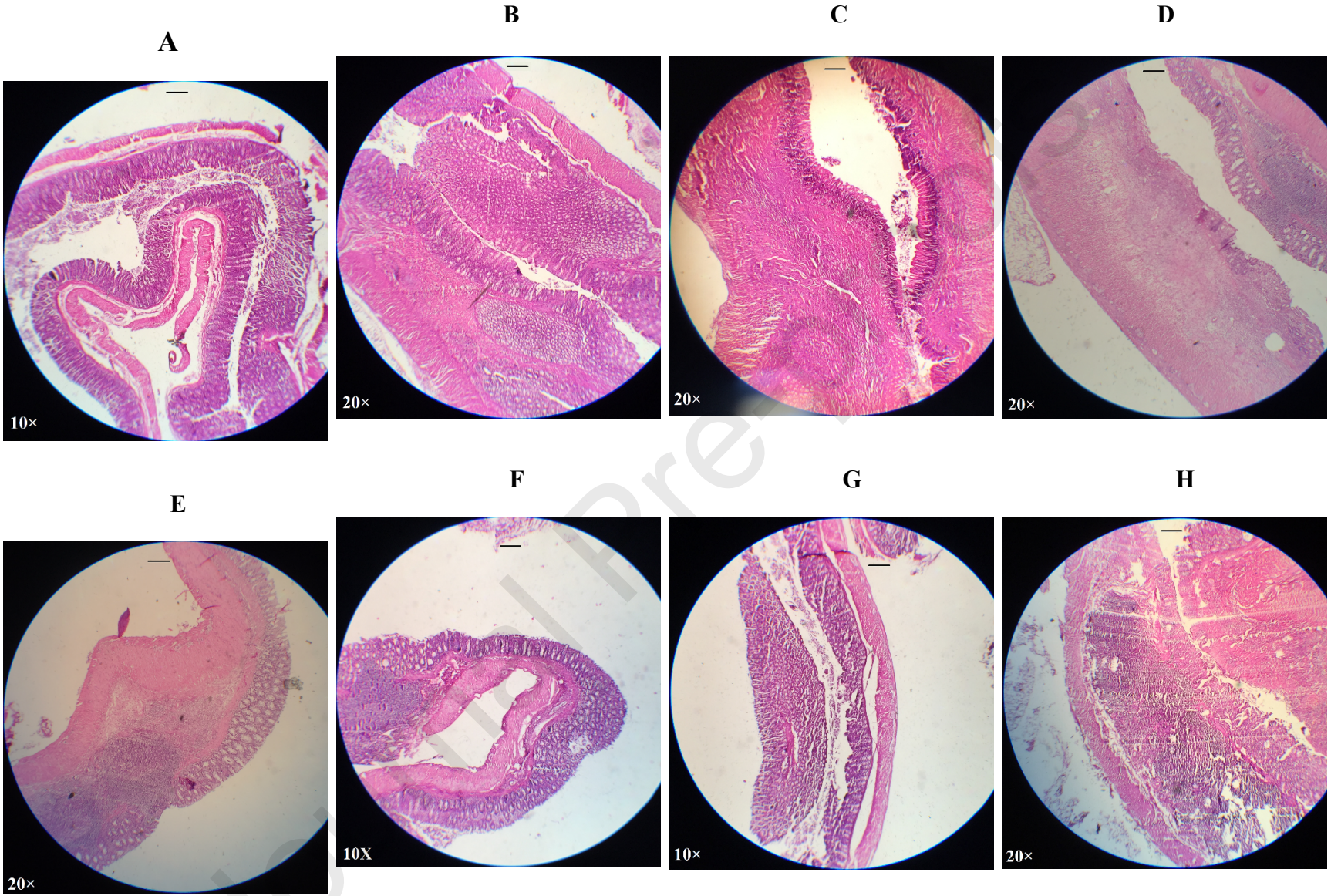


Figure 7. Histopathological characteristics of the colon's tissues taken from the rats subjected to different treatments. (A) shows the normal histology of the colonic tissues. (B) and (C) are the histological sections of the inflammatory colon tissues subjected to the irritating agent of acetic acid. In (B), the rats remained untreated and in (C), they were given only CMC vehicle orally. Others show the colon tissue sections related to the rats treated with uncoated 5-ASA matrix pellet (D), uncoated 5-ASA layered pellet (E), optimally coated 5-ASA layered and matrix pellets, respectively (F & G), and Pentasa® (G). (10 × scale bar 200 μm) and (20 × scale bar 100 μm).

CRediT authorship contribution statement

Hossein Shahdadi Sardou: Data curation, Formal analysis, Methodology, Writing original draft.

Fatemeh Sadeghi: Conceptualization, Methodology, Supervision, Funding acquisition, Review & editing.

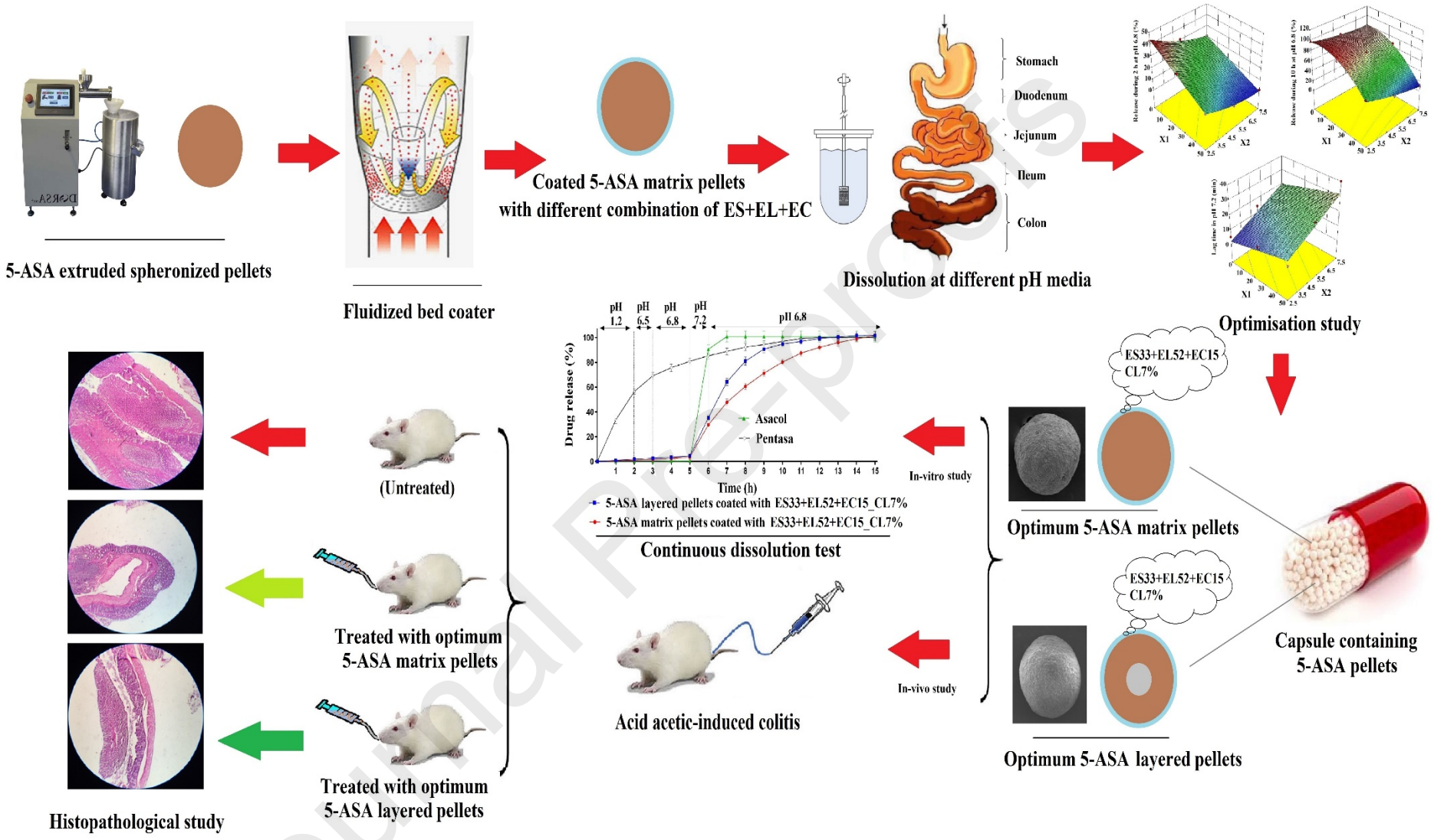
Hadi Afrasiabi Garekani: Conceptualization, Formal analysis, Methodology, Supervision.

Abbas Akhgari: Conceptualization, Formal analysis, Methodology, Supervision.

Amir Hossein Jafarian: Data curation, Methodology.

Mohammadreza Abbaspour: Conceptualization, Formal analysis, Methodology, Resources, Supervision, Funding acquisition, Writing – review & editing.

Ali Nokhodchi: Conceptualization, Writing – review & editing.



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: