Carbohydrate Polymers 87 (2012) 377–382

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Preparation of konjac glucomannan-based pulsatile capsule for colonic drug delivery system and its evaluation in vitro and in vivo

Jing Liu[a], Liangke Zhang[a,*], Wenjing Hu[b], Rui Tian[c], Yongzhen Teng[c], Chengyuan Wang[d]

[a] Chongqing Key Laboratory of Biochemistry & Molecular Pharmacology, School of Pharmacy, Chongqing Medical University, Chongqing 400016, PR China
[b] Chongqing Xijiao Hospital, Chongqing 400016, PR China
[c] Institute of Life Science, Chongqing Medical University, Chongqing 400016, PR China
[d] Research Center of Medical Chemistry & Chemical Biology, Chongqing Technology and Business University, Chongqing 400067, PR China

ARTICLE INFO

Article history:
Received 8 June 2011
Received in revised form 24 July 2011
Accepted 28 July 2011
Available online 5 August 2011

Keywords:
Colon-specific drug delivery
Konjac glucomannan
Pulsatile capsule

ABSTRACT

The current study aims to develop and evaluate a colon-specific, pulsatile drug delivery system based on an impermeable capsule. A pulsatile capsule was prepared by sealing a 5-aminosalicylic acid rapid-disintegrating tablet inside an impermeable capsule body with a konjac glucomannan (KGM)–hydroxypropyl methylcellulose (HPMC)–lactose plug. The drug delivery system showed a typical pulsatile release profile with a lag time followed by a rapid release phase. The lag time was determined by the KGM/HPMC/lactose ratio, the type of HPMC, and the plug weight. The addition of β-glucanase and rat cecal contents into the release medium shortened the lag time significantly, which predicted the probable enzyme sensitivity of the KGM plug. The in vivo studies show that the plasma drug concentration can only be detected 5 h after oral administration of the capsule, which indirectly proves the colon-specific characteristics. These results indicate that the pulsatile capsule may have therapeutic potential for colon-specific drug delivery.

1. Introduction

In the recent years, colon-specific drug delivery has gained increasing importance not only just for its application in the treatment of various colonic diseases, but also for its potential for protein and therapeutic peptide delivery (Sinha & Kumria, 2001; Yang, Chu, & Fix, 2002).

Colon-specific drug delivery systems have been attempted through several approaches, including pH-dependent delivery systems (Gupta, Beckert, & Price, 2001; Khan, Stedul, & Kurjakovic, 2000), time-controlled delivery systems (Gazzaniga, Maroni, Sangalli, & Zema, 2006; Jones, 1996; Peerapattana, Otsuka, & Otsuka, 2004), and microflora-triggered delivery systems (Fan et al., 2008; Freire, Podczeck, Veiga, & Sousa, 2010). Among the three approaches, more interest has been focused on microflora-triggered systems because the abrupt increase in bacterial populations and their associated enzyme activity in the colon represent a non-continuous event unrelated to gastrointestinal transit time.

Natural polysaccharides have received considerable attention in drug delivery systems because they are biodegradable, non-toxic, and easily available. Konjac glucomannan (KGM) is a high-molecular-weight polysaccharide extracted from the perennial plant *Amorphophallus konjac* (syn. *Amorphophallus rivieri*). KGM is water-soluble and it has gelling and swelling properties that contribute to its wide range of applications as films or membranes in coating and packaging, and as controlled release matrices aside from applications in food manufacturing (Williams et al., 2000; Zhang et al., 2001). Recent studies on KGM have demonstrated its potential as a new material for a sustained colon-specific drug release system; it cannot be hydrolyzed by digestive enzymes in the upper gastrointestinal tract, but can be degraded by microbial enzymes produced by colonic microflora (Alvarez-Mancenido, Landin, & Martinez-Pacheco, 2008; Matsuura, 1998; Nakajima & Matsuura, 1997). However, its solubility and swelling properties limit its application in colon-specific drug delivery system.

5-Aminosalicylic acid (5-ASA) is an anti-inflammatory agent for the treatment of inflammatory bowel disease (IBD), especially for ulcerative colitis and Crohn's disease. IBD is a localized inflammation of the small and large intestine (Pertuit et al., 2007). However, 5-ASA was absorbed rapidly and completely through the upper intestine when administered conventionally, not only reduced the dose reached to the colon but also produced some side effects such as nephritic syndrome (Friend, 2005). Therefore, 5-ASA was chosen as model drug for colon-specific delivery in the current work.

In the current work, a capsule-based pulsatile drug delivery system was designed for oral administration. The drug delivery system was adapted from the pulsatile system described by Krogel and

* Corresponding author. Tel.: +86 23 6848 5078; fax: +86 23 6848 5161.
E-mail address: zlkdyx@gmail.com (L. Zhang).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.07.062

Bodmeier (1998, 1999a, 1999b). The novel system consists of an impermeable capsule body filled with a drug containing rapid-disintegrating tablet, and an erodible plug tablet placed in the opening of the capsule body. The lag time can be controlled by varying the properties of the plug tablet. KGM was chosen as the main ingredient of the plug tablet and combined with hydroxypropyl methylcellulose (HPMC) and lactose to achieve a suitable lag time for colon-specific, pulsatile drug delivery. The effects of various parameters such as the formulation of the plug tablet, the type of hydrophilic polymers used in the plug tablet, plug tablet weight, and the different in vitro release media were investigated to optimize the lag time. The release behavior of the pulsatile capsule was also evaluated in beagle dogs.

2. Experimental

2.1. Materials

The following materials were obtained from commercial suppliers and used as received: 5-aminosalicylic acid (5-ASA, Wuhan yuancheng Science and Technology Development Co., Ltd., China), hydroxypropyl methylcellulose (HPM, Methocel® E15-LV, E50-LV, K4M, K15M grade, Colorcon Limited, China), konjac glucomannans (KGM, Chongqing Limao agricultural products development Co., Ltd., China), ethyl cellulose (Ethocel, Colorcon Limited, China), lactose (Meggle Granulac200, Meggle, Germany), polyvinylpyrrolidone (PVP, Sinopharm Chemical Reagent Co., Ltd.), sodium carboxymethyl starch, microcrystalline cellulose, magnesium stearate (Anhui Shanhe Pharmaceutical Excipients Co., Ltd., China). All other reagents were of analytical grade.

Single punch tablet press (Shanghai Yuandong Pharmaceutical Machinery Factory, China) were used for preparation of tablet. A spectrophotometer (UV-3150, Shimazu, Japan) was used to determine the drug contents in solutions. HPLC (LC-2010A, Shimadzu, Japan) was used to determine the drug concentration in plasma. Healthy beagle dogs (12 ± 1 kg), were purchased from Gaoyao Kangda Experimental Animal Technology Co., Ltd. (China). The animals were kept in an environmentally controlled breeding room for one week before the start of the experiments. They were fed standard laboratory chow with water and fasted overnight before the experiment.

2.2. Preparation of impermeable capsule bodies

The bodies and caps of the gelatin capsules were separated manually. Gelatin capsules were made into impermeable capsule bodies using the filling method. The filling was composed of 115 g L^{-1} ethyl cellulose (EC) solution, prepared by mixing ethyl acetate, dichloromethane, and ethanol (4:0.8:0.2). Then, the capsule bodies on a self-made board were refrigerated overnight at 4 °C to evaporate the solvent. Finally, the impermeable capsule bodies were formed after soaking the capsules in water for 15 min.

2.3. Preparation of plug tablet

The plug tablet formulations consisted of KGM, HPMC, and lactose. All materials (KGM, HPMC, and lactose in different ratios) were sieved through a 180 μm-sieve and mixed in a mortar for 10 min. After the addition of 1% talc, each blend was mixed for 5 min. The resultant blends were made into 80, 100, and 120 mg tablets using 6.0 mm shallow, concave punches with a single punch tablet press.

2.4. Preparation of 5-ASA rapid-disintegrating tablets

The rapid-disintegrating tablets containing 5-ASA (40%, w/w), microcrystalline cellulose (20%, w/w), lactose (24%, w/w), and sodium carboxymethyl starch (15%, w/w) were prepared through wet granulation. All materials were passed through a 180 μm-sieve, and 30% ethanol solution was used as wetting agent and slowly added to the blend to prepare wet granules. The resulting granules were spread evenly on a tray and dried at 55 °C for 30 min before 1% magnesium stearate was mixed in. The final blends were passed through a 1.25 mm-sieve and made into 250 mg tablets under an applied force of 5 N using 6.0 mm shallow, concave punches using a single punch tablet press.

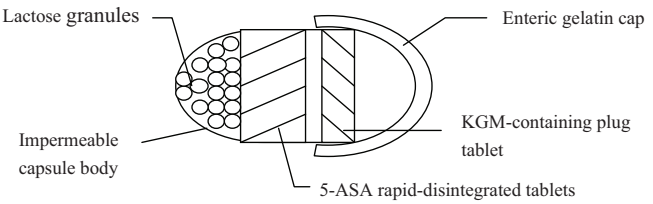

Fig. 1. Configuration of colon-specific plug-controlled pulsatile capsule delivery system.

2.5. Assembly of pulsatile capsule

Assembly of the pulsatile capsule was as follows: 5% polyvinylpyrrolidone solution was used as an adhesive and added to lactose (Spherolac®200) to prepare wet granules. The wet granules were spread evenly on a tray, and dried at 50 °C for 30 min and passed through a 1.25 mm-sieve. The resulting blank lactose granules were placed into the bottom of the capsule, and the rapid-disintegrating tablet was placed on top of the lactose granules. The plug tablet was inserted into the mouth of the capsule and positioned flush with the end of impermeable body (Fig. 1). The capsule body was closed with an enteric cap, and the joint of the capsule body was sealed with a small amount of 5% EC solution. Maintaining the distance between the plug tablet and the rapid-disintegrating tablet is important; otherwise the reproducibility of the lag time and the rapidly drug release in dissolution testing would be significantly affected.

2.6. In vitro dissolution testing

The dissolution testing of the pulsatile capsule delivery system was carried out using a water bath oscillator at 37 ± 0.5 °C. The capsule bodies were immersed at the bottom of 200 mL flask. Samples were withdrawn at predetermined time points and analyzed after appropriate dilution with a UV–visible spectrophotometer. Given that spectrophotometric determination would probably detect the degraded KGM plug, the absorbance of the 5-ASA-loaded pulsatile capsule in the release medium was measured against that of blank pulsatile capsules that correspond to each formulation.

2.7. Preparation of rat cecal content medium

The simulated colonic solution for rat cecal content consisted of 10 mmol L^{-1} $NaHCO_3$, 20 mmol L^{-1} $Na_2HPO_4{\cdot}12H_2O$, 8 mmol L^{-1} NaCl, 6 mmol L^{-1} KCl, 0.5 mmol L^{-1} $CaCl_2{\cdot}2H_2O$, and 0.4 mmol L^{-1} $MgCl_2{\cdot}6H_2O$ in 1000 mL of water, and was stored in a refrigerator (Tozaki et al., 1997).

Four male and female Sprague-Dawley rats with weights ranging from 200 to 300 g were fed with 5% KGM solution at 3 mL/d; through this approach, microbial enzymes were activated in vivo. A week later, the rats were killed using an injection of sodium pentobarbital. Then, the abdomens of the rats were opened and their ceca were ligated at both ends. Immediately, the rat cecal content was suspended in the simulated colonic solution described above and the mixture was continuously aerated with nitrogen to

maintain anaerobic conditions. Finally, the rat cecal content medium was diluted to 5% and stored in the refrigerator for dissolution testing (Gliko-Kabir, Yagen, Baluom, & Rubinstein, 2000; Zhang & Neau, 2002).

2.8. In vivo study

2.8.1. Drug administration

Beagle dogs received a single oral dose of the pulsatile capsule (100 mg plug tablet, HPMC = E50-LV; KGM/lactose/HPMC ratio = 5:4:1, w/w/w) with 200 mL of water. The dogs were fed at 6 h post-dosing, and water were given ad libitum from 2 h post-dosing onwards. Approximately 4 mL blood samples were collected in heparinized tubes using an indwelling cannula at 0, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 h after administration of the test capsules. The blood samples were centrifuged at 3000 rpm for 10 min, and plasma was separated and kept frozen at −20 °C.

2.8.2. Preparation of plasma samples

Plasma sample was prepared by mixing 0.2 mL plasma with 10 μL propanoic anhydride, and then vortexed for 30 min. The resulting sample was mixed with 0.8 mL methanol and vortexed for 3 min. The denatured protein precipitate was separated by centrifugation at 12,000 rpm for 10 min. The supernate was then evaporated to dryness below 40 °C in a vacuum and dissolved in 40 μL of the mobile phase; 20 μL of this sample solution was injected into high-performance liquid chromatography (HPLC) system for analysis. The same procedure was used to determine the recovery and precision in plasma.

2.8.3. HPLC chromatographic conditions

A Shimadzu LC-2010A HPLC system (Shimadzu, Japan) was used in the drug analysis. Chromatography was carried out on an ODS column (Hypersil C18 200 mm × 4.6 mm, 5 μm, Elite, Dalian). The mobile phase consisted of 0.1 mol L^{-1} acetic acid–methanol–triethylamine (500:167:1, v:v:v) and the flow rate was 1.0 mL min^{-1}. Detection was performed at a wavelength of 311 nm under a constant temperature of 35 °C.

2.8.4. Pharmacokinetics analysis

The peak plasma of drug concentration (C_{max}) and the time to reach peak concentration (T_{max}) were obtained from the graph. The area under the plasma concentration versus time curve ($AUC_{0\rightarrow t}$) was calculated using the linear trapezoidal rule from 0 to the last time point.

3. Results and discussion

3.1. Influence of varying KGM/lactose/HPMC proportions on the release of 5-ASA from the pulsatile capsule

Based on a large number of preliminary experiments, different formulations of KGM/lactose/HPMC (5:4:1, 6:3:1, 7:2:1, and 8:1:1) were used to prepare plug tablet. Fig. 2 shows the effect of plug composition on lag time. To achieve the desired gel-forming ability, KGM was formulated with HPMC and lactose. By adjusting the ratio of KGM/lactose/HPMC, a pulsatile drug release with a controllable lag time was achieved in the current study.

The plug tablet would collapse within 10 min in the release medium, with KGM as a single formulation. In contrast, a sufficiently strong gel formed by a mixture of KGM and HPMC might retain the integrity of the tablet longer. With the increased content of KGM, larger gel expansion was achieved, which blocked the capsule mouth. The addition of lactose quickened the erosion of plug tablet. As indicated in Fig. 2, with increased lactose and decreased KGM, less swelling gels were formed in PBS at pH 6.8; in contrast,

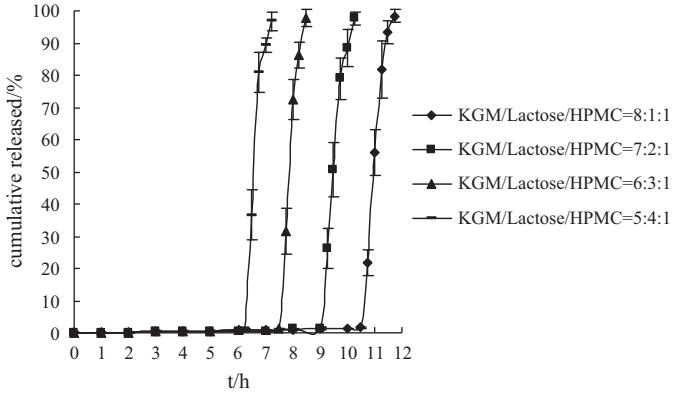

Fig. 2. Influence of the different proportion of KGM/lactose/HPMC on 5-ASA release from pulsatile capsule ($n = 3$). The dissolution study was carried out first in 0.1 N HCl for 2 h, PBS 6.8 for 3 h, then placed in pH 6.0 acetic acid–sodium acetate buffer, containing 0.5% β-glucanase (the plug tablet, HPMC = E50-LV).

the lactose of small molecules accelerated the erosion of gel when attacked by the colonic medium, thereby shortening the lag time.

3.2. Influence of the HPMC viscosity on the release of 5-ASA from the pulsatile capsule

Four different viscosity grades of HPMC (E15-LV, E50-LV, K4M, and K15M) were used to prepare plug tablets as describe above. The results obtained for all types of HPMC are shown in Fig. 3. The lag time using HPMC with higher viscosity (HPMC K15M) was too long (>10 h); a strong gel matrix structure formed by the combination of HPMC K15M with KGM, which took a long time to degrade completely in the enzyme solution. The HPMC with lower viscosity (HPMC E15-LV) produced a weaker gel because of a loose gel structure. Consequently, the plug tablet disintegrated within 3 h, and resulted in an early drug release.

3.3. Influence of the varying weights of plug tablet on the release of 5-ASA from the pulsatile capsule

Plug tablets of different weights (80, 100, and 120 mg) with the corresponding thickness were compressed at a KGM/lactose/HPMC ratio of 5:4:1 and the influence of the weight of the plug tablet on lag time were determined.

Fig. 4 reveals the lag time prior to drug release was prolonged with the increasing plug tablet weight because more time was

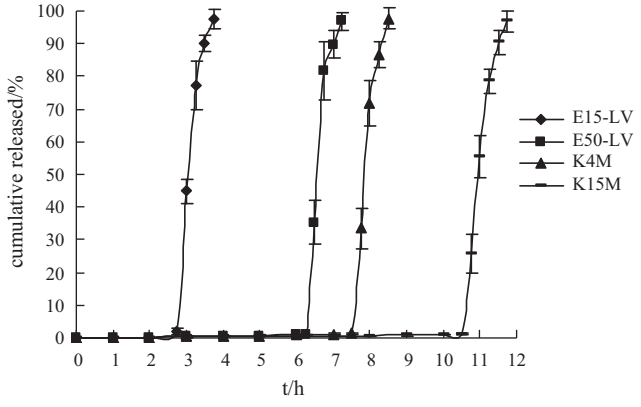

Fig. 3. Influence of the HPMC viscosity grade on 5-ASA release from pulsatile capsule ($n = 3$). The dissolution study was carried out first in 0.1 N HCl for 2 h, PBS 6.8 for 3 h, then placed in pH 6.0 acetic acid–sodium acetate buffer, containing 0.5% β-glucanase (the plug tablet, KGM/lactose/HPMC = 5:4:1, w/w/w).

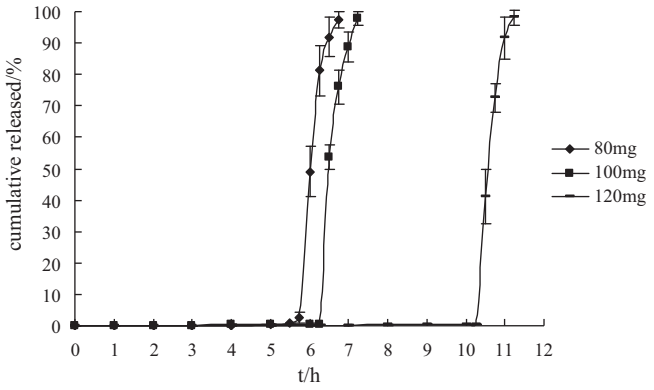

Fig. 4. Influence of the different weight of plug tablet on 5-ASA release from pulsatile capsule ($n = 3$). The dissolution study was carried out first in 0.1 N HCl for 2 h, PBS 6.8 for 3 h, then placed in pH 6.0 acetic acid–sodium acetate buffer, containing 0.1%, 0.3% or 0.5% β-glucanase (the plug tablet, HPMC = E50-LV; KGM/lactose/HPMC = 5:4:1, w/w/w).

necessary for the erosion of the thicker gel layer in PBS pH 6.8. Based on a suitable lag time of 5–6 h through which the drug reached the colon, 80 and 100 mg are possible weights for the plug tablet. Further studies demonstrated that the tablet was too thin and poorly shaped with a lower weight of 80 mg, and fitting them flush with the capsule mouth was difficult, which greatly affected the reproducibility of the lag time. Thus, 100 mg was chosen as the most suitable weight for controlling the lag time.

3.4. Influence of oscillating speed on drug pulsatile release

The dissolution testing was carried out with oscillating speed of 50, 75, and 100 rpm. Fig. 5 shows formulations with higher speed have a tendency to release the drug faster than those with lower speed. The degradation of the plug tablet was related to the mechanical strength of erosion, which is significantly affected by the oscillation speed. The increased speed led to higher flow rates of the release medium around the capsule mouth, which promoted the erosion of plug tablet and consequently reduced the lag time.

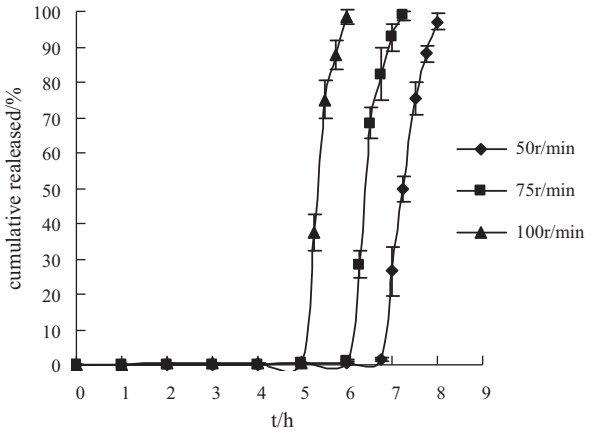

Fig. 5. Influence of rotation rate on the drug pulsatile release from pulsatile capsule ($n = 3$). The dissolution study was carried out first in 0.1 N HCl for 2 h, PBS 6.8 for 3 h, then placed in pH 6.0 acetic acid–sodium acetate buffer, containing 0.5% β-glucanase (the plug tablet, HPMC = E50-LV; KGM/lactose/HPMC = 5:4:1, w/w/w).

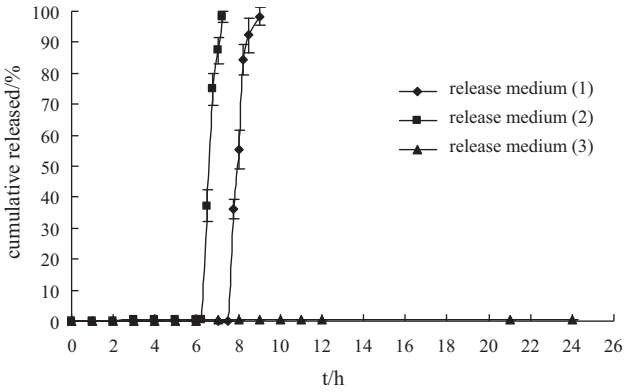

Fig. 6. Influence of the different release medium on 5-ASA release from pulsatile capsule ($n = 3$) (the plug tablet weight 100 mg, HPMC = E50-LV; KGM/lactose/HPMC = 5:4:1, w/w/w).

3.5. Influence of the release medium on pulsatile drug release

3.5.1. Dissolution profiles of the pulsatile capsule in three simulated colonic environment

The dissolution study was carried out in the following release media: (1) 0.1 N HCl for 2 h, PBS 6.8 for 3 h, then placed in rat cecal content release medium, (2) 0.1 N HCl for 2 h, PBS 6.8 for 3 h, then placed in pH 6.0 acetic acid–sodium acetate buffer containing 0.5% β-glucanase, and (3) 0.1 N HCl for 2 h, then PBS 6.8 for the remaining time.

Fig. 6 shows that the plug tablet responded well to the β-glucanase and the rat cecal content, but the effect was more marked with β-glucanase. However, in the absence of enzyme or rat cecal content, the plug tablet still maintained its shape and gel strength for 24 h in PBS 6.8, which indicates that the enzymatically triggered profile of the KGM plug tablet. β-Glucanase played a significant role in the degradation of the KGM gel, which resulted in a drug release 1 h earlier than those with the rat cecal content. This suggests that the enzyme contained in the release media exhibited different enzymatic degradation rates, which effectively controlled the lag time. As purified products, β-glucanase improves the specific degradation over KGM gel; meanwhile, access to the KGM backbone must be hindered to some extent by some non-active enzyme in the rat cecal content.

3.5.2. Dissolution profiles of the pulsatile capsule in the simulated colon environment with varying enzyme content

Different amounts of β-glucanase (0.1%, 0.3%, and 0.5%) were added into the simulated colonic release media of pH 6.0 acid-sodium acetate buffer solution to study the effect of the enzyme quantity on drug release. The lag time decreased with increasing β-glucanase concentration. Fig. 7 illustrates that higher amounts of enzyme accelerated the erosion of the gel layer. In addition, enzymatic degradation was detectable even at the lowest enzyme concentration. At the highest β-glucanase concentration, the degradation of KGM gel was practically complete within 5 h. In contrast, the drug release was delayed to 10 h later with 0.1% β-glucanase.

3.6. Pharmacokinetics analysis

The pulsatile capsule (100 mg plug tablet, HPMC = E50-LV; KGM/lactose/HPMC ratio = 5:4:1, w/w/w) was chosen as the test capsule for the animal study because its lag time was suitable for colon-specific drug delivery.

The validated HPLC method was applied to a pharmacokinetics study, and yielded satisfactory results for 5-ASA determination

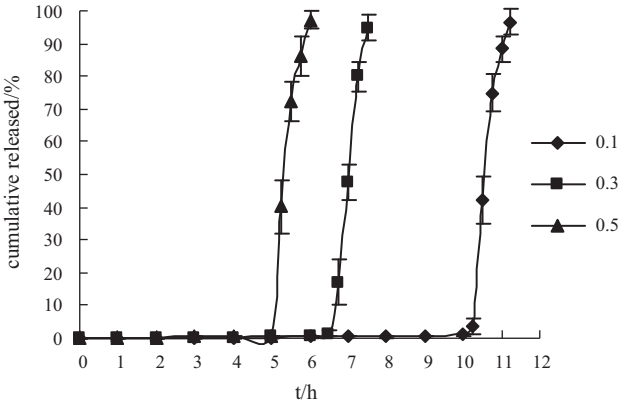

Fig. 7. Influence of the different amount of β-glucanase on 5-ASA release from pulsatile capsule ($n = 3$). The dissolution study was carried out first in 0.1 N HCl for 2 h, PBS 6.8 for 3 h, then placed in pH 6.0 acetic acid–sodium acetate buffer, containing 0.1%, 0.3% or 0.5% β-glucanase (the plug tablet weight 100 mg, HPMC = E50-LV; KGM/lactose/HPMC = 5:4:1, w/w/w).

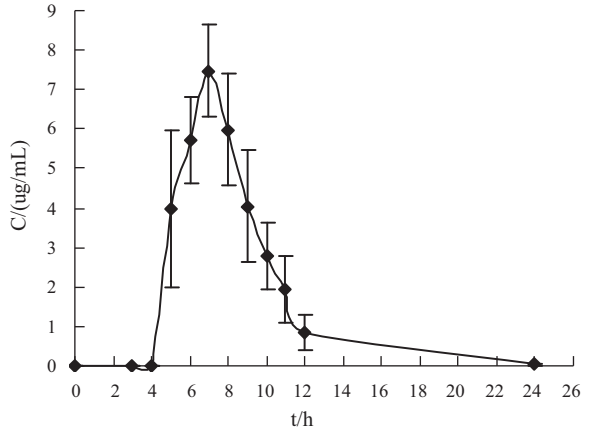

Fig. 8. Drug plasma concentration–time profiles of 3 beagle dogs after oral administration of 5-ASA pulsatile capsule (the plug tablet weight 100 mg, HPMC = E50-LV; KGM/lactose/HPMC = 5:4:1, w/w/w).

of the plasma samples from three beagle dogs following a single oral dose of the pulsatile capsule. Fig. 8 shows the mean plasma concentration–time profile of three beagle dogs. The related pharmacokinetic parameters are as follows: $AUC_{0 \rightarrow t} = 37.70$ μg h/mL, $C_{max} = 7.47$ μg/mL, and $T_{max} = 7$ h.

Based on the drug plasma concentration–time curves for the pulsatile capsule with the KGM plug tablet, the drug can be detected after 5 h, indirectly proving the colon-specific characteristics. At the same time, the pulsatile capsule showed typical pulsatile release profiles with a lag time followed by a rapid release phase.

4. Conclusions

An oral drug delivery system based on an impermeable cylinder and a KGM plug tablet placed within the cylinder was developed, which produces potential colon-specific, pulsatile drug release profiles. When the capsule was swallowed, the enteric cap did not dissolve in the gastric fluid until it reached the small intestines, exposing the plug tablet through peristalsis. KGM cannot be hydrolyzed by digestive enzymes in the upper gastrointestinal tract, but can be degraded by the microbial enzymes produced by the colonic flora. Hence, the capsule went through the small intestines without releasing the drug. In contrast, when the capsule reached the colon, the plug tablet disintegrated by the action of microbial enzyme produced by the colonic flora, and the drug contained in capsule was rapidly released at the expected site. Exhibiting site-specific effect on reducing the total amount of drug administered was possible because of the suitable lag time determined by the degradation of gel layer formed by the plug, thereby reducing the occurrence of side effects. The in vitro and in vivo experiments demonstrated that the drug delivery system can achieve the initial colon-specific characteristics, and the lag time was controlled by the plug tablet.

Acknowledgements

This project was supported by the Chongqing Yuzhong District Science and Technology Project (No. 20100203), Chongqing Municipal Education Commission-funded project (KJ110323), Chongqing Board of Health Project (2010-2-202) and Chongqing Natural Science Foundation (No. CSTC, 2008BB5397).

References

Alvarez-Mancenido, F., Landin, M., & Martinez-Pacheco, R. (2008). Konjac glucomannan/xanthan gum enzyme sensitive binary mixtures for colonic drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*, *69*, 573–581.

Fan, L. F., He, W., Bai, M., Du, Q., Xiang, B., Chang, Y. Z., et al. (2008). Biphasic drug release: Permeability and swelling of pectin/ethylcellulose films, and in vitro and in vivo correlation of film-coated pellets in dogs. *Chemical and Pharmaceutical Bulletin*, *56*, 1118–1125.

Freire, C., Podczeck, F., Veiga, F., & Sousa, J. (2010). Influence of the coating formulation on enzymatic digestibility and drug release from 5-aminosalicylic acid pellets coated with mixtures of high-amylose starch and Surelease intended for colon-specific drug delivery. *Drug Development and Industrial Pharmacy*, *36*, 161–172.

Friend, D. R. (2005). New oral delivery systems for treatment of inflammatory bowel disease. *Advanced Drug Delivery Reviews*, *57*, 247–265.

Gazzaniga, A., Maroni, A., Sangalli, M. E., & Zema, L. (2006). Time-controlled oral delivery systems for colon targeting. *Expert Opinion on Drug Delivery*, *3*, 583–597.

Gliko-Kabir, I., Yagen, B., Baluom, M., & Rubinstein, A. (2000). Phosphated crosslinked guar for colon-specific drug delivery. II. In vitro and in vivo evaluation in the rat. *Journal of Controlled Release*, *63*, 129–134.

Gupta, V. K., Beckert, T. E., & Price, J. C. (2001). A novel pH- and time-based multi-unit potential colonic drug delivery system. I. Development. *International Journal of Pharmaceutics*, *213*, 83–91.

Jones, S. P. (1996). Preparation and evaluation of a time-controlled release capsule made of ethylcellulose for colon delivery of drugs. *Journal of Drug Targeting*, *3*, 477–478.

Khan, M. Z., Stedul, H. P., & Kurjakovic, N. (2000). A pH-dependent colon-targeted oral drug delivery system using methacrylic acid copolymers. II. Manipulation of drug release using Eudragit L100 and Eudragit S100 combinations. *Drug Development and Industrial Pharmacy*, *26*, 549–554.

Krogel, I., & Bodmeier, R. (1998). Pulsatile drug release from an insoluble capsule body controlled by an erodible plug. *Pharmaceutical Research*, *15*, 474–481.

Krogel, I., & Bodmeier, R. (1999a). Development of a multifunctional matrix drug delivery system surrounded by an impermeable cylinder. *Journal of Controlled Release*, *61*, 43–50.

Krogel, I., & Bodmeier, R. (1999b). Evaluation of an enzyme-containing capsular shaped pulsatile drug delivery system. *Pharmaceutical Research*, *16*, 1424–1429.

Matsuura, Y. (1998). Degradation of konjac glucomannan by enzymes in human feces and formation of short-chain fatty acids by intestinal anaerobic bacteria. *Journal of Nutritional Science and Vitaminology*, *44*, 423–436.

Nakajima, N., & Matsuura, Y. (1997). Purification and characterization of konjac glucomannan degrading enzyme from anaerobic human intestinal bacterium, *Clostridium butyricum–Clostridium beijerinckii* group. *Bioscience, Biotechnology and Biochemistry*, *61*, 1739–1742.

Peerapattana, J., Otsuka, K., & Otsuka, M. (2004). Time-controlled pulse-drug release from dry-coated wax matrix tablets for colon drug delivery. *Bio-medical Materials and Engineering*, *14*, 293–301.

Pertuit, D., Moulari, B., Betz, T., Nadaradjane, A., Neumann, D., Ismaili, L., et al. (2007). 5-Amino salicylic acid bound nanoparticles for the therapy of inflammatory bowel disease. *Journal of Controlled Release*, *123*, 211–218.

Sinha, V. R., & Kumria, R. (2001). Polysaccharides in colon-specific drug delivery. *International Journal of Pharmaceutics*, *224*, 19–38.

Tozaki, H., Emi, Y., Horisaka, E., Fujita, T., Yamamoto, A., & Muranishi, S. (1997). Degradation of insulin and calcitonin and their protection by various protease inhibitors in rat caecal contents: Implications in peptide delivery to the colon. *The Journal of Pharmacy and Pharmacology*, *49*, 164–168.

Williams, M. A., Foster, T. J., Martin, D. R., Norton, I. T., Yoshimura, M., & Nishinari, K. (2000). A molecular description of the gelation mechanism of konjac mannan. *Biomacromolecules*, *1*, 440–450.
Yang, L., Chu, J. S., & Fix, J. A. (2002). Colon-specific drug delivery: New approaches and in vitro/in vivo evaluation. *International Journal of Pharmaceutics*, *235*, 1–15.
Zhang, H., & Neau, S. H. (2002). In vitro degradation of chitosan by bacterial enzymes from rat cecal and colonic contents. *Biomaterials*, *23*, 2761–2766.
Zhang, H., Yoshimura, M., Nishinari, K., Williams, M. A., Foster, T. J., & Norton, I. T. (2001). Gelation behavior of konjac glucomannan with different molecular weights. *Biopolymers*, *59*, 38–50.