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

Priscileila Colerato Ferrari[a], Fagner Magalhães Souza[b], Leandro Giorgetti[b], Giselle Faria Oliveira[a], Humberto Gomes Ferraz[b], Marco Vinícius Chaud[c], Raul Cesar Evangelista[a,*]

[a] Department of Drugs and Pharmaceuticals, School of Pharmaceutical Sciences, São Paulo State University – UNESP, Araraquara, SP, Brazil
[b] Department of Pharmacy, School of Pharmaceutical Sciences, São Paulo University – USP, São Paulo, SP, Brazil
[c] Pharmaceutical Sciences Post-Graduate Program, Sorocaba University, Sorocaba, SP, Brazil

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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

* Corresponding author at: Rodovia Araraquara-Jaú, km 01, CEP 14801-902 Araraquara, SP, Brazil. Tel.: +55 16 33016976; fax: +55 16 33220073.
E-mail addresses: revangel@fcfar.unesp.br, raulrasec@yahoo.com.br (R.C. Evangelista).

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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.