Research paper

Novel chewable colon targeted tablets of bumadizone calcium for treatment of ulcerative colitis: Formulation and optimization

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ABSTRACT

The aim of the present study was the formulation of a novel chewable tablet containing the non-steroidal anti-inflammatory bumadizone calcium (BZ) to deliver the drug to the colon for the local treatment of ulcerative colitis. Colon targeted granules were prepared following 3² full factorial design. The effect of two independent variables, namely, polymer type (Eudragit® S100, Eudragit® L100, and a mixture of both in the ratio of 4:1) and drug to polymer ratio (1:1, 1:3 & 1:5) on the % of BZ released for 12 h was studied. In order to produce chewability, candidate formulae were then mixed with different amount of maize starch and mannitol, and compressed into tablets. F11 tablets (composed of drug: Eudragit® S100 in the ratio of 1:3, 250 mg mannitol and 50 mg maize starch with a desirability of 0.925) achieved the required release profile i.e: lowest release before target area (pH 1.2 & 6.8) reaching only 11.00% at the end of the fourth hour, and 100.27% after 12 h (pH7.4). Histopathological studies results declared clearly the ability of the chewable colon targeted tablets F11 to locally treat acetic acid induced colitis. Furthermore, the measurements of myeloperoxidase enzyme activities in colon specimens showed that F11 achieved a significantly lower levels in comparison to both untreated group and group that received the marketed tablets (p<0.05).

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1. Introduction

Inflammatory Bowel Diseases (IBD) are an immune mediated chronic or relapsing disorders of the gastrointestinal (GI) tract. IBD which is comprised of two main types, ulcerative colitis (UC) and Crohn’s disease (CD), affects approximately 3.6 million people in the United States and Europe. An alarming rise in previous low-incidence areas, such as Asia, is currently being observed. They are gaining more and more attention. Although considerable progress has been made the last few years, a major gap in knowledge of the pathogenesis of UC remains [1]. UC is considered as an idiopathic inflammatory disorder involving the mucosa and submucosa of the colon [2,3]. It is characterized by chronic or relapsing immune activation and inflammation within the gastrointestinal (GI) tract that markedly alters GI function [4]. UC causes inflammation and ulcers in the top layer lining the large intestine [5,6] and is characterized by superficial mucosal inflammation, rectal bleeding, diarrhea and abdominal pain [7,8]. When the gut is inflamed, there is breakdown of intestinal barrier function, abnormal secretion, changes in the patterns of motility and visceral sensation, which contributes to symptom generation [9].

The general principle of drug treatment in UC is to induce and maintain remission of outbreaks and to achieve mucosal healing [10]. However, conventional administration of drugs used in treatment of UC are associated with a number of side effects [11,12], decreased efficacy and frequent drug dosing [11,13]. This necessitates the development of colon targeted dosage forms that selectively deliver the drug to the inflamed areas with minimum release of the drug in upper gastrointestinal tract [2,14]. This could help to reduce conventional dose and frequency [15] in addition to reduced incidence of adverse side effects [16]. Colon targeting could be achieved by a wide variety of mechanisms. This ranges from the use of prodrugs that are activated by enzymes in the colon [17,18], microbial triggered systems that depend on the presence of a high bacterial count up to 10¹¹–10¹² C.F.U/ml [13,19,20], the use of pH-sensitive polymers. These polymers have ability to withstand an environment ranging from low pH (~1.2) to neutral pH (~7.5) for several hours [21].

Based on the aforementioned literature and to overcome side effects accompanied with conventional drug administration, the major scientific purpose of this study was to prepare chewable colon targeted tablets containing the NSAID bumadizone calcium (BZ)
using pH-dependent polymers. NSAIDs are widely used in the treatment of chronic inflammatory states. In addition, they showed a promising activity for prevention and treatment of colitis but with tendency to cause gastric bleeding and form ulceration in the gastric lining [22]. Formulation of BZ into colon-targeted dosage forms will avoid these undesirable effects. Some published researches have explored the potential of formulating pH-dependent colon targeted tablets [23–25], yet, none of researchers have tried formulating chewable tablets with the ability of colonic delivery. Chewable tablets offer several advantages over conventional tablets. This is due to the ease of use which leads to improved patient compliance in addition water is not required for swallowing [26]. Geriatric and bedridden patient show inconvenience in swallowing conventional tablets or capsules so chewable tablets offer a better alternative for drug application [27]. Upon searching the literature, no previous studies were carried out to formulate colon-targeted dosage forms containing BZ except our previous attempt where BZ microspheres were formulated using time-dependent polymers and compressed into tablets for colon targeting [28]. Time-depend polymers are generally water-insoluble polymers that act as barrier hindering drug release so drug release takes place by time. These may include Eudragit® RS100, ethyl cellulose and cellulose acetate butyrate [29–31]. However, El-Gazayerly et al. [32] formulated chewable tablets containing verapamil aiming to sustain drug release. Sustenance of drug release was achieved via spray coating sugar beads with different binder solutions containing HPMC, polyox and ethylcellulose. Controlled release properties of the developed formulation didn’t change by chewing or crushing the tablet.

The aim of the present study was the development and evaluation of BZ chewable tablets so to deliver the drug specifically to the colon to avoid its gastrointestinal side effects and to increase the patient compliance. We aimed to decrease the percentage of drug released before target area (pH 1.2 and 6.8) to less than 20% [33] and to maximize the BZ released in target area (pH 7.4) and total BZ released after 12 h. One of the most common side effects of NSAIDs is gastric discomfort, namely, ulcers and erosions. This is due to their ability to inhibit COX I enzyme leading to prostaglandin (PGs) deficiency. PGs have protective role in the stomach as they regulate bicarbonate and mucus production. So generally decreasing drug release before target area will lead to both decreasing side effects of the drug and shifting drug release to the target area. BZ marketed tablets Octomotol® are enteric coated tablets and show up to 30% release in the stomach. The selected colon targeted tablets were examined for its efficacy using acetic acid-induced rabbit colitis model in order to elucidate its usefulness as a specific drug delivery system for the treatment of ulcerative colitis in rabbits compared to marketed tablet.

2. Materials and methods

2.1. Materials

Bumadizone calcium (BZ) and Octomotol® tablets(110 mg) were kindly provided by October Pharma, Egypt. Eudragit® S 100 (EU S100), Eudragit® L100 (EU L100), were kindly provided by Degussa, Rhome and Co. KG, Pharma Polymer, Germany. Polyvinyl pyrrolidone(PVP) K30 was purchased from Fluka chemicals, Switzerland. Mannitol was obtained from Roquette, France. Maize starch was purchased from Sigma-Aldrich Corporation, USA. Isopropyl alcohol, El Nasr pharmaceutical chemical company, Egypt.

2.2. Compatibility studies

2.2.1. Differential scanning calorimetry (DSC)

The DSC studies were performed for bumadizone powder, Eudragit® S100 and Eudragit® L100 and for drug-Eudragit® S100 and drug-Eudragit® L100 physical mixtures (1:1) using Differential scanning calorimeter(DSC), Model -50; Shimadzu, Kyoto, Japan. Samples were heated in a pan at a rate of 5 °C/min in an atmosphere of nitrogen to 200 °C and the thermograms were recorded.

2.2.2. X-ray diffraction

X-ray diffraction patterns of bumadizone powder, pure polymers (Eudragit® S100 and Eudragit® L100) and physical mixtures of drug and each polymer in the ratio of 1:1 were recorded using a Philips X-ray diffractometer (PW1792) Legroupe Interconnexion, Saint -Juire, Clubac, Canada with a copper target at a voltage of 40 kV and a current intensity of 30 mA at a scanning speed of 1 °C/min.

2.3. Formulation of BZ -granules

2.3.1. Experimental design

A 3² full factorial design was adopted to formulate colon targeted BZ granules using two independent variables; (i) polymer type (ii) Drug to polymer ratio. Two pH-dependent polymers were used, namely, Eudragit® S100, Eudragit® L100 and a mixture of both in the ratio of (4:1), respectively [34]. Three drug to polymer ratio namely 1:1, 1:3 and 1:5 were also used. Table 1 summarizes the independent variables along with their levels. The effect of selected factors was studied on release before target area, in target area and total percentage released after 12 h.

2.3.2. Preparation of the granules

Drug and polymers were thoroughly mixed using mortar and pestle for 20 min. Dough mass was then made by addition of few drops of 1% PVP K30 in isopropyl alcohol (binder solution). Granules were obtained by passing the dough mass through sieve number 10. The formed granules were left to dry at room temperature for 24 h [35]. The composition of the prepared granules are shown in Table 2.

2.4. Characterization of formed granules

2.4.1. Content uniformity, angle of repose and Hausner ratio determination

Exactly, one hundred mgs of the prepared granules were weighed, crushed and the drug was allowed to be extracted in phosphate buffer of pH 7.4 overnight using a magnetic stirrer (DAIHAN Scientific Co., USA.) The solution was filtered through a 0.45 μm millipore filter and the drug content was determined by UV spectroscopy at 234 nm after suitable dilution with reference to the calibration curve [36]. For determination of angle of repose, the fixed height cone method was adopted [37] and tan θ was calculated according to equation tan θ = h/d. Regarding Hausner ratio determination, five grams of each of prepared granules were placed in a graduated cylinder and the volume occupied was measured as V1 (initial bulk volume). The cylindrical graduate was then tapped till a constant volume was obtained when the powder was considered to reach the most stable, unchanging arrangement; the volume of the powder was then recorded as the final bulk volume.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Polymer type</th>
<th>Drug: Polymer ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levels</td>
<td>Eudragit® S100</td>
<td>1:1</td>
</tr>
<tr>
<td></td>
<td>Eudragit® L100</td>
<td>1:3</td>
</tr>
<tr>
<td></td>
<td>Mixture(S100:L100)</td>
<td>1:5</td>
</tr>
<tr>
<td></td>
<td>4:1</td>
<td></td>
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</table>
(VF). The Hausner ratio was obtained by dividing Vi by VF [38]. The experiments were done in triplicates.

2.4.2. In-vitro drug release

Release of the BZ from the prepared granules (accurately weighed granules containing 110 mg of the drug) was performed in 900 ml of 0.1 N HCl containing 1% PEG 400 (to maintain sink conditions) for a period of 2 h followed by release in Sorensen’s phosphate buffer pH 6.8 for 2 h then Sorensen’s phosphate buffer pH 7.4 for 8 h to simulate the pHs pertaining to the stomach, proximal and middle small intestine (duodenum and jejenum), and distal small intestine (ileum), respectively [39,40] using USP dissolution tester type I (VK 700 Dissolution Testing Station, Vankel Industries, Inc., NJ, USA). The basket was rotated at 100 rpm. In-vitro release studies were carried out at 37 ± 1 °C. Aliquots were withdrawn from the release media at predetermined time intervals and immediately replaced by equal volume of the release media. Concentration of BZ was assayed spectrophotometrically at λ_{max} 232.6 nm in pH1.2 and λ_{max} 234 nm in pH 6.8 and 7.4 [41] using the regression equation of the standard curve developed in the same media.

The same procedure was conducted for BZ powder and for the marketed tablets Octomotol®. The cumulative drug percent released was plotted against time. The release experiments were performed in triplicate for each sample.

2.4.3. Statistical analysis

The experimental results regarding in-vitro drug release for 12 h of the drug from the prepared granules, were analyzed using Design Expert® 7.0 software (Design-Expert trial version 7; State-Ease Inc., Minneapolis, MN). The data subjected to statistical analysis were the release before target area, in target area and total percentage released after 12 h.

2.5. Preparation of chewable coated colon targeted tablets

Based on the previously mentioned characterization, and the factorial analysis of the main effects and interaction of the release results, two candidate formulae namely, F2 (1:3 drug to EU S100) and F6 (1:5 drug to EU L100) were chosen for further investigation. Both granules formulæ showed the required release profile (<20% before target area (pH1.2 and pH6.8), maximum % of BZ released in target area (pH7.4) and maximum % of BZ released after 12 h). The candidate granules were thoroughly mixed with mannitol and maize starch to improve palatability and produce chewability, and cinnamon powder was used as flavoring agent for F2. The blends were mixed using mortar and pestle for 20 min. Granulation was done as previously mentioned. The formed blends were left to dry at room temperature for 24 h and were then compressed using tabletting machine on a 13 mm punch (tablet single punch press machine, Royal Artist, Bombay, India). The force applied was equivalent to 49.033 KN. The composition of different compressed tablets is shown in Table 3.

2.6. Characterization of the prepared colon targeted tablets

Evaluation was performed to assess the physicochemical properties and release characteristics of developed formulations.

2.6.1. Post compression parameters

Tablet thickness, hardness, friability, weight variation and net content were all done according to the British Pharmacopeia [42].

2.6.2. In-vitro drug release

Release of the drug from the chewable colon targeted tablets was done following the same procedure adopted for the prepared granules. The cumulative percent drug released was plotted against time. Release experiments were performed in triplicate for each sample.

2.7. Kinetic modeling of the dissolution profiles

The drug release profiles were fitted to zero order (cumulative % drug release VS time), first order (log cumulative % drug remaining VS time) and Higuchi diffusion model [43] (cumulative % drug release VS square root of time). The model with the highest correlation coefficient was considered the best fitting. The release profiles were also analyzed according to Korsmeyer-Peppas equation [44].

2.8. In-vivo study

2.8.1. study design

In-vivo study was done to compare the pharmacodynamic activities of BZ from the candidate chewable colon targeted tablet F11 to the commercially available marketed tablet formulation Octomotol® (110 mg, October Pharma) using non-blind, two-treatment, randomized parallel design. The protocol of the study with serial number PI (541) was approved by the Research Ethics Committee of the Faculty of Pharmacy, Cairo University, Egypt. The study was conducted in accordance with EC Directive 86/609/EEC for animal
blends to be compressed and to ensure uniformity of mixing. Nine granules formulation had been prepared using pH- dependant polymers in order to achieve colon targeting. Selected formulations were then compressed into chewable tablets after mixing with maize starch and mannitol. Our aim for colon targeting was to decrease drug release to as low as 20% before target area (pH 1.2 & 6.8) and to maximize release in target area (pH 7.4).

3.1. Compatibility study

3.1.1. Differential scanning calorimetry (DSC)

BZ powder showed a sharp endothermic peak at around 160°C that corresponded to its melting point indicating its crystalline nature. The physical mixtures of the drug with the polymers (Fig. 1 & b) resulted in the disappearance of such fusion peak, replaced by broad endothermic signals exhibiting reduced melting endotherm. The presence of endothermic signals in the physical mixtures confirmed that bumadizone crystals still exist in the physical mixtures. This was previously observed in the physical mixture of 5 aminosalicylic acid and piroxicam with Eudragit® S100 [50,51] and diflunisal with Eudragit® RL100 [52]. For confirmation X-ray diffraction measurements were carried out.

3.1.2. X-ray diffraction (XRD)

X-ray diffraction patterns of BZ, polymers, namely, Eudragit® S100 and Eudragit® L100 and physical mixtures of BZ and polymers in ratio of 1:1 are illustrated in Fig. 2. The drug showed sharp prominent peaks at 2θ = 5.8° and 15.4°. The clear sharp characteristic peak at 2θ = 5.8° remained intact in the physical mixtures indicating that the drug remained in its crystalline structure.

3.2. Content uniformity, angle of repose and Hausner ratio

The average content uniformity of the prepared granules found to be 99.2 ± 0.78%. The allowed limit of the content uniformity is between 95% and 105% [36]. Angle of repose and Hausner ratio are related to friction between particles and can be used to predict the flow properties. The angles of repose of the prepared granules were all close to 25° which corresponded to a very good flow properties [53] and Hausner ratio values were around 1.2 which indicated low inter-particle friction [54].

3.3. In-vitro release

Fig. 3 illustrates the release profiles of the prepared BZ granules in comparison to BZ powder and BZ marketed tablets in HCl (pH 1.2) and Sorensen’s phosphate buffers (pH 6.8 and 7.4). The results revealed that the marketed bumadizone tablet and BZ powder showed fast release when compared to the prepared granules as they showed 100% release after 3 and 4 h, respectively. The percentage of BZ released from the prepared granules was found to be low for the initial 2 h in 0.1N HCl, it was found to vary between 0.45% for F6 prepared using EU L100 in a drug to polymer ratio of (1:5) and 3.57% for F8 prepared using mixture (EU S100:EU L100 4:1) in a drug to polymer ratio of (1:3). Upon replacing 0.1N HCl with phosphate buffer pH 6.8 for another 2 h, the percentage of BZ released varied from 69.04% to 13.1% for F4 and F3, respectively. Some formula (F4 and F5) reached 100% release after 6 and 7 h, respectively. On the other hand, F1, F7, F8 sustained the release till 10 h. Only a few formulations sustained drug release till the end of 12 h, namely, F2 (100.15%), F3 (60.08%), F6 (97.55%) and F9 (91.07%). Fig. 4 shows the percentage of drug release before target area (pH 1.2 & 6.8), in target area (pH 7.4) and total percentage released after 12 h. Statistical analysis revealed that both factors (drug: polymer ratio
3.3. Effect of polymer type

Polymer type had a significant effect on the drug release profile (p<0.05). F4 (BZ: EU L100 1:1) showed faster release in comparison to F1 (BZ: EU S100 1:1) this might be due to the use of EU L100 that dissolved at pH ≥ 6 while EU S100 dissolved at a pH ≥ 7. So F1 was able to sustain drug release to 10 h while F4 sustained drug release only to 6 h. F8 (BZ: Mixture 1:3) showed faster release in comparison to F2 (drug: EU S100 1:3) this might be due to the presence of a percent of Eudragit® L100 that dissolved in a pH ≥ 6. In the same time it showed a slower release than that of F5(drug: EU L100 1:3) due to the presence of Eudragit® S100 that delayed the release till pH of 7.4 as it dissolved in pH > 7. On the other hand F9 (BZ: Mixture 1:5) showed faster release than F3 (BZ: EU S100 1:5) due to the presence of a higher amount of EU L100 that favored the release of the drug in pH > 6.

3.3.2. Effect of drug to polymer ratio

Changing the drug: polymer ratio led to a statistically significant effect on the drug release profile (p<0.05) (Fig. 5). In general, increasing drug to polymer ratio decreased the % of drug released. F1 prepared from drug to EU S100 in the ratio of 1:1 showed 100% release after 10 h. Further increase in polymer concentration (F2 & F3 in the ratio of 1:3 & 1:5, respectively) showed decrease and sustainment in drug release as they reached 100.1% and 60.08% after 12 h, respectively. Saboji et al. [40] used Eudragit® S100 in preparation of colon targeted microspheres, the results showed that increasing Eudragit® S100 concentration caused reduction in drug release in pH = 7.4. Similarly, Wadher et al. [55] working on sustained release metformin tablets declared that with increasing concentration of Eudragit® S100, a decrease in drug release took place. This may be attributed to increasing the barrier in front of the drug to reach the release media which in turn decreased the drug release rate.

This was also shown in case of EU L100, as percentage released decreased as polymer concentration increased. F4 (BZ: EU L100 1:1) showed 101.2% release after 6 h. Increase in polymer concentration to 1:3, drug to polymer ratio revealed 100.07% release after 7 h. F6 (BZ: EU L100 1:5) decreased and sustained release of the drug to 96.7% after 12 h. These results are also in accordance with Ravendra et al. [56] who worked on floating timolol maleate microspheres. Their results declared that increasing Eudragit® L100 concentration led to decrease in release rate of the drug.

Shahzad et al. [57] showed that decreasing concentration of Eudragit® L100 resulted in increase in the release rate of celecoxib, this may be due to the saturation of the release medium with the polymer needing more time to dissolve and delaying drug release. Although the required release profile was achieved in some formulae namely F2, F3 and F6, further optimization was necessary in order to produce chewability and to improve palatability for the prepared granules as they all suffered from unacceptable taste. The aim of optimization of pharmaceutical dosage formulation is generally to determine the levels of variables from which a robust product with high quality characteristics may be produced. Design Expert® software was used to determine the best desirability for
further optimization. An overall desirability function dependent on all the investigated variables was used to predict the ranges of variables where the optimum formulation may occur. We aimed to optimize the responses simultaneously. The criteria selected were minimizing percentage of drug released in pH 1.2 and 6.8 to less than 20% and to maximize release in target area and total percentage released. It is well known that desirability increases as this number closes to 1. F2 and F6 (which were considered the batches

Fig. 2. X-ray diffraction patterns of (i) (a) BZ powder (b) Eudragit® S100 (c) Physical mixture of both in the ratio of 1:1 (ii) (a)BZ powder (b) Eudragit® L100 (c). Physical mixture of both in the ratio of 1:1.

Fig. 3. Release profile of BZ from the prepared granules in gradient pH media.
fulfilling all the constraints favorable for preparation of BZ tablets) were chosen with high desirability values of 0.882 and 0.820, respectively, for further optimization to be compressed into chewable tablets.

3.4. Evaluation of chewable colon targeted tablets

3.4.1. Post compression evaluation

All the chewable colon targeted tablets complied to the pharmacopeial specifications concerning weight variation, friability, hardness, thickness and diameter (Table 4). The average net content was 100.15 ± 0.38%.

3.4.2. In-vitro release

In-vitro release of chewable colon targeted tablets is shown in Fig. 6. Mannitol was incorporated in the tablets as a diluent, in addition, to the sweet cold sensation it leaves the mouth upon chewing. However, the presence of mannitol led to an increase in the percentage release of BZ if compared to the same formula without mannitol. This might be due to the hydrophilic nature of mannitol that dissolved to form pores in the tablets leading to formation of channels and increasing drug release. To evaluate the effect of maize starch concentration in release profiles, statistical analysis revealed that increasing maize starch concentration decreased release of BZ from tablets (Fig. 7). This is in accordance with the results reached by Levina and Rajabi [58] who formulated chlorpheniramine maleate and theophylline tablets using maize starch as one of the excipients. The release studies revealed that maize starch contributed to retardation of both soluble (chlorpheniramine maleate) and slightly soluble (theophylline) drugs. This might be due to the hindrance effect of maize starch as it eroded slowly by the effect of the dissolution medium requiring more time to release the drug which resulted eventually in retardation of drug release. This might be also due to increase in the amount of mannitol, being water soluble it could cause increase in the release rate of the drug.

Kinetic analysis of the release profiles of the prepared chewable colon targeted tablets are shown in Table 5, it varied between zero order and Higuchi diffusion model. In all tested formulations, calculated n values were >0.89 indicating a super case II release. A super case II release mechanism usually indicated that polymer erosion was the technique involved in the release of the drug. It occurred when the erosion rate of the polymer was higher than its swelling rate. Ngwuluka et al. [59] working on paracetamol granules declared that tablets compressed in the presence of maize starch showed a super case II transport.

Based on the above findings, formulæ F10 and F11 had the required release profile i.e: lowest release before target area (pH 1.2 & 6.8) only 13.78% and 11.00% at the end of the fourth hour, respectively. The cumulative drug release reached 99.711% and 100.27% after 10 and 12 h, respectively. In order to choose the candidate formula for further in-vivo investigation, selection was done using Design Expert® software. The same three criteria were taken into consideration, namely, minimizing release before target area, maximizing release in target area and maximizing total release. One formula was chosen, namely, F11 tablets with a desirability of 0.925. This formula composed of drug: Eudragit® S100 in the ratio of 1:3, 250 mg mannitol and 50 mg maize starch. Twenty mg cinnamon was used as flavoring agent. The release of F11 was repeated after being chewed in the mouth and expelled into the dissolution fluid [32] so as to test the effect of chewability on the bumadizone release profile. The repetition of the release showed that the formula of choice (when chewed) still has the required release profile (Fig. 8) (<20% before target area, pH 1.2 & 6.8) and higher % released in target area pH 7.4 when compared to intact tablet.

3.5. Histopathological analysis

Fig. 9 represents the photomicrographs of histopathological sections (hematoxylin and eosin stained) representing rabbit colon of different groups. Fig. 9i reveals normal lamina propia (a) together with normal glands in regards to architecture (b). Upon exposure of the rabbit colon to acetic acid - as to induce ulcerative colitis (Fig. 9ii), severe marked inflammation was observed with extensive inflammatory infiltrate (c) and evidence of gangrenous areas (d) [28]. Treatment with marketed product (gp III) (Fig. 9iii) didn’t result into significant decrease in ulceration as colon suffered from dense inflammatory infiltrate (e). However, upon treatment with F11 (gp IV) (Fig. 9iv), histopathological analysis revealed resolution of the marked inflammation present in the lamina propia (f) in addition to normal glandular architecture and mucin production (g).

3.6. Myeloperoxidase (MPO) measurement

MPO activity, which is an important quantitative index for colonic inflammation, was determined in terms of units per gram tissue weight. Table 6 shows that MPO activity for normal control group (gp I) was 2.724 ± 0.809 U/g tissue weight. However, MPO

![Graph](image-url)

**Fig. 4.** The Percentage of the BZ released from granules before target (pH = 1.2 & pH = 6.8), in target area (pH = 7.4) and total percentage release after 12 h.
Fig. 5. Main Effects of the different factors according to $3^2$ factorial design on release of BZ before target area, in target area and total release: polymer type (A), polymer ratio (B).

### Table 4
Quality control tests results of chewable colon targeted tablets.

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Average weight (mg) Mean ± S.D</th>
<th>Friability (%) Mean ± S.D</th>
<th>Average hardness (Kg) Mean ± S.D</th>
<th>Average thickness (mm) Mean ± S.D</th>
<th>Average diameter (mm) Mean ± S.D</th>
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</thead>
<tbody>
<tr>
<td>F10</td>
<td>754.38 ± 9.8</td>
<td>0.75 ± 0.2</td>
<td>5.096 ± 20.9</td>
<td>6.25 ± 0.291</td>
<td>12.92 ± 0.039</td>
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<tr>
<td>F11</td>
<td>739.4 ± 11.48</td>
<td>0.74 ± 0.1</td>
<td>6.416 ± 25</td>
<td>6.08 ± 0.127</td>
<td>13.00 ± 0.05</td>
</tr>
<tr>
<td>F12</td>
<td>762.5 ± 9.75</td>
<td>0.35 ± 0.09</td>
<td>4.888 ± 46</td>
<td>5.87 ± 0.058</td>
<td>12.98 ± 0.019</td>
</tr>
<tr>
<td>F13</td>
<td>743.16 ± 12.27</td>
<td>0.23 ± 0.11</td>
<td>3.014 ± 88</td>
<td>6.10 ± 0.107</td>
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<tr>
<td>F14</td>
<td>753.58 ± 8.8</td>
<td>0.076 ± 0.02</td>
<td>2.644 ± 58</td>
<td>6.2 ± 0.122</td>
<td>12.95 ± 0.044</td>
</tr>
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<td>F15</td>
<td>757.54 ± 10.23</td>
<td>0.078 ± 0.01</td>
<td>3.2 ± 1.03</td>
<td>5.98 ± 0.162</td>
<td>12.98 ± 0.039</td>
</tr>
</tbody>
</table>
**Fig. 6.** Release Profile of BZ from the chewable colon targeted tablets in gradient pH media.

**Fig. 7.** Main effects of the different factors according to $2^1 \times 3^1$ factorial design on release of BZ before target area, in target area and total release: polymer type (A), amount of maize starch (B).

<table>
<thead>
<tr>
<th>Formulae</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi Model</th>
<th>Korsmeyer -Peppas model</th>
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<td>Correlation coefficient ($R^2$)</td>
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<td></td>
<td>Correlation coefficient ($R^2$)</td>
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<tr>
<td>F10</td>
<td>0.9456</td>
<td>0.8786</td>
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<tr>
<td>F14</td>
<td>0.9232</td>
<td>0.7081</td>
<td><strong>0.9414</strong></td>
<td>0.8417</td>
</tr>
<tr>
<td>F15</td>
<td>0.9612</td>
<td>0.7263</td>
<td><strong>0.9618</strong></td>
<td>0.9074</td>
</tr>
</tbody>
</table>

**Table 5**
Mathematical modeling and release kinetics of BZ from the chewable colon targeted tablets.
activity of the induced colitis group (gp II) was found to be 15.363 ± 1.205 U/g tissue weight. Yamada et al. [60] in their work tried to treat acetic acid induced colitis with misoprostol. They declared that acetic acid could increase MPO activity up to 8.1 folds the normal level. In the present study, MPO was significantly decreased to 11.96 ± 1.30 and 8.45 ± 0.55 U/g tissue weight in the case of the group that received commercial product (gp III) and F11.

Table 6
Mean Myeloperoxidase activity of different animal groups, mean ± S.D. n = 3.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Mean MPO Activity ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2.724 ± 0.809</td>
</tr>
<tr>
<td>Acetic acid induced colitis</td>
<td>15.363 ± 1.205</td>
</tr>
<tr>
<td>Treatment with marketed product</td>
<td>11.96 ± 1.30</td>
</tr>
<tr>
<td>Treatment with F11</td>
<td>8.45 ± 0.55</td>
</tr>
</tbody>
</table>

activity of the induced colitis group (gp II) was found to be 15.363 ± 1.205 U/g tissue weight. Yamada et al. [60] in their work tried to treat acetic acid induced colitis with misoprostol. They declared that acetic acid could increase MPO activity up to 8.1 folds the normal level. In the present study, MPO was significantly decreased to 11.96 ± 1.30 and 8.45 ± 0.55 U/g tissue weight in the case of the group that received commercial product (gp III) and F11.

Fig. 8. Release profile of BZ chewable tablets (intact & chewed) using gradient pH media.

Fig. 9. Photomicrographs of histopathological sections representing. (i) Normal lamina propria and normal glands (gp I). (ii) Marked inflammation with extensive inflammatory infiltrate and evidence of gangrenous areas (gp II). (iii) No marked reduction in inflammation (gp III). (iv) Resolution of inflammation and normal glandular architecture and mucin production (gp IV).
BZ was successfully formulated into chewable tablets that showed a proven in-vivo colon targeted activity. After extensive in-vitro study, F11 (BZ: EU S100 1:3, 250 mg mannitol, 50 mg mae starch and 20 mg cinnamon powder) was chosen as a candidate formula for in-vivo studies. This formula had proper physical properties and was able to adequately modulate drug release so as to achieve minimum drug release before target area (11%) and maximum release in target area (89%). This proves the ability of the formulated tablets to minimize drug release in the upper gastrointestinal tract and to sense the arrival of the dosage form to the colon where it gave the highest release. Histopathological evaluation of colon tissue together with measurement of myeloperoxidase activity in the colon was able to support this claim. Thus, F11 could be a novel formula for future application of BZ to the colon.

5. Declaration of interest

None.

References


