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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

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Graphical Abstract

Abstract

Formulation design for colon-specific delivery of 5-aminosalicylic acid (5-ASA) could bring some therapeutic benefits in the treatment of ulcerative colitis (UC).

In the current study, a 3^2 full factorial design was used to predict optimum coating composed of two enteric (poly methacrylic acid, methyl methacrylates 1:2 and 1:1) and time-dependent (poly ethyl acrylate, methyl methacrylate, trimethylammonio ethyl methacrylate chloride 1:2:0.1) polymethacrylates for colon-specific delivery of 5-ASA pellets. A unique coating composition and coating level predicted by the model was applied onto either inulin-free 5-ASA pellets or inulin-bearing 5-ASA pellets and the coated pellets were examined by dissolution test in-vitro. The coated pellets were also tested in a rat model of UC and compared with the a commercially available colonic delivery system of 5-ASA.

The ratio of the two enteric polymethacrylates and time-dependet polymethacrylate of 16:64:20 w/w at a coating level of 15% was discovered as the optimum coating for delivery of 5-ASA pellets to the colon. In general, the coated pellets offered a better therapeutic outcome compared to commercially available colonic delivery system of 5-ASA and uncoated pellets in terms of colitis activity index and the colon's tissue enzymes of MDA and GSH.

It seems that the coating composed of enteric and pH-dependent polymethacrylates could tune up the rate of drug release from 5-ASA-coated pellets and trigger drug release based on pH and time.

Keywords

5-Aminosalicylic acid pellets, Colonic delivery, pH and time-dependent approach, Controlled release, polymethacrylates, Inulin.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic, episodic mucosal inflammation of the gastrointestinal tract (GIT) and might affect a large area of the GIT from the mouth to the anus (Crohn's disease) or might be limited to the colon and rectum region (Ulcerative colitis) which has been shown to respond to the oral dosages of the anti-inflammatory drug of 5-aminosalicylic acid (5-ASA) (Binienda et al., 2020; Kobayashi et al., 2020). 5-ASA is generally considered the first-line medicine in the treatment of Ulcerative colitis (UC) (Cesar et al., 2018; Chapman et al., 2020; Le Berre et al., 2019). It is believed that a suitable formulation that prevents 5-ASA release in the upper parts of the gastrointestinal tract (GIT) and instead, delivers the drug to the colon can enhance the therapeutic properties of the drug (Foppoli et al., 2019; Shahdadi Sardou et al., 2019a).

The release and absorption of 5-ASA in the upper GIT could result in some undesirable side effects, suboptimal colon delivery, and poor therapeutic efficacy (Amidon et al., 2015; Yan et al., 2019).

There are different platforms for the delivery of drugs through GIT, and specifically to the colon region (Amidon et al., 2015; Maderuelo et al., 2019). These drug delivery systems benefit from some physiological alterations through the GIT, including pH, the residence time, and the microflora to tune the delivery of the drug (Jain and Jain, 2008). In this regard, pH-dependent, time-dependent, and bacterially responsive systems are applied along with the active ingredient to achieve the desired delivery profile in the GIT (Fude et al., 2007).

Considering 5-ASA colon-specific delivery, different categories of polymers have been studied in various combinations to control the drug release in the GIT as intended. These are pH-dependent, time-dependent, and bacterially degradable polymers that are either used in combination or as different separate coating layers on the pellets (Gazzaniga et al., 2006; Kotla et al., 2018; Maderuelo et al., 2019; Thakral et al., 2013; Trenda et al., 2016). The examples of the combinations of polymers as a coating layer are Ethylcellulose/Starch (Karrout et al., 2011), Ethylcellulose/Nutriose (Karrout et al., 2015), Ethylcellulose/Pectin (Ahmed, 2005), Eudragit S/Ethylcellulose (Xu et al., 2014), Eudragit FS/Eudragit RL and RS (Gupta et al., 2001), Eudragit FS/Chitosan (Babazadeh et al., 2007), Ethylcellulose/Eudragit L/Pectin (Fude et al., 2007). Mainly, the drug release from the pellets and the contribution of each polymer to the release profile is studied in these combinations, and the optimal formulation whose effect has been confirmed in clinical studies has not been introduced.

Currently, 5-ASA is given as two common oral dosage forms of Pentasa and Asacol; the first is a controlled-release pellet/tablet formulation and the second is a delayed-release tablet (Goyanes et al., 2015). Both of these formulations have some limitations in the optimal delivery of 5-ASA to the colon region (Williams et al., 2011).

There is some evidence showing that Pentasa with time-dependent delivery, releases 5-ASA gradually from the stomach to small intestine, leaving no or little drug for the colon (Kotla et al., 2018). This leads to insufficient drug delivery to the inflamed tissues of the colon, which may be the reason for the poor treatment of UC with this brand (Shahdadi Sardou et al., 2021). In Asacol with pH-dependent delivery, the tablet triggers 5-ASA release once reaching the ileum with a given pH (Goyanes et al., 2015; Ito et al., 2009; Zeeshan et al., 2019). Like Pentasa, this brand also suffers from poor 5-ASA delivery to the colon region sometimes. Asacol, in some cases, releases almost all 5-ASA abruptly as soon as reaching the ileum (Ito et al., 2009; Xu et al., 2004). Therefore, a

suboptimal 5-ASA payload remains to be delivered throughout the colon. In other cases, when pH in the ileum or colon is lower than the required pH for the dissolution of the coat, the tablet might dissolve partially in the colon and delivers no or just a bit of its drug cargo (Abinusawa and Tenjarla, 2015; Goyanes et al., 2015).

An ideal 5-ASA oral formulation for UC should protect the drug through the upper part of GIT, i.e. stomach and small intestine, and then releases all the drug cargo gradually throughout the diseased colon in UC (Teruel et al., 2020). To reach this point, the formulation needs to be responsive to pH alteration through GIT and triggers the drug release at the colon-specific pH (Wu and Yao, 2013; Zeeshan et al., 2019). Moreover, the residence time of the drug in each part of GIT holds the key to the successful drug formulation design (Hua, 2020).

Based on the literature, pH increases abruptly from 1.2 to 6.5 from the stomach to duodenum and it gradually increases up to 7.2 in the terminal ileum (Zeeshan et al., 2019). The pH slightly drops to 6.8-6.5 in the colon (Hua, 2020). It takes a maximum of 2 h for the tablet to pass through the stomach, 1 h through the duodenum, and 2 h through the jejunum (Akhgari et al., 2006, 2005). It is reported that it takes at least 1 h for the dosage form to pass through the ileum and around 10 hours on average to traverse the colon. In other words, the residence time of the drug in the ileum and colon is about 1 and 10 h, respectively (Shahdadi Sardou et al., 2021). Therefore, to be more specific, the ideal drug formulation should (i) resist drug release at pH of < 6.5, (ii) release 50-70% of the drug within 10 h at pH 6.8, and (iii) present a gradual and sustained release of the drug at pH 6.8 up to 7.2.

Among polymers used in the design of colon delivery formulations, a variety of polymethacrylate (Eudragit) polymers are commonly used; some are known to trigger drug release at a certain pH point, and some others are known for their retarding effect on diffusion of drug if they are used as a coating (Thakral et al., 2013). Poly methacrylic acid, methyl methacrylates 1:2 and 1:1 also known as Eudragit S and Eudragit L (as pH-dependent polymers) or (poly ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride 1:2:0.1) also known as Eudragit RS (as a time-dependent polymer) have been used for delivery of 5-ASA to the colon (Design, 2016; Fude et al., 2007; Kaffash et al., 2019; Wei et al., 2015; Zeeshan et al., 2019). However, none of them alone exhibits the desired above-mentioned features for the colon delivery purposes of 5-ASA pellets (Kotla et al., 2018). For instance, while Eudragit L (L) dissolves at pH 6, Eudragit S (S) starts dissolving once it is subjected to pH 7 and Eudragit RS (RS) as the water-insoluble polymer is too robust to let all the drug release from the pellets across GIT leading to suboptimal drug delivery if either of these polymers is used for coating the pellets (Thakral et al., 2013). Given that S does not dissolve in aqueous media with pH <7, the use of S per se in the coating does not allow the drug to be released completely in the colon region; therefore, the concomitant addition of L is necessary for the coating to ensure drug release at pH <7 (Akhgari et al., 2006; Franco and Marco, 2020). Therefore, we suppose that there might be a perfect coating for 5-ASA pellets for targeting to the colon region if these three polymers were utilized together at an appropriate ratio. We surmise that the films composed of these polymers (S, L and RS) bring about pH-dependent and gradual release features respectively.

Polysaccharides such as guar, pectin, chitosan, inulin, and dextran which are decomposed by the colon flora have long been used in the formulation of colon delivery dosage forms, either as matrix in tablet/pellet structure or as a part of the coating. Among these, we showed in our previous study that inulin was the most susceptible polysaccharide to rat cecal fluid (Shahdadi Sardou et al., 2019b).

In the current study, we attempted to find the best possible combination of S, L and RS as a coating and the coating level to achieve the targeted colon delivery of 5-ASA pellets. For this purpose, a factorial design was employed to reach the point.

To increase the susceptibility of drug release to the colonic environment we also tried the optimized coating on inulin-bearing 5-ASA pellet as well as inulin-free 5-ASA pellets to figure out if there is any facilitation of drug release that the bacterially- degradable polysaccharide could provide in the colon. Moreover, the possible therapeutic benefits were studied in an rat model of UC.

The GIT of the rats resembles closely that of man in anatomy (Bryda, Elizabeth, 2013) and therefore they have been used in many studies as animal models for evaluation of colonic delivery systems (Low et al., 2013; Mladenovska et al., 2007; Mura et al., 2011; Rajendiran et al., 2018; Wei et al., 2008; Xu et al., 2014; You et al., 2009). Other animal models used rarely in the colonic delivery studies including rabbits (Gadalla et al., 2016), dogs (Ji et al., 2007), and pigs (Schoellhammer et al., 2015), were not used in our study as the use of these animals faces multiple problems, such as different dietary requirements as compared to a human, difficulty in the animal care, and some ethical issues (Jiminez et al., 2015).

2. Materials and methods

2.1. Materials

5-ASA was obtained from Arya pharmaceutical co. (Tehran, Iran). Lactose, Polyvinylpyrrolidone (PVP K30), and Microcrystalline cellulose (Avicel PH-101) were purchased from Darupakhsh (Tehran, Iran). Inulin (catalog # I2255) was purchased from Sigma Aldrich (USA). Eudragit S, Eudragit L, and Eudragit RS were procured from Evonik nutrition and care GmbH (Germany). Potassium dihydrogen phosphate, Sodium hydroxide, Isopropanol, Triethyl citrate, Butyl alcohol, Acetonitrile, Methanol, Formalin, Trichloroacetic acid, Phosphoric acid, Ellman's reagent and Thiobarbituric acid were obtained from Merck (Germany). These chemical compounds were of analytical grade. Pentasa (5-ASA controlled-release capsules, 250 mg) were obtained from Shire pharmaceutical company.

2.2. Preparation of pellets

Two combinations of the excipients were used along with 5-ASA to prepare pellets. The first 5-ASA pellet formulation components are composed of 5-ASA (60% w/w), microcrystalline cellulose (25%), lactose (13%), and polyvinylpyrrolidone (2%). The second formulation contained 5-ASA (60%), microcrystalline cellulose (15%), lactose (5%), and inulin (20%). These two pellet formulations were prepared by an extrusion spheronization method as described previously (Shahdadi Sardou et al., 2021).

2.2.1. Design of experiments

A 3^2 full factorial design was applied to achieve the optimized coating composition for delivery of 5-ASA to the colon for inulin-free pellets. The independent variables were the ratio of S to L (X_1 16:64, 32:48 and 48:32) using the constant amount of RS (20%) in the coating composition and the coating level (X_2 5, 10, and 15% w/w). The target points for responses were the drug release of 50-70% within 10 h at pH 6.8 (Y_1), provided that the drug release was <10% within 2 h at this pH (Y2) and the lag time (the time before 2% drug release) <1 h at pH 7.2 (Y_3).

2.2.2. Coating process of pellets

The coating was carried out in a fluidized bed apparatus (Wurster insert, Werner Glatt, Germany). An appropriate amount of S, L, and RS was dissolved in isopropanol/distilled water (9:1) under agitation (Table 1). Triethyl citrate (10%, w/w based on polymer weight) and talc (1%, w/v) were added to the solution as a plasticizer and anti-adhering agent respectively. The coating solution was applied onto 50 g 5-ASA pellets under the following conditions: inlet air temperature 34-38 ^{o}C, outlet air temperature 27-31 ^{o}C, atomization pressure 2 bar and spray rate of 0.5 g/min. Samples of coated pellets were collected when the coating level reached 5, 10 and 15% (w/w).

2.2.3. Dissolution media preparation

Media simulated different parts of the GIT environment were used in dissolution studies. All media were prepared according to the USP XXVI. These were gastric simulated medium with HCl 0.1 N at pH 1.2, small intestine-simulated media with phosphate buffers at pH 6.5, 6.8, and 7.2. Colon-simulated medium (phosphate buffer pH 6.8) supplemented with 4% rat cecal content was also used for testing the inulin-bearing 5-ASA pellets. The rat cecal content was added freshly to the phosphate buffer before conducting the dissolution test. For this purpose, the rats were anesthetized and sacrificed with CO_2 asphyxiation and their abdomens were opened with surgical scissors. The cecum content was collected and transferred to the phosphate buffer pH 6.8 to prepare the simulated colonic fluid (SCF) containing 4% w/v rat cecal content (Kotla et al., 2016). This medium was under continuous aeration with CO2 gas throughout the experiment. The animal experiment was carried out in accordance with the guidelines of the National Institute of Health, and the ethics committee of Mashhad University of Medical Sciences, Mashhad. Iran (IR. MUMS. REC. 1396.195).

2.2.4. Dissolution studies of 5-ASA pellets

The release of 5-ASA was evaluated using the USP apparatus I (Pharma test, PTWS, Germany) in 900 mL dissolution media. Dissolution test was performed for pellets containing 300 mg 5-ASA. The speed of baskets was 100 rpm and samples were withdrawn at several time intervals by a peristaltic pump (Alitea, Sweden) and analyzed by spectrophotometer (Shimadzu, UV/1204, Tokyo, Japan). The amount of 5-ASA was measured at 302 nm for 2 h and 330 nm for 10 h, in the gastric-simulated medium or other media respectively.

The experiment was repeated for the pellets with optimum drug release profile using the continuous mode of the dissolution test at 37 ^{o}C. The 5-ASA delivery was followed successively at the simulated GIT media according to the transit time of the pellets from different regions of the GIT (i.e. stomach, duodenum, jejunum, ileum, and colon). For dissolution study in continuous mode, the baskets containing the pellets were transferred immediately from one dissolution medium to another at the end of the incubation period set for each medium. The dissolution test began with the acidic media containing HCl 0.1 N resembling the gastric medium for 2 h and followed by phosphate buffer (pH 6.5, for 1 h), phosphate buffer (pH 6.8, for 2 h), phosphate buffer (pH 7.2, for 1 h), which were similar to the three parts (duodenum, jejunum, and ileum) of the small intestine media and the residence time of the pellets. The last incubation was done in the phosphate buffer pH 6.8 with/without rat cecal content for 10 h that simulates the colon medium. The sampling of dissolution medium was performed manually and the samples were analyzed by UV spectrophotometry at relevant wavelengths except for the media supplemented by cecum content. In the media supplemented with cecum content, the 5-ASA concentration was determined by high-performance liquid chromatography (HPLC, Knauer) equipped with a Teknokroma column (BRISA LC2, C18 250 mm×4.6 mm, 5 µm). At several intervals, 1 mL of the medium was taken and centrifuged at 15000 rpm at 4 ^{o}C for 30 min, from which the supernatant was withdrawn and mixed with acetonitrile at a ratio of 1:3. Subsequently, this mixture was pumped through a 0.23 µm pore size filter before the

injection to the HPLC column. The column was run under the mobile phase of methanol–deionized water (85:15), with a flow rate of 1.3 mL/min, and the amount of 5-ASA in the filtrate was measured at 211 nm (Kotla et al., 2016; Liu et al., 2012). All experiments were conducted with three samples in each simulated GIT media.

2.2.5. Morphology of pellets

The morphology of the uncoated inulin-free or inulin-bearing pellets was observed under a stereomicroscope (Olympus, DP25, Okura, Japan) at magnification 8× equipped with a digital camera (Sony, Japan). The images were analyzed using an image analysis software (Image.J 1.45f) in terms of the longest and shortest Feret's diameters (d_{max} and d_{min}) of the pellets and the area (A) thereof. The aspect ratio and sphericity of the pellets were calculated as follows:

$$\text{Aspect ratio} = d_{max} / d_{min} \quad \text{(eq. 1)}$$

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

Moreover, the surface and cross-sectional characteristics of the pellets were observed under a MIRA3 scanning electron microscope (TESCAN, Czech Republic) before and after the dissolution test. To view cross-sectional, a few pellets were sliced in half with a surgical blade and then covered with gold.

2.3. Animal model of colitis and treatment groups

The Wistar rats (male, weighing 240–260 g) had been deprived of food, but not of water for 24 h before they were given 2 mL acetic acid (2% v/v in saline solution) enema using a polyethylene tube. The tube was inserted up to 8 cm into the animal's rectum to inject the acetic acid and then quickly (after 30 sec) was removed. The rats were devided into seven groups with four animals in each group. The control group only received normal saline. Subsequently, the rats were fed with a regular diet for the three following days before the start of the treatment study. The colitis-induced rats were randomly divided into different groups. These were the treatment groups that received the 5-ASA uncoated pellets, the optimized coated pellets with/without inulin in the matrix or Pentasa pellets along with 1% w/v Na-CMC as the vehicle. In the control group, the rats received the vehicle only. The treatment groups received equivalent pellets containing 120 mg/kg/day of the 5-ASA through oral gavage for ten consecutive days. Rats were assessed for body weight during the study period. Finally, on the 11th-day post-treatment, animals were sacrificed and their colon tissues were collected (Mura et al., 2011; Xu et al., 2014).

2.3.1. Colitis activity index

The colitis activity index (CAI) which is resulted from averaged scores for weight loss, stool consistency, and rectal bleeding (Lamprecht et al., 2005; Pertuit et al., 2007; Wang et al., 2016) was used to assess the colitis treatment/severity. The score for CAI is in the range of 0 to 4 corresponds to healthy to the maximal activity of colitis respectively. The CAI was 0 for animals with no weight loss, and was from 1 to 4 based on the percent of weight loss (1 for 1-5%, 2 for 5-10%, 3 for 10-20%, and 4 >20%). Similarly, the CAI was considered 0 for healthy defecation and normal consistency of feces, whilst changes in stool consistency to diarrheas correspond to higher scores for CAI. The CAI score was 2 when a loose and semi formed stool with no sign of stickiness to the animals anus was observed, and the score was 4 for the very loose stool, sticking to the anus. In addition, no sign of rectal bleeding corresponds to CAI score of 0, while the CAI was 2 for a trace of bleeding, and 4 for marked rectal bleeding. The mean of these scores was regarded as the final CAI score.

2.3.2. Colon/body weight ratio

The animals were weighed before being sacrificed by CO_2 asphyxiation. Then, their abdomen was opened and the colon was removed and cut across its length. The cecum was removed and the colon was washed and cleaned with an excessive amount of saline solution. The colon tissue, with mucosal surface upward, was placed on a glass plate and was dabbed gently by a paper towel to remove the excess water. Afterward, the colon was weighed precisely and the weight ratio of the colon to the rat body was used as a criterion for the level of colonic tissue edema (Mura et al., 2011).

2.3.3. Length/weight ratio of colon

The part of colon from colo cecal junction to the anal verge was weighed after cecum content had been removed. Also, the length of the colon was determined and the length/weight ratio was calculated as cm/g (Pertuit et al., 2007).

2.3.4. Macroscopic evaluation of colitis severity

Study of histopathological features of the colonic tissue was performed in order to evaluate the severity of colitis. Approximately from 1 cm above the anus, the distal colon for length of 10 cm was opened longitudinally and immersed in formalin solution after being washed with saline solution. Subsequently the colonic tissue was molded in paraffin and sliced with a microtome. The slices were stained with hematoxylin and eosin and observed by an optical microscope equipped with a digital camera and the histopathological properties were examined and scored in terms of pathological severity. When no or mild inflammatory conditions were observed, the severity of colitis was scored 0. When there was a trace region of acute focal inflammatory and colonic crypt abscess, the severity of colitis was scored 1. When the inflammation inflicted the majority of the colon tissue and there was a sign of smooth muscle thickening, the severity of colitis was scored 2. If the inflammation had led to the formation of ulcerated areas and inflammatory cell infiltration in the tissue sections, the severity of colitis was scored 3. Finally, the utmost tissue damage shown as necrosis and gangrene was given the highest score of colitis severity (i.e. 4) (Baldeep Singh Pabla, 2020; Shahdadi Sardou et al., 2021).

2.3.5.Malondialdehyde and glutathione content of the colon tissue

According to previous study, the amount of malondialdehyde (MDA) and glutathione (GSH) were measured in the colon tissue using a spectrophotometric method (Shahdadi Sardou et al., 2021).

2.4. Statistical analysis

Statistical analysis was performed using GraphPad Prism (version 7, San Diego, CA). One-way and two-way (ANOVA) analyses of variance as well as Tukey–Kramer multiple comparisons were carried out at the significance level of 5%.

3. Results and discussions

3.1. Dissolution study of pellets

To find out the best coating composition and level for delivery of 5-ASA to colon, inulin-free pellets were coated at varied compositions and levels according to Table. 1 suggested by factorial design. The release of 5-ASA from coated pellets was examined at the same pHs as those of the different parts of GIT through which these pellets were supposed to pass (Fig.1 A-D)

There was a small drug release from the coated pellets (below 20%) at the acidic pH of 1.2. The drug release was significantly higher from pellets with a low level of coating than others, especially from those with the lowest level (5%) of coating (Fig. 1A). When the pellets were covered with a sufficient layer of coating material (15%), there was no difference in drug release from the coated pellets having varying S/L compositions. However, at the low coating level of 5%, the amount of

drug release became more and more noticeable and higher when the proportion of L in the coating composition increased.

At pH 6.5, the rate of the drug release was increased with an increase in the ratio of the L to S. It seems that L is dissolved at pH 6.5, so 5-ASA is released more from the pellets coated with higher ratio of L to S (Fig. 1B). The level of coating also had considerable influence over the amount of drug release at this pH. With increase in the level of coating from 5% to 15% the rate of the drug release decreased significantly. At pH 6.5 the pellets with a 15% coating level showed a lower release rate than the pellets with a 10 or 5% level of coating, so that they showed a release of less than 20% in 10 hours. In comparison, pellets with the coating level of 5% with the highest and lowest level of L released 75% and 35% of the drug in 10 h respectively (Fig. 1B).

With increase in pH from 6.5 to 6.8 (Fig. 1C), the rate of drug release increased substantially. This denotes the sensitivity of the coating formulations to the change of pH in a way that the pellets coated with a higher level of L displayed a higher rate of drug release. In comparison, the coating formulation with the higher level of S prevented the fast release of the drug. For instance, S48+L32+RS20 with a 15% level of coating released about 15% of drug content, while S16+L64+RS20 with the same coating level released more than 50% of drug content. It is interesting to note that all formulations showed a slow release profile of the drug. These results indicate that the coating formulation is both time and pH-dependent. There was also a significant difference between the pellets with 5, 10, 15% levels of coating. With the increase in the level of coating from 5% to 15%, the amount of drug release decreased almost by 30% in all formulations.

A similar trend was also observed in the rate of drug release from different formulations at pH 7.2 (Fig. 1D). Overall, the lag time of drug release decreased and the rate of drug release increased for all formulations at this pH compared to pH 6.8. It is interesting to note that S48+L32+RS20 with the level of coating 15% released all 5-ASA payload within 8 hours at pH 7.2, while the same formulation released only 20% of the drug content within 10 h at pH 6.8.

Such changes of pH from 6.5 to 6.8 and then 7.2 caused the pellets coated with varying proportions of S or L to behave differently in terms of drug release at different media. For example, the pellets with 48% of S retained almost more than 80% of drug content at pH 6.5 till the end of dissolution time, but they released all their drug content at pH 7.2. With pH increasing to 7.2, all formulations having the lag time of less than 1 h, lost all their drug content within 8 h.

It is worth mentioning that the pH-dependent or time-dependent drug delivery systems has been applied as a layer-by-layer coating over the 5-ASA pellets in some studies (Gupta et al., 2001; Xu et al., 2014). In these studies, the 5-ASA pellets were first coated with a time-dependent layer (e.g. ethylcellulose, RS or RL) and then, coated with an external pH-dependent layer (e.g. S or FS). Upon contact with medium having the pH of dissolution of the coating, the external layer of the coating was rapidly dissolved and the internal coating layer controlled the release rate thereafter. The layer-by-layer coating suffers from some drawbacks such as the need for two-phase coating processes and also the probability of the partial delivery of the drug, as the rate of drug release from time-dependent layer is proportional to the thickness of the coating. In this study, the pellets were coated with a combination of time- and pH-dependent polymers all at once; therefore, the coating provided a sustained drug release at specific pH which corresponds to the dissolution of pH-dependent part in coating and hence, the probability of the drug not to be released became very low.

3.2. Determination of optimum coating composition

The selection of the optimum coating composition and coating level was figured out through running the experiments based on factorial design (Fig.1 and Table 1). For this purpose, the empirical data were analyzed for the best-fitted equations that could estimate the definite mathematical relationships between the independent variables (Xs) and dependent variables (Ys). The equations were as follows:

$$Y_1 = + 133.45977 - 1.70575\ X_1 - 2.12299\ X_2 - 0.012500\ X_1 X_2 + 0.008405\ X_1^2 \quad \text{(eq. 3)}$$
$$Y_2 = + 99.54023 - 1.18592\ X_1 - 8.71034\ X_2 - 0.072500\ X_1 X_2 + 0.185517\ X_2^2 \quad \text{(eq. 4)}$$
$$Y_3 = + 7.98851 - 0.037356\ X_1 - 1.64943\ X_2 + 0.012500\ X_1 X_2 + 0.124138\ X_2^2 \quad \text{(eq. 5)}$$

X_1 and X_2 were the ratio of S to L and the level of coating, respectively. For Y_1 5-ASA release of 50-70% at pH 6.8 in 10 h, for Y_2, a drug release of <10% at pH 6.8 in 2 h and for Y_3, the lag time <1 h at pH 7.2 were considered as target points.

Table 1 lists the experimental runs and the related empirical/predicted responses. In general, all three models fitted perfectly to the empirical data. There was no or little variation between the empirical and the predicted data for these three equations. Similarly, all three models showed a large total sum of squares (TSS) as compared to the residual sum of squares (RSS) indicating an extremely large signal-to-noise and enormously high predictability for the models. In accordance, there was an extremely close to 1 coefficient of determinations (R^2) and a large enough F-ratios for these models, indicating that these models can all explain the variances in the dependent (response) values by the independent factors.

Fig. 1 shows the release profile of 5-ASA from coated pellets and the spectrum of the predicted results for any combinations of independent variables. As can be seen in Fig. 1C and D, S16+L64+RS20 coating composition with 15% coating level met the three target points for 5-ASA release in different media. In other words, the mentioned coating composition offered a 60-70% drug release in 10 h at pH 6.8 (Y1, Fig. 1C, a), while showed a minimum drug release (< 10%) within the first 2 h of incubation at this medium (Y2, Fig. 1C, b), and started releasing the drug with a lag time of less than 1 h (Y3, Fig. 1D, C) at pH 7.2. The data were useful as they contributed to equations 3, 4, and 5 with the full range of probable outcomes for any imagined combinations of independent variables, which are displayed as surface plots in Fig.1 E, F, and G. These figures were similar to those predicted by equations 3, 4, and 5.

With the optimum amounts predicted for X1 and X2 variables, 5-ASA pellets (inulin-free and inulin bearing) were coated under such conditions and put into test.

3.3. Morphological characteristics

The aspect ratio and sphericity of inulin-free 5-ASA pellets were 1.08 ± 0.09 and 0.88 ± 0.09 respectively while the corresponding values for inulin-bearing 5-ASA pellets were 1.12 ± 0.09 and 0.86 ± 0.06. The aspect ratio of below 1.2 and sphericity of above 0.8 are considered as the spherical features for the pellets (Chopra et al., 2002) and therefore these figures indicated that both types of pellets were nearly spherical. Fig. 2 shows the superficial and cross-sectional image of the uncoated and optimized coated pellets. The uncoated pellets showed spherical shape with roughly uneven surfaces (Fig. 2A and D), while the surface of the optimal coated pellets looks smoother (Fig. 2B and E). The coating thickness was uniform and approximately 25 μm (Fig. 2C and F) on both types of pellets.

3.4. Dissolution studies of 5-ASA pellets coated with optimized formulation

Fig. 3A shows the release profile of 5-ASA from the uncoated pellets at pH 1.2 and 6.8. There was a significant difference in the drug release curve between the inulin-free and inulin-bearing 5-ASA pellets at these pHs. The amount of drug release was significantly higher from inulin-free pellets compared to inulin-bearing pellets. This could be due to polysaccharide gelation upon contact with water that might delay diffusion and drug release from the matrix to some extent (Chourasia and Jain, 2004; Mensink et al., 2015; Pachuau, L.aEmail Author, Mazumder, 2013). Overall both sets of pellets released almost all of their drug cargo in a short period indicating that the pellet structural and physical integrity itself was unable to restrict the drug release at these pHs. Moreover, the rate of drug release was higher in the gastric-simulated medium than in phosphate buffer pH of 6.8 for both the inulin-free and the inulin-bearing pellets. Such a difference in the rate of drug release can be attributed to the higher solubility of 5-ASA in the acidic media than in media with neutral pH (Karkossa and Klein, 2018).

Fig. 3B-E shows the dissolution profile of the optimized coated inulin-free pellets in the GIT simulated media. The coated pellets (S16+L64+RS20) showed no drug release at 15% level of coating in the medium with pH 1.2 (Fig. 3B). With the increase in the pH from 6.5 to 6.8, the amount of drug release was enhanced. In the media with pH 6.8, about 60% of the drug was released within 10 h of incubation (with 10% drug release within 2 h, Fig. 3D).

At pH 7.2, the release rate increased even further, and the pellets released all their drug content within 8 h of incubation (Fig. 3E). These pellets started releasing the drug gradually during this period after a lag time of about 30 min. As a result, one can conclude that the rate of drug release increases proportionally with the increase in pH of the media, denoting the pH-sensitivity of the coating layer. This could be attributed to the presence of Eudragit S and L with pH-dependent dissolution in the coating. On the other hand, at pH 6.5 and 7.2 which corresponds to the pH of dissolution of the L and S respectively, the pellet showed a gradual release of 5-ASA, indicating time-dependent characteristic for release of drug due to presence of RS in the coating.

Since the GIT could suffer inflammation from the ileum to the rectum in the UC, it could be beneficial to provide a 5-ASA dosage form that could deliver the drug gradually to all the inflicted areas (Kobayashi et al., 2020). This goal was achieved with the combination of the pH- and time-dependent systems in our study.

Most oral dosage forms of 5-ASA available in the market are formulated based on either pH- or time-dependent systems (Gazzaniga et al., 2006; Tenjarla, 2015; Ye and van Langenberg, 2015). This is probably the reason for the imperfect delivery profile of 5-ASA in the GIT (Lee et al., 2020). To overcome this problem, a combination of S and L (pH-dependent polymer) with RS (a time-dependent polymer) was used to coat the 5-ASA pellets, and it was revealed that this combination could address the undesired 5-ASA delivery profiles observed with marketed formulations.

The optimum coating composition was also applied onto inulin-bearing 5-ASA pellets and both sets of pellets (with and without inulin) and also Pentasa were evaluated by the continuous dissolution testing (Fig 3F). It was observed that both types of 5-ASA pellets coated under optimum coating conditions were able to pass the continuous dissolution test (Fig. 3F). These pellets did not release the drug when they passed through media with pH 1.2, 6.5, and 6.8 for 2, 1, and 2 h residence times, respectively. They started to release the drug at pH 7.2. Both inulin-bearing and inulin-free pellets released all their drug content within 15 h in the continuous dissolution test. In contrast, Pentasa released almost 55% of its drug content within the first 2 h, and the rest in the small intestine- and colon-simulated media. Despite the differences observed for drug release from uncoated pellets (Fig

3A), the drug release profiles were similar at pH 1.2, 6.5, 6.8 and 7.2 for both types of coated pellets. However, there is a difference between these two pellets in terms of drug release in SCF. In SCF, the inulin-bearing pellets released the drug at a higher speed than the inulin-free pellets. Whereas the same pellets showed slower release of drug in phosphate buffer pH 6.8 (with no rat cecal content). This difference indicates that the bacterial enzymes in the SCF might degrade the inulin in the pellets' matrix, resulting in an increased rate of drug release (Imran et al., 2013; Kaur et al., 2017; Kotla et al., 2014; Ravi et al., 2008).

Fig. 4 shows the SEM of the inulin-free and inulin-bearing coated pellets after dissolution test. As can be seen in Fig. 4, the coated inulin-free pellets did not change markedly after dissolution test however some signs of shrinkages were observed in the surface of inulin-bearing pellets after dissolution test in both phosphate buffer pH 6.8 and SCF. The swelling properties of inulin could account for these observations. Besides, inulin-bearing pellets showed more wrinkles on their surface and became smaller in SCF compared to phosphate buffer pH 6.8 which could be due to the degradation of the inulin by the bacterial enzymes in the SCF (Mensink et al., 2015) and confirmed the results of dissolution studies. It is worth mentioning that the inulin-bearing pellets released the drug more slowly than inulin-free pellets in pH 6.8 buffer, whereas in the SCF with the same pH, they released the drug at a faster rate. The difference of release rate in the media with and without rat cecal content might highlight the proportion of the released drug due to the enzymatic activity of the bacteria and the degradation of inulin thereof (Rivière et al., 2018).

Taken together, as both pellets exhibited desirable release behavior and morphological characteristics, they were evaluated on rats with induced UC for their therapeutic efficacy in the final stage.

3.5. Preclinical efficacy of 5-ASA pellets

Administration of coated inulin-free and inulin-bearing pellets improved the treatment of UC in the animals (Fig. 5 and 6). The CAI is an averaged score based on the physical observations of the disease (loss of weight, anal hemorrhage, and stool consistency) (Mura et al., 2011), which was given by a blind investigator to the animal groupings. Following the injection of acetic acid into the colon, the CAI increased up to 3 points within 2 days; however, the CAI decreased afterward following treatment with 5-ASA pellets till day 15^{th}. All the physical symptoms of the disease were relieved to some extent with 5-ASA pellets (Fig. 5A). Compared to Pentasa, the optimized coated pellets enhanced the therapeutic value (Fig. 5A). The weight ratio of colon-to-body and the length/weight ratio of the colon were changed (Fig. 5B and C), and all other physical and histopathological symptoms of the disease were alleviated. It was found that the coated pellets acted better than Pentasa in reducing the CAI. Besides, the molecular markers indicating tissue injury were reduced and optimized coated pellets were superior compared to Pentasa (Fig. 5E and F) in this regard. The level of MDA that is a marker of fatty acid oxidation was reduced and the amount of GSH that demonstrates the anti-oxidative potential of the cells increased upon administration of coated pellets.

Finally, the assessment of the histological sections of the colonic tissues showed also some signs of treatment compared to the control (Fig. 6). The tissues preserved their integrity to a large extent. By contrast, the histopathological evaluation of the colonic tissues showed large scar areas with the development of fibrous connective tissues into the inner muscular layer in the untreated or CMC-vehicle treated groups. Neutrophils and crypt abscesses can also be seen in these sections. Pentasa and the coated pellets demonstrated somehow alleviation in these histopathological features. However, the uncoated pellets failed to relieve these inflammatory features. Some protrusions of

fibrotic scars into the normal tissues were also observed in this group. The uncoated 5-ASA pellets also failed to improve the other disease's features in the animals' colonic tissues. The uncoated pellets only reduced CIA marginally till the end of the treatment period (Fig. 5A). These pellets were also the only treatment groups that improved the colon/body weight ratio (B), the length/weight ratio of the colon (C), MDA (D), and GSH (F) levels marginally, but not significantly. Therefore, it seems that coating of these pellets with proper polymeric layer can offer additional therapeutic values to the 5-ASA pellets.

In our study, we found that the pellets coated based on the combination of the pH- and the time-dependent system could have more therapeutic value as compared to the pellets with a coating based on a single system. In almost all examinations of the UC in animals, our coated pellets acted significantly better than Pentasa and improved the weight ratio of the colon/body (B), the length/weight ratio of the colon (C), and the level of MDA (D), and GSH (F) further. Pentasa with time-dependent release characteristics might give rise to a premature release profile and the release of the majority of the drug before reaching the colon (Karrout et al., 2015). There was no significant difference in terms of therapeutic benefits between 5-ASA pellets with or without inulin having the same optimal coating. This might be related to the release of total drug content for both types of pellets as evidenced by continuous dissolution testing.

4. Conclusion

In the present study, a coating formulation composed of S+L+RS was obtained through a factorial design for colon-targeted delivery of 5-ASA pellets. The optimal coating composition and level were S16+L64+RS20 and 15% w/w respectively. The optimized coating was applied to 5-ASA inulin-free and inulin-bearing pellets and it was found that both types of coated pellets were able to satisfy the requirements for dissolution tests. These pellets were found to be more efficacious than uncoated pellets and Pentasa, in the treatment of the animals suffering from induced UC. This shows that the proposed 5-ASA formulation is highly probable to hit the same therapeutic point in the clinical trial.

Credit author statement

The contribution of authors in the manuscript entitled "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 " is as follow:

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Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1. 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 (S:L:RS)	X_2	Y_1 (% of release)	Y_2 (% of release)	Y_3 (min)	TSS	TSS	TSS
1	16:64:20	5	90/91	45/44	5/4	4393.08	1398.77	419.23
2	16:64:20	10	80/79	21/22	5/6	**RSS**	**RSS**	**RSS**
3	16:64:20	15	65/67	9/8	15/17	10.55	5.22	5.24
4	32:48:20	5	62/65	28/27	5/7	$\mathbf{R^2}$	$\mathbf{R^2}$	$\mathbf{R^2}$
5	32:48:20	10	52 /53	13/13	10/12	0.997	0.996	0.988
6	32:48:20	15	38/40	8/7	20/21	***F*-value**	***F*-value**	***F*-value**
7	48:32:20	5	48/50	12/11	10/9	581.31	373.66	110.52
8	48:32:20	10	30/32	6/5	15/15	***P*-value**	***P*-value**	***P*-value**
9	48:32:20	15	17/18	5/4	25/26	0.0001	0.0001	0.0001

X_1 ratio of S to L with considering the constant ratio of RS in the coating.

X_2 percentage of the pellet's weight increase (coating level).
Y_1 percentage of drug release at pH 6.8 in 10 h (experimental/predicted).
Y_2 percentage of drug release at pH 6.8 in 2 h (experimental/predicted).
Y3 lag time at pH 7.2 (experimental/predicted).
RSS: residual sum of squares.
TSS: the total sum of squares.

Fig. 1. The drug release from the pellets coated under the experimental design condition at varied pH conditions (A-D). The area (a), (b), and (c) indicate the targeted areas for optimum drug delivery. The surface plot for the percent drug released in 10 h at pH 6.8 (E), percent drug released in 2 h at pH 6.8 (F), the lag time before drug release at pH 7.2 (G). Data are shown as mean ± standard deviation (n = 3). * points out the statistical differences between the groups ($p < 0.05$). CL: coating level.

Fig. 2. Scanning electron microscopy of the A) uncoated, B) coated pellets and C) the cross-sectional image of the optimum coated inulin-free pellets. D, E, and F show respectively the uncoated, coated pellets and the cross-sectional image of the optimum coated inulin-bearing 5-ASA pellets.

Fig. 3. The release profile of 5-ASA from the uncoated pellets and the optimized coated pellets are suggested by the experimental design. Release from the uncoated inulin-free or inulin-bearing pellets at two pHs (A), coated inulin-free pellets at different pHs (B-E), coated inulin-free or inulin-bearing pellets, and Pentasa in the continuous dissolution test (F). Data are shown as mean ± standard deviation (n = 3).

Fig. 4. SEM of the optimized coated pellets after continuous dissolution test (t=15h). A) inulin-free pellets, B) inulin-beraing pellets in phosphate buffer pH 6.8, C) inulin-bearing pellets in SCF.

Fig. 5. Therapeutic properties of 5-ASA pellets and Pentasa in animal models of UC. Profile of colitis activity index (CAI) (A). Colon/body weight ratio (B), length/weight ratio of the colon (C), colon damage score (D), the level of MDA (E), and GSH (F). Data are shown as mean ± standard deviation (n = 4). * indicates significant differences between marked groups ($p < 0.05$).

Fig. 6. Histopathological characteristics of the colon's tissues of the rats subjected to different treatments. Rat with normal colonic tissues (A), untreated rat (B), rat treated with CMC vehicle (C), rat treated with uncoated pellets (D), rat treated with optimally coated inulin-free pellets (E) rat treated with optimally coated inulin-bearing pellets (F), and rat treated with Pentasa (G).