Drug Development and Industrial Pharmacy, 2010; 36(1): 81–92

informa
healthcare

ORIGINAL ARTICLE

Development of pH- and enzyme-controlled, colon-targeted, pulsed delivery system of a poorly water-soluble drug: preparation and in vitro evaluation

Huiming Lai[1], Ke Lin[1], Wenbin Zhang[2], Zhirong Zhang[1], Liu Jie[1], Yuna Wu[1] and Qin He[1]

[1]*Key Laboratory of Drug Targeting, Ministry of Education, Sichuan University, Chengdu, PR China and* [2]*Sichuan Cancer Hospital, Chengdu, PR China*

Abstract

Background: As conventional pH-controlled colon-targeted system used for oral drug delivery often shows a poor performance, a more effective way to preserve poorly water-soluble drug from releasing in upper gastrointestinal tract should be researched. *Method*: The objective of this study was to develop a novel colon-targeted drug delivery system using guar gum and Eudragit as enzyme- and pH-based materials. Lansoprazole, a poorly water-soluble drug was used as model drug. Under three different conditions, the in vitro drug release behaviors of this newly developed system was evaluated, using β-mannanase, rat cecal content, and human fecal media to simulate the pH and enzyme during intestinal transit to the colon. *Results*: The released amount of lansoprazole in simulated small intestine fluid (pH 6.8) after 5 hours was less than 10% from the pH- and enzyme-controlled tablets compared with 80.01±0.3% in rat cecal content medium (pH 7.4).The degradation ability of human fecal slurries on PECCT-PT was independent of human age and gender. β-Mannanase did not have a similar effect on the degradation of polysaccharide as rat cecal enzymes and human fecal enzymes in our study. Scanning electron microscope study indicated that the dissolution mechanism of PECCT-PT should be corrosion. *Conclusion*: The above results indicated this system could be served as a potential carrier to deliver poorly water-soluble drug specifically to the colon.

Key words: *Colon targeting; human fecal*; β*-mannanase medium; pH- and enzyme-controlled; rat cecal content*

Introduction

Recently, there is increasing interest in specific delivery of drugs to the colon via the oral route, because there are useful technologies for treating colon-specific diseases. Targeting drugs to the colon not only ensure direct treatment of colon diseases, but also is utilized as a means of achieving chronotherapy for diseases that are sensitive to circadian rhythm.

It is convenient to classify colon-targeted delivery systems into four categories[1]. Widely used colon-targeting delivery systems among them are the pH-based [triggered by a change in local pH as the formulation passes through the gastrointestinal tract (GIT)] and enzyme-based (the enzymes in a local region of the gut, which degrade a prodrug or a formulation to release drug)[2]. However, different physiological conditions and situations, such as diseases, and varied pH of small intestine might pose major barriers for colon targeting. A major disadvantage of these systems is that a considerable amount of drug may be released in the small intestine before the delivery system arrives at the colon. Further, as the pH-difference between the small intestine and the large intestine is not very pronounced, these delivery systems do not allow reproducible drug release[3]. Microflora-activated delivery systems are considered to be preferable and promising approaches as the abrupt increase of bacteria population and associated

Huiming Lai and Ke Lin are co-first authors as they contributed equally to this work.

Address for correspondence: Qin He, Huaxi College of Pharmacy, Sichuan University, Southern Renmin Road, Chengdu, Sichuan, PR China. Tel: +08 28 85502532, Fax: +08 28 85502532. E-mail: qinhe@scu.edu.cn

(Received 19 Mar 2009; accepted 4 Jun 2009)

ISSN 0363-9045 print/ISSN 1520-5762 online © Informa UK, Ltd.
DOI: 10.3109/03639040903092335

enzymatic activities in ascending colon represents a non-continuous event independent of gastrointestinal transit time and pH[4]. The critical component in microflora-activated systems is a series of polysaccharides which evade enzymatic degradation in the small intestine, such as xanthan gum, amylose, dextran, pectin, guar gum, galactomannan, and are predominantly metabolized by colonic bacteria[5]; their solubility and swelling properties in aqueous media prevent them from efficiently avoiding drug release during transit through the upper GIT, thus making the combined use of other strategies, such as coating with pH-sensitive polymers, necessary.

Guar gum was chosen to deliver drug to the colon due to its drug release retarding property, that is, hydrating and swelling in cold water and forming viscous colloidal dispersions or sols[6]. Moreover, its susceptibility to microbial degradation in the colon made it a popular biodegradable material in oral colon-specific drug delivery system studies[7–9].

Lansoprazole, a poorly water-soluble drug, is chemically known as (±)-2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]-sulphinyl]benzimidazole. It is extensively used as an anti-ulcer drug (proton pump inhibitors) through inhibition of H^+, K^+, and ATPase in gastric parietal cells. It reduces the gastric acid secretion regardless of the nature of stimulation. Lansoprazole is a labile acid and thus administered in the form of enteric-coated granules in capsules. However, enteric-coated granules in capsules cannot avoid possible degradation in small intestine and limit solubility of lansoprazole in water[10]. A more effective way to preserve lansoprazole from gastric acid should be researched.

Dissolution testing is probably the most widely used methodology for evaluating oral modified release delivery systems including colon-specific drug delivery. The simulated colonic fluid (SCF) usually contains the enzyme that could degrade polysaccharides used in the delivery system[11–15]. Commercially available β-mannanase preparation has the ability to degrade guar gum. In addition, the variables of the system can be easily controlled so that it is possible to obtain reproducible experimental conditions to allow comparison of results. Such a system would also be useful for initial screening purposes without requiring animal experiments[16]. Therefore, the β-mannanase preparation has been used in the release media to investigate the in vitro release behavior in the initial screening study for guar gum-based colon delivery systems. Rat cecal contents have been commonly utilized as an alternative dissolution medium because of the similarity of human and rodent colonic microflora and its ready availability. Freshly prepared human fecal slurries have been usually used to investigate the fermentation of non-starch polysaccharides, because bacteria consist of approximately 55% of fecal solids[17]. These methods could be adopted for the dissolution testing of colonic drug delivery systems activated by colon microflora. But no comparison of the efficacy to simulate colon environment of the three media on degrading polysaccharide colon delivery systems had been carried out.

The aim of this study was to construct a new, effective colonic drug delivery system by pH- and enzyme-controlled double coatings and improve lansoprazole solubility simultaneously. With all these considerations in mind, this study designed a new pH- and enzyme-controlled, colon-targeted, pulsed tablets (PECCT-PT). The influences of formulation variables on drug release and the drug delivery behavior of optimized PECCT-PT in the simulated in vitro physiological conditions were investigated by using three different SCF media (β-mannanase medium, rat cecal content medium, and human fecal medium). Whether the three media had the same effect on degrading PECCT-PT was studied. Whether the human fecal slurry medium got from volunteers of different age and sex had the same effect on degrading our PECCT-PT was also confirmed. The similarity factor (f_2) was used to evaluate the drug release behavior between different media. Two curves were thought to be statistically similar if the f_2 value was above 50. The surface morphology of PECCT-PT was also examined by scanning electron microscope (SEM).

Materials and methods

Materials

Lansoprazole was a kind gift from Shanxi Hanjiang Pharmaceutical Group Co., Ltd. (Shanxi, China) Guar gum was purchased from Bodie Chemical Co., Ltd. (Tianjin, China). Eudragit II, Eudragit III were provided by Huzhou Zhanwang Pharmaceutical Co., Ltd. (Zhejiang, China); β-mannanase was a generous gift from Bo Shao Biological Technology Co., Ltd. (Beijing, China); β-cyclodextrin was supplied by Yunan Yong-guang Cyclodextrin Limited (Guangdong, China). All other reagents were of analytical grade.

Preparation of PECCT-PT

Figure 1 shows schematic diagram of PECCT-PT, which consists of a fast disintegrating core (containing lansoprazole), an inner enzyme-sensitive compression-coated layer containing guar gum, and an outer enteric coating layer.

Preparation of lansoprazole core tablets

The core tablets of lansoprazole were prepared by wet granulation compression technique. Each core tablet (average weight of 150 mg) for in vitro drug release

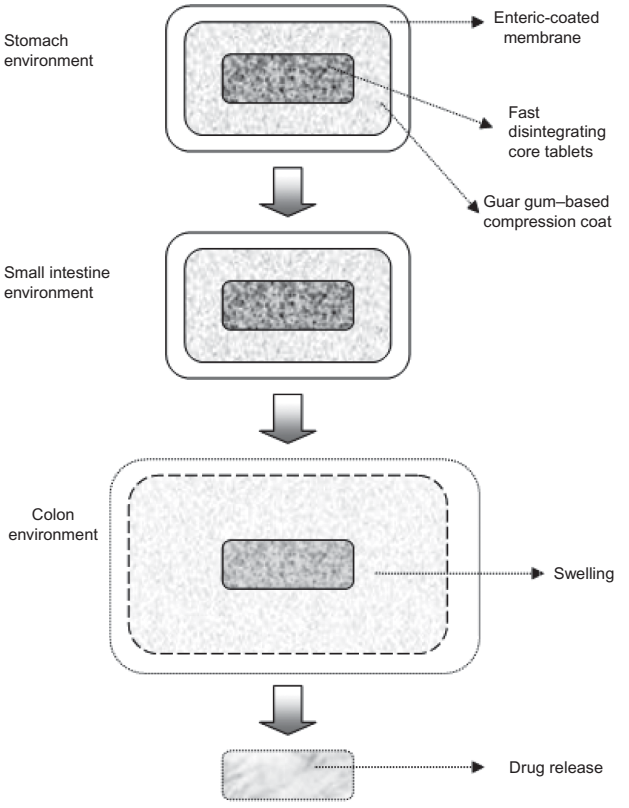

Figure 1. Schematic diagram of PECCT-PT.

studies was consisted of lansoprazole (15 mg), mannitol (75 mg), lactose (50 mg), cross-linked sodium carboxymethyl cellulose (3 mg), and sodium carboxymethyl starch (CMS-Na, 3 mg). Cross-linked sodium carboxymethyl cellulose and sodium carboxymethyl starch were added to obtain fast disintegrated tablets of lansoprazole. The ingredients of 1000 core tablets quantities mentioned above were passed through a mesh (150 μm) and granulated using polyvinylpyrrolidone K-30 (PVP, 6%, w/v) absolute ethanol solution. Formulations of core tablet contained solubilizing agents at a fixed ratio of 4.5% (w/w) of PVP (6%, w/v) absolute ethanol solution, namely, sodium dodecyl sulfate (SDS), poloxamer, and polysorbate 80 (Tween 80®). Granules of the above wet mass were prepared by passing through a sieve of 1.18 mm. The granules were dried for 6 hours at 30°C in the vacuum drying oven. The dried granules were mixed with talc (2 mg/pill) and magnesium stearate (1 mg/pill) and then passed through a sieve with a nominal aperture of 1 mm. The tablets were prepared by compressing the mixed materials using 6.5 mm round, flat, and plain punches on a single station tablet machine (the first pharmaceutical machinery, Shanghai, China). The hardness, content uniformity, friability, and disintegration of the core tablets were tested. These tablets were stored until use.

Table 1. Composition of the guar gum coat formulation for compression tablets of lansoprazole.

	Quality (mg/pill) present				
Composition	F_1	F_2	F_3	F_4	F_5
Guar gum	0	10	20	30	40
β-Cyclodextrin	47	42	37	32	27
Hydroxypropyl-β-cyclodextrin	47	42	37	32	27
PVP K-30	3	3	3	3	3
Talc	2	2	2	2	3
Magnesium stearate	1	1	1	1	1
Total	100	100	100	100	100

100 pills were prepared in each formulation.

Preparation of compression-coated tablets

The core tablets were compression coated with different coat formations F_1, F_2, F_3, F_4, and F_5 containing 0, 10, 20, 30, and 40 mg/pill of guar gum (Table 1). One hundred pills were prepared for each formulation. As guar gum alone gave very soft coats, β-cyclodextrin and hydroxypropyl-β-cyclodextrin (1:1) were included in the coat formulations to yield enough hardness. Talc (2 mg/pill) and magnesium stearate (1 mg/pill) were also used as lubricants. PVP (3 mg/pill) was added as an adhesive material. The coated tablets with different contents of guar gum were prepared by direct compression technique. About 47% of the coating material (47 mg/pill) was placed in the die cavity (diameter 9 mm), the core tablet was carefully positioned in the center of the die cavity, and then the remainder of the coat formulation was filled in the die cavity. The coating material was compressed around the core at an applied force of 5000 kg using 9 mm round, flat, and plain punches. These tablets were stored until use.

Enteric coating of compression-coated tablets

Different weight gains (E_1: 5%, E_2: 7%, and E_3: 9%, respectively, w/w) of enteric layer's materials were coated on the surface of compression coat. A coating solution was prepared by dissolving 6% (w/v) Eudragit II, Eudragit III (at a fixed ratio of 4:1), and 10% dibutylphthalate (w/w) in absolute ethanol. Coating was performed by a coating machine (BY300A, Yellow Sea Test Instrument, Shanghai, China) under the following conditions: inlet temperature, 40°C; rotating speed of pan, 60 rpm. The surface of PECCT-PT exhibited a smooth and uniform appearance. Coated tablets were dried at 35°C for 3 hours.

Preparation of SCF

Preparation of rat cecal content medium

Male Wistar rat weighed 150–200 g and maintained on a normal diet was used. An hour before commencement

of drug release studies, rats were killed by spinal traction. The abdomen were opened, the cecum was traced, ligated at both the ends, dissected, and immediately transferred into pH 7.4 phosphate buffer previously bubbled with nitrogen. The cecal bags were opened, their contents were individually weighed, pooled, and suspended in the buffer continuously bubbled with nitrogen. These were finally added to the dissolution media to give a final cecal dilution of 4% (w/v). All the procedures mentioned above were carried out under nitrogen in order to maintain anaerobic conditions. After centrifuging at 500 × *g* for 15 minutes to remove debris, supernatant were then centrifuged at 15,000 × *g* for another 30 minutes in order to obtain a clear supernatant containing extracellular enzymes[18]. Such a rat cecal and colonic enzyme system was used as one of the release media.

Preparation of human fecal medium

The slurries were prepared by centrifuging fresh feces, which were obtained from healthy human volunteers in phosphate-based anaerobic buffer medium with pH 7.4[19,20]. These volunteers from the same residence usually have no preceding history of gastrointestinal disorder and had not taken antibiotics for at least 3 months prior to the study[21,22]. All the volunteers had standard diet for 2 weeks before the fecal slurries were collected. Thirty healthy volunteers were divided into three groups: gerontism group (five males and five females, age 60–70), majority group (five males and five females, age 16–60), and immature group (five males and five females, age 3–14). The human fecal slurries were weighed, diluted, and centrifuged as mentioned in Section 'Preparation of rat cecal content medium' to obtain a clear supernatant containing colonic enzymes. Such a human colonic enzymes system was used as one of the release media.

Preparation of β-mannanase medium

A series of solution containing β-mannanase were prepared by dissolving β-mannanase in different concentrations (control, N_1: 0.06 U/mL, N_2: 0.09 U/mL, N_3: 0.12 U/mL, N_4: 0.6 U/mL, or N_5: 3 U/mL) with phosphate buffer of pH 7.4. Such a system containing β-mannanase that could specifically degrade guar gum was used as one of the release media.

UV and HPLC–UV analysis of lansoprazole in PECCT-PT tablets and dissolution fluids

Quantitative determination of lansoprazole was performed by both UV spectrophotometer (Varian Cary100; Varian, Palo Alto, CA, USA) and high-performance liquid chromatography (HPLC)–UV.

An RP-C18 column (200 mm × 4.6 mm I.D.; particle size, 5 μm) was used. The mobile phase used was acetonitrile–distilled water (TD water)–triethylamine (380:620:5) (pH adjusted to 7.0 with 5% phosphoric acid). The filtered mobile phase was pumped at a flow rate of 1.0 mL/min. The column temperature was maintained at 40°C. The eluent was detected by UV detection at 284 nm and the data were acquired, stored, and analyzed with the software Class-VP series version 5.03 (Shimadzu, Kyoto, Japan).

Three standard curves were constructed for lansoprazole in the range of 4–20 μg/mL in methanol, phosphate buffer (pH 6.8), and phosphate buffer (pH 7.4) at 284 nm using a UV spectrophotometer. Three more standard curves were also constructed for lansoprazole in the range of 1–20 μg/mL in β-mannanase, rat cecal content, and human fecal media by HPLC–UV. Good linear relationships were observed in the six lines with high correlation coefficient ($r \geq 0.9997$). The UV and HPLC–UV methods used in the study were found to be precise and accurate, as indicated by less than 3.21% RSD (intra- and inter-day) and high recovery of 99.6–100.8% of lansoprazole.

In vitro drug release studies

In order to investigate the ability of the PECCT-PT to remain intact with respect to the pH conditions prevailing in stomach and small intestine, drug release studies were carried out in a basket apparatus[23]. However, slight modification in the procedure was done. The experiments were carried out in 250 mL beaker immersed in water maintained in the jars of dissolution test apparatus. Initial studies were carried out in 150 mL of 0.1 N HCl (pH 1.2) for 2 hours. After this, 50 mL of 0.2 M trisodium phosphate (pH 6.8) was added to the dissolution media for 3 hours[24]. The stirring rates in simulated gastric fluid (SGF) and simulated small intestine fluid (SIF) were 75 rpm, and the temperature was maintained at 37°C. At the end of predetermined period, 3 mL samples were taken and analyzed for lansoprazole content using a UV spectrophotometer and an equal amount of dissolution medium was replenished.

The release behavior of lansoprazole from PECCT-PT in the physiological environment of colon was assessed by continuing the drug release studies in a series of SCF. The tablet that was picked up from dissolution test apparatus after 5 hours was transferred into individual 100 mL batch culture fermenters inoculated with SCF (pH 7.4). The fermenters were sealed under positive nitrogen pressure to establish an anaerobic environment and then placed in an incubator at 37°C and shaken at 100 rpm. Sample (1 mL) was withdrawn from the dissolution medium at hourly intervals over a 7-hour period and 1 mL of SCF, maintained under anaerobic conditions, was replenished into the dissolution media[25]. Methanol (2 mL) was mixed with the sample to ensure solubility of finely suspended drug particles due to breakdown of the coat by the enzymes and then

centrifuged at 12,000 rpm for 15 minutes for analysis of lansoprazole content by HPLC–UV using a validated method. The results were expressed as cumulative percentage of drug release versus time profiles. Each experiment was run in triplicate ($n = 3$).

SEM analysis

The surface morphology of PECCT-PT was examined by SEM (Jeol 6100, JEOL, Tokyo, Japan). The samples were dried at 35°C for 12 hours and stored between the sheets of wax paper in a desiccator before examination. Prior to examination, the samples were sputter coated with gold by using fine coat ion sputter (JFC-1100; JEOL) for 5–10 minutes and examined under SEM. The magnification selected was sufficient to appreciate the general morphology of the samples in detail under study. PECCT-PT morphology was investigated before and during the in vitro drug release study at different time intervals and in different buffers.

Results and discussion

Lansoprazole tablets

The core tablets of lansoprazole were prepared by wet granulation compression technique. The weight of the core tablets was fixed at a low level, that is, 150 mg, to accommodate maximum amount of guar gum coat over the core tablet. The average drug content of the lansoprazole core tablets was found to be 99.6–100.4% of the labeled amount, which indicated the uniformity of drug content in the formulation. The hardness of the core tablets of lansoprazole was found to be in the range of 2.5–3.0 kg/cm^2. The core tablets of lansoprazole were also found to comply with the friability test as the weight loss was found to be less than 0.5%. The core tablets were found to disintegrate within 60 seconds, showing the required fast disintegration characteristics. The combined action of the super disintegrant (sodium carboxymethyl cellulose and sodium carboxymethyl starch) might be contributed to such a fast disintegration property. Lansoprazole was not entirely in solution in all the compacts with SDS, poloxamer, or polysorbate 80 (Tween 80), as the release rates of lansoprazole in tablets with SDS, poloxamer, and Tween 80 were found to be 58.3%, 58.2%, and 74.77%, respectively. The release of water-insoluble lansoprazole in the treatment with Tween 80 was 33.35% higher than those in the treatment without Tween 80 (74.77%, 41.42%, respectively). Thus the core tablets of lansoprazole formulated in the study were found to possess the required characteristics for compression coating with guar gum.

The compression of coat formulations containing various proportions of guar gum were prepared by direct compression because guar gum was found to have poor compressibility and flow properties. The guar gum coat was prepared using β-cyclodextrin, hydroxypropyl–β-cyclodextrin (1:1), and PVP (Table 1). The compression-coated tablets were prepared by applying maximum compression force, and the hardness of the tablets was found to be in the range of 4.5–5.0 kg/cm^2. The difference between the diameter of the core (6.5 mm) and compression-coated tablets (9 mm) was 2.5 mm. As the weight of the compression-coated tablet was constant (100 mg), the thickness of the compression coats around all these formulations remained the same. The total weight of compression-coated tablet was 250 mg. After coating the enteric membrane, PECCT-PT were completely developed.

A batch core tablets with enteric coating only were also prepared as a control group. But the cover color of these tablets turned into yellow from white as soon as the pH coating solution was spurted to the surface of the core tablets. The possible reason might be that lansoprazole was sensitive to acid and the Eudragit was dissolved in absolute ethanol which was a feeble acid.

Formulation aspects of PECCT-PT

Effect of amount of guar gum on drug release

During the past decade, a large number of pH-based delivery systems were developed with the intention of colon-targeted drug delivery. The pH-dependent coating formulation was used following the previous study[26] with slight modification. The core tablets that were prepared as mentioned in Section 'Preparation of lansoprazole core tablets' and enteric materials that were coated as mentioned in Section 'Enteric coating of compression-coated tablets' (coat gaining level 7%) were fixed for the following studies. As the extent of digestion was directly proportional to the amount of guar gum present, five different amounts (F_1: 0, F_2: 10, F_3: 20, F_4: 30, and F_5: 40 mg) of guar gum were taken (Table 1) in compression coat to observe the effect on drug release over 12-hour dissolution studies.

Figure 2 illustrates the influence of different added amounts of guar gum on drug release from PECCT-PT. It clearly indicated that the amount of drug release from these formulations was highly dependent on the amount of guar gum. Since F_1 and F_2 formulations were not intact at the end of 5-hour dissolution study, they failed to be transferred to fermenters and go through the following experiments. The F_3, F_4, and F_5 formulations released 0% of lansoprazole at the end of 5-hour dissolution study. This indicated that a minimal amount of the drug was released from the guar gum compression-coated formulations in the physiological environment of stomach and small intestine. The formulations of compression coating composition F_3, F_4,

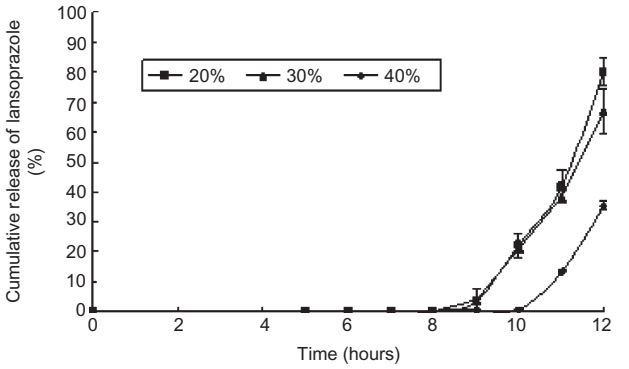

Figure 2. Cumulative percentage of lansoprazole released from PECCT-PT ($n = 3$) containing either 20%, 30%, or 40% of guar gum in 0.1 N HCl (2 hours), pH 6.8 buffer (3 hours), and SCF (4%, rat, male, pH 7.4, 7 hours).

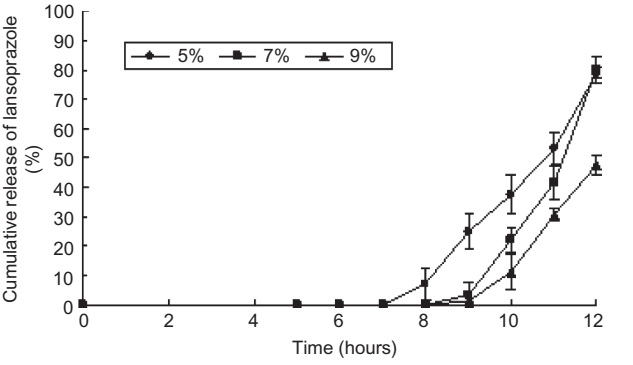

Figure 3. Cumulative percentage of lansoprazole released from PECCT-PT ($n = 3$) with coating levels of 5%, 7%, or 9% in 0.1 N HCl (2 hours), pH 6.8 buffer (3 hours), and SCF (4%, rat, male, pH 7.4, 7 hours).

and F_5 released 80.01 ± 0.3%, 66.71 ± 0.6%, and 35.45 ± 0.1% of lansoprazole, respectively, after complete dissolution studies. Upon increasing the amount of guar gum in the coating of compression-coated tablets, the release of lansoprazole at the end of 12-hour dissolution study decreased.

Another parameter affected by the amount of guar gum was the lag time of drug release. Before SCF dissolution study, it was expected that there was a lag time of 5 hours to reach specific colon region, which meant that the release of drug was only activated by SCF containing colonic bacteria. The amount of guar gum might be the key factor to control the lag time. The formulation with lower amount of guar gum (20%, F_3) showed shorter average lag time (7.37 ± 0.32 hours), whereas the one with higher amount of guar gum (40%, F_5) showed longer average lag time (9.98 ± 0.25 hours) in the whole dissolution setup (SCF, 4%, rat, male). Hence, the relative benefit of the F_3 formulation over the other F_1, F_2, F_4, and F_5 formulations was adopted in further formulation studies.

Sinha et al.[27] had found that as the concentration of the swellable polymer was increased in the formulation, the gel thickness increases upon swelling. This increased the diffusion path length, which in turn decreased the drug release from the tablet. Guar gum hydrates and swells in cold water forming viscous colloidal dispersions or sols. It was thus used as a gelling agent to retard drug release from tablets[13]. Once these tablets reached the colon, guar gum should be broken down by the microflora of the colon and the total amount of drug should be released from the dosage form. So a rapid release of drug could be observed at 2–3 hours after tablets were transferred to rat cecal content medium.

Effect of enteric coat thickness on drug release

Based on the above result, the compression-coated formulation F_3 (guar gum 20%) was selected as optimum for the following studies. The core tablet formulation as mentioned in Section 'Preparation of lansoprazole core tablets' was also fixed. As the extent of digestion was directly proportional to the amount of guar gum present, three different coating levels (E_1: 5%, E_2: 7%, and E_3: 9%) of enteric coat formulation as mentioned in Section 'Enteric coating of compression-coated tablets' were chosen to observe the effect on drug release over 12-hour dissolution studies.

Figure 3 shows the influence of different coating levels of enteric coat on drug release from PECCT-PT. As can be observed, the lag time of drug release was dependent on coating thickness. The lag time was directly related to the coating level of the enteric coat. The lag time of E_1, E_2, and E_3 were 6.62 ± 0.36, 7.37 ± 0.32, and 8.23 ± 0.14 hours, respectively, in the whole dissolution setup (SCF, 4%, rat, male). Because of longer water diffusion pathways and more tortuosity at higher coating levels, as the thickness of membrane increased, the imbibing water rate of guar gum in the compression-coated and the liquefaction rate of the tablet core decreased correspondingly, resulting in the increase of lag time. In addition, more weight gain of pH-coated tablet would make the guar gum less accessible to bacterial degradation, resulting in the slower drug release.

The formulation of compression coating composition E_1, E_2, and E_3 released 78.99 ± 0.4%, 80.01 ± 0.3%, and 47.52 ± 0.6% of lansoprazole, respectively, after complete dissolution studies. The PECCT-PT of E_1 was not integrated; significant difference in release profiles could be found in E_2 and E_3 (the value of f_2 was 45.93). Hence, the relative benefit of the E_2 (7%, w/w) formulation over the other formulations was adopted.

In vitro drug release study

Based on the release studies mentioned above, the compression coating formulation (F_3) and enteric coating

formulation as mentioned in Section 'Enteric coating of compression-coated tablets' (coating level 7%) were selected as optimum for further evaluation. Identification of an enzyme system that could accomplish polysaccharide degradation in a manner similar to that found in the colon environment, such as pectinase for pectin[28] and dextranase for dextran[29], and conduct of an enzymatic degradation test for the polysaccharide-based colon delivery device in that corresponding system would be beneficial for a more mechanistic investigation. Such a system would also be useful for initial screening purposes without requiring rat cecal content or human fecal slurry handling. Despite the simplicity and convenience, dissolution testing with the enzyme system primarily generated essential information on the processing of a colon-specific delivery system rather than being predictive of its in vivo performance. The selection of dissolution media was dependent on the design rationale of the delivery system. The volume and composition of dissolution media and the mixing intensity were not representative of the conditions present in the colon. To overcome the limitation of conventional dissolution testing, rat cecal contents have been widely utilized as an alternative dissolution medium because of the similarity of human fecal slurries. Freshly prepared human fecal slurries had also been commonly used to investigate the fermentation of non-starch polysaccharides.

In vitro drug release study in rat cecal content medium

When rat cecal contents from the same source were used, the release rate of lansoprazole was consistently higher in the medium containing 4% rat cecal contents than that with 2%. Taking the release profile in release media of 10% rat cecal contents as the reference, the released amount of lansoprazole decreased after 8 hours. The observed difference in the cumulative amount of lansoprazole might probably be explained by the instability of lansoprazole and the different contents in rat cecal content medium as concentration changed, which might induce the degradation of lansoprazole. It indicated that varying concentration of rat cecal contents within the range from 2% to 10% had a marked impact. Therefore, the SCF containing 4% rat cecal medium was selected to be compared with the other SCF with human fecal slurries. The data were not shown because some similar studies have done in many other reports.

In vitro drug release study in human fecal slurry medium

The composition of colonic bacteria and corresponding enzymes could be influenced by many factors including age, diet, diseases, medication such as antibiotics, and geographic residences[30]. No previous study was found to compare the release of drug in human fecal slurry medium from volunteers of different age and sex in an in vitro dissolution testing. The volunteers from the same residence who usually had no preceding history of gastrointestinal disorder and did not take antibiotics at least 3 months prior to the study were selected for this study. They were divided into three groups, the gerontism group, majority group, and immature group in order to investigate the effect of age and sex on the release of lansoprazole.

Figure 4 illustrates the release profiles of lansoprazole over 12 hours from PECCT-PT (n = 3) under six different SCF (4%) conditions: 7 hours in SCF with human fecal slurry medium (male, gerontism) or SCF (female, gerontism) human fecal slurry medium, or SCF (male, majority), or SCF (female, majority), or SCF (male, immature), or SCF (female, immature). The appearance of the release profiles, as shown in Figure 4, and the cumulative percentage of lansoprazole released over the entire experiment (12 hours) were not significantly different among them according to the value of f_2, indicating that the ability of human fecal slurries to degrade guar gum formulated in the PECCT-PT tablet was independent of the age and gender in this study.

Intestinal enzymes were used to trigger drug release in various parts of the GIT. Usually, these enzymes were derived from gut microflora residing in high numbers in the colon. In general, the types and activities of bacterial glycosidases were unchanged relative to those in healthy volunteers[31]. This might explain why the human fecal slurries obtained from different people with different age or gender had no significant difference on the ability to degrade guar gum-based colon delivery system.

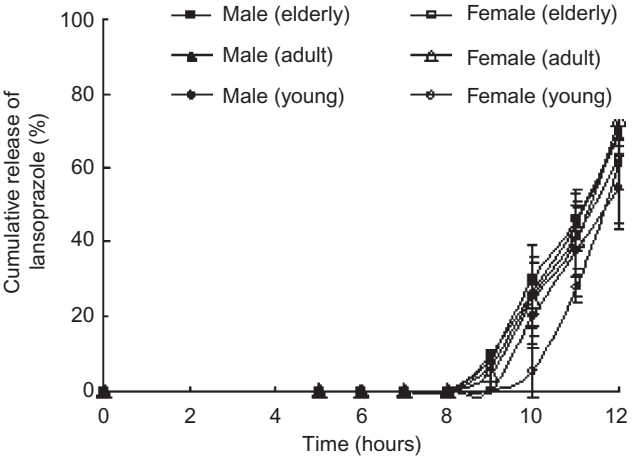

Figure 4. Cumulative percentage of lansoprazole released from PECCT-PT (n = 3) in 0.1 N HCl (2 hours), pH 6.8 buffer (3 hours), then followed by SCF (male, gerontism), SCF (female, gerontism), SCF (male, majority), SCF (female, majority), SCF (male, immature), SCF (female, immature).

In vitro drug release study in β-mannanase medium

Different concentrations of the β-mannanase had used in different studies. It was reported that the appropriate concentration of the β-mannanase solution simulating colon condition was 0.166 U/mL[32]. In another study, to develop sustained release matrix tablets with a mixture of the polysaccharides, konjac glucomannan and xanthan gum, as the sustained release materials, Jiangyang Fan et al.[33] found that the release rate of cimetidine in the media containing 0.220 U/mL β-mannanase was similar to that of the 4% (w/v) of rat cecal content solution. So, we had chosen several concentrations of β-mannanase within the range from 0 to 3 U/mL to investigate the in vitro release behavior of guar gum-based colon delivery systems.

Figure 5 shows the cumulative percentage of lansoprazole released from PECCT-PT in 0.1 N HCl (2 hours), pH 6.8 buffer (3 hours), then followed by six different concentrations of β-mannanase in SCF (control, N_1: 0.06, N_2: 0.09, N_3: 0.12, N_4: 0.6, or N_5: 3 U/mL). The release profiles revealed that, under each condition, from none of formulations, lansoprazole was released during the first 5 hours in SGF and SIF media. The cumulative amount of lansoprazole released over 12 hours in the enzyme-free SCF, control group, was around 10%. As compared with the control, the release of lansoprazole increased markedly over 12 hours when the tablets were exposed to colonic medium with β-mannanase. The lower concentration of β-mannanase (control, N_1, N_2) showed less amount of drug release. Taking the release profile N_3 as reference, the release profiles of N_3 and N_4 were similar (f_2 = 53.36). It indicated that varying concentration of β-mannanase within the range from 0.6 to 3 U/mL did not have a marked impact.

Figure 5 compared the release profiles of PECCT-PT (n = 3) under several different conditions, 0.1 N HCl for 2 hours, pH 6.8 buffer for 3 hours, then followed by 7 hours in different SCF containing β-mannanase ranging from 0.06 to 3 U/mL, or in rat cecal content medium (male, 4%), or in human fecal slurry medium (male, 4%), or in enzyme-free medium as control group. As results shown in Figure 5, the release profile associated with the rat cecal contents was parallel to that of the human fecal slurry medium. No statistically significant difference was observed in lansoprazole release pattern between those two groups over 12 hours, suggesting that PECCT-PT was not susceptible to degradation by these two SCF media f_2 = 66.06. The SCF with rat cecal content or human fecal slurry enzyme was clearly dissimilar when from the release profile associated with β-mannanase (the value of f_2 is 30.18 and 32.78, respectively).

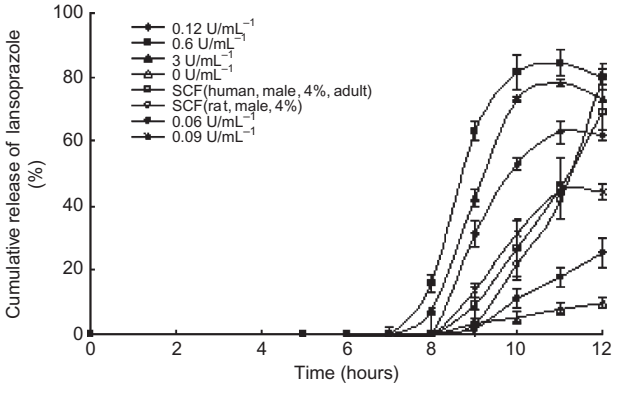

Figure 5. Cumulative percentage of lansoprazole released from PECCT-PT (n = 3) in 0.1 N HCl (2 hours), pH 6.8 buffer (3 hours), then followed by 4% rat cecal content medium (male), 4% human fecal slurry medium (male, adult), or six different concentrations of β-mannanase in SCF (control, 0.06, 0.09, 0.12, 0.6, or 3 U/mL).

It is indicated that β-mannanase medium could not substitute for rat cecal contents and human fecal slurries in in vitro assessment of the performance of PECCT-PT in our study. The possible reason of this phenomenon might be that the release of lansoprazole could be influenced by the β-cyclodextrin in the compression-coated PECCT-PT. Cyclodextrin could be neither hydrolyzed nor absorbed from the stomach and small intestine. However, in the colon, vast microflora broke cyclodextrins into small saccharides and thus were absorbed in the large intestine[24]. β-Mannanase could hydrolyze plant gums (such as carob, guar gum, and konjac) to generate mannose oligosaccharides. However, it did not have or only has a weak ability to hydrolyze cyclodextrin.

As the degradation effect on β-cyclodextrin was different, β-mannanase might not substitute for rat cecal content medium to be suitable medium to mimic the degradation function of PECCT-PT.

SEM analysis

In order to study the changes of the surface morphology throughout the dissolution procedure and the mechanism of drug release from PECCT-PT, PECCT-PT was taken from the dissolution medium at different time intervals. The PECCT-PT appearance was blanc and glossy and the membrane appeared to be integral and smooth with no visible imperfections (Figure 6A), before submitting them to the release test. The PECCT-PT appearance was almost unchanged after 2 hours of exposure to the simulated gastric medium of pH 1.2 (Figure 6B), thus confirming the effectiveness of the pH-sensitive coating. The PECCT-PT appearance was largely intact with slight cracks after 5 hours of exposure to the SIF of pH 6.8 (Figure 6C). The results demonstrated that the enteric coating membrane could protect the compression coating of guar gum with the characteristics of swelling and permeability in the presence of small intestinal fluids.

After exposure to SCF (4%, male, majority), formation of the enteric coating membranes was observed as shown in Figure 6D–G. After 1 hour of exposure to the SCF, as shown in Figure 6D, larger cracks appeared in appearance of pH-sensitive membrane. Figure 6E1–3 shows pH-sensitive membrane and enzyme-sensitive (guar gum) layer, after 2 hours of exposure to the SCF. The PECCT-PT appearance of pH-sensitive membrane had no integrity because of corrosion; therefore, corrosion holes appeared in the enzyme-sensitive (guar gum) layer partly exposed to the SCF. After 3 or 4 hours of exposure to the SCF, as shown in Figure 6F and G, pH-sensitive layer was completely dissolved; the corrosion holes of enzyme-sensitive layer were enlarged. Moreover, the lag time was 7.37 ± 0.32 that had been mentioned in Section 'In vitro drug release study'.

The SEM indicated that the guar gum was accessible to enzymic degradation, which allowed the in situ formation of delivery pores for releasing drug under conditions that might be expected to pertain in the colon. The degradation of guar gum was therefore a rate-limiting factor.

The SEM study indicated that the dissolution and drug release mechanism of PECCT-PT was corrosion. The medium slowly permeated the pH-sensitive layer film in SGF and SIF and then quickly dissolved after being exposed to SCF. When the enzyme-sensitive (guar gum) layer was exposed to SCF, degradation of guar gum could be accomplished by the enzyme in SCF (4%, male, majority).

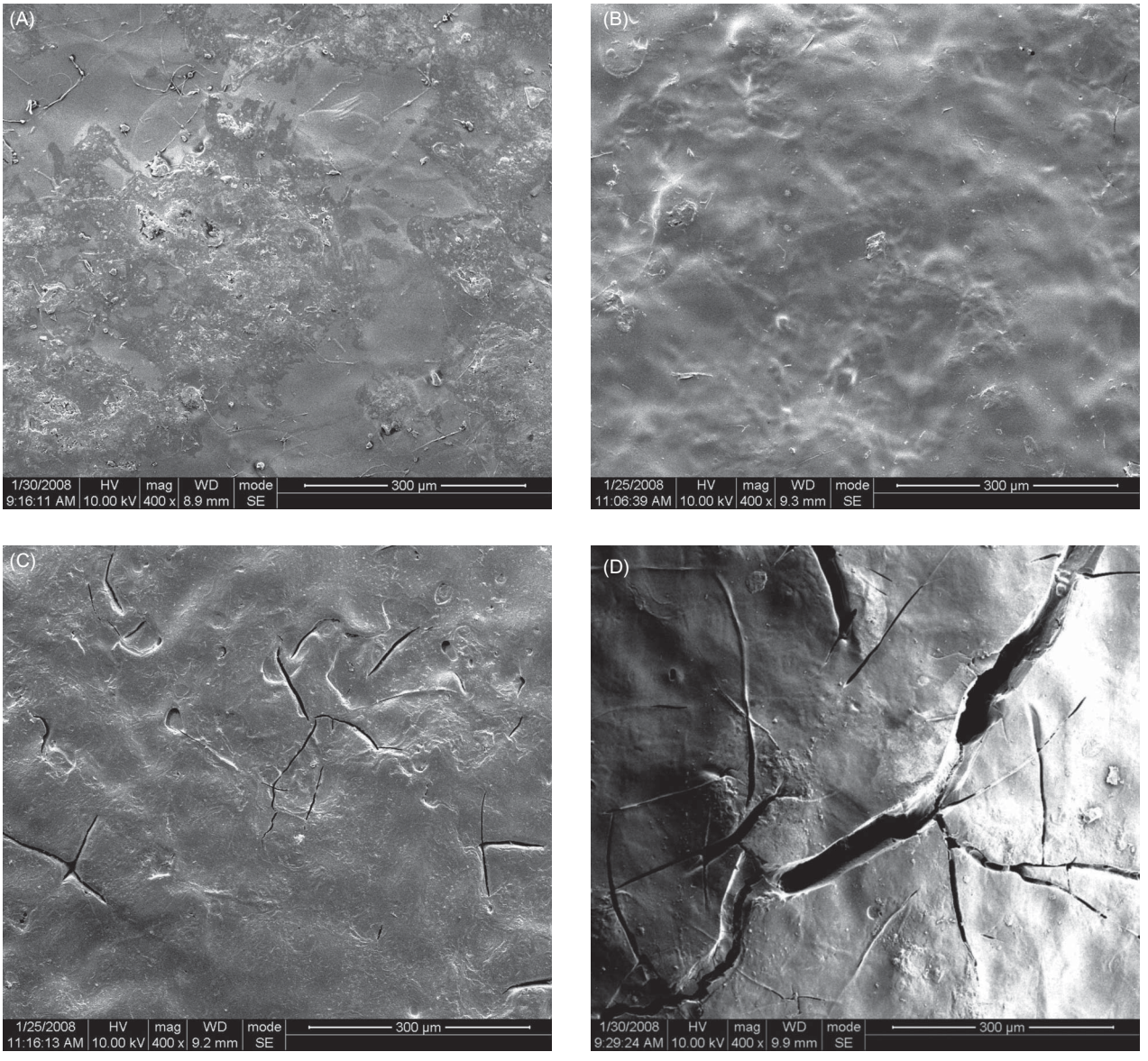

Figure 6. SEM micrographs showing the surface structure of formulation F3 (A) after dissolution in SGF (B), after dissolution in SIF (C), and after dissolution in SCF (4%, male, majority) (D–G).

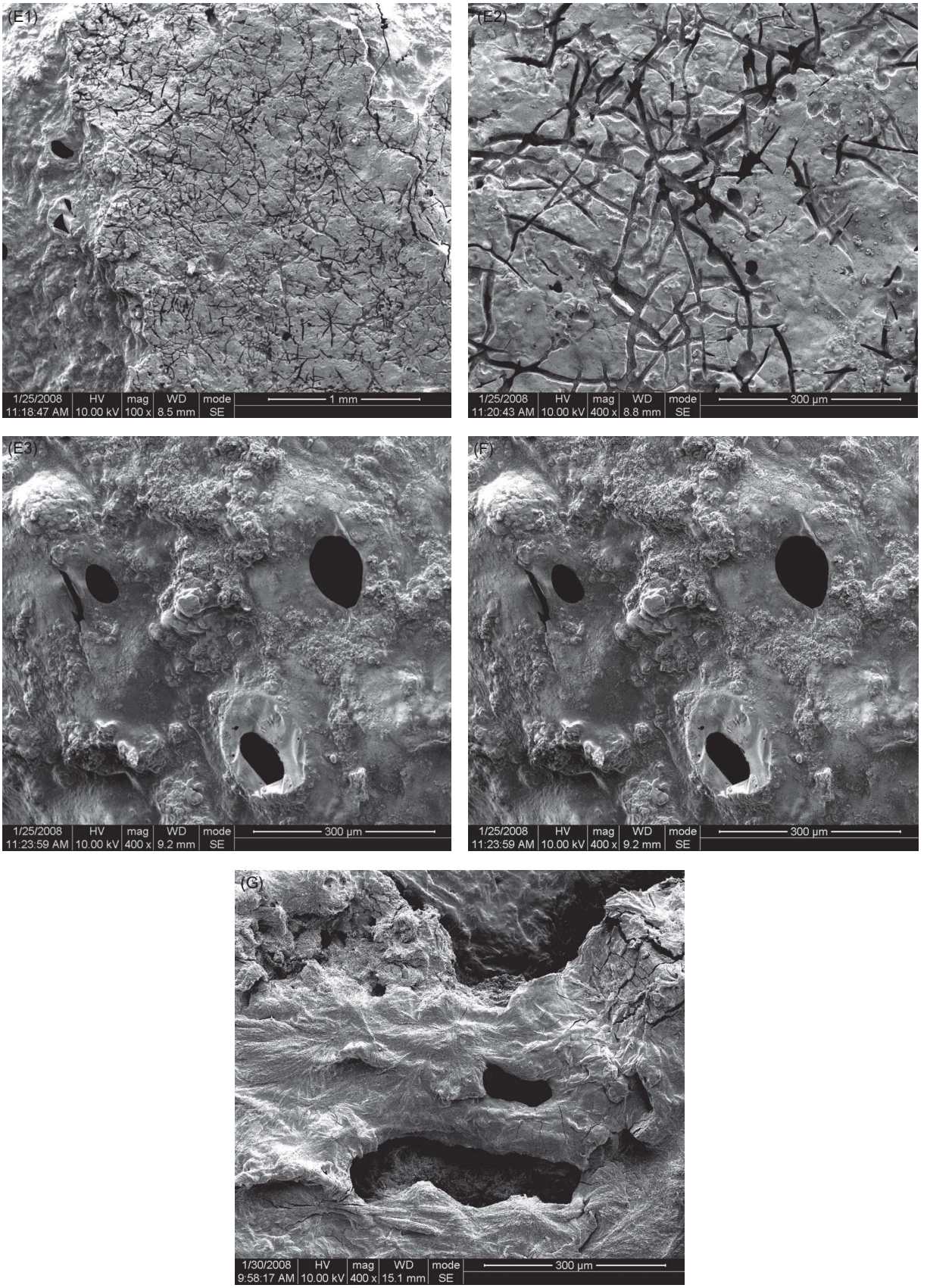

Figure 6. (*Continued*).

With constant degradation and eventual breaking-up of guar gum, large amount of lansoprazole was released.

Conclusion

A novel colon-targeted oral drug delivery system for lansoprazole using pH- and enzyme-sensitive materials was prepared with simultaneously improved drug solubility. The effect of three different media on their microbial degradation was evaluated in dissolution testing for the first time. No lansoprazole was released from PECCT-PT in the physiological environment of stomach and small intestine. However, more than 60% of lansoprazole was released from the PECCT-PT in the physiological environment of colon. And the results indicated that the β-mannanase medium could not substitute for rat cecal contents and human fecal slurries in in vitro assessment of the performance of PECCT-PT in this study. As there were two types of colon-specific polysaccharides in the delivery system, using a single enzyme to simulate the environment of the colon was required for further testing and discussion. Based on the SEM of PECCT-PT and in vitro study, the system formulated using guar gum as coating seemed to be highly site specific due to release of majority of drug only upon degradation effect of the bacterial microflora of the colon. An additional advantage of this system was that it could be formulated easily using common tableting and coating techniques. Further studies are planned to assess the in vivo behaviors of the PECCT-PT compared to conventional lansoprazole therapy.

Acknowledgments

We are thankful for the financial support of the National Basic Research Program (2007CB935801) of China and Program for New Century Excellent Talents in University.

Declaration of interest

The authors report no conflicts of interest.

References

1. Sangalli ME, Maroni A, Foppoli A, Zemaa L, Giordano F, Gazzaniga A. (2004). Different HPMC viscosity grades as coating agents for an oral time and/or site-controlled delivery system: A study on process parameters and in vitro performances. Eur J Pharm, 22:469–76.
2. Yang L-B, Chu JS, Fix JA. (2002). Colon-specific drug delivery: New approaches and in vitro/in vivo evaluation. Int J Pharm, 235:1–15.
3. Sinha VR, Kumria, R. (2003). Microbially triggered drug delivery to the colon. Eur J Pharm, 18:3–18.
4. Maestrelli F, Zerroukb N, Cirri M, Mennini N, Mura P. (2008). Microspheres for colonic delivery of ketoprofen-hydroxypropyl-cyclodextrin complex. Eur J Pharm, 34:1–11.
5. Bajpaia SK, Saxenab SK, Sharma S. (2006). Swelling behavior of barium ions-crosslinked bipolymeric sodium alginate–carboxymethyl guar gum blend beads. Reactive and Functional Polymers, 66:659–66.
6. Brosio E, D'ubaldo A, Verzegnassi B. (1994). Pulsed field gradient spin-echo NMR measurement of water diffusion coefficient in thickening and gelling agents: Guar galactomannan solutions and pectin gels. Cell Mol Biol, 40:569–73.
7. Bayliss CE, Houston AP. (1986). Degradation of guar gum by faecal bacteria. Appl Environ Microbiol, 48:626–32.
8. Tomolin J, Taylor JS, Read NW. (1989). The effect of mixed faecal bacteria on a selection of viscous polysaccharide in vitro. Nutr Rep Int, 39:121–35.
9. Macfarlane GT, Hay S, Macfarlane S, Gibson, GR. (1990). Effect of different carbohydrates on growth, polysaccharidase and glycosidase production of Bacteroides ovatus in batch and continuous culture. J Appl Bacteriol, 68:179–87.
10. Ito Y, Arai H, Uchino K, Iwasaki K, Shibata N, Takada K. (2005). Effect of adsorbents on the absorption of lansoprazole with surfactant. Int J Pharm, 289:69–77.
11. Fetzner A, Böhm S, Schreder S, Schubert R. (2004). Degradation of raw or film incorporated β-cyclodextrin by enzymes and colonic bacteria. Eur J Pharm Biopharm, 58:91–7.
12. Ji C, Xu H, Wu W. (2007). In vitro evaluation and pharmacokinetics in dogs of guar gum and Eudragit FS30D-coated colon-targeted pellets of indomethacin. J Drug Target, 15:123–31.
13. Wong D, Larrabee S, Clifford K, Tremblay J, Friend DR. (1997). USP dissolution apparatus III (reciprocating cylinder) for screening of guar-based colonic delivery formulations. J Control Release, 47:173–9.
14. Ugurl T, Turkoglu M, Gurer US, Akarsu BG. (2007). Colonic delivery of compression coated nisin tablets using pectin/HPMC polymer mixture. Eur J Pharm Biopharm, 67:202–10.
15. Pitarresi G, Casadei MA, Mandracchia D, Paolicelli P, Palumbo FS, Giammona G. (2007). Photocrosslinking of dextran and polyaspartamide derivatives: A combination suitable for colon-specific drug delivery. J Control Release, 119:328–38.
16. Alvarez-Mancenido F, Landin M, Martnez-Pacheco R. (2008). Konjac glucomannan/xanthan gum enzyme sensitive binary mixtures for colonic drug delivery. Carbohydr Polym, 73:241–7.
17. Kalypsys Inc., San Diego. (2008). Biorelevant dissolution testing of colon-specific delivery systems activated by colonic microflora. J Control Release, 125:77–86.
18. Zhang H, Ibrahim AA, Neau SH. (2002). An in vitro evaluation of a guar gum containing multiparticulate system for macromolecule delivery to the colon. Int J Pharm, 239:197–205.
19. Silvester KR, Englyst HN, Cummings JH. (1995). Ileal recovery of starch from whole diets containing resistant starch measured in vitro and fermentation of ileal effluent. Am J Clin Nutr, 62:403–11.
20. Basit AW, Newton JM, Lacey LF. (2002). Susceptibility of the H2-receptor antagonists cimetidine, famotidine and nizatidine, to metabolism by the gastrointestinal microflora. Int J Pharm, 237:23–33.
21. Milojevic S, Newton JM, Cummings JH, Gibson GR, Botham RL, Ring SG, et al. (1996). Amylose as a coating for drug delivery to the colon: Preparation and in vitro evaluation using 5-aminosalicylic acids pellets. J Control Release, 38:75–83.
22. Simonsen L, Hovgaard L, Mortensen PB, Brondsted H. (1995). Dextran hydrogels for colon-specific drug delivery. V. Degradation in human intestinal incubation models. Eur J Pharm, 3:329–37.
23. USP30-NF25 (2007). Pharmacopeial forum, 31(6):1691.
24. Sinha VR, Mittal BR, Kumria R. (2005). In vivo evaluation of time and site of disintegration of polysaccharide tablet prepared for colon-specific drug delivery. Int J Pharm, 289:79–85.
25. Wilson PJ, Basit AW. (2005). Exploiting gastrointestinal bacteria to target drugs to the colon: An in vitro study using amylose coated tablets. Int J Pharm, 300:89–94.
26. Akhgari A, Sadeghi F, Garekani HA. (2006). Combination of time-dependent and pH-dependent polymethacrylates as a

single coating formulation for colonic delivery of indomethacin pellets. Int J Pharm, 320:137–42.
27. Sinha VR, Kumria R. (2002). Binders for colon specific drug delivery: An in vitro evaluation. Int J Pharm, 249:23–31.
28. Ashford M, Fell J, Attwood D, Sharma H, Woodhead P. (1993). An evaluation of pectin as a carrier for colon drug delivery. J Control Release, 26:213–20.
29. Brøndsted H, Hovgaard L, Simonsen L. (1995). Dextran hydrogels for colon specific drug delivery. III. In vitro and in vivo degradation. STP Pharm Sci, 5:60–64.
30. Mueller S, Saunier K, Hanisch C, Norin E, Alm L, Midtvedt T, et al. (2006). Differences in fecal microbiota in different European study populations in relation to age, gender, and country: A cross-sectional study. Appl Environ Microbiol, 72:1027–33.
31. Friend DR. (2005). New oral delivery systems for treatment of inflammatory bowel disease. Adv Drug Deliv Rev, 57:247–65.
32. Burke MD, Park JO, Srinivasarao M, Khan SA. (2005). A novel enzymatic technique for limiting drug mobility in a hydrogel matrix. J Control Release, 104:141–53.
33. Fan J, Wang K, Liu M, He Z. (2008). In vitro evaluations of konjac glucomannan and xanthan gum mixture as the sustained release material of matrix tablet. Carbohy Polym, 73:241–7.