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In vitro drug permeation from chitosan pellets

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ABSTRACT

The purpose of this study was to prepare and characterize coated pellets for controlled drug delivery. The influence of chitosan (CS) in pellets was evaluated by swelling, *in vitro* drug release and intestinal permeation assays. Pellets were coated with an enteric polymer, Kollicoat® MAE 30 DP, in a fluidized-bed apparatus and the coating formulations were based on a factorial design. Metronidazole (MT) released from coated and uncoated pellets were assessed by dissolution method using Apparatus I. Intestinal permeation was evaluated by everted intestinal sac model in rats, used to study the absorption of MT from coated pellets containing CS or not through the intestinal tissue. Although the film coating avoided drug dissolution in gastric medium, the overall drug release and intestinal permeation were dependent on the presence of CS. Thus, pellets containing CS show potential as a system for controlled drug delivery.

1. Introduction

Controlled delivery systems provide an alternative approach to regulating the bioavailability of therapeutic agents, because the drug released can be maintained in an appropriate concentration for an adequate period of time (Jogani, Jinturkar, Vyas, & Misra, 2008). Systems with such characteristics allow achieving more effective therapies while eliminating the potential under- and over-dosing, and reduce the frequency of drug administrations, since the maintenance of drug levels within a desired range is achieved (Petitti, Vanni, & Barresi, 2008). In controlled drug delivery systems, the drug can be incorporated into a polymeric structure and released from the excipient in a predefined form. Depending on the drug delivery system and the administration route, the drug release time may be anywhere from less than a minute to weeks (Jogani et al., 2008; Petitti et al., 2008).

Chitosan (CS) 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 human gastrointestinal tract. (Bhattarai, Gunn, & Zhang, 2010; Park, Saravanakumar, Kim, & Kwon, 2010). The main properties favoring the use of CS in various pharmaceutical preparations include its biological inertness, degradability and adhesive properties (Ibekwe, Fadda, Parsons, & Basit, 2006; Oliveira, Ferrari, Carvalho, & Evangelista, 2010). These attractive properties also make the polymer an ideal candidate for controlled release dosage forms. CS (and several other polysaccharides) has been used for specific drug delivery to the colon because of the active enzymes present there. These polymers are excellent targets for degradation within the gastrointestinal tract because of the large variety of bacteria in the intestine that secrete enzymes, such as glucosidases, galactosidases, amylases, pectinases, xylanases, xylosidases, and dextranases (Chourasia & Jain, 2003; Jain, Gupta, & Jain, 2007; McConnell, Murdan, & Basit, 2008; Sinha & Kumria, 2001).

Several experimental models to investigate the interaction between dosage forms and gastrointestinal tract were already described (Hussain, Jaitley, & Florence, 2001; Ponchel & Irache, 1998). Among the *in vitro* models, the everted intestinal sac model (Wilson & Wiseman, 1954) has been used to study bioadhesive properties and substances uptake and transport in the intestine (Carreno-Gomez, Woodley, & Florence, 1999; Chen, Ping, Guo, Lv, & Gao, 2003; Da Silva, Severino, Martins, Chaud, & Santana, 2009; Mainardes, Chaud, Gremião, & Evangelista, 2006).

The aim of this work was to prepare and characterize coated pellets containing CS to controlled drug delivery. Metronidazole pellets were obtained by extrusion-spheronization and coated with an enteric polymer, Kollicoat® MAE (aqueous dispersion of

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Table 1
2^2 factorial experimental design: factors and levels.

	Factorial design 2^2	Levels	
	Independent factors	−1	+1
X_1	CS presence (%)	0	10
X_2	Kollicoat® MAE (mg/cm^2) coating	0	6

methacrylic acid and ethyl acrylate copolymer). The morphological appearance of pellets, their liquid uptake characteristics, *in vitro* drug release performance and intestinal permeation by everted intestinal sac method were evaluated.

2. Materials and methods

2.1. Materials

Metronidazole and chitosan were purchased from Sigma Aldrich (São Paulo, Brazil). Kollicoat® MAE 30 DP (aqueous dispersion of methacrylic acid and ethyl acrylate copolymer) was 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. Methods

2.2.1. Film coating of pellets

2.2.1.1. 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, 10% PEG 4000 aqueous solution, was mixed to the powder blend until a homogeneous until a homogeneous, cohesive and 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 CS were also prepared as control.

2.2.1.2. Preparation of coated pellets. The pellets were coated with a dispersion of Kollicoat® MAE 30 DP (16%, w/v, solids content) containing propylene glycol and a dye solution (Sicovit® Red 30E172, BASF). Dispersion was sprayed onto the pellets core using a fluidized bed coater Hüttlin® (Mycrolab, Germany). The 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.

2.2.1.3. Experimental design. In order to verify the influence of CS and polymer coating on the MT release from pellets, an experimental design was developed. The variables analyzed were the presence of CS and the enteric coating at two levels each in the range indicated in Table 1. The three responses studied along with their constraint values are listed below:

Amount of drug unreleased after 2 h (Y_1): MT%$_{2\,h}$.
Amount of drug unreleased after 3 h (Y_2): MT%$_{3\,h}$.
Amount of drug unreleased after 6 h (Y_3): MT%$_{6\,h}$.

2.2.2. 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).

2.2.3. 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 was placed on the sintering filter and the volume of liquid 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 percentage 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.

2.2.4. In vitro drug release

The dissolution studies were performed using a Hanson Dissolution Test Station SR8-Plus (Chastworth, USA) based on United States Pharmacopoeia Method I (rotating basket method). The pellets (300 mg of pellets from the size fraction of 1.00–1.18 mm) were poured into hard gelatin capsules (size 0) and placed in the rotating basket immersed in 750 ml of simulated gastric fluid (pH 1.2) for 2 h. Afterwards, 250 ml of PBS was added in the vessel to achieve pH 6.8 (simulated enteric fluid) for 6 h. The acceptor fluid was maintained at 37 ± 0.5 °C and the baskets were submitted to rotation at 100 rpm. At appropriated time intervals, 5 ml of the samples were withdrawn and filtered through cellulose acetate membrane (0.45 μm). The filtrate was analyzed by UV spectrophotometer (Hewlett Packard, Mod. 8453, working with HP UV-Visible ChemStation Software) at 277 nm for simulated gastric fluid and 320 nm for simulated enteric fluid. The related concentrations were calculated using calibration profiles based on absorbance *versus* concentration curves previously designed and standardized. The corresponding drug release profiles were represented by plots of the cumulative temporal percent amount of drug released (calculated from the total amount of MT contained in each sample). The amounts of drug unreleased after 2 h, 3 h, and 6 h, MT%$_{2\,h}$, MT%$_{3\,h}$ and MT%$_{6\,h}$ were used for verify the drug release characteristics.

2.2.5. 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) and Barthe, Woodley, and Houin (1999). The experiment evaluated the permeation of MT from three formulations, across the duodenal segment of rat intestine. The formulations tested were identified as follows: formulation 1: MT powder; formulation 2: Kollicoat® MAE coated pellets without CS; and formulation 3: Kollicoat® MAE coated pellets containing CS. The amount of MT, 1.0 mg, was the same for the three formulations. The assays were carried out along 60 min, to ensure the viability of the intestinal tissue in TC199 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 $NaHPO_4$. The intestinal segment was gently inverted with the aid of a flexible cotton swab with

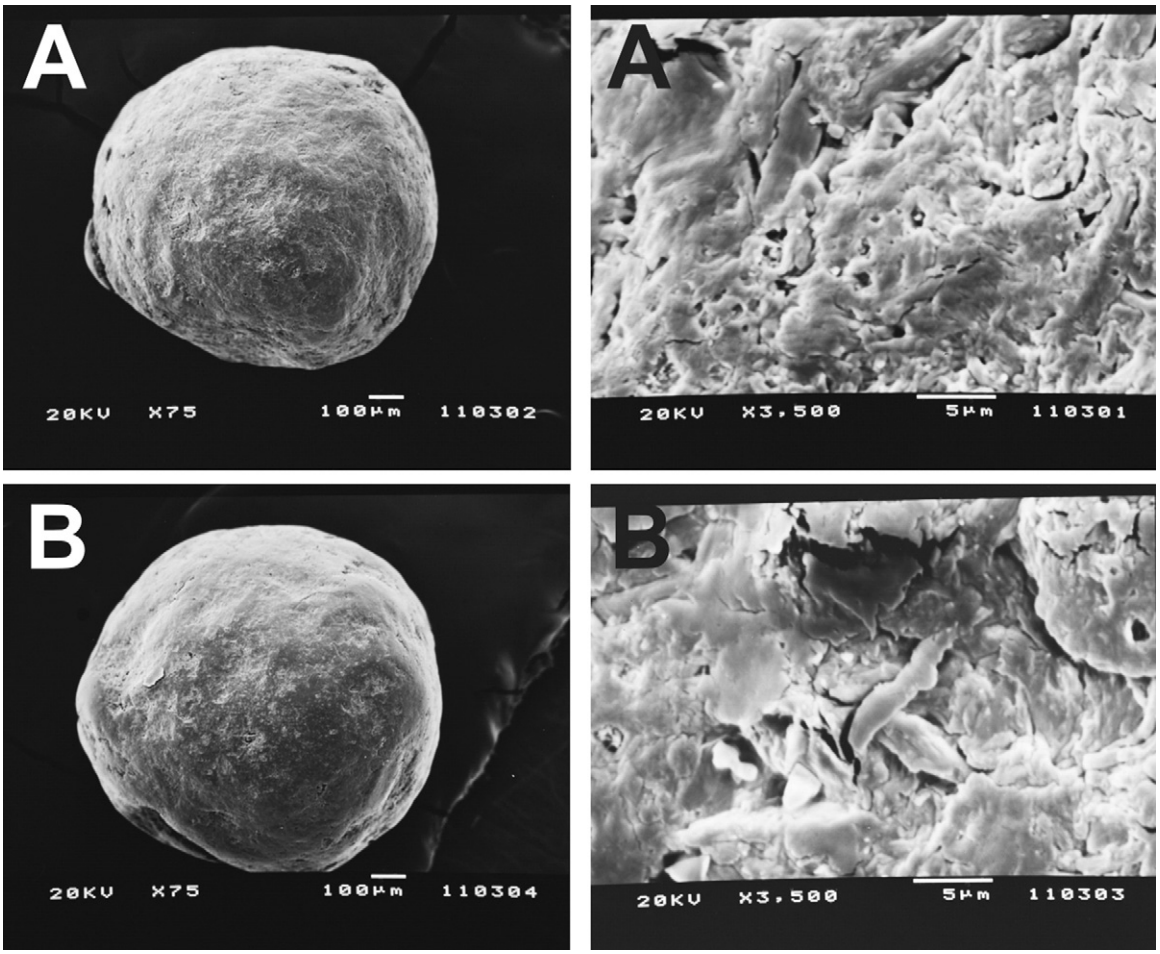

Fig. 1. Scanning electron microscopy of pellets: (A) uncoated pellets without CS; (B) uncoated pellets with CS (75 and 3500×).

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 glucose 10 mM, 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 60 min, and externally washed with fresh TC199 medium. The samples were collected in sextuplicate (six different intestines). Then the sacs were cut and the internal serosal fluid drained into small tubes. The contents were 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 60 min of incubation. The results were expressed as the mean ± standard deviation of six independent experiments and statistical analysis of the results was performed by ANOVA with a significance level α of 0.05.

3. Results and discussion

3.1. Scanning electron microscopy

The morphology of the pellets (Figs. 1 and 2) was analyzed by scanning electron microscopy at magnifications of 75 and 3500, allowing to observe general aspect of sets of pellets as well as details of their surface, such as pores on the coating material and some fractures on the surface of the pellets containing or not CS.

Fig. 1 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% CS seems to have no influence on morphological characteristics of the pellets.

Fig. 2 shows the SEM images obtained from coated MT pellets. SEM micrographs of coated pellets do not show any pores on the surfaces of the film coated pellets, however, the surface of the Kollicoat® MAE coated pellets was not smooth.

3.2. Liquid uptake studies

Fig. 3 represents the swelling profile of the samples in simulated gastric fluid (pH 1.2) and in PBS (pH 6.8) after 2 h. Uncoated pellets containing CS exhibited higher PBS uptake than pellets without CS (p = 0.01252) and than all other samples, confirming that the presence of CS has great influence on the swelling of the pellets.

Kollicoat® MAE coated pellets presented the same swelling profile in both media. In simulated gastric medium, Kollicoat® MAE coated pellets swelled in a similar way to the uncoated pellets regardless of the presence of CS. Kollicoat® MAE coating is an enteric polymer, insoluble in low pH values (1.2) and becomes soluble in pH above 5.5. Thus, in simulated gastric fluid, the insoluble coating resisted against the medium to diffuse the pellets through the pores inwards.

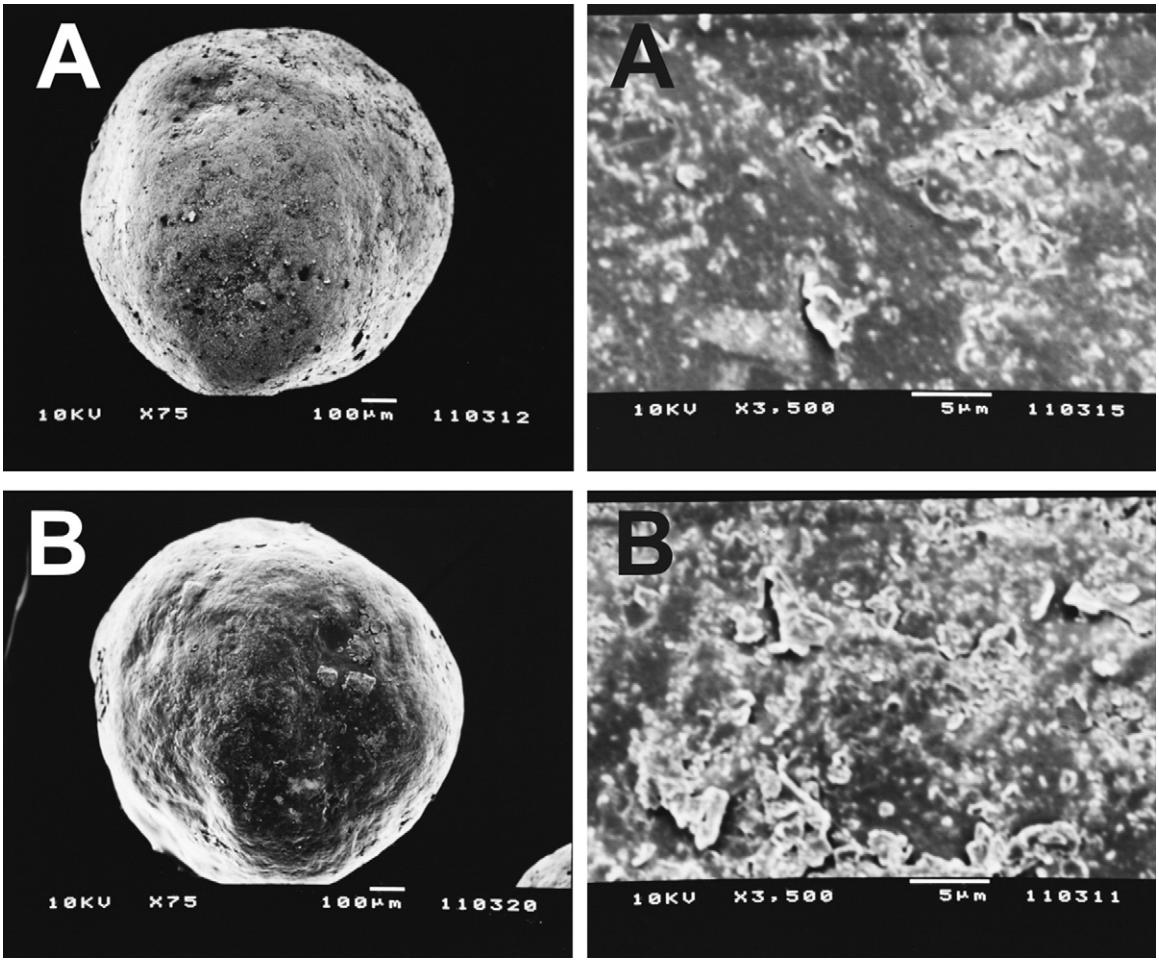

Fig. 2. Scanning electron microscopy of pellets: (A) Kollicoat® MAE coated pellets without CS; (B) Kollicoat® MAE coated pellets with CS (75 and 3500×).

3.3. *In vitro* drug release

The pellets exhibited limited swelling and did not disintegrate during the dissolution assay (8 h). MT was quickly released in 1 h from uncoated pellets containing or not CS in simulated gastric fluid (Fig. 4). 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, MT was promptly released in PBS pH 6.8.

In simulated gastric fluid, MT was released from uncoated pellets; however, in samples containing CS the drug release was slower. The difference in the drug dissolution profile started after 30 min from the beginning of analysis. For example, in 1 h, a

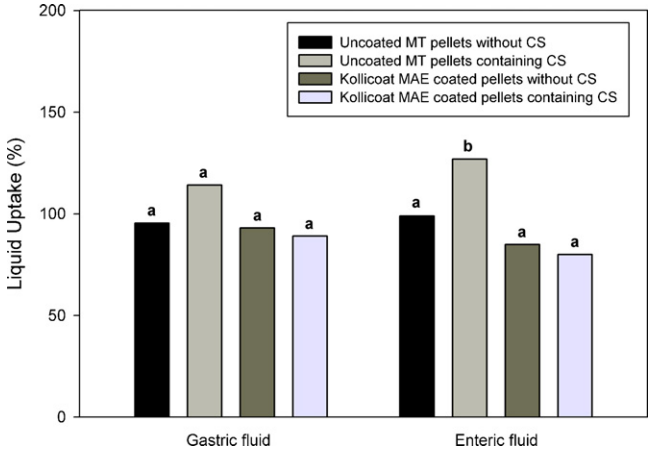

Fig. 3. Swelling studies (same letters represent no significant difference ($p < 0.05$)).

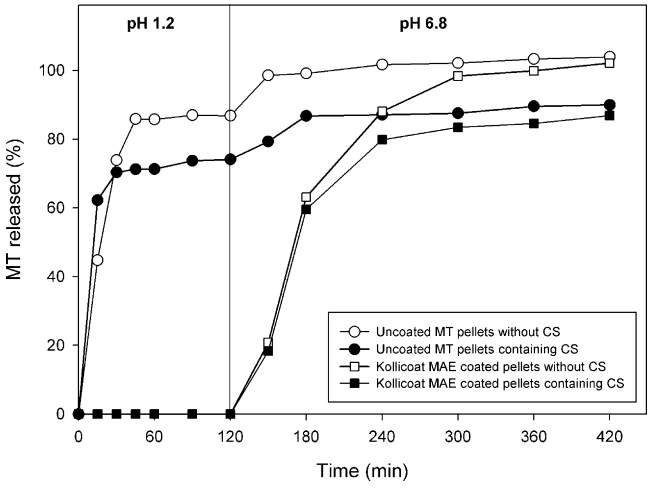

Fig. 4. *In vitro* MT release (for the sake of clarity errors bars are not shown).

difference of 15% of drug was released from pellets without CS in comparison to pellets containing CS. Such behavior remained until the pH of the dissolution medium was changed to 6.8, and the whole drug was released from pellets without CS in less than 1 h under this new condition. Thus, this profile indicates that the presence and the swelling of CS in the pellets influenced the process of drug release, prolonging it.

Enteric coated pellets, containing CS or not, showed the same profile in simulated gastric fluid and the coating was effective in preventing the drug release. After the pH of the dissolution fluid has been modified, MT was fasted released, because of the Kollicoat® MAE solubility in pH 6.8. In this situation, 60% of MT was released from coated pellets within 1 h. However, after that and regarding the presence of CS, the dissolution profile has changed. In samples of pellets containing CS, MT was slowly released, keeping the same release profile of the uncoated pellets, i.e., a difference of 15% of MT released.

The factorial design was carried out to analyze the effect of the variables: presence of CS (X_1) and enteric coated (X_2), which levels were presented in Table 1. The responses analyzed were the remaining amount of MT (still available in the pellets to be released) after 2 h, 3 h and 6 h of dissolution. In other words, the ability of such variables in ensuring gastric protection and in controlling the drug release, respectively. Table 2 shows the conditions of these experiments and their respective responses (Y_1, Y_2 and Y_3).

The analysis of the responses indicated the factors that influenced in the MT release from pellets. Fig. 5A represents Y_1 response, showing that the main factor influencing the drug release in simulated gastric fluid was the X_2 factor, Kollicoat® MAE. The CS presence and their interaction also presented some effect, but with lower magnitude than the enteric coating.

Fig. 5B shows a graphical representation of the Y_2 response. This response evaluates the drug release 1 h after pH modification of dissolution fluid to 6.8. Kollicoat® MAE factor remains the main factor affecting MT release; however, this influence was less pronounced than in the analysis in simulated gastric fluid. The factor "CS presence" also influenced the drug release.

Fig. 5C shows a graphical representation of the Y_3 response, which characterizes final dissolution time, i.e., MT released after 6 h in contact with simulated enteric fluid. In this case, the main factor affecting the drug release was the CS presence. This analysis indicated that the enteric coating protected the pellets, avoiding the drug to be prematurely released in stomach and, after their transfer to enteric fluid, the coating was promptly dissolved. However, the presence of CS decreases the rate of dissolution of the drug, due to the medium uptake by the polysaccharide, prolonging the MT release rate.

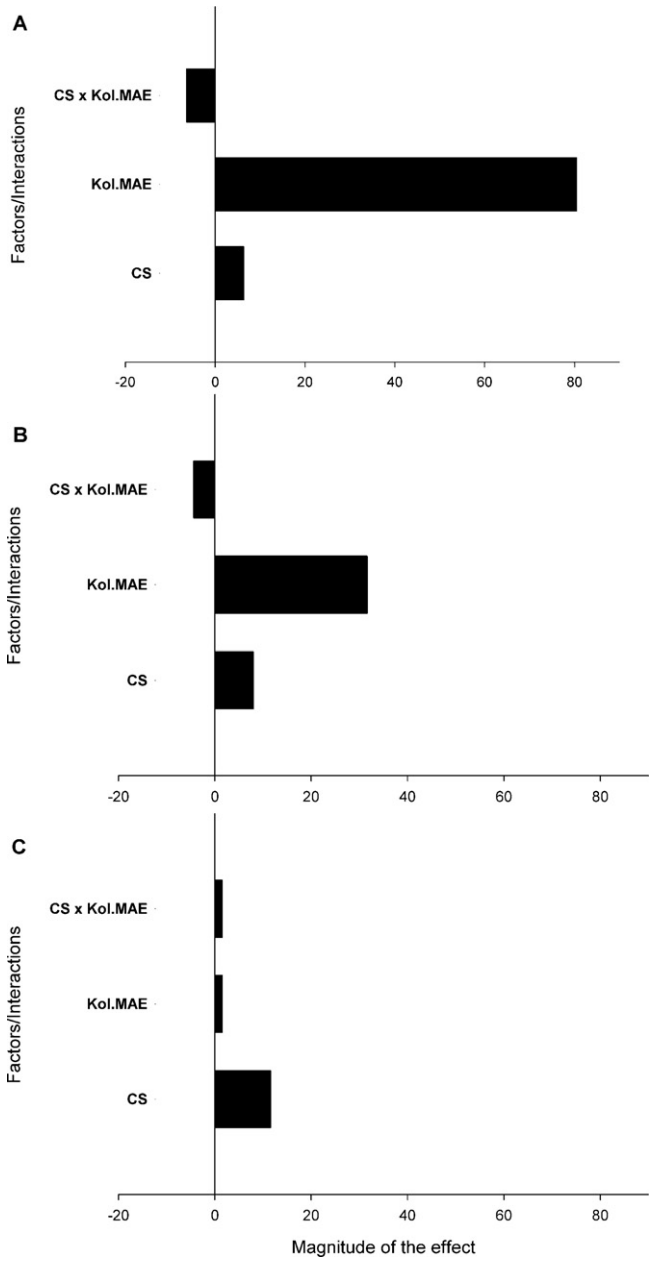

Fig. 5. Graphical representation of effect of factors on responses: (A) Y_1: MT%$_{2\,h}$; (B) Y_2: MT%$_{3\,h}$; and (C) Y_3: MT%$_{6\,h}$.

3.4. Intestinal permeation

The everted intestinal sac technique in rats was used to study the absorption behavior of MT from coated pellets containing CS or not through the intestinal tissues.

Fig. 6 shows the cumulative permeation profile of MT across the duodenal segment. The validated analytical curve used for analysis was linear in the range of MT concentration from 0.3 to 35 μg/ml, showing correlation coefficient of 0.998974.

After 60 min, for formulations 1, 2 and 3, respectively, 96, 56 and 49% of MT permeated through the duodenal segment. Statistical analysis indicated that there was significant difference between formulations 1 and 3 (free drug and coated pellets containing CS). The analysis also showed that the MT intestinal permeation from coated pellets without CS was equivalent to free drug, because the coating is soluble and dissolved in the incubation medium (pH 7.4).

The performance of chitosan as a drug carrier in intestinal permeation was also observed by Guo et al. (2004) and Kotzé et al. (1998). They verified that chitosan interacts with cells and open the tight junctions to allow the paracellular transport as well as improving the intestinal permeation of drugs. However, in this study, the presence of CS in pellets provided controlled drug release and, consequently, slower intestinal permeation. The proportion of CS in samples (10%) could be responsible for these results, whereas after the burst effect, MT was released from pellets containing CS in a prolonged way.

The higher absorbed amount of free MT observed through the intestinal sacs might be attributed to the high percentage of drug available in mucosal fluid because of its easy solubility in this medium. Based on ANOVA results, permeability values of MT in both free MT and coated pellets without CS showed no statistical differences ($p > 0.05$).

Table 2
Coated MT pellets formulation using 2^2 factorial design and results data of mean values of responses, i.e., remaining MT released at 2 h (%, Y_1), at 3 h (%, Y_2) and remaining MT released at 6 h (%, Y_3).

Samples		X_1 CS in pellets	X_2 Kollicoat® MAE coating	Y_1 $MT_{rem}\%_{2h}$	Y_2 $MT_{rem}\%_{3h}$	Y_3 $MT_{rem}\%_{6h}$
1	(−1 −1)	0	0	13.18	0.90	0
2	(+1 −1)	10	0	25.88	13.24	10.04
3	(−1 +1)	0	6	100	36.87	0
4	(+1 +1)	10	6	100	40.47	13.11

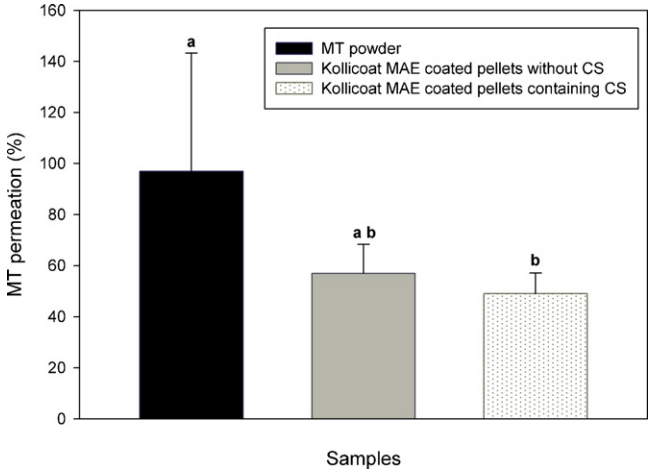

Fig. 6. Intestinal permeation of MT by everted intestinal sac method (same letters represent no significant difference ($p < 0.05$)).

4. Conclusions

Multi-unit delivery systems for controlled drug delivery based on pH dependence and CS presence were developed. The systems constituted by MT pellets containing or not CS were coated with Kollicoat® MAE and an appropriate factorial design was successfully used in the development of coating formulations, for pellets containing or not CS. The dissolution rate of the coated pellets containing CS was lower than that of coated pellets without CS as a consequence of the decreased drug diffusion from pellets caused by CS swelling. It was also demonstrated by *in vitro* model studies that the CS presence caused a decrease on the MT permeability through intestinal segment. The present results suggest the potential use of coated pellets containing CS for control the gastrointestinal tract absorption of MT as colon-targeting oral dosage forms.

Acknowledgement

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