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RESEARCH ARTICLE

Development of multiple-unit colon-targeted drug delivery system by using alginate: *in vitro* and *in vivo* evaluation

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Abstract

Drug delivery systems to the colon are being actively investigated. However, it is difficult to ensure that an oral preparation disintegrates specifically in the human colon. In this study, a pH- and enzyme-controlled, colon-targeted tablets (PECCTT) was established by using outer pH-coated layer and inner alginate-coated compression layer. The influence of the amount of alginate and enteric coat thickness on drug release had been investigated and the formulation that contained 30% alginate in compression layer and 13% weight gain in pH-coated layer was proved to protect the drug release from stomach and small intestine, the lag time was 7.04 ± 0.17 h, and 84.45 ± 1.3% of prednisone was released at 12 h. The results of drug release behaviors and SEM study indicated that drug release mechanism of PECCTT was corrosion. Hybrid scanner combining SPECT and CT was employed to monitor ^{99m}Tc-contained tablets in the human gastrointestinal tract (GIT) and to obtain the images of the disintegration process. The results showed that the tablet remained intact during its transit through the upper GIT, the anatomical site of disintegration was found to be the sigmoidal colon, and the disintegration of the tablet started at 8 h post-dose in the volunteer.

Keywords: pH-coated, alginate, β-mannanase medium, SPECT/CT, colon targeting

Introduction

Inflammatory bowel disease (IBD) such as ulcerative colitis and Crohn's disease is becoming a significant problem as a severe, chronic, and refractory disease and increasing recently[1]. Steroids and immunosuppressive drugs such as prednisolone are usually used as anti-inflammatory medicines in those diseases. However, these agents are often accompanied by toxic side effects, which are mainly based on systemic absorption. 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 such as IBD. Unfortunately, it is difficult to ensure that an oral preparation disintegrates specifically in the human colon. Therefore, it is necessary to develop a new colon-targeted drug delivery system that could protect prednisolone from releasing in stomach and small intestine and cure rheumatic disease or ulcer with higher biological availability.

Various techniques are available for the colon-targeted delivery, which are broadly classified as single-unit and multiple-unit systems[2]. Single-unit drug delivery system may suffer from the disadvantage of unintentional disintegration of the formulation due to manufacturing deficiency or unusual gastric physiology that may lead to drastically reduce drug bioavailability or loss of local therapeutic action. In recent pharmaceutical applications involving colon delivery, multiple-unit dosage forms are gaining much favor over single-unit dosage forms. The potential benefits include increasing bioavailability; predictable, reproducible, and generally short gastric residence time; no risk of dose dumping; reducing risk of local irritation; and the flexibility to blend with different compositions or release patterns in tablets or pellets. So in this study, a multiple-unit tablet was developed to achieve the colon-targeted delivery of prednisolone.

In our previous study, a multiple-unit colon-targeted drug delivery system was developed by using guar gum

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and pH-sensitive material (acrylic resin). Alginate is a good candidate for colon-targeted delivery systems and commonly used for a family of unbranched polymers composed of 1,4-linked β-D-mannuronic and α-L-guluronic acid residues in varying proportions, sequence, and molecular weight[3]. Alginate gelation takes place when divalent cations (usually Ca^{2+}) interact ionically with blocks of guluronic acid residues, resulting in formation of three-dimensional network, which is usually described by egg-box model[4]. And the alginate is predominantly metabolized by colonic bacteria when it travels in the gastrointestinal tract (GIT). All of the above make alginate to be a nice candidate for multiple-unit drug delivery system for local colon-specific drug treatment.

In the field of biopharmaceutical research, it has become evident that *in vitro* studies alone are inadequate when developing modified-release drug formulations. Therefore, *in vivo* behaviors of these dosage forms need to be investigated at an early stage. One of the most appropriate means of studying the fates of site-specific formulations in GI tract nowadays is gamma scintigraphy[5,6]. In most studies, radioactive technetium (^{99m}Tc), indium (^{111}In), or samarium oxide (Sm_2O_3) has been used as a marker[7].

Technetium-99m (^{99m}Tc) has a half-life of 6.03 h and a monoenergetic gamma emission of 140 keV is commonly used because it is safe and inexpensive[8]. However, there are some disadvantages of gamma scintigraphy such as it could not figure out the exact position of the dose after it was swallowed by volunteers. According to this, computed tomography (CT) was introduced in the study to identify the exact position of the tablet because of its density resolution of soft tissue and organs. SPECT/CT was used in this study to identify the exact position of the multiple-unit colon-targeted drug delivery system for local colon-specific drug treatment for the first time.

Materials and methods

Materials

Prednisolone was purchased from Tianyao Pharmaceutical Group Co., Ltd. (Tianjin, China). Alginate was purchased from Kelong Pharmaceutical Group Co., Ltd (Chengdu, China). Acrylic resin was provided by Huzhou Zhanwang Pharmaceutical Co., Ltd.(Hubei, China); β-mannanase was a generous gift from Bo Shao Biological Technology Co., Ltd. (Beijing, China); and all other reagents were of analytical grade.

Preparation of simulated fluid

Simulated gastric fluid (SGF) and simulated small intestinal fluid (SIF) were prepared as following the USP30-NF25.

And a series of solutions containing β-mannanase were prepared by dissolving β-mannanase to different concentrations (N_1: 0 U/mL, N_2: 0.315 U/mL, N_3: 0.601 U/mL, N_4: 1.800 U/mL, or N_5: 3.022 U/mL) with phosphate buffer of pH 7.4 as simulated colon fluid 1 (SCF 1). Such a system containing β-mannanase that could specifically degrade alginate was used as one of the release media. The rat cecal content medium was also prepared as description in our previous study as simulated colon fluid 2 (SCF 2). In brief, rats were killed by spinal traction. The abdomen were opened, and 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, and their contents were individually weighed, pooled, and suspended in the buffer continuously bubbled with nitrogen. After centrifuging at 500 *g* for 15 min to remove debris, supernatants were then centrifuged at 15,000 *g* for another 30 min in order to obtain a clear supernatant containing extracellular enzymes (4%)[9].

UV and HPLC analysis of prednisolone

Quantitative determination of prednisolone was performed by both UV spectrophotometer (Agilent technologies 1200 series) and high-performance liquid chromatography (Agilent Technologies, Santa Clara, CA).

The HPLC analysis was performed on an Diamonsil™ C18 column (200 mm × 4.6 mm, 5 μm) with a column temperature of 35°C. The mobile phase was methanol–distilled water (TD water) (55:45). The filtered mobile phase was pumped at a flow rate of 1.0 mL/min. The eluent was detected by UV detection at 247 nm, and the data were acquired, stored, and analyzed with the software Agilent Chemstation (Agilent).

Three standard curves were plotted for prednisolone with the range of 1–20 μg/mL in methanol, phosphate buffer (pH 6.8), phosphate buffer (pH 7.4), respectively, at 247 nm using a UV spectrophotometer. Two more standard curves were also constructed for prednisolone with the range of 1–20 μg/mL in β-mannanase medium and 4% rat cecal content medium, respectively, by HPLC.

Preparation of pH- and enzyme-controlled, colon-targeted tablets

The pH- and enzyme-controlled, colon-targeted tablets (PECCTT) consisted of a fast disintegrating core (containing prednisolone), an inner enzyme-sensitive compression-coated layer containing alginate, and an outer enteric-coating layer.

Each core tablet (average weight of 150 mg) consisted of prednisolone (15 mg), mannitol (72 mg), lactose (48 mg), cross-linked sodium carboxymethyl cellulose (3 mg), sodium carboxymethyl starch (CMS-Na, 3 mg), talc (3 mg), and magnesium stearate (6 mg). Polyvinylpyrrolidone K-30 (PVP, 6%, w/v), absolute ethanol solution, was used as adhesive. The diameter of the core tablet was 7.0 mm. The hardness, content uniformity, friability, and disintegration of the core tablets were tested.

The core tablets were compression-coated with different coat formations F_1, F_2, F_3, F_4, F_5 containing 0, 15, 30, 45, and 60 mg of alginate (Table 1). Microcrystalline cellulose (MCC), L-HPC lactose, and cross-linked sodium

carboxymethyl cellulose were added as shown in Table 1. PVP (3 mg) was added as adhesive material. The diameter of the compression-coated tablets was 9 mm.

Different weight gains (E_1: 10%, E_2: 12%, E_3: 13%, E_4: 14%, E_5: 15%, and E_6: 17% respectively, w/w) of enteric layer materials were coated on the surface of compression-coated tablet. The procedure of preparation of PECCTT and *in vitro* drug release study was followed our previous study. The fast disintegrating core tablets were prepared by wet granulation compression technique. The inner enzyme-sensitive compression coated layer with different contents of alginate was prepared by direct compression technique. And the outer enteric-coating layer was prepared by spraying coating method.

In vitro drug release studies

In order to investigate the ability of the PECCTT 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 (USP30-NF25, 2007). The same procedure was followed and is shown in Table 2. Three milliliter sample was withdrawn from the dissolution medium at hourly intervals and 3 mL of simulate fluid was added into the dissolution media every hour. Samples were analyzed separately. Each experiment was run in triplicate ($n=3$).

Scanning electron microscopic analysis

The surface morphology of PECCTT was examined by scanning electron microscope (SEM; Jeol 6100, Jeol, Japan). The samples were dried at 35°C for 12 h and stored between 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, Japan) for 5–10 min and examined under SEM. The magnification selected was sufficient to appreciate the general morphology of the samples in detail under study. PECCTT morphology was investigated before and during the *in vitro* drug release study at different time intervals.

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

	Quality (mg/tablet) present				
Composition	F1	F2	F3	F4	F5
Alginate	0	15	30	45	60
MCC	88.67	76.67	67.67	56.67	46.67
L-HPC	43.33	38.33	33.33	28.33	23.33
PVP K-30	15	15	15	15	15
lactose	5	5	5	5	5
Total	150	150	150	150	150

100 tablets were prepared in each formulation.

Gamma scintigraphy

Safety requirements

Gamma spectra and radioactivity were measured to determine the safety of the formulations for use in human studies. Safety requirements were in accordance with the guidelines established by STUK (Finnish Radiation and Nuclear Safety Authority). The as-low-as-reasonably achievable (ALARA) principle was observed and exposure to radiation was minimized in all situations. Total radioactivity of 222 MBq (6 mCi) for the PECCTT corresponds with an effective absorbed dose for the study subject.

In vivo gamma scintigraphy

Sixty microliters of ^{99m}Tc fluid was added on the surface of core table and then dried by air-blower, and the enzyme-sensitive compression-coated layer and enteric-coating layer were prepared as described in the section "Preparation of pH- and enzyme-controlled, colon-targeted tablets." The total radiation dosimetry for the subject was 6 mCi ^{99m}Tc.

A healthy female volunteer (24 years of age) participated in the study. The weight was 53 kg and body mass index (BMI) was 20.7 kg/m^2. Before the study, the volunteer was examined physically and subjected to routine hematological testing and urine analysis. The volunteer was informed about the possible risks and adverse effects of taking the study formulations. Written informed consent to participate in the study was obtained. The investigation was carried out in accordance with International Conference of Harmonization (ICH), Good Clinical Practice Guidelines, and the Declaration of Helsinki (World Medical Assembly, 1964) and subsequent amendments. The study protocol had been approved by the Ethics Committee of Sichuan University. The studies were carried out at West China Hospital of Sichuan University Division of Nuclear Medicine, which has a radiation safety license issued by STUK. Scintigraphic studies were carried out immediately after administration to volunteers. At each time point, the subject was positioned for 25-sec acquisitions in the anterior followed by the posterior aspect. An early planar image (scanning 10 min, matrix 256×256, energy peak 140 keV) and the SPECT acquisition parameters for SLN detection include matrix 128×128, energy peak 140 keV window width 20%, 1 frame per 6, 30 sec per frame, totally

Table 2. Drug release procedure.

	Stomach	Small intestinal	Colon
Simulate fluid	Simulated gastric fluid	Simulated small intestinal fluid	Simulated colon fluid
Volume	150 mL	200 mL	100 mL
pH	1.2	6.8	7.4
Experimental session	2 h	3 h	7 h
Rotation speed	75 r/min	75 r/min	55 r/min
Temperature	37°C	37°C	37°C

30 frames, zoom 1.0, electric current 2.5 mA, slice thickness 10 mm, totally 40 sec lice). The result was analyzed by Philips JETStream workspace software.

Subject was instructed to fast from 10 p.m. the night prior to the study day. Upon arrival at the study center, the subject was questioned on compliance to the study restrictions. The subject was dosed with one ^{99m}Tc-labeled tablet, swallowed with 200 mL water. The imaging schedule was as follows: immediately after dosing, and then every hour interval until complete release of the ^{99m}Tc radiolabel was confirmed. The maximum post-dose time is 24 h in supine position. Between imaging times, the subject was allowed to move freely. The subject received a standard lunch at 4 h post-dose, a snack at 7 h post-dose, and a standard dinner at 10 h post-dose if imaging was ongoing. Decaffeinated fluids were available *ad libitum* after lunch.

Results and discussion

Multiple-unit prednisolone tablets

As a result of UV and HPLC analysis of prednisolone, good linear relationships were observed in the five lines with high correlation coefficient ($r \geq 0.9998$). The UV and HPLC method used in the study was found to be precise and accurate as indicated by <3.21% RSD (intra- and inter-day) and high recovery of 99.6–100.8% of prednisolone.

The weight, average drug content, hardness, friability test, disintegrate of the core tablets of prednisolone formulated in the study were found to possess the required characteristics for compression-coating with alginate.

After coating the enteric membrane, the PECCTTs were completely developed.

In vitro drug release study in SCF

The effect of varying concentration of rat cecal contents within the range from 4% to 38% had been researched. The result concluded that the different concentration of rat cecal contents had the similar effect on the releasing of prednisolone. Therefore, the SCF 2 containing 4% rat cecal was selected to be compared with SCF 1 with β-mannanase (data not shown).

Our previous study indicated that β-mannanase medium could not substitute for rat cecal content and human feces slurries *in vitro* assessment of the performance of degrading guar gum. The possible reason might be that the tablet contained cyclodextrin in the compression-coated layer, and the β-mannanase had a weak ability to hydrolyze cyclodextrin. So cyclodextrin was removed from current study, and alginate was substituted for guar gum. Whether β-mannanase medium had the similar effect with rat cecal content on degraded alginate was investigated. 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 alginate-based colon delivery systems.

Figure 1 showed that cumulative percentage of prednisolone released from PECCTT in SGF (2 h), SIF (3 h), and then followed by five different concentrations of β-mannanase in SCF 1 (N_1: 0 U/mL, N_2: 0.315 U/mL, N_3: 0.601 U/mL, N_4: 1.800 U/mL, or N_5: 3.022 U/mL) and SCF 2 (4%, rat male). The release profiles revealed that under each condition, prednisolone was not released during the first 5 h in SGF (2 h) and SIF (3 h) medium.

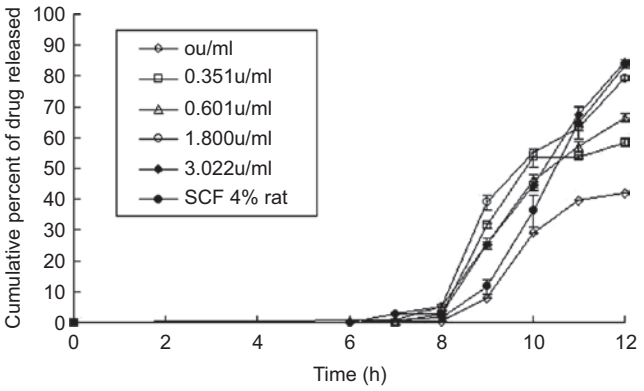

Figure 1. Cumulative percentage of prednisolone released from pH- and enzyme-controlled, colon-targeted tablets (PECCTT) ($n=3$) in simulated gastric fluid (SGF) (2 h), simulated small intestinal fluid (SIF) (3 h), then followed by 4% rat cecal content medium (male), or five different concentrations of β-mannanase in simulated colon fluid (SCF) (0, 0.315, 0.601, 1.800, 3.022 U/mL).

It was also found from Figure 1 that the release of PECCTT had a good response to the addition of the β-mannanase and the microflora of rat cecum. It showed that the drug release rates varied in the media containing different quantities of β-mannanase. When the concentration of enzyme increased, the release rate of drug from PECCTT accelerated. It could be observed that the release rate of the drug in the media of 3.022 U/mL β-mannanase was similar with that in the 4% (w/v) rat cecal content solution ($f_2 = 53.70$), so the media of 3.022 U/mL β-mannanase could be employed to mimic the colon environment in the following studies *in vitro*.

Different concentrations of the β-mannanase had been used in some others studies. It was reported that the proper concentration of the β-mannanase solution simulating colon condition was 0.166 U/mL[10]. However, Fan et al.[11] reported that the media containing 0.220 U/mL β-mannanase was the proper concentration in matrix tablet. Moreover, in our previous study, β-mannanase may not substitute for rat cecal content medium to mimic the degradation in colon, because of β-cyclodextrin in the compression-coated tablets. All the above indicated that the concentration of β-mannanase should be studied in different formulations. The proper concentration of β-mannanase in study was much higher than that was reported in other study. The proper reason might be β-mannanase hydrolyze different plant gums at different rates.

Formulation aspects of PECCTT

Effect of amount of alginate on drug release

Since the extent of digestion was directly proportional to the amount of alginate present, five different amounts

(F_1: 0 mg, F_2: 15 mg, F_3: 30 mg, F_4: 45 mg, and F_5: 60 mg, respectively) of alginate were taken (Table 1) in compression-coat to observe its effect on drug release over 12-h dissolution studies.

Figure 2 illustrated the influence of different amounts of alginate on drug release from PECCTT. It clearly indicated that the amount of drug release from these formulations was highly dependent on the amount of alginate. As shown in Figure 2, the tablets of formulation F_1 (alginate 0%) without any alginate released from 2 h in the SGF and the cumulative percentage of drug release reached 86.95 ± 0.2% at 5 h (lag time was 1.9 h) in the SIF. Formulations F_2 (alginate 10%) and F_3 (alginate 20%) also did not release drug at the end of 2 h in SGF, both of which released drug in the SIF at the end of 5 h (18.89 ± 1.8%, 7.82 ± 0.5%, respectively) in SIF. The lag time of F_2 and F_3 were 2.2 ± 0.11 h and 2.5 ± 0.15 h, respectively. No prednisolone was released from formulations F_4 and F_5 at the end of 5 h dissolution study in SIF. This indicated that a minimal amount of the drug was released from the multiple-unit formulations in the physiological environment of stomach and small intestine. The formulations of compression-coating composition F_4 (alginate 30%) and F_5 (alginate 40%) released 84.45 ± 1.3% and 70.15 ± 1.6% of prednisolone, respectively, after completing the dissolution studies at 12 h. And the lag time of F_4 and F_5 were 7.04 ± 0.17 h and 8.35 ± 38 h, respectively. Upon increasing the amount of alginate in the coating of compression-coated tablets, the release of prednisolone at the end of 12-h dissolution study decreased.

The possible reason could be that as increasing of the content of the swellable polymer in the formulation, the gel thickness increases upon swelling[12]. This increased the diffusion path length, which in turn decreased the drug release from the tablet. Alginate hydrated and swelled forming viscous colloidal dispersions or sols, which increased the diffusion path length. So the alginate retarded the drug release and the lag time was increased as the amount of alginate increased. Once these tablets were transferred into colon condition, alginate shall be disintegrated by β-mannanase and the drug shall be released from the dosage form. So a rapid release of drug could be observed after tablets were transferred to the β-mannanase medium at 8 h. Hence, the relative benefit of the F_4 (alginate 30%) formulation over the other formulations was adopted.

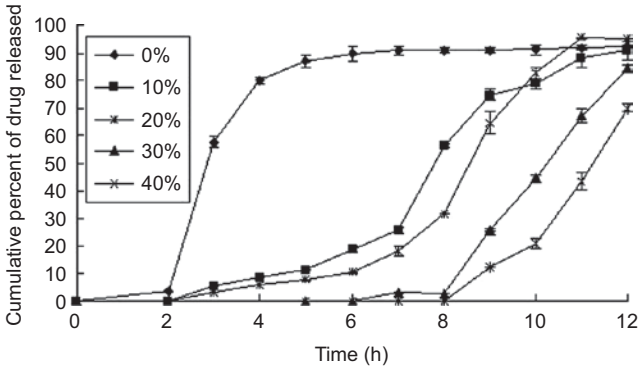

Figure 2. Cumulative percentage of prednisolone released from pH- and enzyme-controlled, colon-targeted tablets (PECCTT) (n=3) containing either 10%, 20%, 30%, or 40% of alginate in simulated gastric fluid (SGF) (2 h), simulated small intestinal fluid (SIF) (3 h), and simulated colon fluid 1 (SCF 1) (3.022 U/mL, 7 h).

Effect of enteric coat thickness on drug release

Based on the above result, the compression-coated formulation F_4 (alginate 30%) was selected as optimum for the following studies. During the past decade, a large number of pH-based delivery systems were developed with the intention of colon-targeted drug delivery. So the pH-dependent coating formulation was used following the previous study[13] with slight modification. Since the extent of digestion was directly proportional to the amount of enteric coat, six different coating levels (E_1: 10%, E_2: 12%, E_3: 13%, E_4: 14%, E_5: 15%, and E_6: 17% respectively, w/w) of enteric coat formulation as mentioned before were chosen to observe the effect on drug release over 12-h dissolution studies.

Figure 3 showed that the influence of different thickness of enteric coat on drug release from PECCTT. As can be observed, the cumulative percentage of drug release was dependent on coating thickness. So did the lag time. The formulation of compression-coating composition released prednisolone 95.44 ± 1.4% in E_1, 98.11 ± 1.2% in E_2, 84.45 ± 1.3% in E_3, 67.05 ± 1.6% in E_4, 69.86 ± 1.2% in E_5, and 39.33 ± 1.9% in E_6, respectively, after complete dissolution studies. The PECTTs of E_1 and E_2 were not integrated at the end of 5 h even though they did not release a large amount of drug, which showed that E_1 and E_2 cannot protect core tablet perfectly. The more weight gain of pH-coated would make the alginate less accessible to bacterial degradation, resulting in the slower drug release. At the end of 12 h, E_3 had released much more drug than E_4, E_5, and E_6. Hence, the relative benefit of the E_3 (13%, w/w) formulation over the other formulations was adopted.

Release mechanisms and mathematical modeling of release profiles

Release data obtained from the tablet formulations was fitted to various mathematical models corresponding to

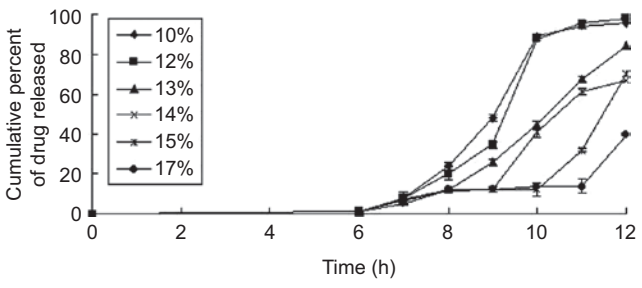

Figure 3. Cumulative percentage of prednisolone released from pH- and enzyme-controlled, colon-targeted tablets (PECCTT) (n=3) with coating levels of 10%, 12%, 13%, 14%, 15%, 17% in simulated gastric fluid (SGF) (2 h), simulated small intestinal fluid (SIF) (3 h), and simulated colon fluid 1 (SCF 1) (3.022 U/mL, 7 h).

possible release mechanisms, such as zero order, first order, Higuchi, Weibull, Hixson-Crowell, Ritger–Peppas, Niebergull. The regression equation of each was showed in Table 3.

To distinguish the models that described the data properly from those that did not fit the data correctly, the sum of the squared residuals (SSR) was obtained. The model that best explains the experimental data is the one that shows the minimal value for the SSR.

$$SSR = \sum_{i=1}^{n} [W_i (y_1 - y_2)^2$$

W_i is an optional weighing factor. The value was 1 in the test; y_1 is the actual value of drug release; y_2: theoretical value of drug release.

However, since a larger number of model parameters could lead to a higher probability of obtaining a smaller SSR value, it was necessary to use a discriminatory criterion that was independent of the number of parameters that each model had. For this reason, the Akaike Information Criterion (AIC) was applied. The model that shows the smallest value for the AIC is the one which, statistically, describes the best in the drug release mechanism.

$$\mathrm{AIC} = n(\ln \mathrm{SSR}) + 2P$$

n is the number of dissolution data points; P is the number of the parameters in the model. The value was 2 in the test[14].

R^2 (coefficient of determination), R^2_{adjusted} (adjusted coefficient of determination), r (correlation coefficient) were also introduced to describe the best of the drug release mechanism. The model that best explains the experimental data is the one that shows the value get close to 1 for the R^2, R^2_{adjusted}, r.

$$R^2_{\text{adjusted}} = 1 - \frac{(n-1)}{(n-P)}(1 - R^2).$$

n is the number of dissolution data points; P is the number of the parameters in the model. The value was 2 in the test; R^2 is the coefficient of determination.

$$r = \sqrt{R^2}$$

R^2 is the coefficient of determination.

The values of SSR, AIC, R^2, R^2_{adjusted}, r were showed in Table 3.

As seen from Table 3, Ritger–Peppas could be the optimization equations that described the drug release from the PECCTT.

The Ritger–Peppas semi-empirical equation was applied to the tablet swelling profile to determine the nature of the drug release process:

$$\mathrm{M_t}/\mathrm{M_\infty} = kt^n$$

M_t/M_∞ is the fraction of the solvent diffused into the polymer matrix after time t; k is a constant; and n is the diffusion exponent.

The double logarithmic plot of M_t/M_∞ against time of the initial stage of swelling gives a straight line with a slope of n. For cylindrical-shaped devices such as tablets, $n < 0.45$ corresponds to Fickian diffusion, whereas $0.45 < n < 0.89$ indicates anomalous or non-Fickian diffusion. However, when $n > 0.89$, the drug-releasing mechanism is corrosion[15,16]. The value of n was 1.197 in study so that the mechanism of drug release of PECCTT could be corrosion.

SEM analysis

In order to study changes of the surface morphology throughout the dissolution procedure and the mechanism of drug release from PECCTT, PECCTT was taken from the dissolution medium at 2, 5, 6, 7, 8, and 9 h, respectively, for SEM. The PECCTT had a regular and smooth surface and the membrane appeared to be integral and with no visible imperfections (Figure 4, 0 h), before submitting them to the release test. No evident morphological differences was observed after 2-h exposure to the pH 1.2 simulated gastric medium (Figure 4, 2 h), thus confirming the effectiveness of the pH-sensitive coating. The PECCTT appearance was largely intact with slight cracks after 5 h of exposure to the pH 6.8 simulated SIF (Figure 4, 5 h). The results demonstrated that the enteric-coating membrane could protect the alginate in the compression-coating from swelling in SIFs. Some distinct began to be visible starting from 6 h of exposure to the SCF 1, as shown in Figure 4, 6 h, larger cracks appeared in appearance of pH-sensitive membrane. Figure 4, 7 h had showed the appearance of pH-sensitive membrane and enzyme-sensitive (alginate) layer, after 2 h of exposure to the SCF 1. The pH-sensitive membrane had no longer integrity because of corrosion; therefore, corrosion holes appeared in the enzyme-sensitive (alginate) layer partly exposed in the SCF. After 3 or 4 h of exposure to the SCF 1, as shown in Figure 4,

Table 3. The regression equation of prednisolone release profile and parameters from the PECCTT.

Model	Regression equation	SSR	AIC	R^2 adjusted	R^2	r
Zero order	$y = 0.186$ (t – Tlag) – 0.082	0.00689	–17.8428	0.9860	0.9930	0.9965
First order	$\ln(1-y) = -0.428$ (t – T lag) + 0.468	0.03297	–6.2365	0.8600	0.9300	0.9644
Higuchi	$y = 0.591$ (t – Tlag)1/2 – 0.514	0.003334	–13.1108	0.9320	0.9660	0.9829
Weibull	$\ln\ln(1/(1-y)) = 1.604 \ln(t - \mathrm{Tlag}) - 2.114$	0.000251	–20.8681	0.9520	0.9760	0.9879
Hixson-Crowell	$(1-y)1/3 = -0.105$ (t – Tlag) + 1.094	0.01051	–9.6651	0.9240	0.9620	0.9808
Ritger–Peppas	$\ln y = 1.197 \ln(t - \mathrm{Tlag}) - 2.089$	0.001897	–14.8021	0.990	0.9950	0.9975
Neibergull	$(1-y)1/2 = -0.137$ (t–Tlag) + 1.108	0.005824	–11.4375	0.9460	0.9730	0.9864

8 h and 9h, the corrosion holes of enzyme-sensitive layer were enlarged after almost complete dissolution of the pH coating.

The SEM study indicated that the dissolution and drug release mechanism of PECCTT was corrosion. The water permeated into the pH-sensitive film in SGF and SIF slowly, and then the film quickly dissolved after being exposure to SCF 1. When the enzyme-sensitive (alginate) layer was exposed to SCF 1, degradation of alginate could be accomplished by enzyme in SCF 1. The degradation of alginate was therefore a rate-limiting factor. With constant degradation and eventual breaking-up of alginate, large amount of prednisolone was released.

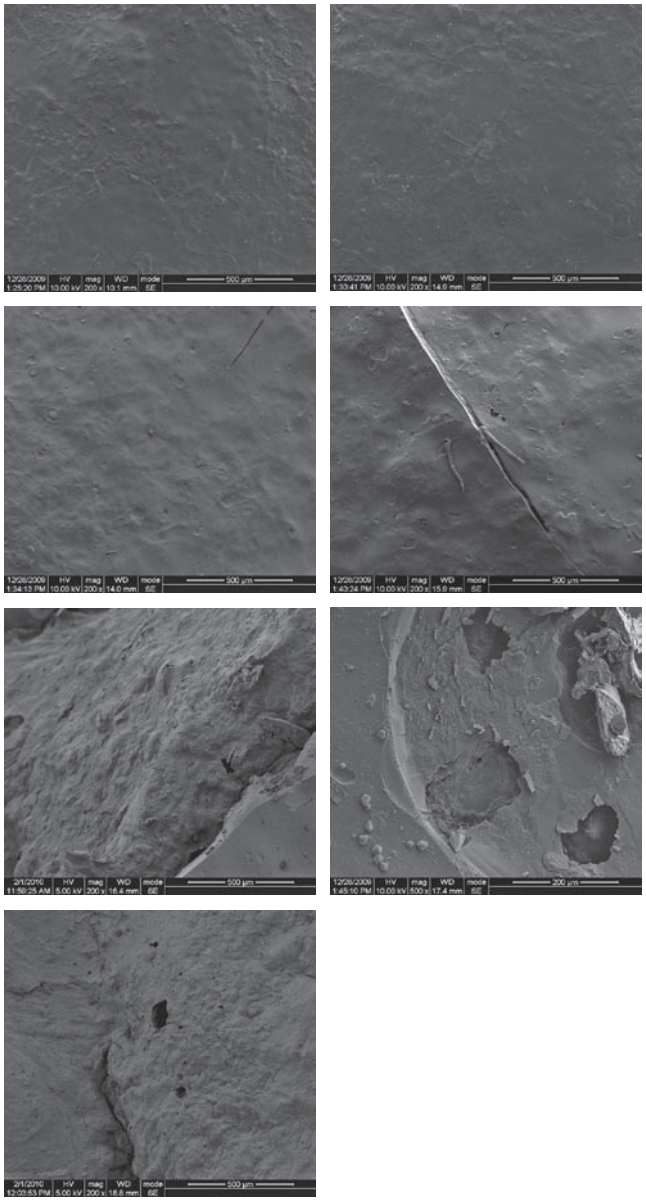

Figure 4. SEM micrographs showing the surface structure of formulation F3 (0 h), in simulated gastric fluid (SGF) (2 h), in simulated small intestinal fluid (SIF) (5 h), and in simulated colon fluid 1 (SCF 1) (3.0 U/mL, 6—9 h), respectively.

In vivo gamma scintigraphy

It was reported that irradiation can accelerate drug release, especially from formulations that contain polymeric excipients[17–19]). So the release of drug from PECCTT with or without ^{99m}Tc was compared *in vitro*. However, we found that the change in dissolution profile was so minimal that PECCTT contained ^{99m}Tc could still represent the PECCTT without ^{99m}Tc as determined by means of gamma images, which may be explained by the limited amount of ^{99m}Tc in the tablets (Figure 5).

As soon as a tablet was swallowed with 200 mL water, a image was taken with SPECT/CT. Figure 6, which was a picture of SPECT/CT imaging at 0 h, indicated that the tablet was on the top of stomach. The tablet was still in the stomach after 1 h. Figure 7 (2 h) showed that the tablet reached the small intestine, so the gastric emptying time was 1–2 h. The gastric emptying is affected not only by the physical properties of the dosage form such as size and density[20], but also by the dietary condition or amount and composition of the meal[21].

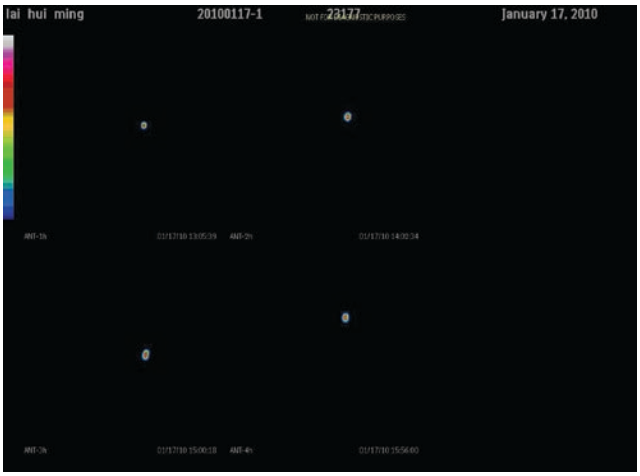

Figure 5. Scintigrams of the volunteer after administration of PECCTT 1–4 h.

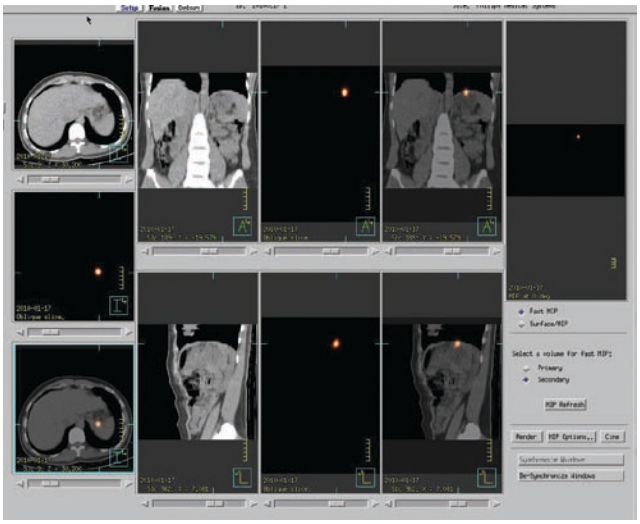

Figure 6. Co-registered SPECT/CT images demonstrate regional heterogeneity of radioisotope uptakes in stomach (0 h).

As seen from Figure 8, 6 h after administration, the tablet had already reached the ileocecal junction, that is, the transit time through the small intestine was about 4 h. Transit time through the small intestine typically takes 3–5 h, is fairly constant, and is unaffected by food[22]. Figure 9 showed that the PECCTT had already arrived at sigmoid colon at 8 h.

From 9- and 12-h images in Figure 10, it can be seen that the tablet had disintegrated and the radiation detected was spread to the sigmoid colon. Progress through the GIT would have subjected the tablet to higher pH, which would result in the physical breakdown of the tablet pH-coated layer, exposing the compression-coated tablet. Formation of a hydrated gel was crucial to enable inward diffusion of enzymes and subsequent breakdown of the compressed coat. This spreading was most likely caused by the action of the bacterial enzymes in the colon degrading the compressed coat and accelerating the release of the radioactivity. It is evident from the present study that the more the time the tablets stay in the colon, the better the degradation of the gum by the colonic bacterial enzymes thereby resulting in effective distribution of the released tracer in the colon. This clearly indicated that the drug release from the PECCTT was a consequence of enzymatic degradation rather than by simple time-dependent disintegration. However, it appeared that disintegration of the PECCTT in the present study could be result of a combination of time dependent on pH-coated in SGI and SIF and enzymatic degradation by colonic bacteria in colon.

The disintegration lasts for 4 h; this may be a consequence of the colonic environment, the lack of agitation, tightly packed and viscous contents, and low fluid volume[23,24].

Gamma scans at 18 h scanning in Figure 11 showed that tablet was disintegrated completely and occupied cecum, ascending, transverse, and descending colons. In the present study, ^{99m}Tc, which was adsorbed onto sodium chloride, was used as a tracer to monitor the *in vivo* behavior of PECCTT. Due to the high solubility of the sodium chloride, containing the tracer, ^{99m}Tc was adsorbed quickly and was expelled out of body with urine. So in the 18-h image, there were much ^{99m}Tc in the bladder. As seen from Figure 11, the tablet cannot be visible at 24 h after administration.

This *in vivo* study of PECCTT had shown that alginate, in the form of multiple-unit colon-targeted drug delivery system, was capable of protecting the drug from being released in the physiological environment of stomach and small intestine and was susceptible to the enzymatic action of colonic bacteria.

The study formulation behaved in similar manner *in vitro* and *in vivo*. The PECCTT had remained intact in stomach *in vivo* and SGF *in vitro* in 2 h. It also had not released drug from small intestine *in vivo* and simulated intestinal fluid *in vitro* in 5 h. Drug released began when it reached the colon *in vivo* and SCF *in vitro* in 8 h. The

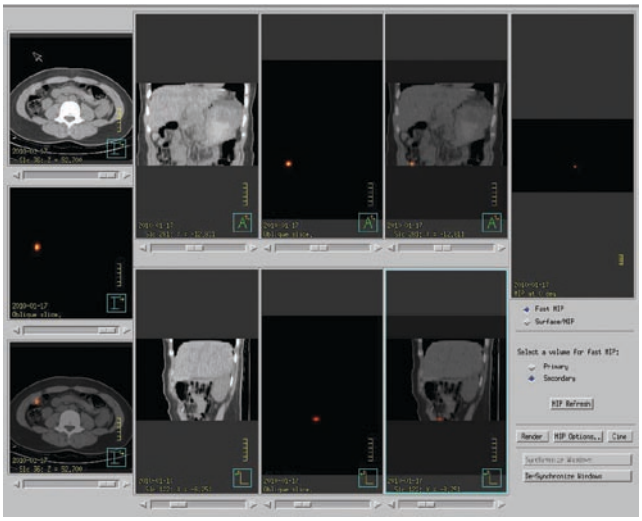

Figure 7. Co-registered SPECT/CT images demonstrate regional heterogeneity of radioisotope uptakes in upper gastrointestinal tract (2 h).

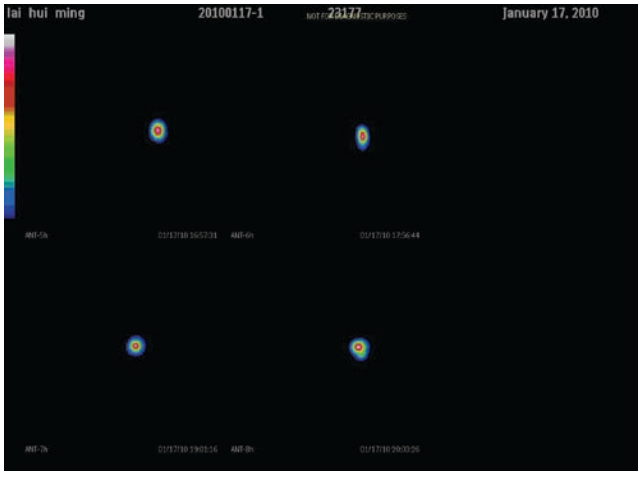

Figure 8. Scintigrams on the volunteer after administration of pH- and enzyme-controlled, colon-targeted tablets (PECCTT) 5–8 h.

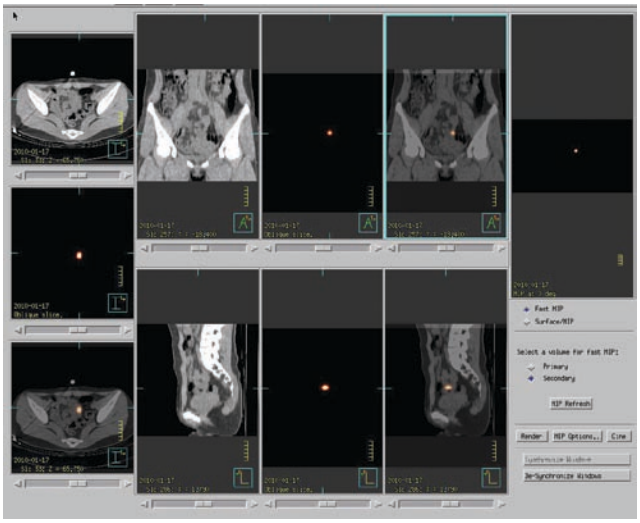

Figure 9. Co-registered SPECT/CT images demonstrate regional heterogeneity of radioisotope uptakes in hypogastrium (8 h).

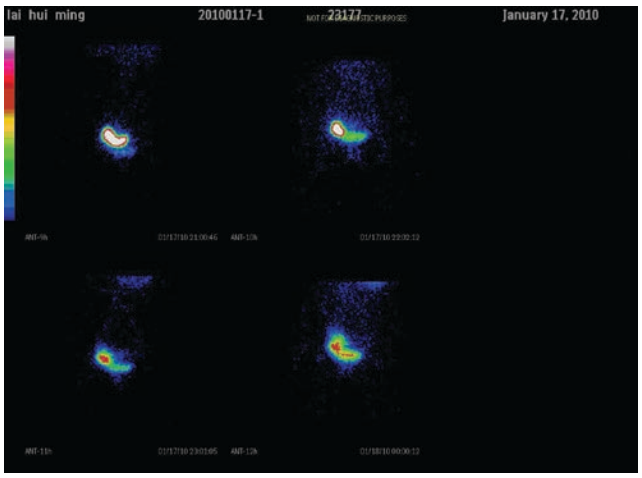

Figure 10. Scintigrams on the volunteer after administration of pH- and enzyme-controlled, colon-targeted tablets (PECCTT) 11–12 h.

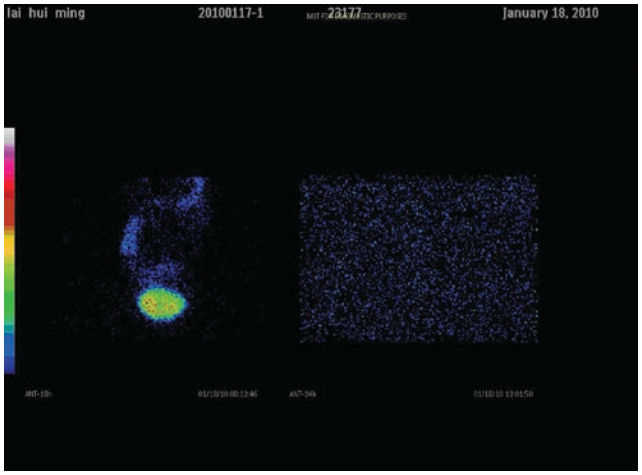

Figure 11. Scintigrams on the volunteer after administration of pH- and enzyme-controlled, colon-targeted tablets (PECCTT) 18 and 4 h.

highly similar behavior indicated that correlations exist between *in vitro* and *in vivo* characteristics.

In previous reports, anterior anatomical markers containing ^{99m}Tc were taped onto the skin where the midclavicular line meets the right costal margin to direct the position of tablets or pellets[25]. That method cannot provide precise information of transformation of tablets or pellets in the GIT. In some other reports, water labeled with ^{99m}Tc DTPA was given to subjects, which allowed the outline of the stomach to be visualized to view the transformation of tablets or pellets in GIT[26]. However, the more radioactivity markers was used, the more harm would suffer to subjects. A more precise and safe way should be discovered.

The development of a technique that would accurately recover the 3D activity distribution in a specific area or organ of interest from SPECT scans will allow for the conventional nuclear medicine diagnostic and therapy procedures to move to a new, fully quantitative level. As the increased availability of hybrid systems (SPECT/CT), the targeted organ can be segmented into few regions[27]. Hybrid scanners combining SPECT and CT offer physicians the opportunity to acquire spatially correlated functional and morphological information in a single session. These hybrid systems have greatly improved the diagnostic accuracy and have therefore met with broad clinical acceptance[21]. This new precise and safe hybrid system was introduced in the colon-targeted delivery system for the first time, and it could be an very useful system in future colon-targeted studies.

Conclusion

A novel multiple-unit colon-targeted drug delivery system for prednisolone using pH- and enzyme-sensitive materials was prepared with acrylic resin and alginate. The effect of β-mannanase on degradation was evaluated in dissolution testing in the range of 0 U/mL to 3.0 U/mL. The release rate of the drug in the media of 3.022 U/mL β-mannanase was similar with that in the 4% (w/v) rat cecal content solution (f_2 = 53.70). So the media of 3.022 U/mL β-mannanase was adopted to mimic the colon environment. With the protection of pH- and enzyme-sensitive coated layer, no prednisolone was released from PECCTT in the physiological environment of stomach and small intestine. However, 84.45% of prednisolone was released from the PECCTT in the physiological environment of colon at 12 h. The lag time was 7.04 ± 0.17 h, and the disintegration of the PECCTT in the present study could be result of a combination of time delayed of pH-coated layer in the environment of stomach and small intestine and enzymatic degradation by colonic bacteria of the alginate-coated layer in colon. The result of release mechanisms of different mathematical modeling and SEM study indicated that the dissolution and drug release mechanism of PECCTT in colon was corrosion. As seen from the results on volunteer, the PECCTT could protect drug from release in stomach and small intestine. It began to disintegrate and release drug in colon 8 h after administration as indicated from SPECT/CT images. The system formulated using alginate as coating seems to be highly site-specific due to release of majority of drug only upon degradation effect of the bacterial microflora of the colon.

Declaration of interest

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References

1. Fiocchi C. (2002). Inflammatory bowel disease. Current concepts of pathogenesis and implications for therapy. Minerva Gastroenterol Dietol, 48:215–226.

2. Bodmeier R. (1997). Tableting of coated pellets. Eur J Pharm Biopharm, 43:1–8.
3. Bajpai SK, Sharma S. (2004). Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca2t and Ba2t ions. React Funct Polym, 59:129–140.
4. Grant GT, Morris ER, Rees DA, Smith PJ, Thom D. (1973). FEBS Lett, 32:195.
5. Newman SP, Hirst PH, Wilding IR. (2003). New developments in radionuclide imaging for assessing drug delivery in man. Eur J Pharm Sci, 18:19–22.
6. Mentula S, Harmoinen J, Koski P, Westermarck E, Rautio M, Huovinen P et al. (2004). Inhibition of ampicillin-induced emergence of resistance in intestinal coliforms by targeted recombinant beta-lactamase. Int J Antimicrob Agents, 24:555–561.
7. Marvola J, Kanerva H, Slot L. (2009). Neutron activation-based gamma scintigraphy in pharmacoscintigraphic evaluation of an Egalet® constant-release drug delivery system. Int J Pharm, 281:3–10.
8. Hodges LA, Connolly SM, Band J, O'Mahony B, Ugurlu T, Turkoglu M et al. (2009). Scintigraphic evaluation of colon targeting pectin-HPMC tablets in healthy volunteers. Int J Pharm, 370:144–150.
9. Zhang H, Ibrahim AA, Neau SH. (2002). An *in vitro* evaluation of a guar gum containing multi-unit system for macromolecule delivery to the colon. Int J Pharm, 239:197–205.
10. Burke MD, Park JO, Srinivasarao M, Khan SA. (2005). A novel enzymatic technique for limiting drug mobility in a hydrogel matrix. J Control Release, 104:141–153.
11. 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. Carbohydr Polym, 73:241.
12. Sinha VR, Kumria R. (2002). Binders for colon specific drug delivery: an *in vitro* evaluation. Int J Pharm, 249:23–31.
13. 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, 320:137–142.
14. Serra L, Domenech J, Peppas NA. (2006). Drug transport mechanisms and release kinetics from molecularly designed poly(acrylic acid-g-ethylene glycol) hydrogels. Biomaterials, 27:5440–5451.
15. Ritger PL, Peppas NA. (1987). Simple equation for description of solute release II. Fickian and anomalous release from swellable devices. J Control Release, 5:37–42.
16. Maltais A, Remondetto GE, Subirade M. (2010). Tabletted soy protein cold-set hydrogels as carriers of nutraceutical substances. Food Hydrocolloids, 24:518–524.
17. Watts PJ, Atkin BP, Wilson CG, Davies MC, Melia CD. (1991). Radiolabeling of polymer microspheres for scintigraphic investigations by neutron activation. 1. Incorporation of samarium oxide and its effects on the properties of Eudragit RS:sulfasalazine microspheres. Int J Pharm, 76:55–59.
18. Waaler T, Sande SA, Müller BW, Schüller-Lisether G. (1997). The influence of thermal neutron irradiation on the *in vitro* characteristics of ASA oral dosage forms. Validation of neutron activation. Eur J Pharm Biopharm, 43:159–164.
19. Säkkinen M, Marvola J, Kanerva H, Lindevall K, Ahonen A, Marvola M. (2004). Scintigraphic verification of adherence of a chitosan formulation to the human oesophagus. Eur J Pharm Biopharm, 57:145–147.
20. Sugito K, Ogato H, Goto H, Noguchi M, Kogure T, Takano M et al. (1990). Gastrointestinal transit of non-disintegrating solid formulations in humans. Int J Pharm, 60:89–97.
21. Han J, Kostler H, Bennewitz C, Kuwert T, Hornegger J.(2008). Computer-aided evaluation of anatomical accuracy of image fusion between X-ray CT and SPECT. Comput Med Imaging Graph 32:388–395.
22. Billa N, Yuen K-H, Khader MAA. (2000). Gamma-scintigraphic study of the gastrointestinal transit and *in vivo* dissolution of a controlled release diclofenac sodium formulation in xanthan gum matrices. Int J Pharm, 201:109–120.
23. McConnell EL, Fadda HM, Basit AW. (2008). Gut instincts: explorations in intestinal physiology and drug delivery. Int J Pharm, 364:213–226.
24. Parr AF, Sandefer EP, Wissel P, McCartney M, McClain C, Ryo UY et al. (1999). Evaluation of the feasibility and use of a prototype remote drug delivery capsule (RDDC) for non-invasive regional drug absorption studies in the GI tract of man and beagle dog. Pharm Res, 16:266–271.
25. Goto T, Tanida N, Yoshinag T. (2004). Pharmaceutical design of a novel colon-targeted delivery system using two-layer-coated tablets of three different pharmaceutical formulations, supported by clinical evidence in humans. J Control Release, 97:31–42.
26. Macleod GS, Fell JT, Collett JH, Sharma HL, Smith AM. (1999). Selective drug delivery to the colon using pectin:chitosan:hydroxypropyl methylcellulose film coated tablets. Int J Pharm, 187:251–257.
27. Shcherbinin S, Celler A. (2009). An enhancement of quantitative accuracy of the SPECT/CT activity distribution reconstructions: physical phantom experiments. Comput Med Imaging Graph, xxx:8–16.