

Design and preparation of a novel colon-targeted tablet of hydrocortisone

Yachao Ren^{1,†}, Lei Jiang^{1,†}, Shuman Yang², Sainan Gao¹, Hui Yu¹, Jie Hu¹, Dandan Hu¹, Wenbin Mao³, Haisheng Peng^{1,*}, Yulong Zhou^{3,*}

¹Harbin Medical University-Daqing, Daqing, China, ²Department of Environmental Health, Division of Epidemiology and Biostatistics, University of Cincinnati Medical Center, Cincinnati, Ohio, US, ³College of Animal Science and Technology, Heilongjiang Bayi Agricultural University, Daqing, China

The objective of this research was to design a new colon-targeted drug delivery system based on chitosan. The properties of the films were studied to obtain useful information about the possible applications of composite films. The composite films were used in a bilayer system to investigate their feasibility as coating materials. Tensile strength, swelling degree, solubility, biodegradation degree, Fourier transform infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Scanning electron microscope (SEM) investigations showed that the composite film was formed when chitosan and gelatin were jointly reacted jointly. The results showed that a 6:4 blend ratio was the optimal chitosan/gelatin blend ratio. *In vitro* drug release results indicated that the Eudragit- and chitosan/gelatin-bilayer coating system prevented drug release in simulated intestinal fluid (SIF) and simulated gastric fluid (SGF). However, the drug release from a bilayer-coated tablet in SCF increased over time, and the drug was almost completely released after 24 h. Overall, colon-targeted drug delivery was achieved by using a chitosan/gelatin complex film and a multilayer coating system.

Uniterms: Chitosan/gelatin complex film/drugs coating. Chitosan/multilayer coating system/drugs coating. Drugs/delivery system/colon-targeted. Tablets/bilayer-coated.

O objetivo desta pesquisa foi planejar um novo sistema de liberação de fármacos direcionado ao cólon, utilizando quitosana. Estudaram-se as propriedades dos filmes a fim de obter informações úteis sobre a aplicação desses filmes compósitos. Utilizaram-se os filmes compósitos em sistema de bicamada para investigar a sua viabilidade como materiais de revestimento. Estudos de resistência à tração, grau de intumescimento, solubilidade, grau de biodegradação, no infravermelho por transformada de Fourier (FTIR), de calorimetria diferencial de varredura (DSC) e de microscopia eletrônica de varredura (SEM) mostraram que o filme compósito se formou quando a quitosana e a gelatina reagiram entre si. Os resultados mostraram que a mistura de proporção ótima foi de 6:4 de quitosana:gelatina. Resultados da liberação do fármaco *in vitro* indicaram que o sistema de revestimento de Eudragit e bicamada de quitosana/gelatina impediu a liberação de fármaco em fluido intestinal simulado (SIF) e em fluido gástrico simulado (SGF). Entretanto, a liberação de fármaco do comprimido revestido em bicamada no SCF aumentou ao longo do tempo e o fármaco foi quase completamente liberado após 24 h. Em geral, se obteve a forma de liberação dirigida ao cólon, utilizando filme complexo de quitosana/gelatina e sistema de revestimento multicamada.

Unitermos: Quitosana/filme de complexo de gelatina. Quitosana/sistema multicamadas de revestimento/ revestimento de fármacos. Fármacos/sistema de liberação/dirigido ao cólon. Comprimidos/revestidos com bicamada.

*Correspondence: Y. Zhou. College of Animal Science and Technology, Heilongjiang Bayi Agricultural University, Daqing, 163319, China. E-mail: zhouyulong1980@163.com. H. Peng. Harbin Medical University-Daqing, Daqing, 163319, China. E-mail: fisher@163.com

INTRODUCTION

Colon-targeted drug delivery systems have been increasing interest for treating colonic diseases such as Crohn's disease, irritable bowel syndrome, and ulcerative colitis (Cao, Fu, He, 2007; Chourasia, Jain, 2004; Jain, Jain, 2008; Luo *et al.*, 2011). Colon-targeted drug delivery increases efficacy and reduces side effects by delivering high drug concentrations topically at the colonic disease site. An ideal colon-specific drug delivery system should release most of the drug locally into the colon, and not into the stomach or small intestine (Rubinstein, 1995). Several strategies are currently being investigated for colon-specific delivery, including prodrugs (Dhaneshwar *et al.*, 2007) and pH-sensitive (Yin *et al.*, 2008; Vaghani, Patel, Satish, 2012), time-dependent (Fude *et al.*, 2007), and microflora-activated systems (Dev, Bali, Pathak, 2011; Yang, 2008).

The pH of the gastrointestinal tract gradually increases as one moves down the gastrointestinal tract from the stomach (pH 1.5–3) to the terminal ileum (pH 7–8) (Gupta, Beckert, Price, 2001). pH-sensitive delivery systems takes advantage of polymeric materials that are insoluble in the low pH environment of the upper gastrointestinal tract, but dissolve at the higher, near neutral pH of the distal gut. Microflora-activated delivery systems are preferable and promising (Fan *et al.*, 2009). The human colon is a dynamic and ecologically diverse system consisting of over 400 distinct bacterial species (Cummings, Macfarlane, 1991) that secrete a variety of enzymes (Guarner, Malagelada, 2003). The primary sources of nutrition for these bacteria are non-starch polysaccharides which are fermented during their transit through the colon and which are negligibly broken down in the stomach and small intestine (Englyst, Cummings, 1985). Currently, natural polysaccharides are extensively used for the development of solid dosage forms for the delivery of drugs to the colon (Nath, Nath, 2013).

However, it was reported that a colon-specific delivery system which is based only on pH-sensitive (Gupta, Beckert, Price, 2001) or microflora-activated systems have been reported as unreliable due to the inherent variability in pH or water swelling from the gastrointestinal tract. Fortunately, a bilayer system would serve as a unique drug delivery device that is compatible with conventional manufacturing methods. Similarly, multi-layered tablets have been developed to control drug delivery using a predefined release profile for different active ingredients. Therefore, we intend to design a new drug delivery system by a combining pH-sensitive system with microflora-activated system.

Polysaccharides, which are metabolized by the

colonic flora, have gained widely attention as potential colon targeting carriers for their non-toxic, hydrophilic, gel-forming, biodegradable, inexpensive, and abundant properties and they are available in various grades of varying properties. Chitosan, which is a high molecular weight natural polysaccharide, is completely biodegradable, non-toxic, low-cost, and has favorable gelation characteristics (Singla, Chawla, 2001). Chitosan cannot be digested in the upper gastrointestinal tract by human digestive enzymes (Bhattarai, Gunn, Zhang, 2010; Park *et al.*, 2010). However, chitosan is prone to glycosidic hydrolysis by microbial enzymes in the colon (Chourasia, Jain, 2003; McConnell, Murdan, Basit, 2008; Muzzarelli, 2011). Furthermore, chitosan has been applied in colon-targeted drug delivery systems in several forms such as capsules (Tozaki, *et al.*, 2002), matrices (Zambito, Di Colo, 2003), hydrogels (Zhang, Alsarra, Neau, 2002) and microspheres (Lorenzo-Lamosa *et al.*, 1998). Therefore, the potential for chitosan to be used as a coating material is obvious. However, chitosan dissolves in the acidic solutions, i.e., a factor that would limit the applications of chitosan in colon-targeted drug delivery. Gelatin is a soluble protein compound obtained by partially hydrolyzing collagen. It has a number of applications in the biomedical field, such as in tissue engineering, wound dressing, drug delivery and gene therapy (Mano *et al.*, 2007). Furthermore, gelatin films, which are thin, flexible and transparent biodegradable materials, are available for engineering food, packaging, drug delivery and other applications (Bergo, Sobral, 2007). Unfortunately, gelatin exhibits poor mechanical properties, limiting its possible applications. Blending is an easy way to improve the properties of polymer materials. Based on the above, a chitosan/gelatin complex film was prepared as an inner coat. Eudragit® L100, anionic copolymers, dissociates above pH 6.0 and was used as the outer coat.

The objective of this study was to develop a multi-unit delivery system of a model drug so that the majority of the drug release occurs in the colon in a sustained-release fashion. A schematic of the delivery system is shown in Figure 1. Hydrocortisone is more extensively used as a corticosteroid to cure inflammatory bowel disease. Hydrocortisone was used as a model drug in this study. Tablets were chosen for development because they are more economical and easier to industrialize.

MATERIAL AND METHODS

Material

Hydrocortisone was purchased from Jinjin Pharmaceutical Co. Ltd. (Tianjin, China). The following

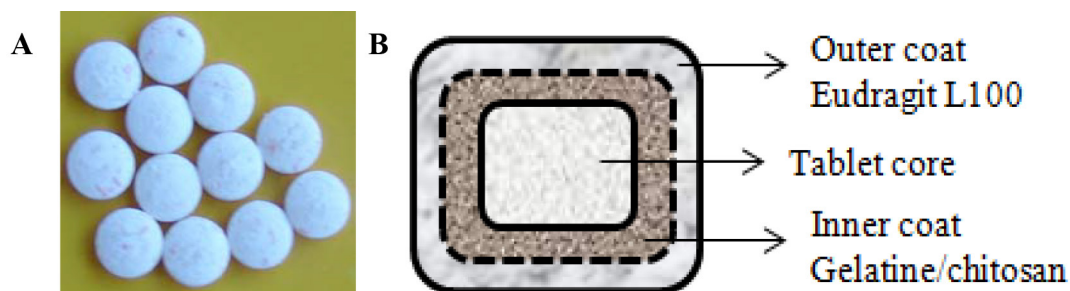


FIGURE 1 - Bilayer coated tablet. Bilayer coated tablet was prepared in this study (A); schematic of the bilayer coated tablet delivery system (not to scale) (B).

excipients and chemicals were purchased from commercial sources and used as received: chitosan (deacetylation degree 96%, Haidebei Co. Ltd., Shandong, China), microcrystalline cellulose (MCC, Huzhou Zhanwang Medical Company, Huzhou, China), Eudragit® L100 (Röhm Pharm, Darmstadt, Germany), triethylcitrate (TEC, Johnson Matthey company, USA), polyethylene glycol (PEG) 6000 (Pudong Chemical Co., Shanghai, China), talc and magnesium stearate (Shanghai Yunhong Pharmaceutical Excipients & Technology Co. Ltd., Shanghai, China), lactose and gelatin (Beijing Aoboxing Biotechnology, Beijing, China), and pepsin, lipase and pectinase (Beijing Kebio Biotechnology, Beijing, China). The solvents were reagent grade; double distilled deionized water was used in all experiments.

Film preparation

Gelatin was dissolved in ultra-pure water, and chitosan was dissolved in a 2% acetic acid solution. The gelatin and chitosan solutions were mixed at different weight ratios (Table I) and PEG6000 was added as a plasticizer. Subsequently, the mixed solution was stirred for 15 min at room temperature. In this reaction, the gelatin and the chitosan array formed a complex. Subsequently, the mixtures were cast onto silanized glass Petri dishes and dried at room temperature for 5-7 days. After drying, the gelatin/chitosan films were removed from the Petri dishes and stored in desiccators at room temperature.

Preparation of the simulated gastric/intestinal fluid

Preparation of the simulated gastric/intestinal fluid with enzyme

A simulated gastric fluid (SGF) was prepared comprising an aqueous solution containing 12,000 U/L pepsin adjusted to pH 1.5 using 0.1 M HCl. A simulated intestinal fluid (SIF) was prepared comprising an 8400

TABLE I - Formulation design of film

Formulation No. (FN)	Composition	Weight Rate
1	Gelatin/chitosan/PEG	10:0:1
2	Gelatin/chitosan/PEG	9:1:1
3	Gelatin/chitosan/PEG	8:2:1
4	Gelatin/chitosan/PEG	7:3:1
5	Gelatin/chitosan/PEG	6:4:1
6	Gelatin/chitosan/PEG	5:5:1
7	Gelatin/chitosan/PEG	4:6:1
8	Gelatin/chitosan/PEG	3:7:1
9	Gelatin/chitosan/PEG	2:8:1
10	Gelatin/chitosan/PEG	1:9:1
11	Gelatin/chitosan/PEG	0:10:1

U/L lipase solution in a phosphate buffer (pH 7.4). A simulated colonic fluid (SCF) was prepared comprising a 6 g/L pectinase solution in a phosphate buffer (pH 6.8).

Preparation of the simulated colonic fluid with rat colonic medium (RCM)

The extracellular enzymes released from the microflora in rat colonic contents had more profound degradation effects on chitosan than their membrane enzymes (Zhang, Alsarra, Neau, 2002). Therefore, rat colonic medium was prepared. Male Wistar rats (200-300 g) were lightly anesthetized using ether, and sacrificed by decapitation. Their caeca were exteriorized, cauterized at both ends (2.0 cm apart), cut loose, and immediately removed from the rat body. The formed cecal bag was opened, and its contents were weighed, pooled, and suspended in two volumes of cold bicarbonate-buffered saline to give a final cecal dilution of 33% (w/v). The suspension was filtered through a 400 mesh grit twice to remove any debris. The supernatant was then centrifuged at 15,000 x g for 30 min to obtain a

clear supernatant containing the extracellular enzymes (Zhang, Alsarra, Neau, 2002). The animal study protocol was approved by the Animal Care and Use Committee of Harbin Medical University.

Film characteristics

Appearance

The surface conditions and color of the gelatin/chitosan films were observed.

Thickness

The thickness of the gelatin/chitosan films was determined with a micrometer, and the average value of 10 measurements taken at random points was reported.

Tensile strength measurement

The gelatin/chitosan films were cut into rectangular samples. Four tests were performed with each film at room temperature using an electronic universal testing machine BLD 200S (Jinan, China). The characteristic parameters of the rectangular samples included a 40-mm gauge length and a 10-mm width. Average values from at least three measurements were reported. The tensile strength (TS) was calculated using the following expression:

$$\delta = P / (b \times d) \quad (\text{Equation 1})$$

where δ is the tensile strength, P is the maximal tension needed to break the film, b is the width of the film and d is the thickness of the film.

Swelling of film

The degree of swelling was determined gravimetrically. Films 20 mm long and 20 mm wide were put into SGF, SIF and SCF without enzymes to swell at the experimental temperature. The samples were removed at regular intervals from the buffers, blotted with filter paper, weighed and replaced in the same immersion bath. This process was repeated until a swelling equilibrium was reached. The swelling degree was defined as the weight of water imbibed by the sample per unit weight of film. Each film was analyzed three times. The equilibrium swelling degree Q_{∞} was calculated in grams of water per gram of film using the following expression:

$$Q_{\infty} (\%) = (m_{\infty} - m_0) \times 100 / m_0 \quad (\text{Equation 2})$$

where m_0 is the initial weight of the dried film, and m_{∞} is the weight of film at swelling equilibrium.

Solubility of film

Dried films ($n = 3$) were weighed (W_1) and immersed in SGF, SIF and SCF without enzyme for 24 h, dried in vacuo overnight, and weighed again (W_2). The solubility of each sample was determined three times. Solubility (S) was calculated using Equation (3):

$$S (\%) = (W_1 - W_2) \times 100 / W_1 \quad (\text{Equation 3})$$

Fourier transform infrared spectroscopy (FTIR)

The films were cut into small pieces and KBr discs were prepared. Infrared spectra were recorded on an 8400S FTIR spectrometer (Shimadzu, Japan) in the 4000–400 cm^{-1} region.

Differential Scanning Calorimetry (DSC)

DSC assays of gelatin/chitosan/PEG mixtures and gelatin/chitosan films were performed by using a DZ3335 DSC (Nanjingdazhan, China) instrument. A sample (5 mg) was scanned in an aluminum pan over a temperature range between 35 °C to 340 °C under nitrogen at a scanning rate of 5 °C/min. All scans were performed in triplicate.

Scanning electron microscope (SEM) analysis

Surface morphologies were examined by SEM using a S3400N scanning microscope from Hitachi Ltd. Co. (Tokyo, Japan) at an accelerating voltage of 15 kV. Samples were mounted on metal stubs and coated with gold by ion sputtering instrument E-1010.

Characterization of biodegradation

The gelatin/chitosan films with thicknesses averaging 0.6–0.8 mm were cut into 2 cm × 2 cm pieces. The film samples were degraded in 20 ml SGF (2 h), SIF (24 h), SCF (24 h) or RCM (24 h). Each sample was tested three times. The degradation degree (D_t) at time t was calculated using the following expression:

$$D_t = (m_t - m_0) / m_0 \quad (\text{Equation 4})$$

where m_0 is the initial weight of the dried film (at $t = 0$), and m_t is the weight after a time t .

Preparation and evaluation of colon-targeted tablets

Core Tablet Preparation

The formulation of prototypical core tablets containing 10 mg hydrocortisone were prepared using a wet granulation technique. The tablets contained 46% MCC, 46% lactose, 2% PVPP and 1% magnesium

stearate. The drug and excipients were mixed. The dry blend was granulated with 3% aqueous PVPK₃₀ solution as the binding solution, passed through a no. 16 mesh, and dried at 55 °C on a tray drier. The dried granules were passed through a no. 20 mesh before they were blended with 1.0% (w/w) magnesium stearate and compressed using an 8-mm punch on a single punch tablet press (DP30A, Beijing Gylongli Sci. & Tech. Co., Ltd., China). Core tablets were evaluated based on friability, hardness and disintegration.

Preparation of Coating Tablets

Three layers of polymeric coatings were applied to all core tablets in the following order: HPMC, gelatin/chitosan and Eudragit L100. The HPMC coat formulation was prepared by dissolving 2% (w/v) HPMC in 80% ethanol HPMC, the gelatin/chitosan coat formulation was gelatin (2%, w/v), chitosan (3%, w/v), PEG (0.5%, w/v), and 3% talc (3%, w/v) in 2% acetic acid solution, and the Eudragit L100 coat formulation was Eudragit L100 (6%, w/v), triethyl citrate (1%, w/v), and talc (3%, w/v) in an ethyl alcohol/water mixture (96/4). The coating weight gains for the HPMC, Eudragit L100 and gelatin/chitosan formulations were 2%, 4% and 20%, respectively.

In Vitro Dissolution Testing

Hydrocortisone release profiles from uncoated, gelatin/chitosan-coated, Eudragit-coated and bilayer-coated tablets were assessed by three-step dissolution experiments (Liu *et al.*, 2007), which were carried out in RC-6 dissolution tester (Tian Jin Optical Instrument Factory, China) to simulate the GIT under physiological conditions (250 mL per vessel): 2 h in SGF, 4 h in SIF and finally, 24 h in SCF. The stirring rates in SGF, SIF and SCF were 20, 20, and 5 rpm, respectively, and the temperature was maintained at 37 °C. Aliquots of the dissolution medium were withdrawn at predetermined times and replaced with the same volume of corresponding release medium to maintain a consistent volume. Each test was performed in triplicate and the release of hydrocortisone was monitored at 243 nm (UV 2000 UV-vis spectrophotometer).

Stability studies

The bilayer-coated tablets were stored in a high-density polyethylene plastic bottle with silica gel. A stability study of the bilayer-coated tablet was carried out according to ICH guidelines at 40 ± 2 °C/75% ± 5% RH. Physical attributes of the tablets, i.e., appearance, percentage drug content and dissolution profiles were tested over a period of 6 months.

Data analysis

The Rank Sum Ratio (RSR) method was used to evaluate the effect of the formulation composition on films using seven indices: tensile strength (TS), swelling degrees (Q) in SIF and SCF without enzyme, solubility (S) in SIF and SCF without enzyme, and biodegradation degree (D) in SIF and in SCF. High TS, Q in SCF without enzyme (QC), S in SCF without enzyme (SC) and D in SCF (DC), and low Q in SIF (QI), S in SIF without enzyme (SI) and D in SIF (DI) facilitate better film performance. Therefore, TS, QC, SC and DC were the high optimization indices. QI, SI and DI were the low optimization indices. The best film was identified by Equation (5):

$$RSR_n = \frac{1}{7 \times 11} \sum_{j=1}^7 R_{nj} \quad (\text{Equation 5})$$

where n represents the line, j represents the array and R_{nj} is the rank of n among the j indices.

SPSS11.5 software was used for all statistical analyses. A value of p < 0.05 was considered significant.

RESULTS AND DISCUSSION

Film characteristics

Film appearance and thickness

The gelatin/chitosan films were homogeneous, transparent and slightly brittle. The color of the films deepened as the chitosan content increased.

The average thicknesses of the films are listed in Table II. The thickness of the films significantly increased from 0.058 to 0.079 mm when the chitosan content increased. The film thickness was likely to be dependent on the film nature and composition. This result was similar to the result reported by Sebti *et al.* (2007) and Di Pierro *et al.* (2006) who showed possible relationships between film thickness and film-forming polymer content and nature.

Tensile strength measurement

The tensile strengths (TS) of the gelatin/chitosan films are presented in Table II. The TS of the composite membrane increased compared with the chitosan film, and the mechanical behavior of the composite film improved due to the added gelatin. A few studies have suggested that films formed from mixtures likely improved their physicochemical properties (Di Pierro *et al.*, 2006; Prodpran, Benjakul, Artharn, 2007). Chitosan contains more functional groups such as hydroxyls, amines and amides, whereas gelatin has more carboxyl and

TABLE II - Physicochemical properties of the gelatin/chitosan films

FN	Average thickness (mm)	Tensile strength (MPa)
1	0.058	29.8
2	0.060	31.2
3	0.061	31.5
4	0.064	33.3
5	0.065	34.9
6	0.067	36.5
7	0.069	33.5
8	0.072	32.5
9	0.073	30.1
10	0.075	23.7
11	0.079	17.4

amino groups (Celasco *et al.*, 2008). These groups may form electrostatic and hydrogen bonding interactions. Furthermore, when the gelatin and chitosan films were prepared, the non-covalent interactions, such as hydrogen bonding and hydrophobic interactions, likely formed between the gelatin and chitosan molecules (Cao, Fu, He, 2007). Therefore, the tensile strength of the composite film increased due to the non-covalent interactions between gelatin and chitosan. However, when the chitosan content continued to increase up to and beyond a 60% mass fraction, the TS of the composite film decreased. There may not have been enough gelatin to form the non-covalent interactions with chitosan.

Influence of the simulated gastrointestinal tract environment on the complex film

The swelling behavior, solubility and the in vitro degradation of the films are reported in Table III. The Q_{∞} values revealed that all the films had water retention capabilities. The Q_{∞} values of the blended films depended on the chitosan/gelatin blend ratios and the physical structure of the films. The composite films had improved the mechanical strength, thermal stability, and swelling capability of films. The Q_{∞} slightly decreased with the chitosan content increasing in SIF and SCF; Q_{∞} was smaller when the chitosan content was 50%-80%. These results agreed with studies that reported composite films improved mechanical strength (McConnell, Murdan, Basit, 2008) and swelling capability of films (Rueda, Secall, Bayer, 1999).

The ability to regulate the solubility of

biodegradable films presents a number of potential advantages in biomaterials. Therefore, the solubility of the films at gastrointestinal pH was studied. As shown in Table III, the solubility of the blended films in SIF and SCF decreased rapidly as the chitosan content increased. The solubility of the chitosan film was 14% and 18% in SIF and SCF when treated for 24 h at room temperature, respectively. It reveals that the blend ratio had a significant effect on the solubility of the film. This may be because gelatin is a water-soluble polymer and because chitosan is insoluble in pure water at neutral pH but soluble in acidic solutions.

The films were easy to prepare by casting or spraying methods. The properties of the composite films were evaluated and these films are experimentally economical and statistically efficient (Pinotti *et al.*, 2007). The degradation behaviours of the chitosan/gelatin complexes were investigated as films. In vitro degradation tests of the chitosan/gelatin films were conducted by immersing the films in the SGF, SIF and SCF. The effect of chitosan content on degradation is presented in Table III. The biodegradability of the composite film in SIF rapidly decreased with the increasing chitosan content. The biodegradability of the blended film in SCF also declined when the chitosan content increased, but the trend was not obvious when the blended film ratio was 1:1. This phenomenon was in line with studies that reported that chitosan is susceptible to glycosidic hydrolysis by microbial enzymes in the colon (Chourasia, Jain, 2003; McConnel, Murdan, Basit, 2008; Vargas *et al.*, 2009).

Results of the RSR Test to Evaluate Preparations with Different Compositions

Because all indices were not simply positively correlated or negatively correlated, the RSR method was applied to evaluate all the films. In Table III, the chitosan/gelatin/PEG (6:4:1, w/w/w) film was relatively optimal.

Evaluation of the optimal film

FTIR

The changes in the structure of chitosan film were examined by FTIR spectroscopy as shown in Figure 2. The FTIR spectra of the gelatin, gelatin/chitosan and chitosan films showed a broad peak between 2800 and 3500 cm^{-1} , attributed to the stretching of OH bonds and to the hydrogen bridges in films with PEG. The gelatin/chitosan films had wider peaks in that region, possibly due to strong interactions between gelatin and chitosan. This result agreed with those reported by Rueda, Secall, and

TABLE III - Results of the RSR Test

FN	TS(MPa)		QI (%)		QC (%)		SI (%)		SC (%)		DI (%)		DC (%)		RSR
	X ₁	R ₁	X ₂	R ₂	X ₃	R ₃	X ₄	R ₄	X ₅	R ₅	X ₆	R ₆	X ₇	R ₇	
1	29.8	3	-	1	-	1	90	1	91	11	93	1	94	11	0.34
2	31.2	5	-	1	-	1	80	2	86	10	82	2	87	10	0.34
3	31.5	6	272	3	339	11	58	3	83	9	66	3	85	9	0.49
4	33.3	8	204	4	279	10	42	4	70	8	48	4	72	8	0.49
5	34.9	10	176	5	253	9	30	5	36	7	45	5	55	7	0.49
6	36.5	11	102	10	157	6	24	6	25	5	39	7	48	4	0.49
7	33.5	9	100	11	164	7	24	6	29	6	44	6	52	6	0.55
8	32.5	7	114	6	168	8	23	8	24	4	30	9	49	5	0.52
9	30.1	4	104	9	113	3	20	10	21	3	31	8	36	3	0.47
10	23.7	2	107	7	117	4	21	9	19	2	25	10	31	1	0.43
11	17.4	1	105	8	119	5	14	11	18	1	17	11	32	2	0.49

X₁-X₇ are the values for the TS, QI, QC, SI, SC, DI, and DC, respectively. R₁-R₇ are the ranks of the TS, QI, QC, SI, SC, DI, and DC, respectively.

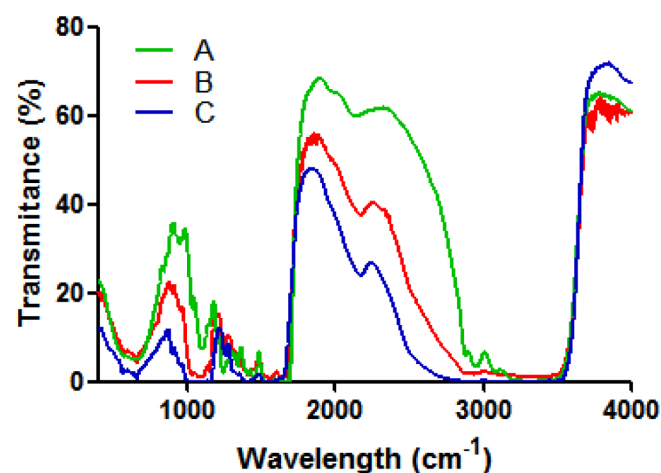


FIGURE 2 - FTIR spectra of gelatin (A), gelatin/chitosan (B) and chitosan (C) films.

Bayer (Mansur, Oréface, Mansur, 2004) who observed a broadened peak around 3400 cm⁻¹.

DSC

To investigate whether each ingredient of the complex film interacted with each other, the DSCs of the gelatin, chitosan, PEG, gelatin/chitosan/PEG mixture and gelatin/chitosan films were performed. As shown in Figure 3, the gelatin/chitosan/PEG powder mixture had an endothermic transition at approximately 200 °C. However, the complex film did not show any endothermic or exothermic transition. This result suggested that gelatin may have interacted with chitosan or PEG when the film was formed.

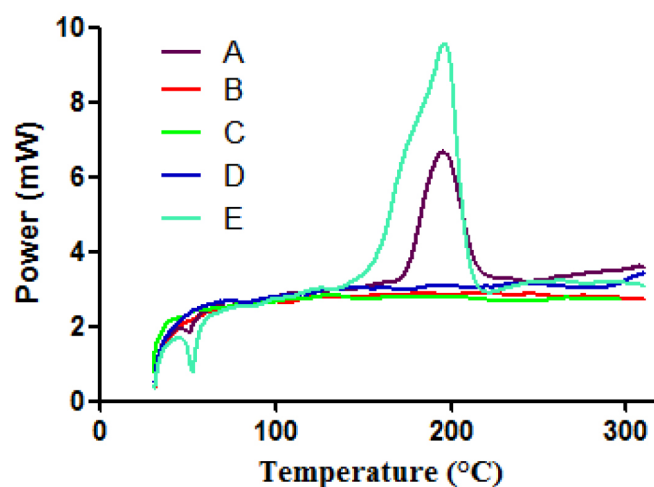


FIGURE 3 - DSC curves of gelatin/chitosan/PEG mixture (A) gelatin/chitosan films (B), chitosan (C), gelatin (D) and PEG (E).

SEM

Scanning electron microscopy images of the gelatin, chitosan and gelatin/chitosan films are shown in Figure 4. It shows that the structure of the gelatin film was homogeneous, convex and continuous. The chitosan and gelatin/chitosan films were smooth and had compact structures, as shown in Figures 4B and 4C. These observations agree with reports that the surface of chitosan films presents as a smooth, continuous and compact structure (Ghaffari *et al.*, 2007; Yu *et al.*, 2011). This indicates that the presence of gelatin hardly caused structural changes in the chitosan film when the chitosan/gelatin blend ratio was 6:4.

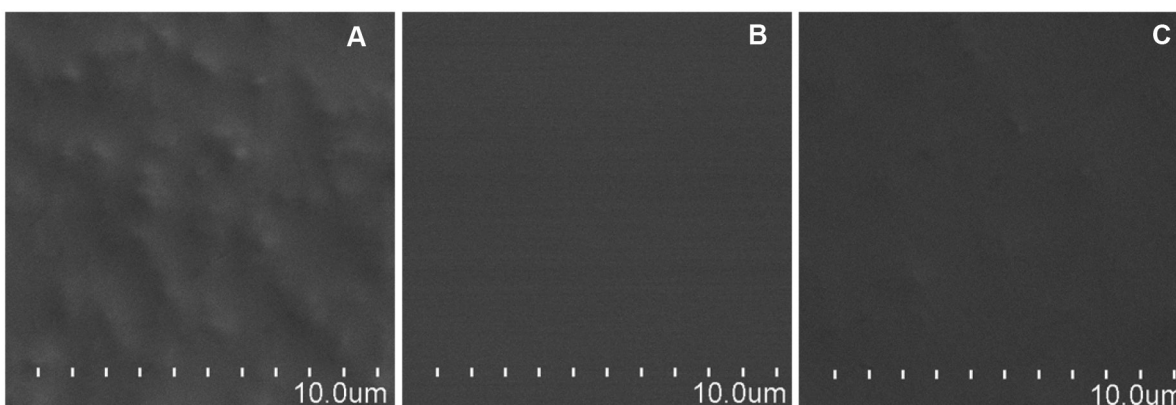


FIGURE 4 - SEM images of the surfaces of gelatin (A); chitosan (B) and gelatin/chitosan (C) films.

Biodegradation of the Optimal Film in Rat Colonic Medium

The study of the degradation rate of the optimal film was conducted by immersing the film samples in rat colonic medium. The degradation profile of the optimal film is shown in Figure 5. The biodegradability of the optimal film in rat colonic medium increased sharply to 65% after 24 h. Under the same conditions, the degradation was only 32% in SCF. A significant difference between the weight losses of the optimal film in rat colonic medium and in SCF was observed. Rat cecal contents contained more enzymes secreted by a variety of bacterial species. Chitosan is easily degraded by glycosidic hydrolysis by microbial enzymes in the colon (Finegold *et al.*, 1997; Gamelin *et al.*, 1998; Yang, 2008). Salyers and Leedle (1983) demonstrated that more than one bacterial enzyme was involved in the digestion of polysaccharides. Thus, the rat bacterial enzymes were more effective *in degrading* chitosan film than pectinase enzyme, promoting the degradation of the film by bacterial enzymes.

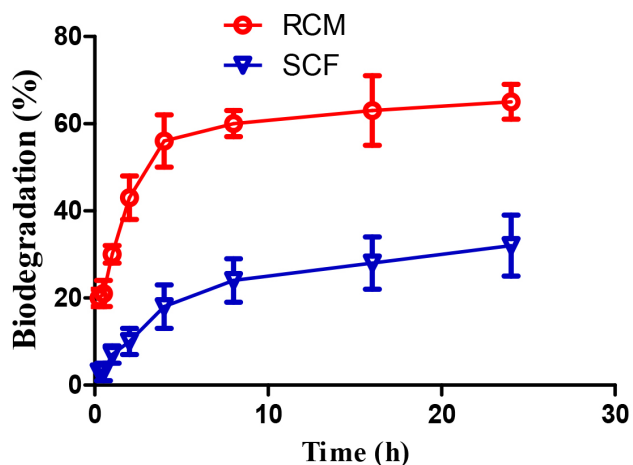


FIGURE 5 - Biodegradation of the optimal film in SCF and RCM.

Attributes of core tablets

The average weight of the uncoated core tablets was 200 mg. The hardness of the tablets was 4.6 kg/cm², and their friability ranged from 0.08% to 0.20% (w/w). The uncoated tablets were prepared using 1% PVPP (w/w).

In vitro drug release studies in simulated gastrointestinal fluids

The *in vitro* release studies were performed in medium at different pHs at 37 °C ± 0.5 °C. As was shown in Figure 6A, the uncoated and gelatin/chitosan-coated tablets showed immediate release characteristics in SGF, compared with the Eudragit and bilayer-coated tablets. However, the Eudragit coating prevented drug release in SGF at pH 1.2 but allowed for rapid and complete drug release in SIF when the pH was raised to pH 7.4. There was no measurable drug release for bilayer-coated tablets up to 2 h in SGF (pH 1.2). In SIF at pH 7.4, the drug release was negligible (< 5%) up to 4 h. Thus, the bilayer coating prevented drug release in SGF and SIF. After dissolution, the bilayer-coated tablet was tested in SGF for 2 h and SIF for 4 h, and its dissolution was further detected in SCF. The drug release in the bilayer-coated tablet in SCF increased over time, and the drug was almost completely released after 24 h (Figure 6B). Therefore, the Eudragit and chitosan/gelatin-coated tablets would likely resist release in the upper GI tract and thereby, prevent drug release at non-targeted sites.

Stability studies

Accelerated stability studies were carried out for a duration of 6 months as per ICH guidelines. As shown in Table IV, the formulation was stable according to the evaluation of the formulation for physical appearance, drug content and drug release profile.

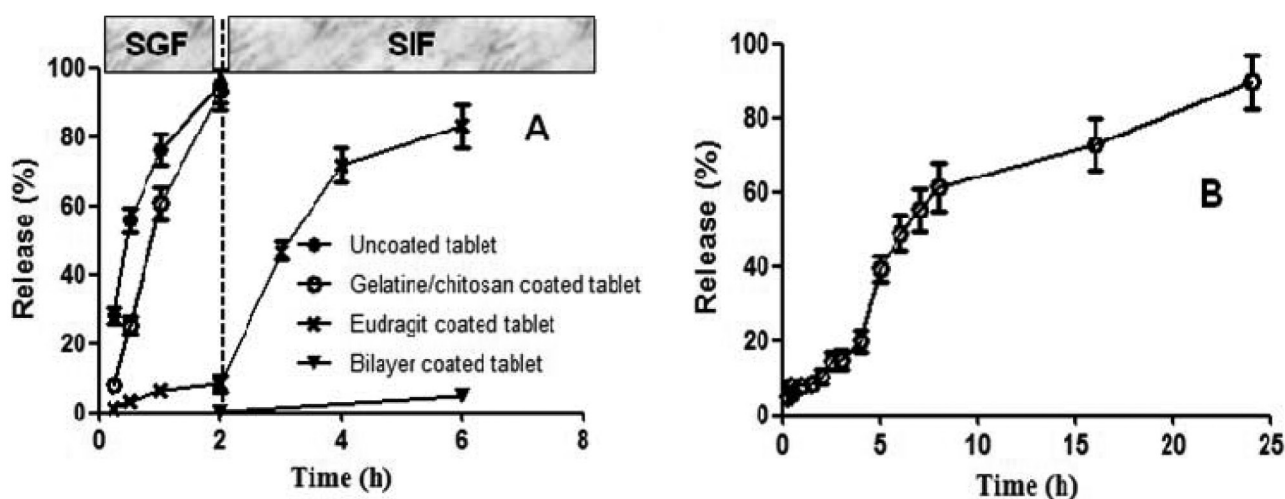


FIGURE 6 - Dissolution profiles of uncoated tablet and gelatin/chitosan, Eudragit and bilayer-coated tablets in SGF and SIF (A), and bilayer-coated tablet in SCF after it was tested in SGF for 2 h and SIF for 4 h (B).

TABLE IV - Physicochemical properties of bilayer-coated tablet during 6-month accelerated stability studies

Time (months)	Appearance	Drug content (%)	Release (%)		
			SGF (2 h)	SIF (4 h)	SCF (24 h)
1	no change	98.67 ± 0.73	–	3.83 ± 0.05	92.35 ± 0.78
2	no change	98.21 ± 0.35	–	2.98 ± 0.07	91.88 ± 0.54
3	no change	98.32 ± 0.48	–	3.32 ± 0.06	92.12 ± 0.69
6	no change	97.72 ± 0.69	–	3.58 ± 0.05	91.36 ± 0.42

CONCLUSION

We have designed a new microbially triggered system based on chitosan for colonic drug delivery. The characteristics of the designed complex film depend on the chitosan/gelatin blend ratio; a 6:4 blend ratio was optimal. The Eudragit and chitosan/gelatin bilayer coating system prevented drug release in SGF and SIF. The drug was almost completely released in SCF after 24 h. Colon-targeted drug delivery was achieved by using a chitosan/gelatin complex film and a multilayer coating system.

ACKNOWLEDGEMENTS

Our study was supported by the Wu Liande Fund for the Youth of Harbin Medical University (WLD-QN1111).

REFERENCES

BERGO, P.; SOBRAL, P. J. A. Effects of plasticizer on physical properties of pigskin gelatin films. *Food Hydrocolloid*, v.21, p.1285-1289, 2007.

BHATTARAI, N.; GUNN, J.; ZHANG, M. Chitosan-based hydrogels for controlled, localized drug delivery. *Adv. Drug. Deliv. Rev.*, v.62, n.1, p.83-99, 2010.

CAO, N.; FU, Y.; HE, J. Preparation and physical properties of soy protein isolate and gelatin composite films. *Food Hydrocolloid*, v.21, p.1153-1162, 2007.

CELASCO, G.; MORO, L.; BOZZELLA, R.; MANGANANGANO, K.; QUATTROCCHI, C.; AIELLO, C.; DONIA, M.; FAGONE, P.; DI MARCO, R. Efficacy of intracolonic administration of low-molecular-weight heparin CB-01-05, compared to other low-molecular-weight heparins and unfractionated heparin, in experimentally induced colitis in rat. *Dig. Dis. Sci.*, v.53, n.12, p.3170-3175, 2008.

CHOURASIA, M.K.; JAIN, S.K. Pharmaceutical approaches to colon targeted drug delivery systems. *J. Pharm. Pharm. Sci.*, v.6, n.1, p.33-66, 2003.

- CHOURASIA, M. K.; JAIN, S. K. Polysaccharides for colon targeted drug delivery. *Drug. Deliv.*, v.11, n.2, p.129-148, 2004.
- CUMMINGS, J. H.; MACFARLANE, G. T. The control and consequences of bacterial fermentation in the human colon. *J. Appl. Bacteriol.*, v.70, n.6, p.443-459, 1991.
- DEV, R. K.; BALI, V.; PATHAK, K. Novel microbially triggered colon specific delivery system of 5-Fluorouracil: Statistical optimization, in vitro, in vivo, cytotoxic and stability assessment. *Int. J. Pharm.*, v.411, n.1-2, p.142-151, 2011.
- DHANESHWAR, S. S.; GAIROLA, N.; KANDPAL, M.; VADNERKAR, G.; BHATT, L. Colon-specific, mutual azo prodrug of 5-aminosalicylic acid with L-tryptophan: synthesis, kinetic studies and evaluation of its mitigating effect in trinitrobenzenesulfonic acid-induced colitis in rats. *Bioorg. Med. Chem.*, v.15, n.14, p.4903-4909, 2007.
- DI PIERRO, P.; CHICO, B.; VILLALONGA, R.; MARINIELLO, L.; DAMIAO, A.E.; MASI, P.; PORTA, R. Chitosan-whey protein edible films produced in the absence or presence of transglutaminase: analysis of their mechanical and barrier properties. *Biomacromolecules*, v.7, n.3, p.744-749, 2006.
- ENGLYST, H. N.; CUMMINGS, J. H. Digestion of the polysaccharides of some cereal foods in the human small intestine. *Am. J. Clin. Nutr.*, v.42, n.5, p.778-787, 1985.
- FAN, L. F.; HE, W.; CHANG, Y. Z.; XIANG, B.; DU, Q.; WANG, F.; QIN, M.; CAO, D. Y. Studies of chitosan/Kollicoat SR 30D film-coated tablets for colonic drug delivery. *Int. J. Pharm.*, v.375, n.1-2, p.8-15, 2009.
- FINEGOLD, S. M.; SUTTER, V. L.; SUGIHARA, P. T.; ELDER, H. A.; LEHMANN, S. M.; PHILLIPS, R. L. Fecal microbial flora in Seventh Day Adventist populations and control subjects. *Am. J. Clin. Nutr.*, v.30, n.11, p.1781-1792, 1977.
- FUDE, C.; LEI, Y.; JIE, J.; HONGZE, P.; WENHUI, L., DONGMEI, C. Preparation and in vitro evaluation of pH, time-based and enzyme-degradable pellets for colonic drug delivery. *Drug. Dev. Ind. Pharm.*, v.33, n.9, p.999-1007, 2007.
- GAMELIN, E.; BOISDRON-CELLE, M.; DELVA, R.; REGIMBEAU, C.; CAILLEUX, P.E.; ALLEAUME, C.; MAILLET, M.L.; GOUDIER, M.J.; SIRE, M.; PERSON-JOLY, M.C.; MAIGRE, M.; MAILLART, P.; FETY, R.; BURTIN, P.; LORTHOLARY, A.; DUMESNIL, Y.; PICON, L.; GESLIN, J.; GESTA, P.; DANQUECHIN-DORVAL, E.; LARRA, F.; Robert, J. Long-term weekly treatment of colorectal metastatic cancer with fluorouracil and leucovorin: results of a multicentric prospective trial of fluorouracil dosage optimization by pharmacokinetic monitoring in 152 patients. *J. Clin. Oncol.*, v.16, n.4, p.1470-1478, 1998.
- GHAFFARI, A.; NAVAEE, K.; OSKOUI, M.; BAYATI, K.; RAFIEE-TEHRANI, M. Preparation and characterization of free mixed-film of pectin/chitosan/Eudragit RS intended for sigmoidal drug delivery. *Eur. J. Pharm. Biopharm.*, v.67, n.1, p.175-186, 2007.
- GUARNER, F.; MALAGELADA, J. R. Gut flora in health and disease. *Lancet*, v.361, n.9356, p.512-519, 2003.
- GUPTA, V. K.; BECKERT, T. E.; PRICE, J. C. A novel pH- and time-based multi-unit potential colonic drug delivery system. I. Development. *Int. J. Pharm.*, v.213, n.1-2, p.83-91, 2001.
- JAIN, S.K.; JAIN, A. Target-specific drug release to the colon. *Expert. Opin. Drug. Deliv.*, v.5, n.5, p.483-498, 2008.
- JOSE, S.; PREMA, M.T.; CHACKO, A.J.; THOMAS, A.C.; SOUTO, E. Colon specific chitosan microspheres for chronotherapy of chronic stable angina. *Colloids. Surf. B. Biointerfaces*, v.83, n.2, p.277-283, 2011.
- LIU, H.; YANG, X.; NIE, S.F.; WEI, L.L.; ZHOU, L.L.; LIU, H.; TANG, R.; PAN, W.S. Chitosan-based controlled porosity osmotic pump for colon-specific delivery system: screening of formulation variables and in vitro investigation. *Int. J. Pharm.*, v.332, n.1-2, p.115-124, 2007.
- LORENZO-LAMOSAS, M. L.; REMUNAN-LOPEZ, C.; VILAJATO, J. L.; ALONSO, M. J. Design of microencapsulated chitosan microspheres for colonic drug delivery. *J. Control. Release*, v.52, n.1-2, p.109-118, 1998.
- LUO, J.; ZHONG, Y.; CAO, J.; CUI, H. Efficacy of oral colon-specific delivery capsule of low-molecular-weight heparin on ulcerative colitis. *Biomed. Pharmacother.*, v.65, n.2, p.111-117, 2011.

- MANO, J.F.; SILVA, G.A.; AZEVEDO, H.S.; MALAFAYA, P.B.; SOUSA, R.A.; SILVA, S.S.; BOESEL, L.F.; OLIVEIRA, J.M.; SANTOS, T.C.; MARQUES, A.P.; NEVES, N.M.; REIS, R.L. Natural origin biodegradable systems in tissue engineering and regenerative medicine: present status and some moving trends. *J. R. Soc. Interface.*, v.4, n.17, p.999-1030, 2007.
- MANSUR, H.S.; ORÉFICE, R.L.; MANSUR, A.A.P. Characterization of poly(vinyl alcohol)/poly(ethylene glycol) hydrogels and PVA-derived hybrids by small-angle X-ray scattering and FTIR spectroscopy. *Polymer*, v.45, n. 21, p.7193-7202, 2004.
- MCCONNELL, E. L.; MURDAN, S.; BASIT, A. W. An investigation into the digestion of chitosan (noncrosslinked and crosslinked) by human colonic bacteria. *J. Pharm. Sci.*, v.97, n.9, p.3820-3829, 2008.
- MUZZARELLI, R. A. Biomedical exploitation of chitin and chitosan via mechano-chemical disassembly, electrospinning, dissolution in imidazolium ionic liquids, and supercritical drying. *Mar. Drugs*, v.9, n.9, p.1510-1533, 2011.
- NATH, B.; NATH, L.K. Studies on stercuria gum formulations in the form of osmotic core tablet for colon-specific drug delivery of azathioprine. *PDA J. Pharm. Sci. Technol.*, v.67, n.2, p.172-184, 2013.
- PARK, J. H.; SARAVANAKUMAR, G.; KIM, K.; KWON, I. C. Targeted delivery of low molecular drugs using chitosan and its derivatives. *Adv. Drug. Deliv. Rev.*, v.62, n.1, p.28-41, 2010.
- PINOTTI, A.; GARCÍA, M. A.; MARTINO, M. N.; ZARITZKY, N. E. Study on microstructure and physical properties of composite films based on chitosan and methylcellulose. *Food Hydrocolloid*, v.21, n.1, p.66-72, 2007.
- PRODPRAN, T.; BENJAKUL, S.; ARTHARN, A. Properties and microstructure of protein-based film from round scad (*Decapterus maruadsi*) muscle as affected by palm oil and chitosan incorporation. *Int. J. Biol. Macromol.*, v.41, n.5, p.605-610, 2007.
- RUBINSTEIN, A. Approaches and opportunities in colon-specific drug delivery. *Crit. Rev. Ther. Drug. Carrier. Syst.*, v.12, n2-3, p.101-149, 1995.
- RUEDA, D. R.; SECALL, T.; BAYER, R. K. Differences in the interaction of water with starch and chitosan films as revealed by infrared spectroscopy and differential scanning calorimetry. *Carbohydr. Polym.*, v.40, n.1, p.49-56, 1999.
- SALYERS, A. A.; LEEDLE, J. A. *Carbohydrate metabolism in the human colon*, Academic Press, New York, , 1983. p. 129-146.
- SEBTI, I.; CHOLLET, E.; DEGRAEVE, P.; NOEL, C.; PEYROL, E. Water sensitivity, antimicrobial, and physicochemical analyses of edible films based on HPMC and/or chitosan. *J. Agric. Food Chem.*, v.55, n.3, p.693-699, 2007.
- SINGLA, A. K.; CHAWLA, M. Chitosan: some pharmaceutical and biological aspects--an update. *J. Pharm. Pharmacol.*, v.53, n.8, p.1047-1067, 2001.
- TOZAKI, H.; ODORIBA, T.; OKADA, N.; FUJITA, T.; TERABE, A.; SUZUKI, T.; OKABE, S.; MURANISHI, S.; YAMAMOTO, A. Chitosan capsules for colon-specific drug delivery: enhanced localization of 5-aminosalicylic acid in the large intestine accelerates healing of TNBS-induced colitis in rats. *J. Control. Release*, v.82, n.1, p.51-61, 2002.
- VAGHANI, S.S.; PATEL, M.M.; SATISH, C.S. Synthesis and characterization of pH-sensitive hydrogel composed of carboxymethyl chitosan for colon targeted delivery of ornidazole. *Carbohydr. Res.*, v.347, n.1, p.76-82, 2012.
- VARGAS, M.; ALBORS, A.; CHIRALT, A.; GONZÁLEZ-MARTÍNEZ, C. Characterization of chitosan-oleic acid composite films. *Food Hydrocolloid*, v.23, n.2, p.536-547, 2009.
- YANG, L. Biorelevant dissolution testing of colon-specific delivery systems activated by colonic microflora. *J. Control. Release*, v.125, n.2, p.77-86, 2008.
- YIN, H.Q.; LEE, E.S.; KIM, D.; LEE, K.H.; OH, K.T.; BAE, Y.H. Physicochemical characteristics of pH-sensitive poly(L-Histidine)-b-poly (ethylene glycol)/poly(L-Lactide)-b-poly(ethylene glycol) mixed micelles. *J. Control. Release*, v.126, n.2, p.130-138, 2008.
- YU, Q.; SONG, Y.; SHI, X.; XU, C.; BIN, Y. Preparation and properties of chitosan derivative/poly(vinyl alcohol) blend film crosslinked with glutaraldehyde. *Carbohydr. Polym.*, v.84, n.1, p.465-470, 2011.

ZAMBITO, Y.; DI COLO, G. Preparation and *in vitro* evaluation of chitosan matrices for colonic controlled drug delivery. *J. Pharm. Pharm. Sci.*, v.6, n.2, p.274-281, 2003.

ZHANG, H.; ALSARRA, I. A.; NEAU, S. H. An *in vitro* evaluation of a chitosan-containing multiparticulate system for macromolecule delivery to the colon. *Int. J. Pharm.*, v.239, n.1-2, p.197-205, 2002.

Received for publication on 04th January 2015

Accepted for publication on 10th March 2016