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European Journal of Pharmaceutics and Biopharmaceutics 63 (2006) 198–204
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Preparation, characterization, and in vivo anti-ulcer evaluation of pantoprazole-loaded microparticles

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Received 10 October 2005; accepted in revised form 26 January 2006
Available online 13 March 2006

Abstract

Pantoprazole is an important drug in the treatment of acid-related disorders. This work concerns the preparation and characterization of gastro-resistant pantoprazole-loaded microparticles prepared using an O/O emulsification/solvent evaporation technique. The in vivo activity of the pantoprazole-loaded Eudragit® S100 microparticles was carried out in rats. Furthermore, tablets containing the microparticles were also investigated. Microparticles presented spherical and smooth morphologies (SEM) and they remained intact in the inner surface of tablets. DSC and IR analyses showed that pantoprazole was physically and molecularly dispersed in the polymer. In vivo anti-ulcer evaluation showed that the microparticles were able to protect rat stomachs against ulcer formation, while the drug aqueous solution did not present activity. Drug dissolution profiles from tablets demonstrated slower release than untabletted microparticles. Weibull equation was the best model for describing the drug release profiles from microparticles and tablets. As regards the acid protection, tablets showed a satisfactory drug protection in acid medium ($61.05 \pm 8.09\%$ after 30 min).

Keywords: Microparticles; Pantoprazole; Emulsification/solvent evaporation; Polymer; Gastric resistance; Tablet; Release profile; In vivo ulcer evaluation

1. Introduction

Pantoprazole is an important drug in the treatment of acid-related disorders [1] and it is also very effective against *Helicobacter pylori* infections alone or associated to other drugs, like metronidazole, clarithromycin or amoxicillin [2,3]. This drug was the first water-soluble benzimidazole, 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-benzimidazole (Fig. 1), which could be administered intravenously in the form of sodium pantoprazole sesquihydrate. In low pH values, pantoprazole turns into a cationic sulfenamide, which is its active form [2,4]. This drug accumulates in the highly acidic environment of the parietal-cell canalicular lumen and it is activated. The active form, a tetracyclic cationic sulfenamide, reacts with thiol group of cysteines 813 and 822 of the transmembranal H^+/K^+ATPase [1,5]. This conversion must occur inside the gastric parietal cells, so pantoprazole must be absorbed intact by gastrointestinal tract [2].

Pantoprazole has several advantages compared to its analogues (e.g., omeprazole and lansoprazole) such as specific site of binding, greater stability in neutral pH environment, and longer duration of action [6]. Besides, it presents no potential to induce or inhibit the CYP 450 [1,2,7]. It is a more selective inhibitor of acid secretion than other proton pump inhibitors [8].

Due to the necessity to pass intact through the stomach for reaching the duodenum for absorption, the pantoprazole is formulated as solution for intravenous administration (lyophilized powder for reconstitution) or as gastric-resistant tablets (oral delayed-release dosage form). In the case of oral administration, the enteric coating prevents

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doi:10.1016/j.ejpb.2006.01.013

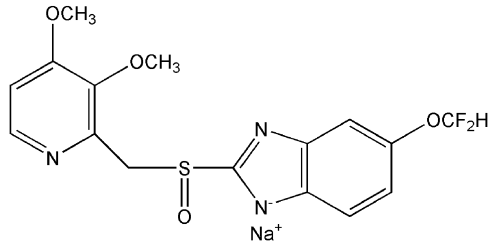

Fig. 1. Chemical structure of sodium pantoprazole.

pantoprazole from degradation in the gastric juice (at pH 1–2, pantoprazole degrades in few minutes) [9].

Up to now, no multiple-unit pharmaceutical dosage forms containing pantoprazole have been developed. As a general rule, the multiple-unit products show large and uniform distribution; they are less affected by pH and there is a minor risk of dose dumping [10]. Besides, these new drug delivery systems, as the polymeric microparticles, are also proposed to improve absorption, distribution, and bioavailability of acid labile drugs [11,12]. As they rapidly disperse in the gastrointestinal tract, they can maximize drug absorption, minimize side effects, and reduce variations in gastric emptying rates and intersubject variability [13].

The emulsification/solvent evaporation is a technique very well described in the literature [10,14] to prepare polymeric microparticles, which are useful for stabilizing drugs and to improve their distribution. This technique is an easy method, compatible with several polymers [15], and it is suitable for encapsulating both lipophilic and hydrophilic drugs [11]. Another advantage is the possibility of producing microparticles with a wide range of sizes, porosities, and shapes [16].

Eudragit® S100 is a gastro-resistant polymer used for colonic delivery, protecting drugs from pH of upper gastrointestinal tract [17]. This polymer is an anionic copolymer formed by methacrylic acid and methyl methacrylate (ratio 1:2). It is insoluble in acids and pure water, whereas it is soluble in aqueous solution presenting pH higher than 7 [18]. Microparticles prepared with this polymer can be tabletted, offering the advantages of the particulate-controlled release dosage forms [19].

Taking all the above into account, this study concerns the characterization of gastro-resistant microparticles containing sodium pantoprazole prepared using an O/O emulsification/solvent evaporation technique. Furthermore, the present study is devoted to evaluate the in vivo activity of the pantoprazole-loaded Eudragit® S100 microparticles. Additionally, tablets containing the pantoprazole microparticles were also prepared and characterized.

2. Methods

2.1. 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). Sorbitan monooleate was obtained from Lipo Chemicals (New Jersey, USA) and mineral oil, USP grade, obtained from Fraccionata (Porto Alegre, Brazil). Acetonitrile, HPLC grade, was obtained from Fisher Chemicals (New Jersey, USA). All other chemicals were of analytical grade.

2.2. Preparation of microparticles

After dissolving the Eudragit® S100 (750 mg) in acetone (80 mL), pantoprazole sodium sesquihydrate (350 mg) was added. This suspension was emulsified (1000 rpm) with mineral oil (350 mL) containing sorbitan monooleate (1500 mg). The O/O emulsion was mechanically stirred for 2.5 h to remove the acetone. The microparticles were collected by vacuum filtration, washed with cyclohexane (2× 50 mL), and kept overnight in a desiccator.

2.3. Characterization of microparticles

2.3.1. Drug loading

An amount of the microparticles, equivalent to 10 mg of pantoprazole, was weighed and stirred with 40 mL of 0.05 M NaOH for 12 h. The volume was completed to 50 mL and drug concentration was determined after filtration (0.45 μm, Millipore®) by HPLC (Perkin-Elmer series 200; UV detector, $\lambda = 290$ nm, Shelton, USA), using a Waters® Nova Pak® column C_{18} (3.9×150 mm). Mobile phase consisted of acetonitrile/phosphate buffer, pH 7.4 (35:65 v/v).

HPLC method for pantoprazole quantification was validated [20]. Linearity (coefficient correlation) presented values higher than 0.99. The accuracy was $105 \pm 1\%$, the repeatability presented a RSD = 0.47, and the intermediate precision showed a RSD = 1.17. The detection limit was 0.5 μg/mL. To determine the reproducibility of the method and the drug purity, a liquid chromatographic system Shimadzu (LC-10ADVP, Kyoto, Japan) with a Diode Array Detector (SPD-M10AVP) was used.

2.3.2. Optical and electronic microscopies

The shape and the surface of the microparticles were analyzed by optical microscopy (Olympus®, Model BX-41, coupled with a photographic camera, Olympus®, Model PM-20, Tokyo, Japan) and scanning electronic microscopy (SEM) (Jeol Scanning Microscope JSM-5800®, Japan).

For optical microscopy analyses, samples were suspended in mineral oil. The SEM analyses were carried out using an accelerating voltage of 20 kV after they were gold sputtered (Jeol Jee 4B SVG-IN®, Peabody, USA).

2.3.3. Determination of surface area and pore diameter

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 capillar mercury barometer and the results were compared to an alumina pattern. The specific surface areas of microparticles were determined by the BET multipoint technique [21] and the pore size distribution was obtained using BJH method [22].

2.3.4. Determination of particle size

The particle size distribution was determined by laser diffractometry (Mastersizer 2000, Malvern Instruments, London, UK) after dispersion in *iso*-octane. The mean diameter over the volume distribution $d_{4.3}$ was used. The span was calculated using the following equation:

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

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.

2.3.5. Determination of water content

The water content of the sample was determined by Karl Fisher titrimetry (Mettler DL 37 KF Coulometer, Switzerland). Samples were analyzed in duplicate and compared to pantoprazole.

2.3.6. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed (DSC-60 Shimadzu, Kyoto, Japan) after sealing the samples (pantoprazole, Eudragit® S100, the physical mixture, and microparticles) in aluminum pans. Calibration was carried out using indium. DSC tracings were performed from −140 to 250 °C at a rate of 10 °C/min.

2.3.7. Fourier transform infrared spectroscopic analysis

FTIR spectroscopic analysis (Shimadzu spectrometer, FTIR 8101, Kyoto, Japan) of raw materials, their physical mixture, and microparticles was carried out using KBr pellets.

2.3.8. Dissolution profile

The dissolution study was performed in flow-through cell apparatus at 37 °C using a peristaltic pump (Desaga, Heidelberg, Germany), flow rate of 1 mL/min, and phosphate buffer, pH 7.4 (PBS) as media. The samples were collected at predetermined time intervals and analyzed spectrophotometrically at 295 nm (Unicam 8625 UV/Vis spectrometer, Cambridge, England). The methodology for UV quantification was validated [20]. The profiles were analyzed by model-dependent methods (monoexponential, biexponential, and Weibull) and by model-independent method (dissolution efficiency) [23,24]. The selection of model-dependent method was based on the best correlation coefficient, the best model selection criteria (MSC), provided by Scientist® software, and the best graphic adjustment.

2.3.9. Bulk density and tapped density of microparticles

Bulk density was determined by weighing 500 mg of microparticles and measuring its volume. Tapped density was measured using a J. Engelsmann volumeter (Ludwigshafen, Germany).

2.4. In vivo anti-ulcer activity

Ulcers were induced by the oral administration of absolute ethanol (5 mL/kg) to 24 h fasted Wistar male rats ($n = 8$), weighing 200 g [25]. The groups are described in Table 1. Formulations (20 mg/kg of drug) were administered orally 1 h before the administration of ethanol. After 2 h of ethanol administration, animals were sacrificed; the stomachs were removed, opened along the greater curvature, and examined for lesion measurements. Ulcer indexes (UI) were calculated using the equation:

$$\text{UI} = \frac{10}{x},$$

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).

2.5. Preparation and characterization of tablets

2.5.1. Tabletting microparticles

Microparticles were tabletted with microcrystalline cellulose (Avicel® PH101) (28%) and magnesium stearate (0.5%). Tablets (170 mg theoretically containing 30 mg of drug) were prepared in double punch tablet machine (Korch EK0, Berlin, Germany) by individual weighing and direct compression using 8.0 mm punches.

2.5.2. Drug loading and dissolution profile

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.

Table 1
Groups of rats (Control 1, Control 2 and Treatment) for the in vivo anti-ulcer activity

Groups	Administered samples
Control 1	42% sodium bicarbonate solution
Control 2	Drug dissolved in water (2 mg/mL)
Treatment	Microparticles dispersed in water (mass equivalent to 2 mg/mL of pantoprazole)

2.5.3. Determination of the gastric resistance

Tablets were placed in 0.1 M HCl (3 mL) for 30 min at 37 ± 1 °C. Then, the medium was neutralized with 150 μL of 2 M NaOH and the volume was completed to 100 mL using 0.05 M NaOH. Collected samples were diluted, filtered, and analyzed by HPLC.

3. Results

Pantoprazole-loaded Eudragit® S100 microparticles were obtained using an O/O emulsion, whose anhydrous condition could prevent the degradation of drug. SEM images showed smooth spheres without crystals on the surface (Fig. 2a), presenting a hollow core after being frozen and broken (Fig. 2b). Optical microscopy analysis corroborated the capsular structure of the microparticles (Fig. 2c).

The microparticles presented drug content of 229.5 ± 20.1 mg/g, surface area of 41 m^2/g, and pore volume of 0.04 cm^3/g. The pore size distribution in the range of 2–50 nm was close to zero, meaning the absence of mesopores [26]. The water content was 6.89% for microparticles and 6.59% for pure pantoprazole sodium sesquihydrate. The microparticles presented average size of 56.25 μm and span of 3.45.

DSC analyses were carried out for pantoprazole, Eudragit® S100, their physical mixture, and microparticles (Fig. 3). The tracing for microparticles showed a glass transition at −71.2 °C, a melting peak at 69.3 °C, and no event for pantoprazole. The results suggest that pantoprazole-loaded Eudragit® S100 microparticles are composed by a homogeneous phase, in which the polymer presents a lower degree of crystallinity than the raw material and the drug is molecularly dispersed in the polymer.

Infrared analyses (Fig. 4) were performed in order to qualify the interaction between the polymer and the drug in microparticles. The spectra of raw materials showed their characteristic bands [27–29]. When this polymer is ionized, the carboxylate band shifts from 1728 to 1560 cm^{-1}, corresponding to the anti-symmetrical vibration of COO^- [29]. The bands observed in the microparticle spectrum did not show any shift, suggesting that no new chemical bond was formed after preparing the formulation

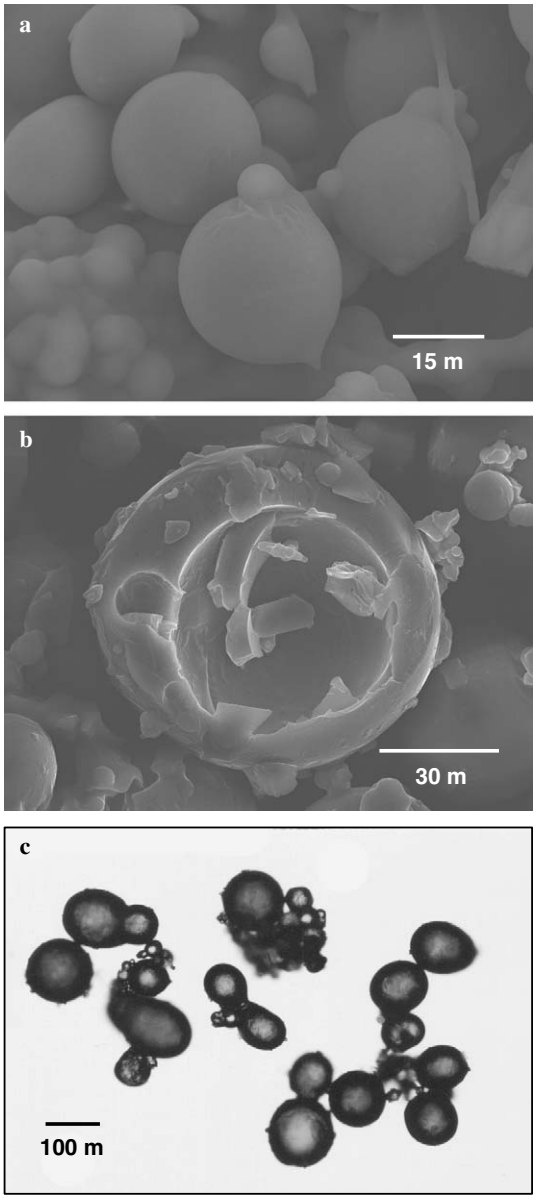

Fig. 2. SEM photomicrographs: (a) microparticles and (b) broken microparticle, and (c) optical microscopy image of microparticles.

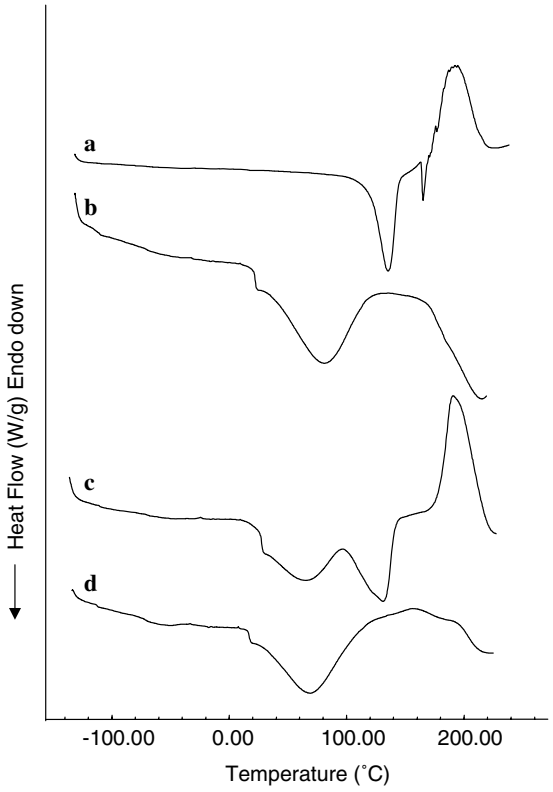

Fig. 3. DSC tracings of (a) pantoprazole, (b) Eudragit® S100, (c) physical mixture of pantoprazole and Eudragit®, and (d) microparticles.

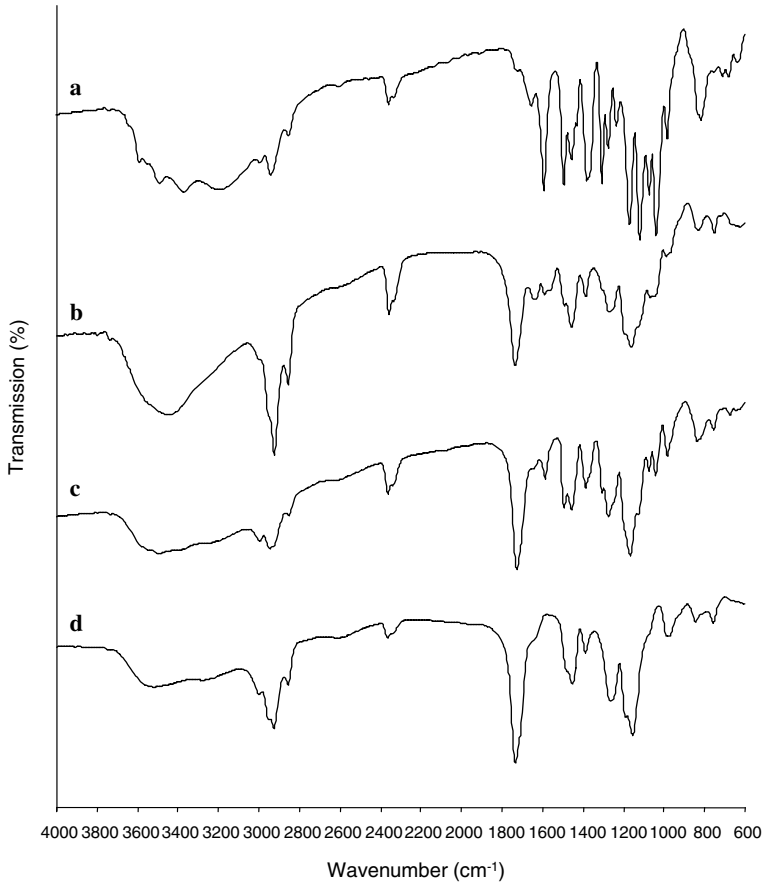

Fig. 4. Infrared spectra: (a) sodium pantoprazole sesquihydrate, (b) Eudragit® S100, (c) physical mixture of drug and polymer, and (d) microparticles.

and the results confirmed that the drug is physically dispersed in the polymer.

Regarding the gastric ulcer indexes (Fig. 5), the values were 0.42 ± 0.15 for the sodium bicarbonate solution (Control 1), 0.46 ± 0.17 for pantoprazole water solution (Control 2), and 0.07 ± 0.04 for pantoprazole-loaded Eudragit® S100 microparticles (Treatment). The Kruskal–Wallis test detected statistical differences ($p = 0.002$) among these indexes. The multiple analysis (Student–Newman–Keuls) showed that the pantoprazole-loaded Eudragit® S100 microparticles presented a gastric ulcer index statistically lower ($p < 0.05$) than those of the sodium bicarbonate aqueous solution and the pantoprazole solution groups. These results showed that the microparticles were able to protect ulcer formation by ethanol.

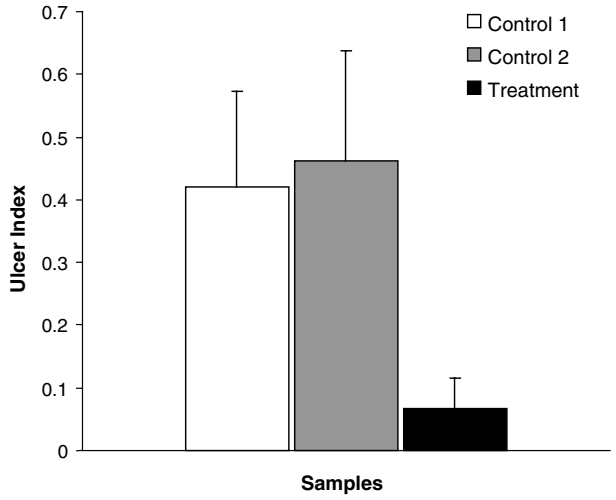

Fig. 5. Gastric ulcer indexes after administration of ethanol and sodium bicarbonate solution (Control 1), sodium pantoprazole solution (Control 2) or pantoprazole-loaded microparticles dispersed in water (Treatment).

The bulk and tapped densities of microparticles were performed and the Carr ratio and Hausner index were calculated as previously described [30]. Microparticles showed Carr ratio of 33.3 and Hausner index of 1.5. These flowability indexes demonstrated that the microparticles were cohesive and, in consequence, it was necessary to use excipients to prepare viable tablets.

The tablets presented mean weight of 169.8 ± 2.8 mg/unit corresponding to an amount of 27 mg of pantoprazole sodium sesquihydrate (equivalent to 24 mg of drug). With the aim of verifying the presence of intact microparticles in the inner of the tablets, a similar tablet formulation was prepared in the absence of the microparticles. Both tablets were broken and their insides were analyzed by SEM. Microparticles can be clearly observed in the inner of the microparticle tablets (Fig. 6).

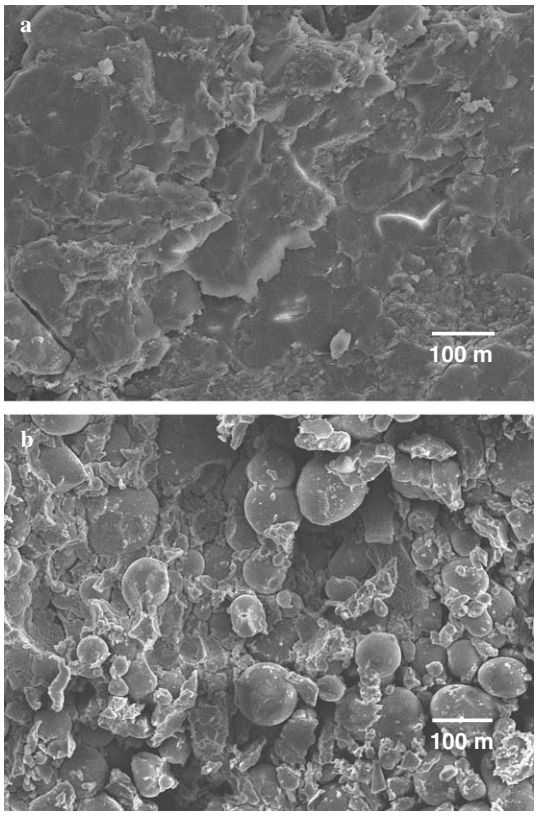

Fig. 6. SEM images. (a) Inner of a tablet prepared with the physical mixture of raw materials and (b) inner of a tablet prepared with the pantoprazole-loaded Eudragit® S100 microparticles.

Regarding the dissolution profiles (Fig. 7), 92.6 ± 2.4% of the pure pantoprazole was dissolved whereas 100.3 ± 3.9% was released from microparticles after 120 min. Drug dissolution from tablets achieved 102.1 ± 4.7% in 240 min. Dissolution efficiencies were statistically different ($p = 0.002$, ANOVA) for microparticles (81.4%) in comparison to the pure drug (73.7%). The dissolution efficiency for tablets was 67.1%.

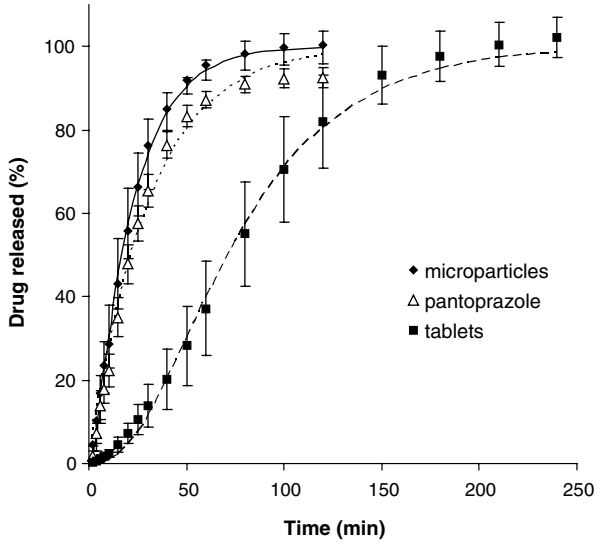

Fig. 7. Dissolution profiles (in PBS) of sodium pantoprazole sesquihydrate, pantoprazole-loaded microparticles, and tablets prepared with the microparticles. Lines show mathematical modeling for the three curves.

Considering the mathematical modeling, for pantoprazole dissolution the first-order model described the experimental data ($r = 0.9953$, MSC = 4.22, and $k = 0.032\ \text{min}^{-1}$). For the drug release from the microparticles and the tablets, the best model was that of Weibull ($D = 100[1 - e^{-(t/T_d)\beta}]$) presenting sigmoidal curves ($\beta = 1.02$ and 3.02, respectively). For microparticles r was 0.9987, MSC was 5.43, and the time at which 63.2% of the drug was dissolved (T_d) was 19.99 min. For tablets r was 0.9985, MSC was 5.27, and T_d was 44.89 min.

The acid resistance experiment carried out with tablets showed that 61.05 ± 8.09% of pantoprazole remained stable presenting a satisfactory drug protection.

4. Conclusions

Pantoprazole-loaded Eudragit® S100 microparticles were successfully obtained by O/O emulsion/solvent evaporation technique. Microparticles presented spherical and smooth morphologies. DSC and FTIR analyses demonstrated that pantoprazole was physically and molecularly dispersed in the polymer forming a homogeneous phase. In vivo anti-ulcer activity evaluation showed that the microparticles were able to protect rat stomachs against ulcer formation by ethanol, while the drug aqueous solution did not present activity.

The flowability indexes (Carr ratio and Hausner index) demonstrated that the microparticles were cohesive. In this way, tabletting microparticles was possible after addition of excipients. SEM analysis showed the microparticles remained intact in the inner surface of tablets.

Drug dissolution profiles from tablets demonstrated slower release than untabletted microparticles. Weibull equation was the best model for describing the drug release profiles from microparticles and tablets. Concerning the acid protection, tablets showed a satisfactory drug protection after 30 min in acid medium. In this way, tablets obtained from pantoprazole-loaded microparticles seem to be an interesting alternative for the administration of this drug due to their ability to improve the acid protection and to control the drug release.

Acknowledgments

The authors thank Dr. M.I. Ré (IPT-USP, São Paulo) for the particle size determination and Dr. E.V. Benvenutti (IQ-UFRGS, Porto Alegre) for the surface area and pore distribution measurements. This work was supported by CNPq/MCT, Rede Nanobiotec CNPq. R.P.R. and L.C. thank CAPES (Brazil).

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