AAPS PharmSciTech, Vol. 10, No. 2, June 2009 (© 2009)
DOI: 10.1208/s12249-009-9214-5

Research Article

Agglomerates Containing Pantoprazole Microparticles: Modulating the Drug Release

Renata P. Raffin,[1,4] Paolo Colombo,[2] Fabio Sonvico,[2] Alessandra Rossi,[2] Denise S. Jornada,[1] Adriana R. Pohlmann,[1,3] and Silvia S. Guterres[1]

Received 1 October 2008; accepted 21 February 2009; published online 25 March 2009

Abstract. Pantoprazole-loaded microparticles were prepared using a blend of Eudragit® S100 and Methocel® F4M. The accelerated stability was carried out during 6 months at 40°C and 75% relative humidity. In order to improve technological characteristics of the pantoprazole-loaded microparticles, soft agglomerates were prepared viewing an oral delayed release and gastro-resistant solid dosage form. The agglomeration was performed by mixing the pantoprazole microparticles with spray-dried mannitol/lecithin powders. The effects of factors such as the amount of lecithin in the spray-dried mannitol/lecithin powders and the ratio between pantoprazole microparticles and spray-dried mannitol/lecithin powders were evaluated. The pantoprazole-loaded microparticles present no significant degradation in 6 months. The agglomerates presented spherical shape, with smooth surface and very small quantity of non-agglomerated particles. The agglomerates presented different yields (35.5–79.0%), drug loading (58–101%), and mechanical properties (tensile strength varied from 44 to 69 mN mm^{-2}), when the spray-dried mannitol/lecithin powders with different lecithin amounts were used. The biopharmaceutical characteristics of pantoprazole microparticles, i.e., their delayed-release properties, were not affected by the agglomeration process. The gastro-resistance of the agglomerates was affected by the amount of spray-dried mannitol/lecithin powders. The ratio of lecithin in the spray-dried mannitol/lecithin powders was the key factor in the agglomerate formation and in the drug release profiles. The agglomerates presenting better mechanical and biopharmaceutical characteristics were prepared with 1:2 (*w*/*w*) ratio of pantoprazole-loaded microparticles and mannitol/lecithin (80:20) powder.

KEY WORDS: agglomerates; delayed release; gastro-resistance; microparticles.

INTRODUCTION

Polymeric drug delivery systems have potential therapeutic advantages in comparison with conventional dosage forms such as the reduction of drug side effects (1), the improvement of therapeutic effect (2), and the control of drug release (3), decreasing the administration frequency (4). Among the different techniques used to prepare drug-loaded microparticles, spray drying is a one-stage continuous process of easy scaling up, which is barely dependent on the solubility of drugs and polymers (5,6). This technique provides microparticles, diameters of which range from few to several tens of micrometers with relatively narrow size distribution (5). The production of a spray-dried powder involves the droplet formation from the atomized suspension, solution or emulsion followed by their solidification driven by the solvent evaporation (7). The desired microstructure can be created from the complex mixture of polymers, surfactants and the appropriate drug that gives the in-use properties of the product (8). The particle size of spray-dried powders depends on the viscosity of the feed solution, design of the atomizer, air pressure, and air/spray contact (9,10). The boiling point of the solvent, as well as the spray-drier scale, can affect particle sizes (10,11). Microparticulated dried powders, granules, or agglomerates can be used as oral dosage forms (4,9,12) and nasal powders (13,14).

Among the various drug delivery devices used to sustain the drug release, the hydrophilic matrix systems are generally preferred because of their ability to swell, coalesce, and form a viscous gel. The viscous layer acts as a diffusional barrier to the drug (15). Cellulose derivatives are frequently chosen to develop such systems due to their low toxicity and cost. Hydroxypropylmethylcellulose (HPMC) is a nonionic cellulose derivative that presents different grades of substitution. Methocel® F4M is a HPMC with viscosity of 3800 mPa s (2% solution in water, 20°C). Methacrylate copolymers (Eudragit®) are interesting candidates for the production of microparticles by spray drying since they are inert and freely soluble in organic solvents (16,17). Eudragit® S100 is a pH-dependent enteric copolymer composed of methacrylic acid and methylmethacrylate mono-

[1] Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752/405, 90610-000, Porto Alegre, Brazil.
[2] Dipartimento Farmaceutico, Università degli Studi di Parma, Parma, Italy.
[3] Departamento de Química Orgânica, Instituto de Química, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.
[4] To whom correspondence should be addressed. (e-mail: renata.raffin@ufrgs.br)

mers, rendering it soluble in aqueous solutions presenting pH higher than 7 (18). In this way, it is insoluble in the mouth and in the stomach but starts to dissolve in the duodenum (pH around 6). Eudragit® S100 has in its structure a hydrophobic monomer unit (methyl methacrylate) and another monomeric unit (methacrylic acid), the behavior of which is dependent on its protonation state. At pH over 7, the carboxylic groups became ionized and the polymer soluble; at lower pH, the carboxylates groups become not ionized and the polymer precipitates (19). In previous works, Methocel® F4M was blended with Eudragit® S100, and its aqueous solution was spray-dried to produce pantoprazole-loaded microparticles (10,12).

Pantoprazole (5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl] sulfinyl]-benzimidazole) is a prodrug used in the treatment of digestive ulcers, gastroesophageal reflux disease, as well as an adjuvant in the eradication of the *Helicobacter pylori* (20). In acid medium, pantoprazole turns into a cationic sulfenamide, which is its active form. It accumulates in the highly acidic environment of the parietal-cell canalicular lumen and it is activated (20). The active form, a tetracyclic cationic sulfenamide, reacts with thiol group of cysteines 813 and 822 of the transmembranal H+/K+ ATPase. This conversion must occur inside the gastric parietal cells, so pantoprazole must be absorbed intact by the gastrointestinal tract (20).

Pantoprazole-loaded microparticles showed both characteristics of gastro-resistance and controlled release (10). Eudragit® S100 was responsible for the gastro-resistance, whereas the presence of hydroxypropylmethylcellulose (HPMC) delayed the release in comparison with formulations prepared without this polymer (9). These microparticles were formed by a solid solution of the polymers, and the pantoprazole was molecularly dispersed in the blend (12). Those microparticles also demonstrated an anti-ulcer activity in rats in an ethanol-induced ulcer *in vivo* model (12). The scaling up of the spray-drying process was validated according to the following parameters: total solid concentration in the solution feed, type of atomizer, air pressure, and air/spray contact (10). The more adequate conditions were 6.6% of solids, two fluid nozzle atomizer, co-current flow, and air pressure of 196 kPa (10).

Unfortunately, the attainment of biopharmaceutical attributes by microparticles is opposed by the small size of the particles that leads to powders with bulk volume and problematic flow for dosage forms manufacturing (14,21). In several pharmaceutical applications, particles might be fine for drug delivery but coarse enough to facilitate the solid dosage form preparation.

Often, the transformation of microparticles in solid dosage forms involves granulation and compaction, provoking irreversible modifications of the microparticle range size and structure (22). In particular, this technological problem could be solved using soft agglomeration, a process in which the powder size is enlarged by constructing weak clusters of primary microparticles (14). Soft agglomerates are easily broken down by air turbulence or water uptake, reconstituting the original size of the microparticles. Weak cohesion bonds due to capillary, van der Waals, or electrostatic forces hold together the primary particles in soft structures (23). The quantity and the nature of these interactions, as well as the method of production, determine the agglomerate structures (24). Recently, a new procedure for agglomerating microparticles has been described (25). Morphine crystals have been agglomerated in soft clusters by processing the physical mixture of the drug with spray-dried mannitol/lecithin powders (25). The lecithin was used as a binder to improve the interparticle cohesion reinforcing the internal structure of agglomerates (26). Taking those findings into account, we hypothesized that the soft agglomeration procedure could be applied in preparing stable soft agglomerates of pantoprazole-loaded microparticles formulated using a blend of Eudragit® S100 and Methocel® F4M. Additionally, the purpose of this research was to study the accelerated stability of Eudragit® S100 and Methocel® F4M blended microparticles, as well as to study the release control of pantoprazole by changing the composition of the spray-dried mannitol/lecithin powders in the agglomerates.

MATERIALS AND METHODS

Materials

Sesquihydrate sodium pantoprazole (M_w=432.4, pKa_1=3.92, pKa_2=8.19, freely soluble in water, very slightly soluble in phosphate buffer at pH 7.4, and practically insoluble in *n*-hexane) was purchased from Henrifarma (São Paulo, Brazil). Eudragit® S100 [(poly(methacrylic acid-co-methyl methacrylate) 1:2, average M_w=135,000, pKa approximately 6, soluble in methanol, ethanol, in aqueous isopropyl alcohol, and acetone, as well as in 1 N sodium hydroxide] was a kind gift from Almapal® (São Paulo, Brazil, produced by Rohm®, Germany). Methocel® F4M [HPMC, methoxyl content of 27–30%, hydroxypropyl content of 4–7.5%; soluble in cold water, practically insoluble in chloroform, ethanol (95%), and ether] was provided by Colorcon® (São Paulo, Brazil, produced by Dow Chemical, USA). Mannitol (Ph. Eur.) was a gift of Lisapharma (Como, Italy), and lecithin (Lipoid S45) was supplied by Lipoid AG (Ludwigshafen, Germany). Acetonitrile was of high-performance liquid chromatography (HPLC) grade and supplied by Tedia (Fairfield, USA). Sodium hydroxide was obtained from Merck (Darmstadt, Germany). Ethanol, hydrochloric acid, monohydrate sodium phosphate, and hexane were of analytical grade.

Preparation of Pantoprazole-Loaded Microparticles

Pantoprazole-loaded microparticles were prepared in pilot scale as previously described (10). Briefly, the feed solution consisted of 36 g of Eudragit® S100 in 2,000 mL NaOH solution (3 g L^{-1}). After its complete dissolution, Methocel® F4M (18 g) was added, and the solution was kept at 10°C for 24 h. Sodium pantoprazole (9 g) was added just before spray drying. The solution was then spray dried in pilot scale equipment (Model PSD 52 APV1Anhydro, Denmark) presenting the cylindrical dryer chamber of 1.0 m diameter and 2.3 m of total height. A two-fluid pneumatic atomizer with external mixing was used. In this nozzle, the liquid to be atomized is discharged through a central hole diameter of d_o=1.5 mm, whereas the atomizing air is injected through a ring area around the liquid hole. The atomizing air pressure was 196 kPa, the inlet temperature was 170±1°C, and the flow rate was 2 L h^{-1}. Room temperature and humidity were controlled (24±1°C and 54±2% of relative humidity). One batch was prepared and used for the

accelerated stability. Another batch was produced and used in the preparation of all agglomerates.

Accelerated Stability Tests of Pantoprazole-Loaded Microparticles

Transparent glass vials containing 0.5 g of microparticles were stored for 6 months in a stability chamber at 40°C and 75% relative humidity (RH) (27,28). During 180 days, sealed and non-sealed vials were evaluated every 30 days for their drug content. Humidity was gravimetrically determined.

Drug Loading

The drug loading was assayed by a validated HPLC method (29). Briefly, an amount of agglomerates, equivalent to 10 mg of pantoprazole, was weighed and magnetically stirred with 40 of 0.05 mol·L^{-1} NaOH solution for 1 h in a volumetric flask. The volume was completed to 50 mL, and drug concentration was determined after filtration (0.45 μm) by HPLC (Perkin Elmer serie 200, detector UV-Vis) using a LiChrospher RP18 (Merck) column. The mobile phase consisted of acetonitrile/phosphate buffer pH 7.4 (35:65 *v*/*v*). The flow rate was 1 mL·min^{-1}, and the drug was detected at 290 nm. Retention time was 6.8 min. Linearity was achieved between 0.5 and 20 μg mL^{-1} and presented a correlation coefficient of 0.9994. The relative standard deviation for its precision was 0.47% and for its reproducibility 1.13%. Accuracy was between 95.4% and 101.4% for different concentrations.

Preparation and Characterization of Spray-Dried Mannitol/Lecithin Powders

The feed solution of mannitol and lecithin was prepared as follows: lecithin (15%, 17.5%, and 20%) was dissolved in 60 mL ethanol. In another beaker, mannitol (85%, 82.5%, and 80%) was dissolved in water (340 mL). Both solutions were then mixed, and the final ratios between them were 85:15, 82.5:17.5, and 80:20 (*w*/*w*). The resulting solutions had 15% of ethanol and 4.5% of solids. The solutions were spray-dried in a laboratorial spray-drier Buchi Mini Spray Dryer B-191 (Buchi Laboratoriums-Tecnik, Flawil, Switzerland) using flow rate of 6.5 mL min^{-1}, inlet temperature of 90±2°C, aspiration set in 100%, and air flow of 500 NL h^{-1}. The three spray-dried mannitol/lecithin powders were used as excipients for the agglomeration with pantoprazole-loaded microparticles. They were characterized in terms of yield, humidity content, morphology, and specific surface area.

The yield, expressed in percent, was calculated by the ratio between the mass obtained and the mass of mannitol and lecithin added to the solution. The particle size distributions of spray-dried mannitol/lecithin powders were measured using a laser light diffraction apparatus (series 2600 Malvern Instruments Ltd, Spring Lane South Malvern, Worcestershire, UK). Particles were suspended in ethyl acetate, a non-solvent for these materials. Particle size was expressed as median volume diameter. After gold sputtering, the morphology of the spray-dried mannitol/lecithin powders was assessed by scanning electron microscopy (SEM) using accelerating voltage of 15 kV (JSM 6400, Jeol Ltd, Tokyo, Japan).

The specific surface areas of spray-dried mannitol/lecithin powders were determined by the Brunauer, Emmet, and Teller multipoint technique (BET) (30). 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.

Water content was assayed by Karl Fisher titration (Titro Matric 1S, Crison, Alella, Spain). Different test methods to determine flowability, which vary in nature and degree of force and energy transmission, quantify the macroscopic properties of a powder in different ways. Therefore, different test procedures provide different answers and powder classifications (31). Flowability, as well as bulk and tapped densities, were measured according to the European Pharmacopoeia (32). The compressibility index was calculated according to USP (33). The differential scanning calorimetry (DSC) was performed in a DSC-60 (Shimadzu, Kyoto, Japan) at 10°C min^{-1} from −140°C to 200°C.

Preparation of the Agglomerates

The agglomerate preparation is summarized in Fig. 1. Pantoprazole-loaded microparticles and spray-dried mannitol/lecithin powders were mixed in a Turbula apparatus (Wab, Basel, Switzerland) for 3 h. Five grams of the mixture of pantoprazole-loaded microparticles and spray-dried mannitol/lecithin powders were put on the top of two sieves stack with nominal apertures of 106 and 850 μm, respectively (10 cm sieves, Endecotts Ltd, London, UK). The mixture was vibrated for 10 min on a laboratory sieve shaker (amplitude 2–3; Analysette 3 Fritz model, Fritsch GMBH, Idar-Oberstein, Germany). Agglomerates between 106 and 850 μm were collected. The reprocess of the non-agglomerated powder and the crushing of larger agglomerates was repeated eight times. The ratios tested were 1:1, 1:2, and 1:3 (*w*/*w*) (Table I). The drug content measured by HPLC was used to determine powder homogeneity.

Characterization of the Agglomerates

The yield of the agglomeration process was calculated by dividing the weight of the agglomerates (106–850 μm) by the total weight of powder before agglomeration, multiplied by 100.

The agglomerates were examined by SEM, as described before. Mean size distribution was verified by sieving (106, 250, 425, 500, 600, 710, 850 μm, ∅ 10 cm, Endecotts Ltd, London, UK). The average diameter was calculated by determining the mass retained in each sieve. The specific surface area was calculated by BET method (30). The nitrogen adsorption–desorption isotherms of previous degassed organic solids were obtained, under vacuum at 40°C, at liquid nitrogen boiling point in a homemade volumetric apparatus, using nitrogen as probe. The pressure measurements were made using a capillary Hg barometer and also an active Pirani gauge. The results were systematically compared with an alumina standard reference.

Flowability and compressibility index were determined using the same procedure described above for the spray-dried mannitol/lecithin powders.

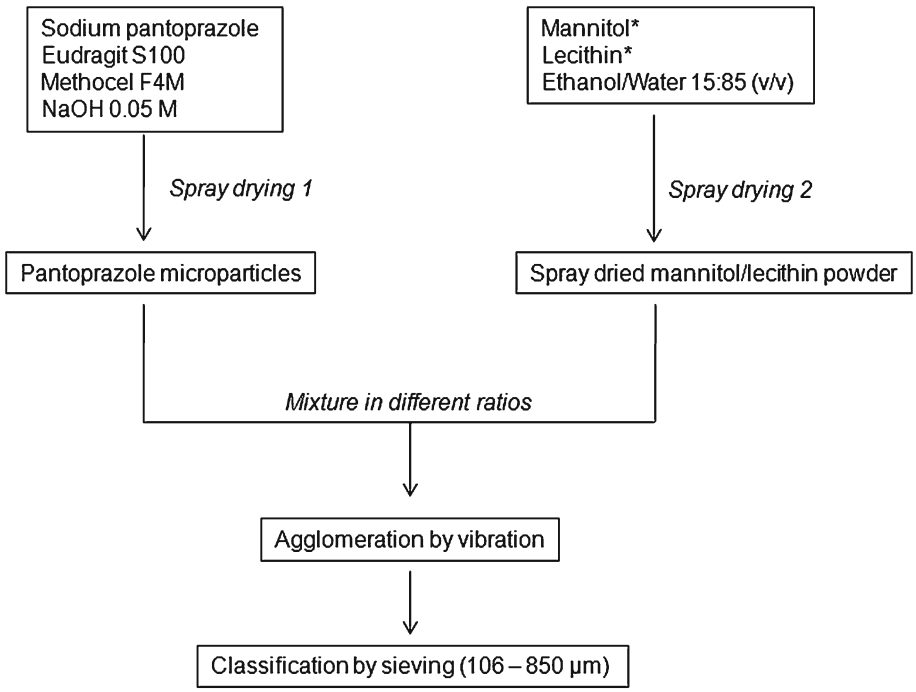

Fig. 1. Preparation of the pantoprazole microparticles, spray-dried mannitol/lecithin and agglomerates. *Asterisk* indicates variable concentrations

Agglomerates (2 g) were tested for resistance using a friabilometer (Model 300, Nova Ética, Brazil) operating at 25 rpm for 4 min (32). The agglomerates were separated from the powder during the test using a 106-µm sieve. The recovered agglomerates were weighed, and the percentage of powder loss was calculated.

In order to determine the tensile strength, a single agglomerate (n=8) was placed on a mobile platform under the measuring head of a calibrated load cell (514 QD, DS Europe, Milan, Italy) (14). The very slow movement of the platform caused the compression of the agglomerate against the measuring head. The force–time curve was recorded using the Scope v 3.5 software (AdInstruments Ltd, Oxfordshire, UK). From the crushing force (F), the tensile strength (σ) was calculated (Eq. 1) (14).

$$\sigma = \frac{2.8F}{\pi d^2} \quad (1)$$

where d is the agglomerate diameter.

Table I. Composition of the agglomerates, as well as the final amount of lecithin present in the agglomerates

Agglomerates	Lecithin in excipient microparticles (%)	Pantoprazole/ excipient microparticles ratio	Percentage of lecithin in the agglomerates (%)
A	15.0	1:3	11.25
B	17.5	1:2	11.67
C	17.5	1:3	13.12
D	20.0	1:1	10.00
E	20.0	1:2	13.33

The agglomerate disintegrations in aqueous media (phosphate pH 7.4 or 0.1 M HCl) were recorded under an optical stereomicroscope (magnification of ×20; Citoval 2, Jena, Germany) connected to a video camera (JVC, Tokyo, Japan). The disintegration tests were performed by placing the agglomerates (425–500 µm) over a microscope glass and wetting them with 50 µL of each medium: phosphate buffer pH 7.4 or 0.1 M HCl at 37°C. The disintegration time was measured on 25–30 agglomerates as referred to the time for deagglomeration of the globular structure.

To determine the drug release profile, size 00 hard gelatin capsules without coloring agent were filled with a mass of agglomerates corresponding to 15 mg of pantoprazole. *In vitro* drug release tests were conducted in USP Dissolution Apparatus II at 150 rpm at 37°C. To evaluate the gastro-resistance, the hard gelatin capsules containing the agglomerates (A to E) were exposed to 300 mL of 0.1 M HCl. After 1 h, an aqueous solution (600 mL) composed of NaOH (2.6 g) and KH_2PO_4 (6.12 g) was added into the dissolution vial in order to reach pH 7.4 (13,34,35). At this step, sampling began from 0 up to 600 min.

In order to determine if the agglomerates were able to release 100% of the encapsulated drug, the dissolution was evaluated in phosphate buffer pH 7.4 for 480 min in the same apparatus and conditions as referred for the gastro-resistance test.

Pantoprazole concentrations were determined by UV at 295 nm (Vankel UV/Vis spectrometer). The analytical method was previously validated (36). Gastro-resistance profiles were fit to monoexponential and biexponential models, using the MicroMath Scientist® software (Salt Lake City, UT, USA). The best fit was chosen considering the highest model selection criteria (MSC, given by the software), the highest determination coefficient, and the best graphic adjustment.

The Korsmeyer–Peppas model was used (Eq. 2) to determine the drug release mechanism.

$$f_t = at^n \quad (2)$$

where f_t (dimensionless) is the fraction of pantoprazole released at time t (min), a (min^{-1}) is a constant that incorporates structural and geometric characteristics of the carrier, and n (dimensionless) is the release exponent that indicates the release mechanism (37).

The adequacy of the models was evaluated considering the best correlation coefficient and the best model selection criteria (MSC), both provided by the software, as well as the best graphic adjustment.

The contact angle was assessed using a Dynamic Contact Angle Meter and Tensiometer (DCAT 11, Dataphysics, Filderstadt, Germany). The capillary constant was measured using *n*-hexano, and the contact angle of the pantoprazole-loaded microparticles and the agglomerates was measured with water (38).

Statistical Analysis

A one-way analysis of variance was employed in the comparison of the experimental data for significance at p values lower than 0.05.

RESULTS AND DISCUSSION

Accelerated Stability Tests for Pantoprazole-Loaded Microparticles

One batch of pantoprazole-loaded microparticles presenting mean particle size of 22.0±1.2 μm were obtained as off-white powders (62%) with drug content of 128 mg of pantoprazole per gram of microparticles and the residual moisture of 2%. Within 180 days of storage, no variation in the weight was verified either to sealed or non-sealed vials, indicating that pantoprazole microparticles are not hygroscopic in spite of the use of NaOH in the preparation. Regarding the drug content, both samples (sealed and non-sealed vials) were stable (Fig. 2). The decay in pantoprazole content was less than 5% after 6 months of storage.

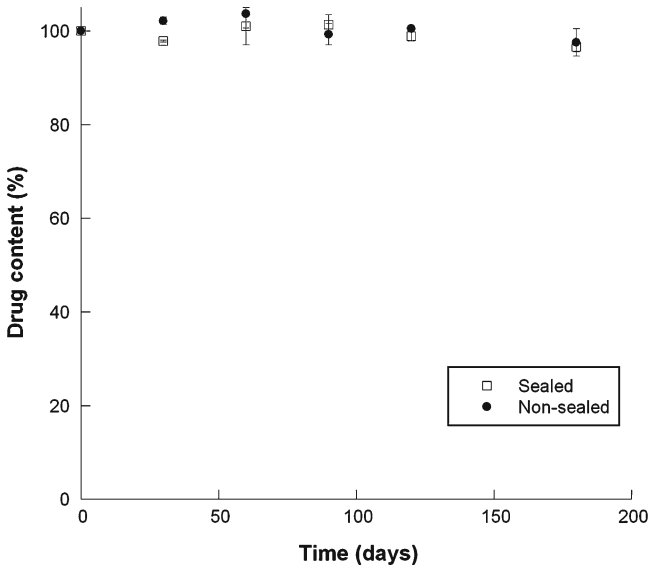

Fig. 2. Drug content of the microparticles during 180 days of accelerated conditions storage. Sealed and non-sealed vials were evaluated (n=3). *Error bars* indicate standard deviations

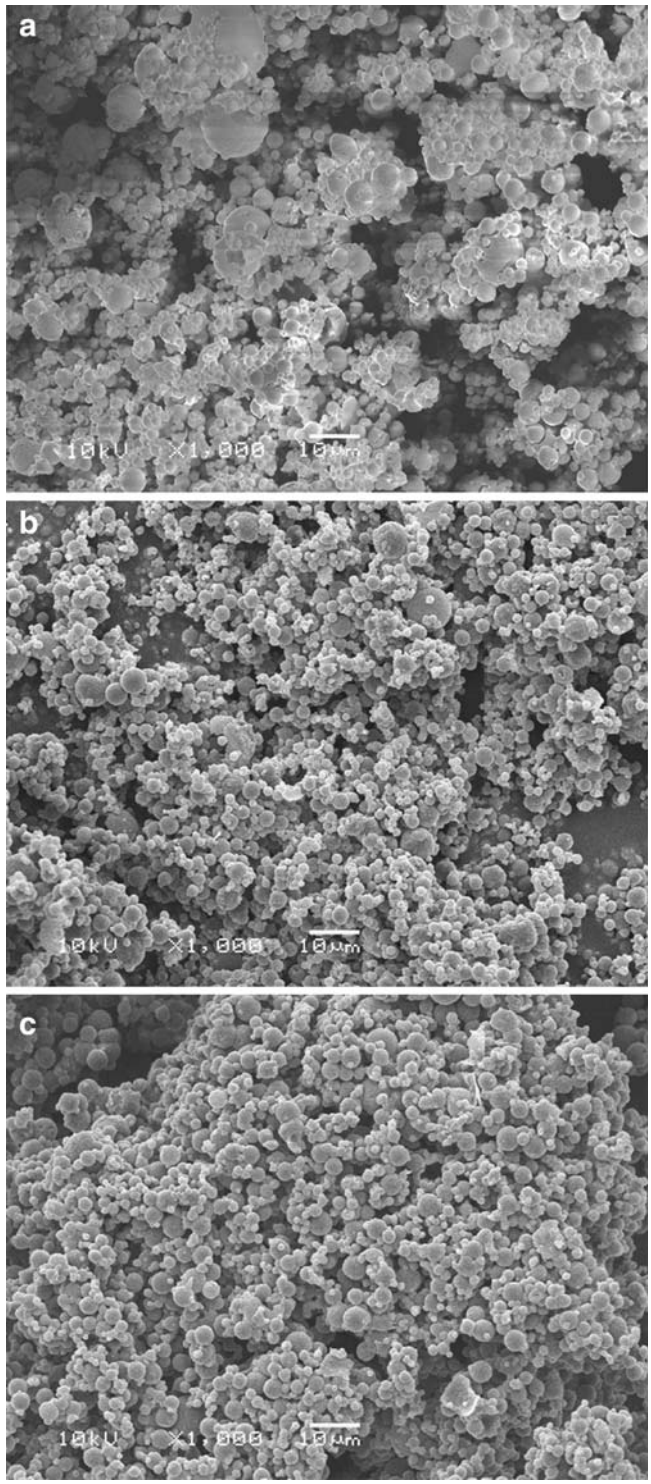

Fig. 3. The spray-dried mannitol/lecithin powders prepared with 15.0% **a**, 17.5% **b**, and 20% **c** of lecithin (magnification, ×1,000)

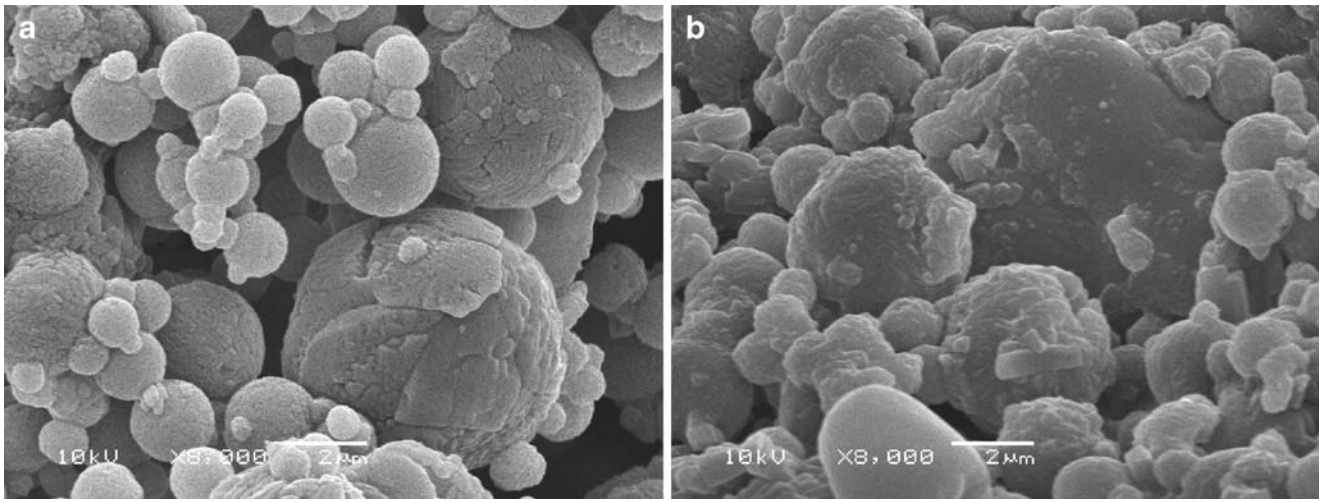

Fig. 4. Spray-dried mannitol/lecithin containing 17.5% of lecithin **a** and agglomerates C **b** (magnification, ×8,000)

Characterization of the spray-dried mannitol/lecithin powders

The spray-dried mannitol/lecithin powders presented lecithin contents of 15.0%, 17.5% and 20.0% (*w*/*w*). The yield of the spray-drying process was not affected by the lecithin concentration (approximately 55% for all formulations). In addition, the particle sizes were not influenced by the lecithin concentration, and all the three spray-dried mannitol/lecithin powders had mean diameters of 3.7 μm, evidently smaller than the size of the pantoprazole-loaded microparticles (mean particle size of 22.0±1.2 μm). The spray-dried mannitol/lecithin powders had mean diameters compatible to those described in the literature for powders prepared using the same type of equipment (11,39), in which a model plasmid was encapsulated in PLGA/stearylamine microparticles by spray drying (39). The microparticles became more uniform in size and less aggregated and more flowable with the increase of stearylamine. Particle size ranged from 2.1 to 8.3 μm, depending on the stearylamine concentration (39). In another research, DNA microparticles were prepared with polyethylenimine as a complexation agent for DNA (11). The size of the microparticles obtained by spray drying were similar, ranging from 2.5 to 8.1 μm. SEM revealed microparticles with regular shapes and smooth surfaces (11).

All the powders were composed of spherical particles (Fig. 3). The powders showed a tendency to form aggregates as the content of lecithin increased. Their moisture content were similar for all samples (lower than 2.0%). Particles were spherical shaped (Fig. 4a), and the mannitol was crystalline after the spray-drying process (verified by DSC, data not shown). Those spray-dried powders presented bulk density between 0.2 and 0.3 g.cm^{-3}, poor packing (compressibility index between 22 and 31) and did not flow according to the European Pharmacopoeia test (32).

Characterization of the Agglomerates

Agglomerates were obtained with yields from 35% to 79% (Table II), presenting drug loading from 58% to 100%. The lecithin content used in the present work was higher than the one described in our previous work (40) due to the lower density and cohesiveness of the pantoprazole-loaded Methocel/Eudragit microparticles. In the present work, the incomplete drug loading occurred because of the segregation of the two powders (spray-dried mannitol/lecithin powders and pantoprazole-loaded microparticles). In these cases, the lecithin content (used as a binder) was not sufficient to aggregate all the amount of pantoprazole-loaded microparticles in larger clusters (the agglomerates). Using the spray-dried mannitol/lecithin powder containing 15% of lecithin, agglomerates were prepared with 1:3 (*w*/*w*) ratio; otherwise, a complete segregation of the two powders was observed. Agglomerates A presented satisfactory yield and drug loading of 85.3%.

Mannitol/lecithin powders containing 17.5% of lecithin were also prepared. However, the 1:1 (*w*/*w*) ratio of mannitol/lecithin powder and pantoprazole-loaded microparticles also presented segregation of powders. Agglomerates B prepared with a 1:2 (*w*/*w*) ratio presented lower yield and lower drug loading than agglomerates A, and less than 60% of the drug was incorporated in the clusters. The 1:3 (*w*/*w*) ratio was also tested (agglomerates C). In this case, the agglomerates presented the highest agglomeration yield (79%) and complete drug loading (100%).

Table II. Characteristics of the agglomerates (n=3)

Agglomerates	Yield (%)	Drug loading (%)	Bulk density (g cm^{-3})	Tapped density (g cm^{-3})	Compressibility (%)	Flowability (s)
A	59.4±1.6	85.3±4.6	0.24±0.01	0.28±0.01	11.8±1.0	122.2±22.0
B	35.3±4.8	57.9±0.8	0.21±0.01	0.26±0.01	18.1±0.9	135.6±33.8
C	79.0±0.9	101.0±2.3	0.22±0.02	0.23±0.03	11.3±2.8	131.6±14.8
D	62.4±2.5	100.3±3.2	0.15±0.01	0.17±0.01	11.8±0.5	237.9±29.8
E	76.9±3.7	95.5±1.3	0.19±0.01	0.24±0.01	19.5±0.1	276.8±30.2

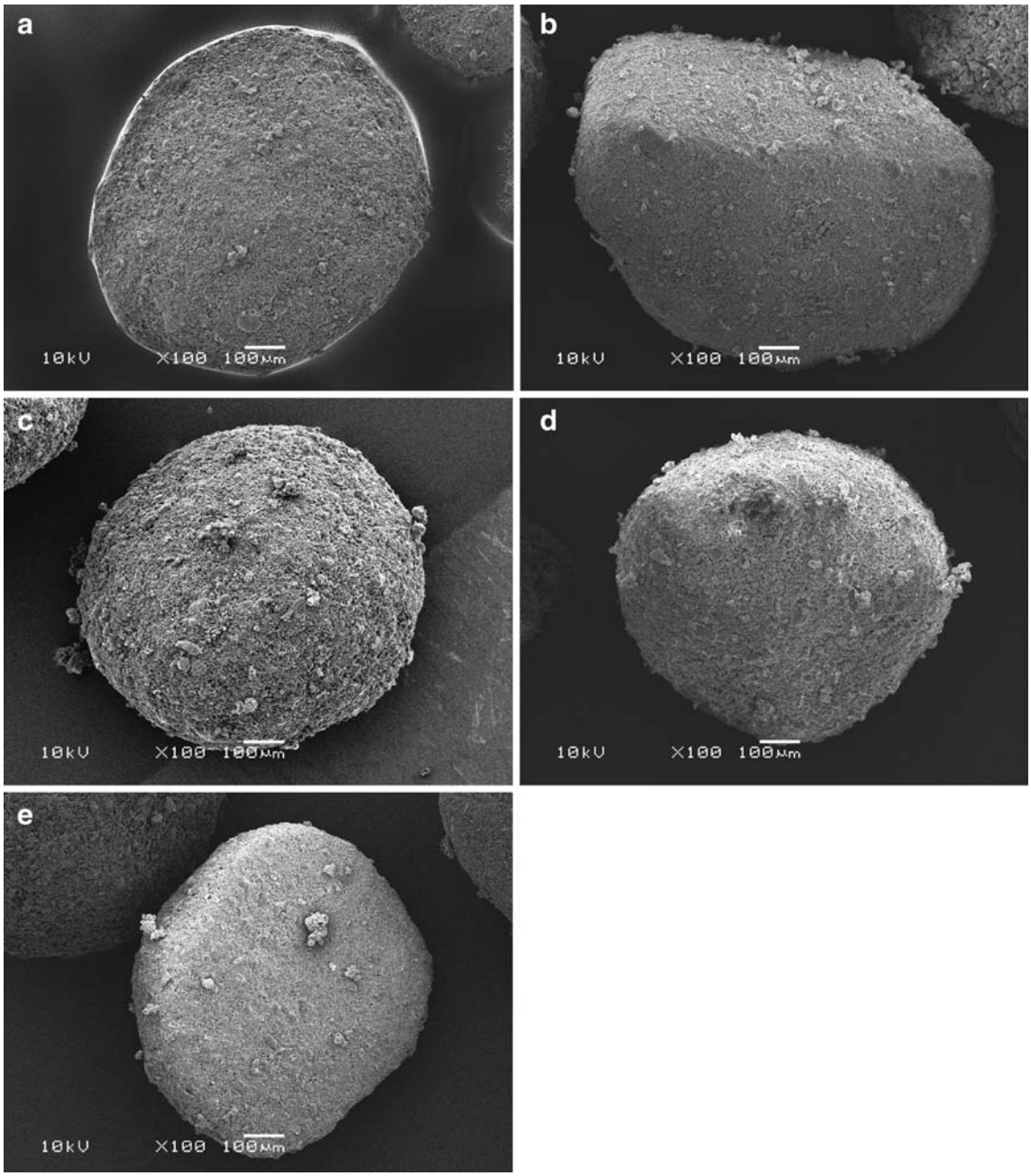

Fig. 5. SEM images of agglomerates a, b, c, d, and e (magnification, ×100)

In order to maintain the ethanol proportion in the spray-drying solution, the maximum concentration of lecithin tested was 20% in the spray-dried mannitol/lecithin powders due to the lecithin solubility in this solvent. The agglomeration of this powder with the pantoprazole-loaded microparticles at the ratios of 1:1 (*w*/*w*) and 1:2 (*w*/*w*) showed yields of 62 % and 77%, respectively (Table II). Furthermore, agglomerates D and E presented complete drug loading. Using the spray-dried mannitol/lecithin powder with 15% of lecithin, it was not possible to agglomerate all the amount of pantoprazole-loaded microparticles. In the same way, agglomerates B showed the highest segregation, as well as the lowest yield.

The agglomerates presented spherical shape (Fig. 5). The globule surface was smooth with a very small quantity of non-agglomerated particles on the surface. The agglomerate surface was characterized by small spray-dried mannitol/lecithin powders embedding larger pantoprazole microparticles without evident bridges among them (agglomerate C was chosen as an example in Fig. 6). In detail, some material, likely lecithin, was spread out over the particles leading to particles more closely connected (Fig. 4b), which was particularly evident for the agglomerates containing the mannitol/lecithin powder prepared using higher content of lecithin (agglomerates E). This result suggested that, in the mannitol/lecithin powder, lecithin could be located at the surface acting as a binder. The specific surface area of the agglomerates was measured (Table III). The surface areas of the microparticles (close to 98 $m^2 g^{-1}$) and of the mannitol/lecithin powders (close

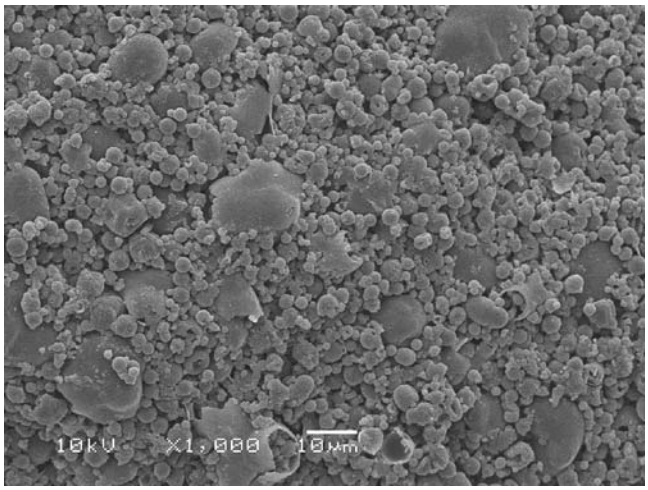

Fig. 6. Photomicrograph of the surface of agglomerate C (magnification, ×1,000)

to 60 $m^2 g^{-1}$) were used to calculate the expected surface area of the agglomerates, considering the ratio between the components. In all cases, the measured and the expected areas were very close, indicating that no changes in the particle structure occurred during the agglomeration process.

The agglomerates showed values of bulk densities around 0.20 g cm^{-3}, whose values were higher than those observed for the pantoprazole microparticles (0.06 g cm^{-3}) but still corresponding to a loose packing arrangement of particles (Table II). The density values were determined by the ratio between pantoprazole-loaded microparticles and mannitol/lecithin powders. The bulk densities were higher for the agglomerates prepared with higher amounts of spray-dried mannitol/lecithin powder. The compressibility indexes were around 11 for agglomerates A, C, and D. On the other hand, agglomerates B and E presented slightly higher values of compressibility index (18 and 19, respectively).

The agglomeration process, determining the organization of particles in the globular structure, favored the packed arrangement of powder bed over primary microparticle powders. The compressibility index that is related to the powder flowability was improved by agglomeration. Pantoprazole microparticles, as well as the three different spray-dried mannitol/lecithin powders, showed flow in infinite time (the entire samples failed to flow under conditions prescribed for the flowability test). In contrast, the agglomerates flowed well (Table II). Agglomerates A, B, and C presented higher flowability than agglomerates D and E ($p<0.01$). However, all products were classified as free-flowing powders. In summary, the agglomerates showed characteristics of packing arrangement and flowability more adequate for handling and dose metering than the pantoprazole-loaded microparticles.

In order to evaluate the resistance of the agglomerates during transportation, the friability test was performed for the agglomerates. Values were statistically similar ($p=0.32$) from 1.06% to 2.48% (Table III). Increasing the amount of mannitol/lecithin powders,l the friability increased, demonstrating that there were some particles not embedded in the globular structure. Pantoprazole agglomerates had a very low resistance to crushing, and the tensile strength values (Table III) were between 44 and 69 mN mm^{-2}, similar to those reported by Russo and co-workers (20). The samples prepared with 1:2 (*w*/*w*) ratio of pantoprazole microparticles and spray-dried mannitol/lecithin powders had higher tensile strength values. The agglomerates prepared with 1:1 and 1:3 (*w*/*w*) ratios presented lower values of tensile strength. The 1:2 (*w*/*w*) ratio seemed the optimal composition to improve resistance. Therefore, the agglomerates presented good resistance while flowing and poor resistance when compressed. Based on these features, they are suitable for filling hard gelatin capsules viewing oral administration of these agglomerates containing pantoprazole microparticles.

Disintegration and Dissolution of the Agglomerates

In phosphate buffer pH 7.4, the agglomerates were slowly penetrated and slightly swollen by the solvent, maintaining the globular structure. Only agglomerates prepared with 1:3 (*w*/*w*) ratio (A and C) disintegrated after 2 min. The soluble spray-dried mannitol/lecithin powders in large quantity influenced the gel layer formation of HPMC in the pantoprazole-loaded microparticles, facilitating the water penetration and the drug diffusion. The mannitol acted as a disintegrant, and increasing mannitol amount resulted to faster disintegration. Agglomerates B and E prepared with 1:2 (*w*/*w*) ratio disintegrated more slowly within 5–10 min. Agglomerates D did not disintegrate within 20 min. In order to understand the influence of lecithin and mannitol on the disintegration behavior, agglomerates constituting exclusively of spray-dried mannitol/lecithin powder were tested at pH 7.4. These agglomerates disintegrated within 2 min, indicating that the slow disintegration is dependent of HPMC and that the spray-dried mannitol/lecithin powder acted as a disintegrant in the agglomerates. Varying the amount of the spray-dried mannitol/lecithin powders in the agglomerates, the modulation of the disintegration time was achieved.

The stability of all samples (pantoprazole-loaded microparticles and agglomerates) in phosphate buffer pH 7.4 was evaluated, showing that the pantoprazole-loaded microparticles and the agglomerates reached 100% of pantoprazole dissolution after 500 min. The drug did not degrade in this experimental condition. These results indicated that the spray drying and the agglomeration techniques, as well as the medium used in the release experiments, did not affect the stability of pantoprazole.

Concerning the gastro-resistance evaluation, the agglomerates showed different results from the pantoprazole microparticles in terms of dissolution profile and total amount after the acid step (Fig. 7). Pantoprazole microparticles showed

Table III. Specific surface area and mechanical properties of the agglomerates

Agglomerates	Specific surface area ($m^2 g^{-1}$)	Friability (%) ($n=3$)	Tensile strength (mN mm^{-2}) ($n=8$)
A	76	2.44±1.37	44.0±8.6
B	85	1.06±0.67	61.6±4.2
C	70	2.47±0.36	54.1±7.7
D	73	1.67±0.44	52.3±6.7
E	78	1.14±0.60	69.3±5.5

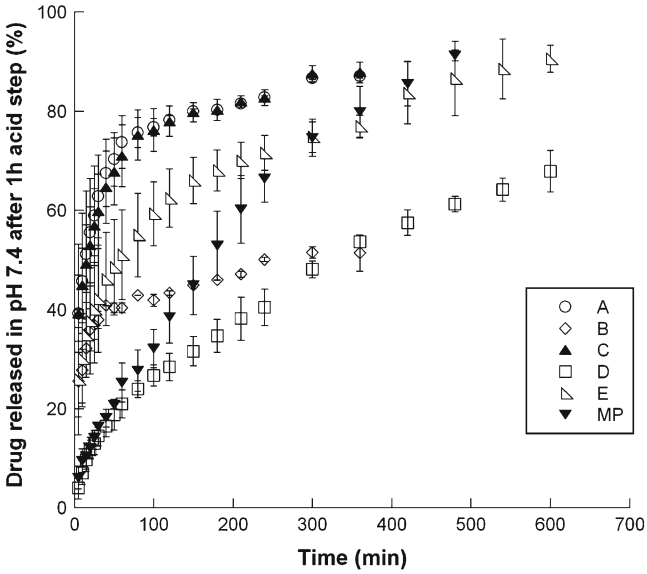

Fig. 7. Gastro-resistance of pantoprazole microparticles and agglomerates (A to E) (n=3) *Error bars* indicate standard deviations. Drug release is reported in phosphate buffer pH 7.4 after exposure of 1 h in 0.1 M HCl

92% of pantoprazole content after exposure to acid medium. Agglomerates D prepared with 1:1 (*w*/*w*) ratio and 20.0% of lecithin, presented 70% of pantoprazole after the acid step. This percentage is similar to that reported for the correspondent pantoprazole-loaded microparticle formulation prepared in laboratory scale (12). Agglomerates B prepared with 1:2 (*w*/*w*) ratio, and 17.5% of lecithin presented the lowest gastro-resistance (51%). This formulation also showed low agglomeration yield and incomplete drug loading. Agglomerates E prepared with 1:2 (*w*/*w*) ratio and 20.0% of lecithin showed 91% of gastro-resistance. This formulation showed a gastro-resistance similar to that observed for the pantoprazole-loaded microparticles before agglomerating. Agglomerates A and C presented very similar profiles, and the amount of drug stabilized was approximately 87%.

The gastro-resistance profiles were mathematically modeled to fit mono- or biexponential equations. The profile of the pantoprazole-loaded microparticles fit the monoexponential model (MSC=4.38; r^2=0.996), and the half-life of drug release was 155.8 min. In general, drug delivery systems containing water-soluble drugs follow the monoexponential model (41). On the other hand, all agglomerate profiles fit the biexponential model (Table IV).

The biexponential release profiles have two different release rates (burst and sustained release phases). The initial burst was higher for agglomerates A and C, which presented A_b parameters close to 0.4. The burst phase half-lives were 16.1 and 19.2 min for agglomerates A and C, respectively. These agglomerates contained a higher amount of mannitol (in the form of spray-dried mannitol/lecithin powders) that acted as a disintegrant, facilitating the influx of water inside the agglomerates and reducing the gel layer formation by the swelling of HPMC. Those agglomerates presented sustained phase half-lives of 330.1 and 301.4 min, respectively. Otherwise, agglomerates B, D, and E showed A_b values of 0.21, 0.12, and 0.25, respectively. The B_s values were between 0.5 and 0.9 (Table IV). The amount of spray-dried mannitol/lecithin powders influenced the amount of pantoprazole released in the burst phase, as well as the release rate, modulating the drug release.

In order to access the release mechanism, the profiles were modeled to fit the Korsmeyer–Peppas model. The pantoprazole-loaded microparticle profile showed n value of 0.68 (Eq. 2), demonstrating that the release mechanism is the anomalous transport. The anomalous transport has intermediate characteristics between the Fickian diffusion and the swelling/controlled release (37).

Agglomerates A, B, C, and E presented n values of 0.27, 0.15, 0.25, and 0.28, respectively, indicating the Fickian diffusion release mechanism for polydisperse systems. On the other hand, agglomerates D presented the same release mechanism of the pantoprazole-loaded microparticles (n=0.54). In this way, the drug release mechanism was also changed with the concentration of spray-dried mannitol/lecithin powders.

In order to evaluate the wettability, the contact angle between the microparticles and water and the agglomerates and water were determined. The contact angle between pantoprazole-loaded microparticles and water was 89.9°, while the contact angles between water and the agglomerates A, B, C, D, and E were 76.2°, 75.1°, 65.5°, 87.6°, and 70.2°, respectively. The presence of mannitol/lecithin powders strongly influenced the contact angle and, as a consequence, the release mechanism of the agglomerates. The hydrophilic powder of mannitol and lecithin increased the wettability of the agglomerates, facilitating the disintegration of the globular structure. The agglomerates presented faster release as the ratio between pantoprazole-loaded microparticles and mannitol/lecithin powders increased. The high solubility and prompt disintegration of these excipients altered the gel layer formation around the pantoprazole-loaded microparticles, facilitating the water penetration inside the agglomerates. It was possible to maintain the release mechanism and the release rate of the primary pantoprazole microparticles when 1:1 (*w*/*w*) ratio was used. However, those agglomerates were not capable of stabilizing more than 90% of pantoprazole as required by the Pharmacopoeia (33). Furthermore, agglomerates E containing 1:2 (*w*/*w*) ratio of mannitol/lecithin powders (80:20) presented high gastro-resistance and an intermediate release rate (half-life of release of 108.8 min).

CONCLUSIONS

The spray-drying process was reproducible, and the pantoprazole-loaded microparticles were stable under accelerate condition within 6 months. Indeed, pantoprazole microparticles were not hygroscopic. The agglomeration of

Table IV. Mathematical model of the agglomerate dissolution profiles and fit to the biexponential equation (n=3)

	Biexponential equation parameters					
Agglomerates	A_b	α	B_s	β	r^2	MSC
A	0.42	0.043	0.27	0.0021	0.999	5.9
B	0.21	0.075	0.61	0.0007	0.992	3.8
C	0.39	0.036	0.29	0.0023	0.999	5.8
D	0.12	0.057	0.87	0.0017	0.999	6.7
E	0.25	0.034	0.53	0.0026	0.998	5.5

pantoprazole-loaded microparticles blended with spray-dried mannitol/lecithin powders was successfully applied to size enlargement of micronized products that could be damaged by granulation or compaction. The composition and quantity of the spray-dried mannitol/lecithin powders resulted to be the crucial factors for the agglomerate quality, in terms of process yield, drug loading, resistance, friability, and flowability. Therefore, adjusting the content of lecithin used as a binder, it was possible to agglomerate microparticulated materials that have poor flowability. The presence of mannitol/lecithin powders strongly influenced the disintegration and the drug release from the agglomerates. The agglomerates with more adequate mechanical and biopharmaceutical characteristics were prepared with 1:2 (*w*/*w*) ratio of pantoprazole-loaded microparticles and mannitol/lecithin powder (80:20).

ACKNOWLEDGEMENT

The authors are grateful for the financial support of CNPq/MCT, Universal 2007 CNPq and for the CAPES fellowship. The financial support of the Italian Ministry for University and Research is also gratefully acknowledged. We thank Prof. Edilson Benvenutti for the BET analysis.

REFERENCES

1. N. S. Barakat, and A. A. E. Ahmad. Diclofenac sodium loaded-cellulose acetate butyrate: Effect of processing variables on microparticles properties, drug release kinetics and ulcerogenic activity. *J. Microencapsul.* **25**(1):31–45 (2008).
2. Y. C. Huang, A. Vieira, M. K. Yeh, and C. H. Chiang. Containing betamethasone pulmonary anti-inflammatory effects of chitosan microparticles. *J. Bioact. Compat. Polym.* **22**:30–41 (2007).
3. S. Tamilvanan and B. Sa. *In vitro* and *in vivo* evaluation of single-unit commercial conventional tablet and sustained-release capsules compared with multiple-unit polystyrene microparticle dosage forms of ibuprofen. *AAPS PharmSciTech*. 7(3):Article 72 (2006).
4. K. P. R. Chowdary and Y. Srinivasa Rao. Design and *in vitro* and *in vivo* evaluation of mucoadhesive microcapsules of glipizide for oral controlled release: A technical note. *AAPS PharmSciTech*. **4** (3):Article 39 (2003).
5. K. Masters. *The spray drying handbook*, Longman, New York, 1991.
6. R. C. R. Beck, A. R. Pohlmann, and S. S. Guterres. Nanoparticle-coated microparticles: preparation and characterization. *J. Microencapsul.* **21**:499–512 (2004).
7. A. Goula, K. Adamopoulos, and N. Kazakis. Influence of spray drying conditions on tomato powder properties. *Dry. Technol.* **22** (5):1129–1151 (2004).
8. M. I. Ré. Microencapsulation by spray drying. *Dry. Technol.* **16**:1195–1236 (1998).
9. R. P. Raffin, S. S. Guterres, A. R. Pohlmann, and M. I. Ré. Powder characteristics of pantoprazole delivery systems produced in different spray-dryer scales. *Dry. Technol.* **24**:339–348 (2006).
10. R. P. Raffin, D. S. Jornada, M. I. Ré, A. R. Pohlmann, and S. S. Guterres. Sodium Pantoprazole-loaded enteric microparticles prepared by spray drying: effect of the scale of production and process validation. *Int. J. Pharm.* **324**:10–18 (2006).
11. C.G. Oster, and T. Kissel. Comparative study of DNA encapsulation into PLGA microparticles using modified double emulsion methods and spray drying techniques. *J. Microencapsul.* **22**:235–244 (2005).
12. R. P. Raffin, L. M. Colomé, S. E. Haas, D. S. Jornada, A. R. Pohlmann, and S. S. Guterres. Development of HPMC and Eudragit S100® blended microparticles containing sodium pantoprazole. *Pharmazie*. **62**:361–364 (2007).
13. S. Harikarnpakdee, V. Lipipun, N. Sutanthavibul, and G. C. Ritthidej. Spray-dried mucoadhesive microspheres: Preparation and transport through nasal cell monolayer. *AAPS PharmSciTech*. **7**(1):Article 12 (2006).
14. P. Russo, F. Buttini, F. Sonvico, R. Bettini, G. Massimo, C. Sacchetti, P. Colombo, and P. Santi. Chimeral agglomerates of microparticles for administration of caffeine nasal powders. *J. Drug Deliv. Sci. Technol.* **14**:449–454 (2004).
15. T. D. Reynolds, S. A. Mitchell, and K. M. Balwinsky. Investigation of the effect of tablet surface area/volume on drug release from hydroxypropylmethylcellulose controlled-release matrix tablets. *Drug Dev. Ind. Pharm.* **28**:457–466 (2002).
16. E. Esposito, F. Cervellati, E. Menegatti, C. Nastruzzi, and R. Cortesi. Spray dried Eudragit microparticles as encapsulation devices for vitamin C. *Int. J. Pharm.* **242**:329–334 (2002).
17. G. F. Palmieri, G. Bonacucina, P. Di Martino, and S. Martelli. Gastro-resistant microspheres containing ketoprofen. *J. Microencapsul.* **19**:111–119 (2002).
18. D. B. Beten, M. Gelbcke, B. Diallo, and A. J. Moes. Interaction between dipyridamole and Eudragit S. *Int. J. Pharm.* **88**:31–37 (1992).
19. H. P. de Oliveiraa, J. J. F. Albuquerque Jr., C. Nogueirasa, and J. Rieumonta. Physical chemistry behavior of enteric polymer in drug release systems. *Int. J. Pharm.* **366**:185–189 (2009).
20. S. Cheer, A. Prakash, D. Faulds, and H. Lamb. Pantoprazole—an update of its pharmacological properties and therapeutic use in the management of acid-related disorders. *Drugs*. **63**:101–132 (2003).
21. B. K. Kim, S. J. Hwang, J. B. Park, and H. J. Park. Characteristics of felodipine-located poly(ε-caprolactone) microspheres. *J. Microencapsul.* **22**:193–203 (2005).
22. Y. J. Fu, F. L. Mi, T. B. Wong, and S. S. Shyu. Characteristic and controlled release of anticancer drug loaded poly (D,L-lactide) microparticles prepared by spray drying technique. *J. Microencapsul.* **18**:733–747 (2001).
23. S. Tsantilis, and S. E. Pratsinis. Soft- and hard-agglomerate aerosols made at high temperatures. *Langmuir.* **20**:5933–5939 (2004).
24. R. Boerefijn, Z. Ning, and M. Ghadiri. Disintegration of weak lactose agglomerates for inhalation applications. *Int. J. Pharm.* **172**:199–209 (1998).
25. P. Russo, C. Sacchetti, I. Pasquali, R. Bettini, G. Massimo, P. Colombo, and A. Rossi. Primary microparticles and agglomerates of morphine for nasal insufflation. *J. Pharm. Sci.* **95**:2553–2561 (2006).
26. R. Moreno-Atanasio, and M. Ghadiri. Mechanistic analysis and computer simulation of impact breakage of agglomerates: Effect of surface energy. *Chem. Eng. Sci.* **61**:2476–2481 (2006).
27. Stability testing guideline RE 1, Brazilian National Health Surveillance Agency, July 29, 2005.
28. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Stability Testing of New Drug Substances and Products (Q 1A (R2)), 2003.
29. R. P. Raffin, L. M. Colomé, S. S. Guterres, and A. R. Pohlmann. Validation of analytical methodology by HPLC for quantification and stability evaluation of sodium pantoprazole. *Quim. Nova*. **30**:1001–1005 (2007).
30. S. Brunauer, P. H. Emmet, and E. Teller. Adsorption of gases in multimolecular layers. *J. Am. Chem. Soc.* **60**:309–319 (1938).
31. Y. S. L. Lee, R. Poynter, F. Podczeck, and J. M. Newton. Development of a Dual Approach to Assess Powder Flow from Avalanching Behavior. *AAPS PharmSciTech* 1(3):Article 21 (2000).
32. European Pharmacopoeia. 5th edn., Council of Europe, Strasbourg, 2005.
33. US Pharmacopeia XXX, US Pharmacopeial Convention, Rockville, MD, 2007.
34. Farmacopéia Brasileira 4ª ed., Atheneu, São Paulo, 2005.
35. H. X. Guo, J. Heinamaki, and J. Yliruusi. Amylopectin as a subcoating material improves the acidic resistance of enteric-coated pellets containing a freely soluble drug. *Int. J. Pharm.* **235**:79–86 (2002).

36. R. P. Raffin, L. M. Colomé, A. R. Pohlmann, and S. S. Guterres. Preparation, characterization, and *in vivo* anti-ulcer evaluation of pantoprazole-loaded microparticles. *Eur. J. Pharm. Biopharm.* **63**:198–204 (2006).
37. R. W. Korsmeyer, R. Gurny, E. Doelker, P. Buri, and N. A. Peppas. Mechanism of solute release from porous hydrophilic polymers. *Int. J. Pharm.* **15**:25–35 (1983).
38. Z. Wong, and E. Schaffer. Experimental study of contact line dynamics by capillary rise and fall. In K. L. Mittal (ed.), *Contact Angle, Wettability and Adhesion*, Brill, Boston, 2003, pp. 25–37.
39. K. N. Atuah, E. Walter, H. P. Merkle, and H. O. Alpar. Encapsulation of plasmid DNA in PLGA-stearylamine microspheres: a comparison of solvent evaporation and spray-drying methods. *J. Microencapsul.* **20**:387–399 (2003).
40. R. P. Raffin, P. Colombo, F. Sonvico, F. S. Polleto, G. Colombo, A. Rossi, A. R. Pohlmann, and S. S. Guterres. Soft Agglomerates of pantoprazole gastro-resistant microparticles for oral administration and intestinal release. *J. Drug Deliv. Sci. Technol.* **17**:407–413 (2007).
41. P. Costa, and J. M. S. Lobo. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.* **13**:123–133 (2001).