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Development and *in vitro* evaluation of coated pellets containing chitosan to potential colonic drug delivery

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ABSTRACT

In this work pellets containing chitosan for colonic drug delivery were developed. The influence of the polysaccharide in the pellets was evaluated by swelling, drug dissolution and intestinal permeation studies. Drug-loaded pellets containing chitosan as swellable polymer were coated with an inner layer of Kollicoat® SR 30 D and an outer layer of the enteric polymer Kollicoat® MAE 30 DP in a fluidized-bed apparatus. Metronidazole released from pellets was assessed using Bio-Dis dissolution method. Swelling, drug release and intestinal permeation were dependent on the chitosan and the coating composition. The drug release data fitted well with the Weibull equation, indicating that the drug release was controlled by diffusion, polymer relaxation and erosion occurring simultaneously. The film coating was found to be the main factor controlling the drug release and the chitosan controlling the drug intestinal permeation. Coated pellets containing chitosan show great potential as a system for drug delivery to the colon.

1. Introduction

The colon is showing increasing relevance as a target for drug delivery, because of the therapeutic benefits to be gained from topical treatment of local disorders, such as inflammatory bowel disease, irritable bowel disease and carcinoma (Basit, 2005). Colon-specific drug delivery is intended to improve the efficacy and reduce side effects by delivering high drug concentrations topically at the disease site. An ideal colon-specific drug delivery system should prevent drug release in the stomach and small intestine and, eventually, release the drug into the colon (Rubinstein, 1995). This requires a triggering mechanism built in the delivery system responsive to the physiological changes particular to the colon. Commonly used pharmaceutical strategies to achieve a colon-specific drug delivery include timed-release similar to the gastrointestinal (GI) transit time, pH-sensitive polymer coatings, prodrugs, and delivery systems activated by the colonic microflora (Yang, Chu, & Fix, 2002).

The approach based on pH variation for colonic drug delivery focuses on the pH differential along the GI tract, with values increasing from about 1 to 2.5 in the stomach, 6.6 in the proximal small bowel until a peak of about 7.5 in the terminal ileum, followed by a fall to pH 6.8 in the colon (Evans et al., 1988). This concept utilizes polymeric carriers that are insoluble in the low pH media of the upper GI tract, but dissolve at the higher, near neutral pH of the distal gut.

Chitosan is a functional linear polymer derived from chitin, the most abundant natural polysaccharide on the earth after cellulose, and it is not digested in the upper GI tract by human digestive enzymes (Bhattarai, Gunn, & Zhang, 2010; Park, Saravanakumar, Kim, & Kwon, 2010). Chitosan (CS) is added in delivery systems because it is susceptible to glycosidic hydrolysis by microbial enzymes in the colon (Chourasia & Jain, 2003; McConnell, Murdan, & Basit, 2008; Muzzarelli, 1993, 2011). The main properties favoring the use of chitosan in various pharmaceutical preparations include its biological inertness, biodegradability and bioadhesive properties (Ferrari et al., 2011; Ibekwe, Fadda, Parsons, & Basit, 2006; Oliveira, Ferrari, Carvalho, & Evangelista, 2010).

The objective of this study was to develop a multi-unit system for colonic drug delivery based on both solubility dependent upon pH and specific bacterial enzymatic erosion. Pellets were chosen as dosage form because they can spread out over a large area of intestine, making them more effective for the treatment of local

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diseases of colon (Amighi, Timmermans, Puigdevall, & Moës, 1998; Gupta, Beckert, & Price, 2001; Yang, 2008).

The purpose of the study was to obtain a colonic delivery system based on pellets containing chitosan, coated with Kollicoat® SR, an aqueous colloidal polyvinyl acetate dispersion used for extended release coatings, and/or an enteric polymer dispersion, Kollicoat® MAE, used to provide an outer coating to the pellets to avoid premature gastric drug delivery.

2. Materials and methods

2.1. Materials

Metronidazole and chitosan (low molecular weight; 75–85% deacetylated; 20–300 mPa s) were purchased from Sigma Aldrich (São Paulo, Brazil). Kollicoat® SR 30 D (aqueous dispersion of polyvinyl acetate; MW 450,000; 65–85 mPa s), Kollicoat® MAE 30 DP (aqueous dispersion of methacrylic acid and ethyl acrylate copolymer; MW 250,000; 5–10 mPa s) and Kollidon® 30 were a gift from BASF (São Paulo, Brazil). Microcrystalline cellulose, PVP K30, PEG 4000, propylene glycol and talc were obtained from Synth (Diadema, Brazil). All other reagents and solvents were of analytical grade.

2.2. Film coating of pellets

2.2.1. Preparation of drug-loaded pellet cores

Drug-loaded pellet cores were prepared by extrusion–spheronization. Metronidazole (MT) (30%), chitosan (10%), microcrystalline cellulose (55%) and PVP K30 (5%) were mixed in a planetary mixer (Model K5SS, Kitchen Aid, USA) for 20 min. The granulating liquid, a 10% PEG 4000 aqueous solution, was added slowly to the powder blend, which was then mixed until a homogeneous, cohesive, plastic mass was obtained. The resulting wet mass was extruded at a speed of 18 rpm (Model 20, Caleva, England), through perforations of 1.0 mm in diameter. Spheronization was performed in a spheronizer (Model 250, Caleva, England) with a rotating plate of regular cross-hatch geometry, at a speed of 1000 rpm, for 3 min. Pellets were then dried on a fluidized bed (Hüttlin®, model Mycrolab, Germany) at 40 °C for 10 min. Samples without chitosan were also prepared as control.

2.2.2. Preparation of coated pellets

For the inner coat, a dispersion of Kollicoat® SR 30 D (6%, w/v) containing propylene glycol, talc and Kollidon® 30 was sprayed onto the pellets core using a fluidized bed coater Hüttlin® (Mycrolab, Germany). A dye solution (Sicovit® Gelb 10E172, BASF) was included in the formulation to differentiate the coatings.

For the outer layer, the pellets containing the sub-coating layer of Kollicoat® SR 30 D were further coated with a dispersion of Kollicoat® MAE 30 DP (12%, w/v solids content) containing propylene glycol and a dye solution (Sicovit® Red 30E172, BASF), using the same fluidized-bed processor.

Coating conditions were: batch size = 100 g, inlet temperature = 60 °C, product temperature = 40 °C, air flow = 15 m^3/h, nozzle diameter = 1.2 mm, spray pressure = 8.0 psi, spray rate = 1.8 g/min, final drying at 40 °C for 15 min.

2.2.3. Experimental design

In order to verify the influence of chitosan and polymers coatings on the MT release at colonic region an experimental design was developed. The variables analyzed were (i) the presence of chitosan, (ii) the enteric coating and (iii) the sustained release coating at two levels each in the range indicated in Table 1.

The two responses studied along with their constraint values are listed below:

Amount of drug remaining after 30 min (Y_1): $MT\%_{0.5\,h}$
Amount of drug remaining after 6 h (Y_2): $MT\%_{6\,h}$

2.3. Pellets characterization

2.3.1. Sieve analysis

The particle size distribution of the pellets was determined using a set of test sieves (1.25; 1.18; 1.12; 1.0; 0.9; 0.8; 0.71 mm) attached to a sieve shaker (Haver & Bocker Model EML Digital Plus, Westfalen, Germany) operated for 2 min at a frequency of 50 Hz and an amplitude of 2 mm. The percentage of weight retained was plotted against the mean size of pellets in each fraction.

2.3.2. Pellets size and shape

Pellet size and shape were determined using an image analysis system. Morphological examination of the pellets shape was carried out using a Leica MZ APO stereoscope. The capture of images associated with MOTIC Image Advanced 3.2 software allows the analysis of different parameters, including morphological characterization, *i.e.* shape factors.

2.3.3. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to visualize the surface morphology of the coated pellets. For the assay, dry samples were placed on a double face tape adhered to a metal support and coated with colloidal gold under vacuum. Photomicrographs were taken with a scanning electron microscope (JEOL JSM–T330A, Jeol, Tokyo, Japan).

2.3.4. Liquid uptake

Liquid uptake measurements were carried out using an Enslin apparatus (Ferrari, Oliveira, Chibebe, & Evangelista, 2009). For these studies, each sample was analyzed in simulated gastric fluid without enzymes (pH 1.2) and simulated enteric fluid (PBS pH 6.8). For the assay, 0.5 g of pellets samples were placed on the sintering filter and the volume of water absorbed after 15, 30, 60, 90 and 120 min was measured on the graduated pipette. The assays were carried out in triplicate and the results expressed as % of liquid uptake in relation to the initial mass of the samples. Statistical analysis of the results was performed by ANOVA with a significance level α of 0.05.

2.3.5. In vitro drug release

The dissolution studies were carried out in triplicate on a Bio-Dis III reciprocating cylinder (Varian Inc., Cary, USA) apparatus coupled to a sampler (Varian, model VK 8000, with peristaltic pump, Cary, USA) and set with an oscillation rate of 8.0 dips per minute (dpm). The temperature was kept at 37 °C and buffer solutions with different pH values (250 ml per vessel) were used as dissolution media. At first, the drug dissolution was determined in simulated gastric fluid pH 1.2 for 30 min. Afterwards, pellets were transferred to acetate buffer pH 4.5 for 1 h, PBS pH 6.0 for 2 h, PBS pH 6.8 for 3 h and then, finally, to PBS pH 7.2 during 2 h, totaling 8 h of experiment. The amount of drug release from pellets (300 mg of pellets from the size fraction of 1.00–1.18 mm) was measured at the suitable time interval and the MT released from pellets was then determined spectrophotometrically (Hewlett Packard, Mod. 8453, coupled with HP UV-Visible ChemStation Software) at 277 and 320 nm. $MT\%_{0.5\,h}$ and $MT\%_{6\,h}$ were used for verify the drug release characteristics.

2.3.6. Kinetics mechanisms

Mathematical models were applied to verify the mechanisms of drug release from pellets and the *in vitro* drug release data were fitted (SigmaPlot 10.0 software) to Weibull release kinetic model (Papadopoulou, Kosmidis, Vlachou, & Macheras, 2006), which presented the highest adjusted coefficient of determination.

Table 1
Coated MT pellets formulation using 2^3 factorial design and results data of mean values of responses, *i.e.* remaining MT released at 30 min (%, Y_1), remaining MT released at 6 h (%, Y_2) and MT transport mechanism from coated pellets according to the Weibull model.

Samples	X_1	X_2	X_3	Y_1	Y_2	Weibull Model		
	CS in pellets	Kollicoat® SR coating	Kollicoat® MAE coating	MT_{rem}%0,5 h	MT_{rem}%6 h	R^2 adjusted	b	$MT\%_{8\,h}$
1 (−1 −1 −1)	0	0	0	11.55	0	–	–	–
2 (+1 −1 −1)	10	0	0	0	0	–	–	–
3 (−1 +1 −1)	0	3	0	99.68	72.30	0.9921	1.4014	59.6
4 (+1 +1 −1)	10	3	0	99.68	22.25	0.9895	1.1851	79.7
5 (−1 −1 +1)	0	0	6	98.58	0	–	–	–
6 (+1 −1 +1)	10	0	6	93.46	0	–	–	–
7 (−1 +1 +1)	0	3	6	100	93.72	0.9193	3.8601	14.4
8 (+1 +1 +1)	10	3	6	100	92.94	0.9702	1.2718	12.5

2.3.7. Intestinal permeation

The intestinal permeation of MT was evaluated using the everted gut sac model, according to modifications introduced by Barthe, Bessouet, Woodley, and Houin (1998), Barthe, Woodley, and Houin (1999). The experiment evaluated the permeation of MT from coated and uncoated pellets, across the duodenal segment of rat intestine. The amount of MT, 1.0 mg, was the same for all formulations. The total assays were carried out along 270 min (4.5 h), in three steps, with each segment being analyzed during 90 min to ensure the viability of the intestinal tissue in TC 199 solution, according to Barthe et al. (1999).

Firstly, male adult Wistar rats (250 ± 10 g) were handled in accordance with the provisions of the Guide to care and use of experimental animals in all experimental procedures (CEUA/UFSCar Ethics in Research Committee, # 003/2011). Animals were kept in a fasting condition for 8 h before the assay. Afterwards, they were anesthetized with sodium pentobarbital and a duodenum segment (6 cm length) of the rat intestine was immediately dissected and flushed with TC199 solution at 10 °C. The flushing TC199 solution was composed by 145 mM NaCl; 4.56 mM KCl; 1.25 mM $CaCl_2{\cdot}2H_2O$; 5 mM $NaHPO_4$ (pH 7.4). The intestinal segment was gently inverted with the aid of a flexible cotton swab with its extremity protected by a fine fabric (mini brush). One end of the segment was clamped and filled with fresh TC199 medium and sealed with a second clamp, in order to obtain the closed sac. Then, the everted sacs were placed in Erlenmeyer flasks containing the formulations and 20 ml of the TC199 medium with the addition of 10 mM glucose, oxygenated (O_2:CO_2 = 95:5) and incubated at 37 °C. The everted sacs were collected by removing the sacs from the flasks, after 90 min, and externally washed with fresh TC199 medium. After the first sac was removed, a novel segment was put in the same medium of incubation and maintained for further 90 min. After that, a third segment was put in the same medium totalizing 270 min (or 4.5 h). The samples were collected in triplicate (three independent intestines). Then each sac was separately cut, and the internal serosal fluid drained into small tubes. The content was filtered through a cellulose membrane filter (MilliPore, 0.22 μm). The internal medium was analyzed by UV spectrophotometer (Hewlett Packard, Mod. 8453, coupled with HP UV–Visible ChemStation Software) set at 320 nm for evaluating the MT concentration after each 90 min of incubation. The results were expressed as the mean ± standard deviation of three independent experiments and statistical analysis of the results was performed by ANOVA with a significance level α of 0.05.

3. Results and discussion

Pellets were only possible to be prepared with a maximal chitosan concentration of 10%. Higher chitosan concentrations result in pellets with lack of both an adequate sphericity and homogeneous size distribution, both features being of crucial importance for controlling drug release.

3.1. Pellets characterization

The analysis of pellets included the aspect, morphological quality and circularity degree. The pellets obtained were almost spherical and very uniform in shape. It can be observed that the presence of 10% chitosan did not influence the morphological characteristics of the pellets. The average diameter of the pellets from the two samples was very similar, being 0.962 ± 0.193 and 0.961 ± 0.134 mm for samples with and without chitosan, respectively. For this parameter, a value close to 1.0 mm would be expected, since a screen whit this gap was used during the extrusion process. The final value is very acceptable because the volume of the dried pellets frequently is shorter, and in this case the difference was only 0.04 mm.

Concerning the degree of circularity of the pellets, it was observed that the samples with or without chitosan showed similar values, *i.e.* the presence of this polymer in the proportion of 10% did not affect this property. The values obtained (0.688 ± 0.062 and 0.696 ± 0.062 for samples with and without chitosan, respectively) indicate that the pellets are not perfectly circular and, consequently, not perfectly spherical, since the results are not so close to 1.0, a value that characterizes a perfect circle (or a perfect sphere) (Andréo-Filho, Pessole, Issa, Villela, & Ferraz, 2009; Podczeck, Rahman, & Newton, 1999).

The results of the particle size distribution for pellets with and without chitosan are graphically presented in Fig. 1. They were determined by the percentage of weight retained in each sieve. There was no significant statistical difference between the sizes of pellets containing or not chitosan (ANOVA: $p = 0.4983$; $\alpha = 0.05$). The majority of the pellets (more than 60%) stood in the range size from 0.9 to 1.2 mm.

The individual size of the pellets is an important parameter when a drug release system is being designed because the smaller the particles are, the faster the drug release rate will be and *vice versa*. Similarly, pellets with a narrower size distribution will assure a more homogeneous drug release rate.

The morphology of the pellets analyzed by scanning electron microscopy is shown in Figs. 2 and 3. Some fractures on the surface of the pellets containing or not chitosan can be observed. Analyses were performed under magnifications of 75 and 3500-fold. It was possible to observe the general aspect of sets of pellets as well as details of their surface, such as pores.

Fig. 2A and B shows the SEM images obtained from the uncoated pellets. Under the smaller magnification, the surface seems to be very rough. Under the greatest magnification, some pores can be seen on the pellets surface and its roughness becomes more evident. The presence of 10% chitosan had no influence on morphological characteristics of the pellets.

Fig. 3 shows the SEM images obtained from coated MT pellets containing chitosan. SEM micrographs of coated pellets do not show any pores on the surfaces of the film coated pellets.

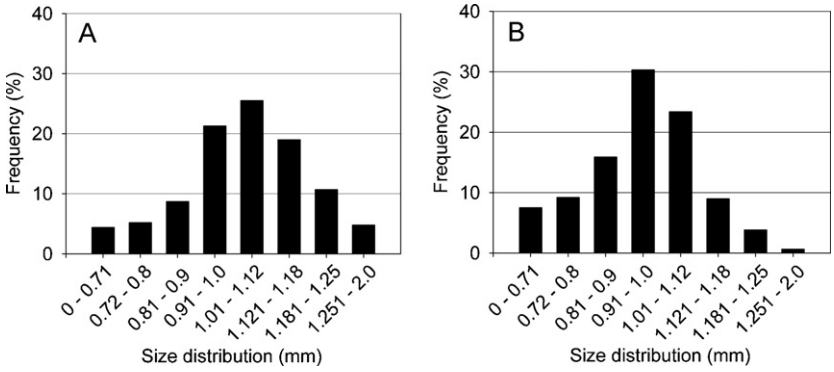

Fig. 1. Size distribution of pellets: (A) without chitosan; (B) with 10% chitosan.

On Kollicoat® SR coated pellets, a uniform layer of polymer deposited during the coating process can be seen, as well as for the pellets coated with Kollicoat® MAE alone and with both polymers (Kollicoat® SR and MAE).

3.2. Liquid uptake

The samples swelled quickly during the first 15 min of analysis, followed by a further slight volume increase, reaching a steady-state before the completion of the experiment in 2 h. Fig. 4 represents the swelling profile of the samples in simulated gastric fluid (pH 1.2) and in simulated enteric fluid (pH 6.8) after 2 h.

The swelling profile of pellets changed according to (i) the medium assayed, (ii) to the type of coating and (iii) to the presence of chitosan.

The most samples presented the same swelling profile in both media, except for the uncoated pellets containing chitosan, which exhibited higher enteric fluid uptake, and the sample coated with Kollicoat® SR but also containing chitosan, which exhibited higher gastric fluid uptake.

In simulated gastric medium, Kollicoat® SR coated pellets containing chitosan swelled in a similar way to uncoated pellets, due to the pore-forming agent present in these coating formulations, Kollidon® 30, more soluble in acid solutions, allowing the medium penetration through the pores built after its dissolution.

In simulated enteric fluid, pellets containing chitosan and coated with Kollicoat® SR showed lower medium uptake, indicating that the pore-forming agents present in this coating were less soluble in this fluid.

The type of the coating also affected the swelling of the pellets. In both media, samples coated with Kollicoat® MAE showed significantly higher swelling degree than samples with this polymer as outer coating. Additionally, pellets coated with both Kollicoat® SR and Kollicoat® MAE swelled less than pellets coated with only

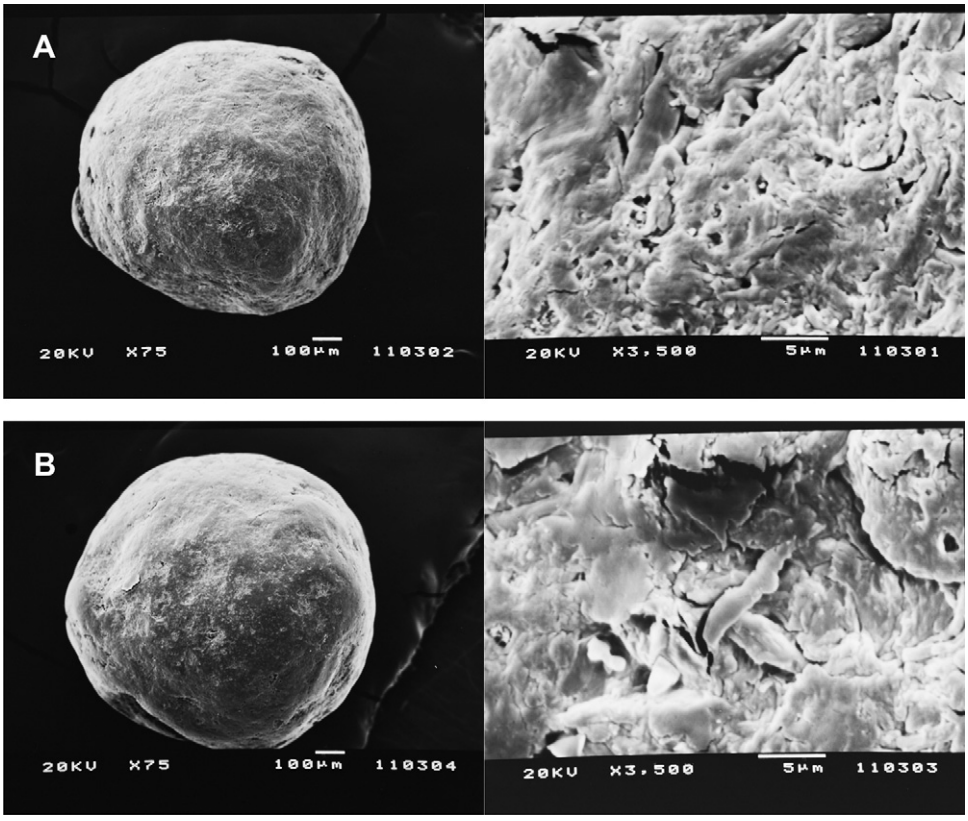

Fig. 2. Scanning electron microscopy of pellets: (A) uncoated pellets without chitosan; (B) uncoated pellets with chitosan (75 and 3500×).

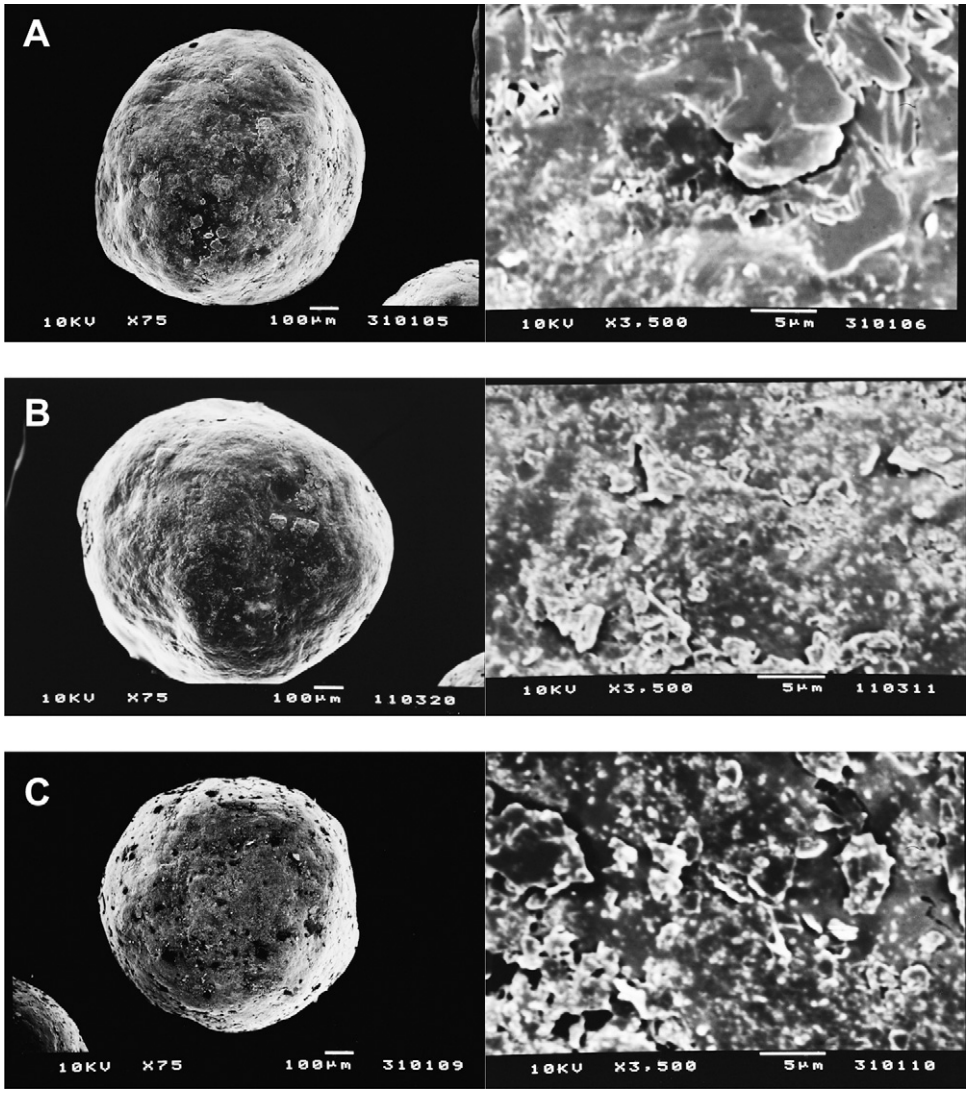

Fig. 3. Scanning electron microscopy of pellets: (A) Kollicoat® SR coated pellets with chitosan; (B) Kollicoat® MAE coated pellets with chitosan; (C) Kollicoat® SR and MAE coated pellets with chitosan (75 and 3500×).

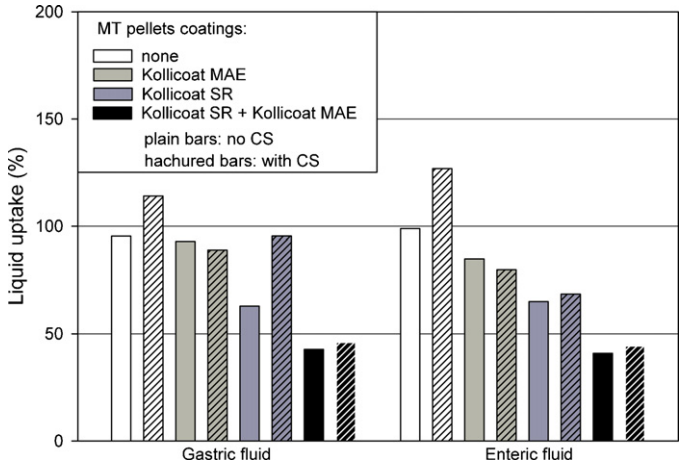

Fig. 4. Swelling studies (same letters represent no significant difference ($p < 0.05$) (CS = chitosan).

one of the polymers. This combination of polymers prevented the uptake of more liquid because of the protection given by the enteric coating (Kollicoat® MAE), avoiding the pore-forming agents to dissolve and, consequently, the medium to diffuse the pellets through the pores inwards. Pellets containing or not chitosan coated with both Kollicoat® SR and MAE, and pellets without chitosan coated with only Kollicoat® SR showed lower liquid uptake than uncoated pellets in both media.

Finally, uncoated pellets containing chitosan exhibited higher enteric fluid uptake than pellets without chitosan ($p = 0.0125$) and than all other samples, confirming that the presence of chitosan has great influence on the swelling of the pellets.

3.3. In vitro drug release

The Bio-Dis is the most attractive apparatus for the study of modified drug release because it simulates the passage of the dosage form through the GI tract by varying the pH easily and reproducibly.

The pellets exhibited limited swelling and did not disintegrate during the dissolution assay (8 h). MT was completely released in

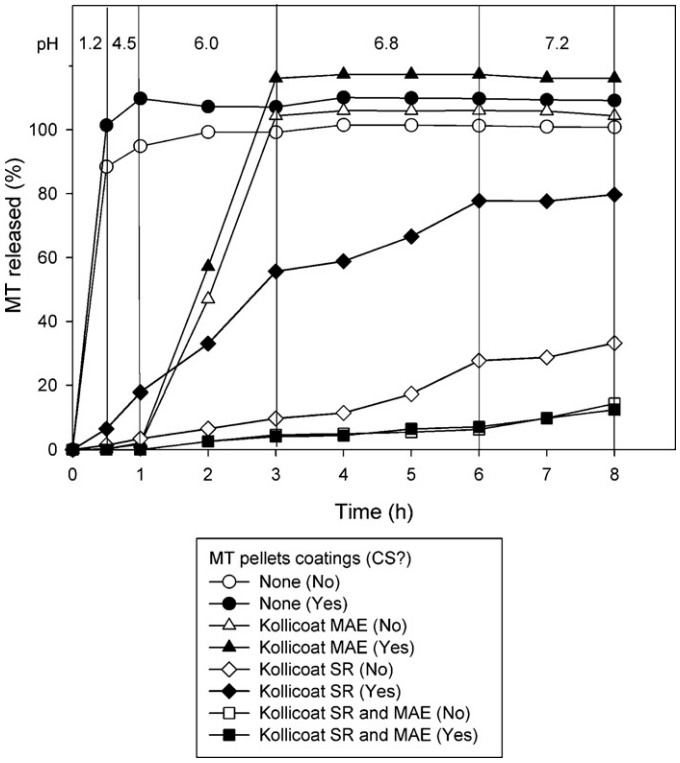

Fig. 5. *In vitro* MT release (for the sake of clarity errors bars are not shown) (CS = chitosan).

1 h from uncoated pellets containing or not chitosan in simulated gastric fluid and acetate buffer pH 4.5 (Fig. 5). For this burst release contributed the porous nature of the pellets' surface, as could be observed by scanning electron microscopy, into which the medium penetrated easily and dissolved the entrapped drug.

Kollicoat® MAE was successfully used as enteric coating since no drug was released in simulated gastric fluid. However, as this coating contains acidic functional groups and, consequently, is soluble at pH above 5.5, all MT was released after 2 h in PBS pH 6.0.

Kollicoat® SR coating enabled an extended release of MT at least during 8 h irrespective of pH variation. About 60% of MT was released from coated pellets containing chitosan after 3 h, while the pellets without chitosan released 10% of the drug during the same time period. This behavior was kept unchanged until 8 h of assay: about 80% of MT was released from chitosan-pellets and only about 30% from pellets without chitosan, supporting the fact that the presence of this polysaccharide and its higher swelling ability contributed to a faster drug release. The liquid uptake studies (Fig. 4) indicated that the pore-forming agents dissolved preferentially in simulated gastric fluid, forming pores on the coating, into which the penetrant flows and the resultant swollen chitosan increases the MT release rate. Samples without chitosan did not swell significantly, allowing the drug to remain longer in the pellets without being released.

The coating with Kollicoat® SR and MAE had no influence on the MT release, regardless to presence of chitosan or not. The association of these polymers resulted in an extended MT release from pellets, since about only 20% of the drug was released until 8 h. The release was prolonged because the enteric coating prevented the dissolution of the pore-forming agent and, consequently, the drug diffusion was slower.

The factorial design was carried out to analyze the effect of the variables: presence of chitosan, enteric coated and insoluble coated, which levels are presented in Table 1. The responses analyzed were the remaining amount of MT (still available in the pellets to be released) after 0.5 h and 6 h of dissolution, *i.e.* the ability of such variables in ensuring gastric protection and colonic drug release, respectively. Table 1 shows the conditions of these experiments and their respective responses (Y_1 and Y_2).

The main effects were the variables X_2 and X_3, Kollicoat® SR and MAE coating, respectively, for both responses. However, the major influence related to gastric protection was the Kollicoat® MAE coating, while X_2 (Kollicoat® SR) was the main factor influencing the colonic drug release.

Fig. 6 shows a graphical representation of the Y_1 response. The main effects affecting gastric protection, *i.e.* the factors that avoided the MT release in simulated gastric fluid were X_2 and X_3. The interaction between these factors also influenced the gastric protection, because the drug release from pellets coated with both polymers was prolonged, as showed by the drug dissolution profile in Fig. 5.

Fig. 6 also shows a graphical representation of the Y_2 response, the ability of MT to reach the colonic region. This response evaluated the factors influencing the drug release by assessing the amount of drug present in the pellets, not released, until 6 h of dissolution. MT incorporated in pellets coated with Kollicoat® SR and MAE was not released in simulated gastric and enteric fluid. This leads to conjecture that such systems should not allow the drug to be released into the upper GI tract.

The factor that caused the main influence on the Y_2 was the Kollicoat® SR coating (X_2), which prevented the MT release in media with different pH values. This coating is an insoluble polymer able to sustain the drug release for a long time. The factor X_3 (enteric coating) and its interaction with the factor X_2 also influenced the MT release.

The factor X_1, chitosan presence, also had influence on the Y_2 response. Chitosan is well known as a highly swellable substance. This swelling ability allows chitosan to act as the main responsible for the release rate, since the swelling process is the driven force in systems that enable the drug diffusion only through swollen polymer regions. This behavior restricts the drug diffusion, which can be controlled by varying the relative amount of the swellable polymer. Thereby, the drug is released from pellets by specific degradation of chitosan by colonic bacterial enzymes, allowing the drug to be available for local intestinal action.

Moreover, the *in vitro* MT dissolution assay showed that chitosan-pellets coated with Kollicoat® SR plus both Kollicoat® SR and MAE are promising to release MT in the colonic region.

3.4. Kinetics mechanisms

Coated pellets were analyzed by Weibull model in order to better characterize the drug release. The model was applied on the dissolution curves and the drug release mechanism was identified by some characteristic parameters. The Weibull equation expresses the cumulative fraction of the drug at time t:

$$m = 1 - \exp\left[\frac{-(t - t_i)^b}{a}\right] \quad (1)$$

where a defines the time scale of the process, t_i represents the interval time before the dissolution or release process (zero in most cases) starts, the b parameter characterizes the shape of dissolution curve as exponential ($b = 1$), sigmoidal ($b > 1$) or parabolic ($b < 1$). The term a may be replaced by the more informative parameter T_d, which is the time required to release 63.2% of drug, indicating the release mechanism of the drug transport within the polymer matrix (Costa & Lobo, 2001).

According to the Weibull model, values of $b > 1$ are indicative of a complex release mechanism. For values smaller than 0.75,

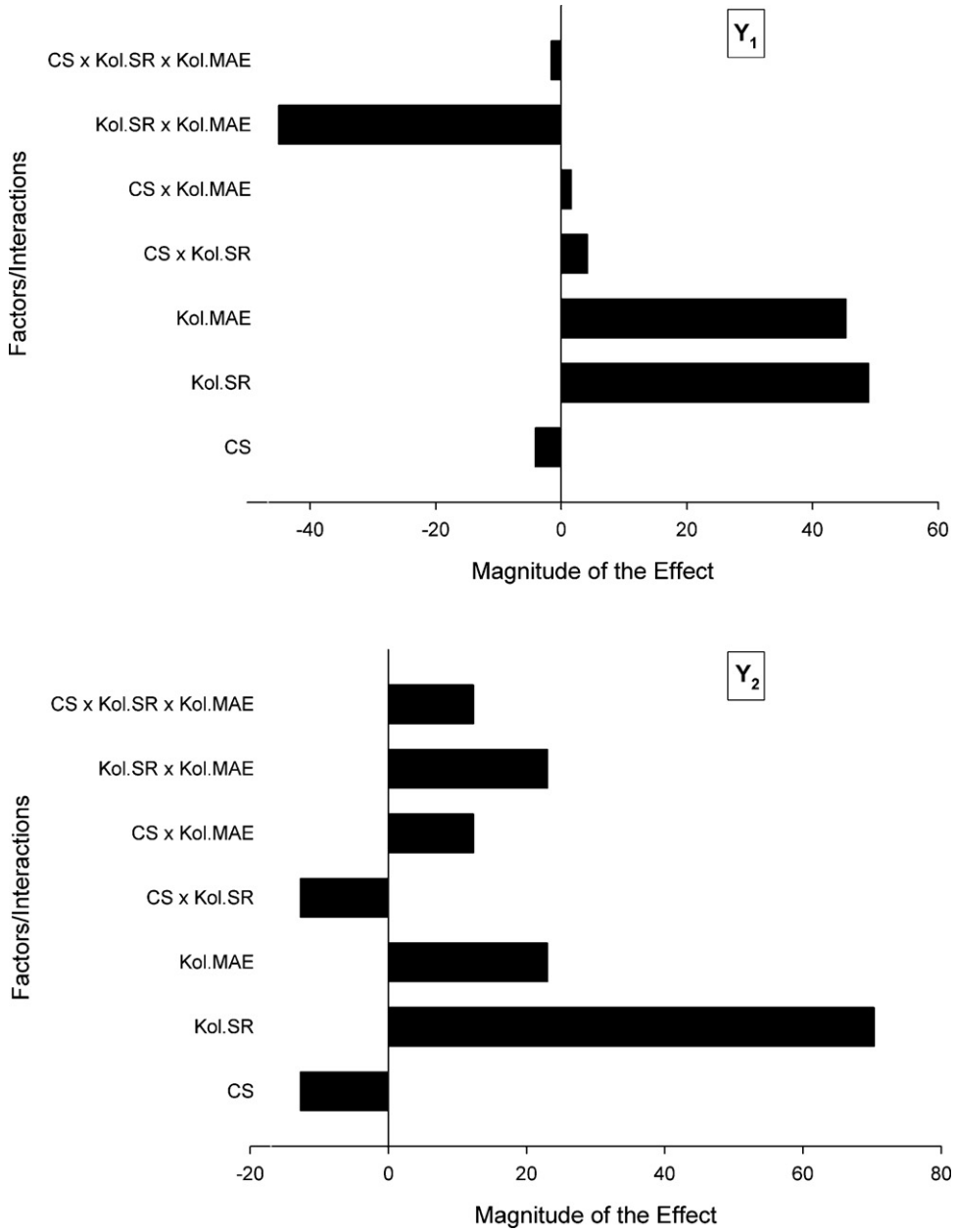

Fig. 6. Graphical representation of effect of factors on responses (CS = chitosan).

the diffusion occurs according to Fick's law. Intermediate values (0.75–1) indicate the so-called transport case II, in which the contribution of another mechanism has to be considered (Kosmidis & Macheras, 2007). The results of the regression analysis after applying the Weibull equation to the dissolution data indicated that the MT release from pellets is satisfactorily described by this model (Table 1).

The results shows that *b* values were greater than 1.0 in all samples, indicating that the MT release from pellets occurred by a complex mechanism in which several processes occur simultaneously.

Kollicoat® SR coated pellets show the best adjusted R^2 values. MT release from pellets coated with Kollicoat® SR and MAE also indicated a complex release mechanism, involving diffusion, swelling and erosion.

Both the coating and the internal structure of the pellets (chitosan presence) affected the drug release. These factors changed the dissolution rate but not the mechanism of drug release.

From uncoated pellets, the MT release was very fast and the corresponding correlation coefficient values were low, making unfeasible the application of this model.

3.5. Intestinal permeation

The everted intestinal sac technique in rats was used to study the absorption behavior of MT from coated pellets containing chitosan or not through the intestinal tissues. The drug concentration range within which the analytical curve remained linear was from 0.3 to 35 μg/ml and the correlation coefficient was 0.9989.

Fig. 7 shows the intestinal permeation profile and the cumulative permeation of MT across the duodenal segment after 270 min of incubation. Samples of free MT and uncoated pellets showed similar profile and no significant difference of total quantity of MT absorbed (100%). The higher absorbed amount of MT observed through the intestinal sacs might be attributed to the high percentage of drug available in mucosal fluid because of its easy solubility

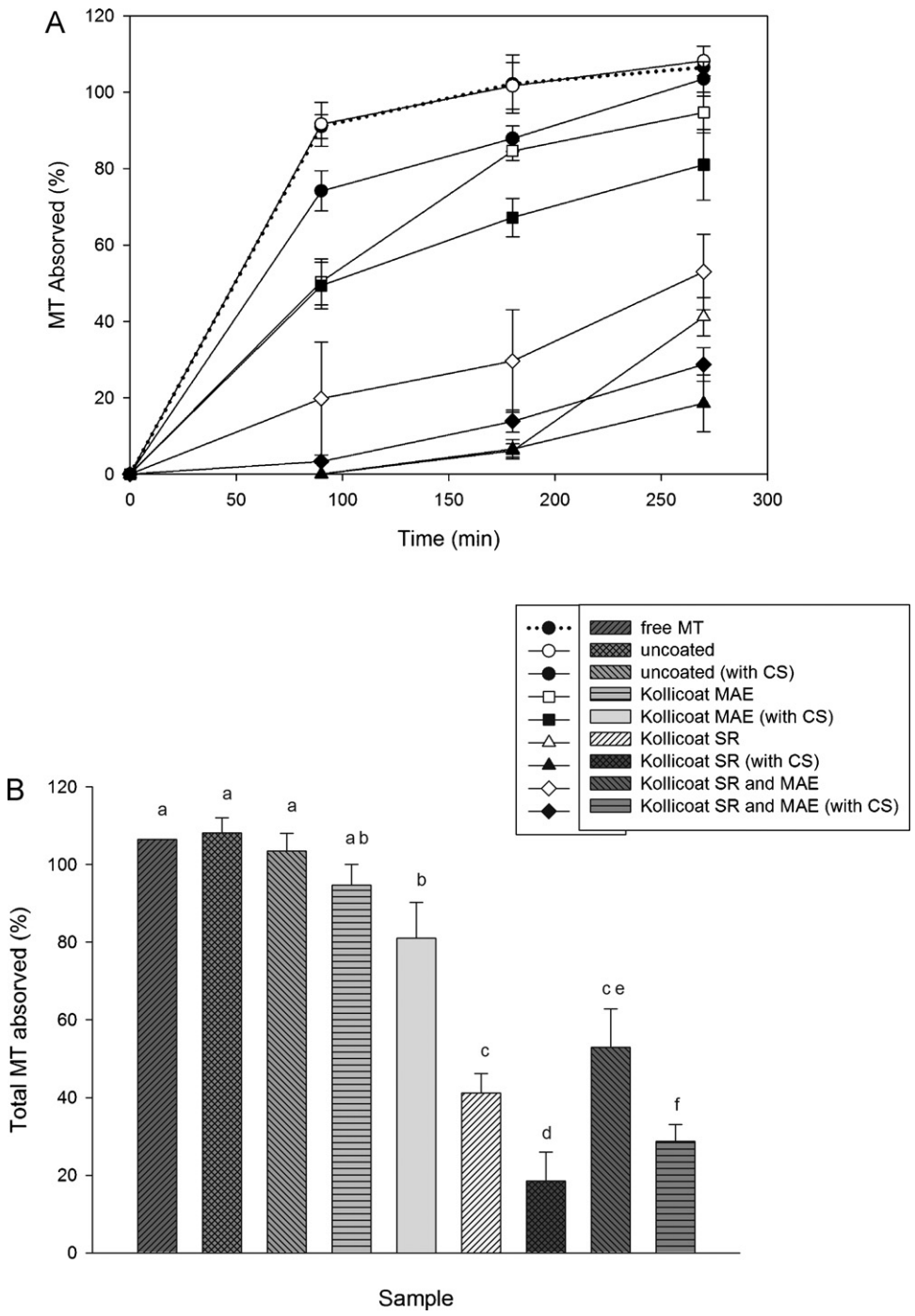

Fig. 7. Intestinal permeation of MT by everted intestinal sac method (CS = chitosan). (A) MT absorption profile during 270 min; (B) cumulative permeation of MT (same letters represent no significant difference ($p < 0.05$).

in this medium. For coated samples, statistical analysis indicated that the type of coating and the chitosan presence modify the MT absorption by intestinal mucosa.

More than 80% MT of samples coated by Kollicoat® MAE was permeated, because the film coating was dissolved in the medium (pH 7.4), and the observed behavior was the same of the uncoated pellets. The chitosan did not influence this permeation in these samples. However, the presence of chitosan interfered with the MT absorption by pellets coated by Kollicoat® SR and Kollicoat® SR and MAE. The assay of pellets coated by Kollicoat® SR showed that less than 50% of MT was absorbed in the time analyzed, due to the pore-forming agents dissolved preferentially in acid fluid, forming pores on the coating, into which the penetrant flows. Pellets with chitosan permeated a little quantity of MT by intestinal mucosa, indicating that chitosan in pellets provided controlled drug release and, consequently, slower intestinal permeation, according to what was observed by Ferrari et al. (2011). Pellets coated with Kollicoat® SR and MAE without chitosan presented the same permeation behavior as pellets coated only with Kollicoat® SR, because of the solubility of the enteric polymer.

For pellets containing chitosan the statistical analysis showed significant difference among all samples, indicating that the presence of chitosan associated with the different film coating modified the intestinal permeation of MT.

Chitosan has attracted a lot of attention in recent years as a potential absorption enhancer across epithelia in acidic conditions. The increase in the transport of these compounds is believed to be a result of an interaction of the positively charged amino groups on the C-2 position of chitosan with negatively charged sites on the cell membranes and tight junctions, thereby altering the integrity of the tight junctions to allow the paracellular transport. In solutions where the pH is less or in the order of the pK_a (6.0–6.5) of chitosan (Yalpani & Hall, 1984), the charge density of chitosan is an important factor for the enhancement of mucosal transport (Kotzé, Lueßen, de Boer, Verhoef, & Junginger, 1999; Schipper et al., 1997). Chitosan is a weak base and in neutral and basic environments the chitosan molecules will lose its charge and precipitate from solution. At these conditions, chitosan will be ineffective as an absorption enhancer, because at these higher pH values the chitosan molecules exist in a more coiled conformation.

In this study, the intestinal permeation from pellets with chitosan was slower than pellets without chitosan, because the potential use of chitosan as absorption enhancer in the more basic environments of the large intestine and colon are limited. Chitosan is able to allow paracellular transport by opening the tight junctions of epithelial cells, but this absorption enhancing effect is only possible in acidic environments due to the insolubility of this polymer in neutral and basic environments.

4. Conclusion

Pellets for colonic drug delivery based on chitosan and pH-dependency were developed. The systems constituted by MT pellets containing or not chitosan were coated with Kollicoat® SR and/or MAE. The techniques used lead to foresee an ease preparation of such systems in a larger scale.

An appropriate factorial design was successfully used in the development of coating formulations, for pellets containing or not chitosan. These systems show to be promising to colon-specific drug delivery, since they were able to retain the MT release until 8 h of dissolution in media with different pH values. According to the mathematical model, the drug release from the coated pellets follows a complex release mechanism, in which several processes, including diffusion, swelling, and erosion should be involved and occurs simultaneously. Intestinal permeation assay indicated that the presence of chitosan in coated pellets controlled the permeation of the MT, becoming a potential colonic drug delivery system.

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References

Amighi, K., Timmermans, J., Puigdevall, J., & Moës, A. J. (1998). Peroral sustained-release film-coated pellets as a means to overcome physicochemical and biological drug-related problems. I. *In vitro* development and evaluation. *Drug Development and Industrial Pharmacy*, *24*, 509–515.

Andréo-Filho, N., Pessole, L., Issa, M. G., Villela, C., & Ferraz, H. G. (2009). Gastro-resistant pellets of didanosine obtained by extrusion and spheronization: Assessing the production process. *Latin American Journal of Pharmacy*, *28*, 32–40.

Barthe, L., Bessouet, M., Woodley, J. F., & Houin, G. (1998). The improved everted gut sac: A simple method to study intestinal P-glycoprotein. *International Journal of Pharmaceutics*, *173*, 255–258.

Barthe, L., Woodley, J., & Houin, G. (1999). Gastrintestinal absorption of drugs: Methods and studies. *Fundamental and Clinical Pharmacology*, *13*, 154–168.

Basit, A. W. (2005). Advances in colonic drug delivery. *Drugs*, *65*, 1991–2007.

Bhattarai, N., Gunn, J., & Zhang, M. (2010). Chitosan-based hidrogels for controlled, localized drug delivery. *Advanced Drug Delivery Reviews*, *62*, 83–99.

Chourasia, M. K., & Jain, S. K. (2003). Pharmaceutical approaches to colon targeted drug delivery systems. *Journal of Pharmacy and Pharmaceutical Science*, *6*, 33–66.

Costa, P., & Lobo, J. M. S. (2001). Modeling and comparison of dissolution profiles. *European Journal of Pharmaceutical Science*, *13*, 123–133.

Evans, D. F., Pye, G., Bramley, R., Clark, A. G., Dyson, T. J., & Hardcastle, J. D. (1988). Measurement of gastrointestinal pH profiles in normal ambulant human subjects. *Gut*, *29*, 1035–1041.

Ferrari, P. C., Oliveira, G. F., Chibebe, F. C. S., & Evangelista, R. C. (2009). In vitro characterization of coevaporates containing chitosan for colonic drug delivery. *Carbohydrate Polymers*, *78*, 557–563.

Ferrari, P. C., Souza, F. M., Giorgetti, L., Oliveira, G. F., Chaud, M. V., Ferraz, H. G., et al. (2011). In vitro drug permeation from chitosan pellets. *Carbohydrate Polymers*, *87*, 2526–2531.

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.

Ibekwe, V. C., Fadda, H. M., Parsons, G. E., & Basit, A. W. (2006). A comparative *in vitro* assessment of the drug release performance of pH-responsive polymers for ileo-colonic delivery. *International Journal of Pharmaceutics*, *308*, 52–60.

Kosmidis, K., & Macheras, P. (2007). Monte Carlo simulations for the study of drug release from matrices with high and low diffusivity areas. *International Journal of Pharmaceutics*, *343*, 166–172.

Kotzé, A. F., Lueßen, H. L., de Boer, A. G., Verhoef, J. C., & Junginger, H. E. (1999). Chitosan for enhanced intestinal permeability: Prospects for derivatives soluble in neutral and basic environments. *European Journal of Pharmaceutical Sciences*, *7*, 145–151.

McConnell, E. L., Murdan, S., & Basit, A. W. (2008). An investigation into the digestion of chitosan (noncrosslinked and crosslinked) by human colonic bacteria. *Journal of Pharmaceutical Sciences*, *97*, 3820–3829.

Muzzarelli, R. A. A. (1993). Biochemical significance of exogenous chitins and chitosans in animals and patients. *Carbohydrate Polymers*, *20*, 7–16.

Muzzarelli, R. A. A. (2011). Biomedical exploitation of chitin and chitosan via mechano-chemical disassembly, electrospinning, dissolution in imidazolium ionic liquids, and supercritical drying. *Marine Drugs*, *9*, 1510–1533.

Oliveira, G. F., Ferrari, P. C., Carvalho, L. Q., & Evangelista, R. C. (2010). Chitosan-pectin multiparticulate systems associated with enteric polymers for colonic drug delivery. *Carbohydrate Polymers*, *82*, 1004–1009.

Papadopoulou, V., Kosmidis, K., Vlachou, M., & Macheras, P. (2006). On the use of Weibull function for the discernment of drug release mechanisms. *International Journal of Pharmaceutics*, *309*, 44–50.

Park, J. H., Saravanakumar, G., Kim, K., & Kwon, I. C. (2010). Target delivery of low molecular drugs using chitosan and its derivatives. *Advanced Drug Delivery Reviews*, *62*, 28–41.

Podczeck, F., Rahman, S. R., & Newton, J. M. (1999). Evaluation of a standardized procedure to assess the shape of pellets using image analysis. *International Journal of Pharmaceutics*, *192*, 123–128.

Rubinstein, A. (1995). Approaches and opportunities in colon-specific drug delivery. *Critical Reviews in Therapeutic Drug Carrier Systems*, *12*, 101–149.

Schipper, N. G. M., Olsson, S., Hoogstraate, J. A., de Boer, A. G., Varum, K. M., & Artursson, P. (1997). Chitosans as absorption enhancers for poorly absorbable drugs. 2. Mechanism of absorption enhancement. *Pharmaceutical Research*, *14*, 923–929.

Yalpani, M., & Hall, L. D. (1984). Some chemical and analytical aspects of polysaccharide modifications III. Formation of branched-chain, soluble chitosan derivatives. *Macromolecules*, *17*, 272–281.

Yang, L. (2008). Biorelevant dissolution testing of colon-specific delivery systems activated by colonic microflora. *Journal of Controlled Release*, *125*, 77–86.

Yang, L.-B., 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.