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Development of HPMC and Eudragit S100® blended microparticles containing sodium pantoprazole

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Pantoprazole is used in the treatment of acid related disorders and *Helicobacter pylori* infections. It is activated inside gastric parietal cells binding irreversibly to the H^+/K^+-ATPase. In this way, pantoprazole must be absorbed intact in gastro-intestinal tract, indicating that enteric delivery systems are required. The purpose of this study was to prepare pantoprazole-loaded microparticles by spray-drying using a blend of Eudragit S100® and HPMC, which can provide gastro-resistance and controlled release. Microparticles presented acceptable drug loading (120.4 mgg^{-1}), encapsulation efficiency (92.3%), surface area (49.0 m^2g^{-1}), and particle size (11.3 μm). DSC analyses showed that the drug is molecularly dispersed in the microparticles, and *in vivo* anti-ulcer evaluation demonstrated that microparticles were effective in protecting stomach against ulceration. Microparticles were successfully tabletted using magnesium stearate. *In vitro* gastro-resistance study showed that microparticles stabilized pantoprazole in 62.0% and tablets in 97.5% and provided a controlled release of the drug.

1. Introduction

Multi-particulate drug delivery systems based on polymer blends have shown several advantages over single unit ones, such as more uniform transit times through the gastro-intestinal tract, less variability among individuals, smaller risk of dose dumping and high local concentrations of drug (Lin and Kao 1991; Beckert et al. 1996). In this sense, polymer blend formulations have been widely studied because they can improve polymer mechanical properties, reduce drug toxicity and control drug delivery (Vega-Gonzalez et al. 2004). Eudragit S100 [poly(methacrylic acid-co-methyl methacrylate) (1 : 2)] is an enteric polymer and hydroxypropylmethylcellulose (HPMC) is a hydrophilic derivative of cellulose commonly used as drug release rate-controlling polymer. Both polymers are largely used in pharmaceutical formulations (Alvarez-Fuentes et al. 2004; Li et al. 2005). However, up to now, blends of these polymers have not been reported as microparticulate drug delivery systems.

Pantoprazole is a prodrug used in the treatment of digestive ulcers, gastro-esophageal reflux disease and *Helicobacter pylori* infections (Cheer et al. 2003). This prodrug is activated inside gastric parietal cells binding irreversibly to the H^+/K^+-ATPase. In this way, it must be absorbed intact in the gastro-intestinal tract, indicating that an enteric drug delivery system is required for its oral administration (Sachs et al. 2003). In consequence, enteric tablets containing pantoprazole are commercially available.

In our previous work, pantoprazole-loaded microparticles have been prepared with Eudragit S100 by an O/O emulsification/solvent evaporation technique (Raffin et al. 2006a). These microparticles were able to protect the stomachs of rats against ulcer formation. The main drawback of this technique is the difficulty of scaling up the production of microparticles as well as controlling their shape and size. So, recently, we have studied the spray-drying technique for the preparation of microparticles containing pantoprazole (Raffin et al. 2006b). This technique was adequate for the preparation of microparticles in both laboratory and pilot scales. Besides, the shape and the size of the particles have been controlled by changing the composition of the formulation and the spray-drier operational conditions.

As far as we know, no multiple-unit system based on HPMC and Eudragit S100 blended microparticles containing pantoprazole has already been developed. Taking into account all this, the purpose of the present study was to prepare pantoprazole-loaded microparticles by a spray-drying technique using the blend of Eudragit S100 and HPMC. The blend would be able to provide simultaneously gastric protection and controlled release of the drug, due to the physico-chemical characteristics of those polymers. Eudragit S100 is insoluble in acid media and pure water, whereas it is soluble in intestinal media from pH 7 upwards. HPMC hydrates, swells, coalesces, and forms a viscous phase around the exterior of the particle. This viscous layer can control both the influx of water and the efflux of drugs. Additionally, this work was dedicated to characterize the microparticles by DSC, HPLC, SEM, and surface area, as well as to verify the effectiveness of this multiparticulate system in protecting the gastro-intestinal tract against ulceration induced by ethanol in rats. The *in vitro* gastro-resistance of microparticles and tabletted microparticles was also evaluated.

2. Investigations, results and discussion

It has already been described in the literature (Riegel and Leopold 2005) that omeprazole is unstable in some solutions and suspensions containing enteric polymers. As pantoprazole is a benzimidazole analog to omeprazole its stability in the polymeric solution must be evaluated before the preparation of the microparticles. The solution containing both polymers and pantoprazole was kept at room temperature and absence of light for 24 h. No degradation was detected in 24 h demonstrating that this formulation can be used to prepare microparticles. Microparticles prepared by spray-drying were obtained with a yield of $36 \pm 1\%$ as off-white powders.

Microparticles presented concave spherical shape with visible folding and shrivelling (Fig. 1a). This morphology is formed by uneven shrinkage forces during the drying of droplets, depending on the viscosity of the liquid feed (Foster and Laetherman 1995). The addition of HPMC to the formulation increases its viscosity and the tendency to shrive or fold is also increased.

The drug content assayed by HPLC was $120.4 \pm 11.9\ mgg^{-1}$ of pantoprazole in the microparticles resulting in an encapsulation efficiency of 92.3%. The microparticle surface area was $49\ m^2g^{-1}$ at an average particle size of 11.3 μm (span = 2.6). Microparticles prepared with Eudragit S100 containing pantoprazole (Raffin et al. 2006b) presented an increase in particle size with the increase of feed's viscosity. The microparticles produced with a feed with viscosity of 6.1 cP presented an average size of 6.7 μm and those produced with a viscosity of 10.4 cP presented a mean size of 9.0 μm. The formulation produced with HPMC presented a viscosity of 15.6 cP and as expected a higher average size. In accordance to the relationship between size and surface area, larger particles presented lower surface area. The surface area decreased from 86 to $49\ m^2g^{-1}$ comparing the formulations without and with HPMC.

In DSC analysis, pantoprazole (Fig. 2) showed an endothermic peak at 130 °C, followed by its degradation above 170 °C. The peak at 130 °C corresponds to the melting and the dehydratation of pantoprazole, which are parallel processes (Zupancic et al. 2005). In pantoprazole DSC, the position of the melting endotherm strongly depended on the heating rate (Rosenblatt et al. 2005), even though this effect is less pronounced than with omeprazole presenting a melting range only slightly above the onset temperature for decomposition. Eudragit S100 presented an endothermic peak at 69 °C (melting) and HPMC showed an endothermic peak at 67 °C, which corresponds to the loss of adsorbed moisture or solvent from the macromolecule (Jug and Becirevic-Lacan 2004). Regarding the physical mixtures of drug and polymers the tracings showed two endothermic peaks, one correlated with the polymers

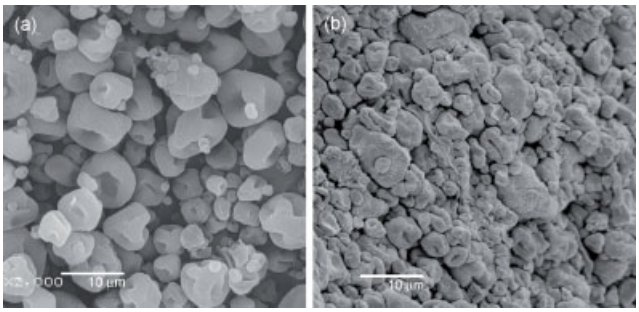

Fig. 1: SEM photomicrographs of (a) microparticles and (b) tabletted microparticles (broken section)

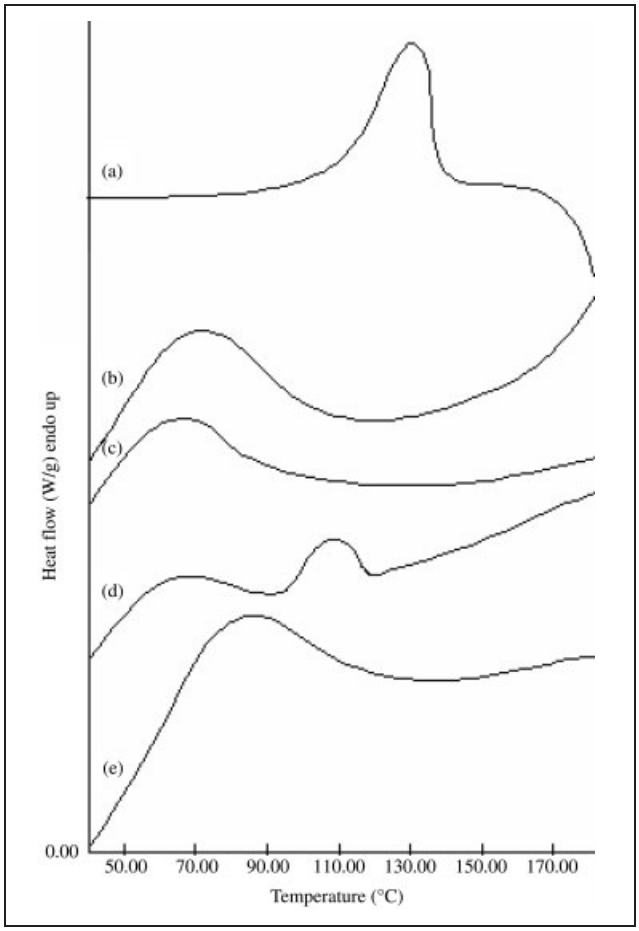

Fig. 2: DSC tracings of (a) sodium pantoprazole sesquihydrate, (b) Eudragit S100, (c) HPMC, (d) physical mixture of raw materials and (e) microparticles

(64 °C) and the other one with the drug (108 °C). On the other hand, for the microparticles only one peak at 83 °C was observed which corresponds to the melting of the blend. These results suggest that pantoprazole is molecularly dispersed in the blend. According to the literature, the disappearance of any event of the drug indicates its encapsulation (Ford and Timmins 1999).

Ulcers were induced by ethanol which induced large hemorragic bands that were evaluated measuring the affected area. The *in vivo* evaluation showed that ulcer index values were 0.74 ± 0.34 for the sodium bicarbonate solution, 0.46 ± 0.17 for sodium pantoprazole solution and 0.06 ± 0.07 for pantoprazole-loaded microparticles. Student-Neuman-Keuls analyses showed that the pantoprazole-loaded microparticles presented a gastric ulcer index statistically lower than the sodium bicarbonate solution ($p = 0.007$) and the sodium pantoprazole solution ($p = 0.013$). These results demonstrated that the microparticles were able to protect the stomach against ulceration induced by ethanol.

Microparticles were successfully tabletted using magnesium stearate (0.5%) as excipient and the tablets presented hardness of 34 N. Furthermore, SEM analyses showed intact microparticles inside the broken tablets (Fig. 1b).

The stability evaluation in phosphate buffer pH 7.4 showed that the pure pantoprazole, the microparticles and the tablets reached 100% of pantoprazole dissolution after 500 min. These results indicate that neither the spray-drying technique nor the medium used in the release experiments affect the stability of the drug.

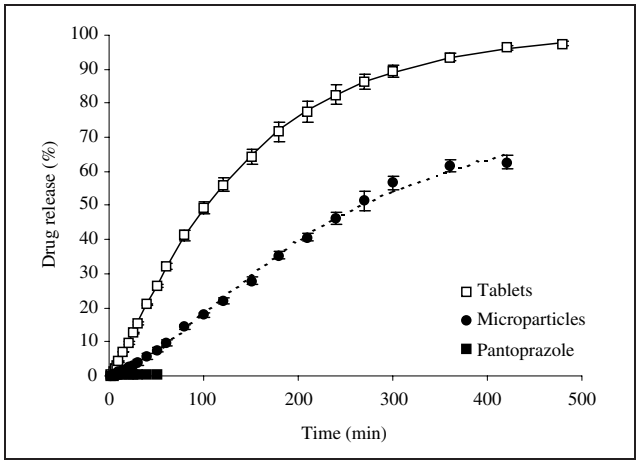

Fig. 3: Gastro-resistance of pantoprazole: drug release after acid stage (1 h). Lines show mathematical modeling for the three curves

As regards the gastro-resistance study, after the acid stage, 0.5% of pure pantoprazole remained stable, whereas the microparticles protected pantoprazole in 62.7% and tablets in 97.5% (Fig. 3).

The dissolution efficiencies were $0.40 \pm 0.10\%$ for pure pantoprazole, $43.73 \pm 1.58\%$ for microparticles and $71.41 \pm 1.37\%$ for tablets. ANOVA test indicated statistical differences ($p = 2.10^{-8}$) among the groups.

Mathematical modeling of the pantoprazole release showed that microparticle and tablet profiles fit the Weibull model (Eq. (1)).

$$C = X[1 - e^{-(t/Td)\beta}] \quad (1)$$

where C is the percentage dissolved at time t, X is the maximum percentage dissolved after the acid stage, Td is the time at which 63.2% of the material is dissolved and β is the shape parameter. Both profiles showed sigmoidal curves ($\beta = 1.67$ and 1.14 for microparticle and tablet profiles, respectively). The parameters X and Td were 77.35% and 180.7 min for the microparticles and 100.63% and 129.9 min for the tablets, respectively. These results indicate that microparticles presented a slower release than the tablets, since it lasts 180 min to reach 49% of drug release from microparticles and 129 min to reach 64% of drug release from the tablets.

The percentage of drug dissolved is proportional to the initial concentration of the drug after the acid stage. In this sense, the tablets were able to protect the pantoprazole in a higher extension due to the smaller surface area than the microparticles. Even though the amount of drug was the same before the acid stage for each sample, after 1 h in acid medium, the drug concentration was different for pure pantoprazole, tablets and microparticles. The tablets release pantoprazole faster than the microparticles because the initial drug concentration was higher in the former than in the latter. These results are due to the lower surface area of the tablets in comparison with the microparticles, which caused a higher protection of the drug in the tablets during the acid stage.

In conclusion, pantoprazole-loaded microparticles presented acceptable drug loading, encapsulation efficiency and particle size distribution. DSC analysis showed that microparticles are formed by a blend of Eudragit S100 and HPMC, as well as that pantoprazole is molecularly dispersed in the particles. The *in vivo* anti-ulcer evaluation in rats showed that microparticles provided a significant protection of stomach against ulcer formation. Furthermore, the *in vitro* gastro-resistant study showed that the microparticles and the tablets were able to stabilize the pantoprazole and provide a controlled drug release.

3. Experimental

3.1. Materials

Pantoprazole sodium sesquihydrate was obtained from Henrifarma (São Paulo, Brazil). Eudragit S100 was kindly given by Almapal® (São Paulo, Brazil produced by Rohm®, Germany). Methocel® F4M was provided by Colorcon® (São Paulo, Brazil, produced by Dow Chemical, USA). Acetonitrile, HPLC grade, was obtained from FisherChemicals (New Jersey, USA). All other chemicals were analytical grade.

3.2. Microparticle preparation

Eudragit S100 (1.2 g) was dissolved in 0.05 M NaOH (75 mL). Subsequently, HPMC (0.6 g) was added and the mixture was magnetically stirred. The solution was kept at 10 °C for 24 h. Sodium pantoprazole sesquihydrate (0.3 g) was added in the mixture before spray drying (MSD 1.0, LabMaq, Brazil). The experimental conditions were: 0.8 mm nozzle, inlet temperature of 150 °C and flow of 0.44 Lh^{-1}.

3.3. Drug loading and encapsulation efficiency

An amount of the microparticles, equivalent to 10 mg of pantoprazole, was weighed and stirred with 40 mL of 0.05 M NaOH for 1 h. The volume was completed to 50 mL and drug concentration was determined after filtration (0.45 μm, Millipore®) by HPLC (Perkin Elmer serie 200; UV detector, $\lambda = 290$ nm, Shelton, USA), using a LiChrospher® 100 RP_{18} (5 μm) (Merck®). Mobile phase consisted of acetonitrile/phosphate buffer pH 7.4 (35 : 65 v/v) and the flow used was 1 $mLmin^{-1}$. The HPLC method for pantoprazole quantification was previously validated in terms of linearity, precision, reproducibility, accuracy and specificity. The concentration range was 0.5 to 20.0 μgmL^{-1}. Linearity was 0.999 and the detection limit was 0.55 μgmL^{-1}. The accuracy was $95.39 \pm 3.77\%$ to 6 μgmL^{-1}, $101.13 \pm 1.71\%$, to 9 μgmL^{-1} and $101.38 \pm 1.46\%$ to 14 μgmL^{-1}. The reproducibility presented a RSD = 0.47, and the intermediate precision showed a RSD = 1.17.

3.4. Scanning electron microscopy

The shape and the surface of the microparticles were analyzed by scanning electron microscopy (SEM) (Jeol Scanning Microscope JSM-5200®, Japan). The SEM analyses were carried out using an accelerating voltage of 15 kV after they were gold sputtered (Jeol Jee 4B SVG-IN®, Peabody, USA).

3.5. Determination of surface area and pore size distribution

The nitrogen adsorption-desorption isotherms of previous degassed organic-solids, under vacuum at 40 °C were determined at liquid nitrogen boiling point in a homemade volumetric apparatus using nitrogen as probe. The pressure was measured using capilar mercury barometer and the results were compared to an alumina pattern. The specific surface areas of microparticles were determined by the BET multipoint technique (Brunauer et al. 1938) and the pore size distribution was obtained using BJH method (Barrett et al. 1951).

3.6. Determination of particle size

The particle size distribution was determined by laser diffractometry (Mastersizer 2000, Malvern Instruments, London, UK) after dispersion of powders in *iso*-octane. The mean diameter over the volume distribution $d_{4,3}$ was used. The span was calculated using the Eq. (2).

$$\text{span} = \frac{d_{(v,90)} - d_{(v,10)}}{d_{(v,50)}} \quad (2)$$

where $d_{(v,90)}$, $d_{(v,10)}$ and $d_{(v,50)}$ are the diameters at 90%, 10% and 50% cumulative volumes, respectively. Thus, the span gives a measure of the range of the volume distribution relative to the median diameter.

3.7. Differential scanning calorimetry (DSC)

DSC was performed (DSC-4 Shimadzu, Kyoto, Japan) after sealing the samples (pantoprazole, Eudragit S100, HPMC, the physical mixture and the microparticles) in aluminum pans. Calibration was carried out using indium. DSC tracings were performed from 40 °C to 180 °C at a rate of 10 °Cmin^{-1}.

3.8. In vitro gastro-resistance evaluation

The gastro-resistance study (37 °C) was performed in flow-through cell apparatus at 37 °C using a peristaltic pump (Desaga, Heidelberg, Germany). The samples were collected at predetermined time intervals and

Table: Groups of rats (control 1, control 2 and treatment) for the *in vivo* anti-ulcer activity test

Groups	Administered samples
Control 1	4.2% sodium bicarbonate solution
Control 2	Pantoprazole dissolved in water (2 mgmL^{-1})
Treatment	Microparticles dispersed in water (mass equivalent to 2 mgmL^{-1} of pantoprazole)

analyzed spectrophotometrically at 295 nm (Unicam 8625 UV/Vis spectrometer, Cambridge, England). The methodology for UV quantification was validated in terms of linearity, precision, reproducibility, accuracy and specificity. The concentration range was 4.0 to 30.0 μgmL^{-1}. Linearity was 0.9999. Accuracy was 102 ± 2.09%, 97.24 ± 1.61% and 100.37 ± 2.56%, for the concentrations of 8, 11 and 17 μgmL^{-1}, respectively. The reproducibility presented RSD of 0.68, and the intermediate precision showed RSD of 0.36. The samples were placed in the cells and treated with 0.1 M HCl (1 mLmin^{-1}) (acid stage). Then, after 1 h, the medium was replaced by phosphate buffer pH 7.4 and samples were collected at predetermined time intervals and analyzed. The profiles were analyzed by model-dependent methods (monoexponential, biexponential, power law and Weibull) and by a model-independent method (dissolution efficiency) (Costa and Lobo 2001; Beck et al. 2005). The selection of the model-dependent was based on the best correlation coefficient, the best model selection criteria (MSC), provided by Scientist® software, and the best graphic adjustment.
In order to verify the stability of pantoprazole in phosphate buffer pH 7.4, a dissolution experiment was conducted.

3.9. In vivo anti-ulcer activity

Ulcers were induced by the oral administration of absolute ethanol (5 mLkg^{-1}) to 24 h fasted Wistar male rats (n = 8), weighing 200 g (Gombosova et al. 1993; Shah et al. 2003). The groups are described in the Table. Formulations (20 mgkg^{-1} of drug) were administered orally 1 h before the administration of ethanol. Two hours after ethanol administration, the animals were sacrificed; the stomachs were removed, opened along the greater curvature and examined for lesion measurements.
Ulcer indexes (UI) were calculated using the Eq. (3).

$$UI = \frac{10}{x} \quad (3)$$

where x is the total mucosal area divided by the total ulcerated area.
This protocol was approved by the Ethical Committee (deliberation number 2003247, Universidade Federal do Rio Grande do Sul, Brazil).

3.1. Preparation and characterization of tablets

Microparticles were tabletted with magnesium stearate (0.5%). Tablets (theoretically containing 40 mg of drug) were prepared in a double punch tablet machine (Korch EK0, Berlin, Germany) by individual weighing and direct compression using 8.0 mm punches. In order to evaluate the integrity of the microparticles after the compression, one tablet was fractured and the inner face was analyzed by SEM.
For drug loading determination, tablets were milled by a mortar and pestle. An amount of sample equivalent to 10 mg of pantoprazole was diluted with 0.05 M NaOH (40 mL) and magnetically stirred. After 12 h, the volume was completed to 50 mL and aliquots were analyzed by HPLC as described above for the microparticles. The dissolution profile was determined using a flow through cell apparatus as described above for microparticles.

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