Enhancement of Felodipine Dissolution Rate Through Its Incorporation into Eudragit® E–PHB Polymeric Microparticles: *In Vitro* Characterization and Investigation of Absorption in Rats

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ABSTRACT: In this study, felodipine was incorporated into microparticles prepared with Eudragit® E and it blended with poly(3-hydroxybutyrate) (PHB) using the emulsion–solvent evaporation technique, with the aim of improving the dissolution rate of the drug. The formulation prepared with Eudragit® E showed irregular and fragmented microparticles, with a loading efficiency (LE) of 82.6%. When the microparticles were prepared with a blend of Eudragit® E and PHB, they had a spherical form with a LE of 103.9%. X-ray diffraction and differential thermal analysis indicated a reduction in the crystallinity of felodipine after its incorporation into the microparticles, which caused a significant increase in the felodipine dissolution rate. An investigation into the absorption in rats was carried out using high-performance liquid chromatography analysis of the blood collected 20 and 60 min after the animals were administered felodipine [30 mg/Kg, orally (p.o.)] or felodipine microparticles (30 mg/Kg, p.o.). Animals that were given felodipine showed mean plasmatic levels of 0.0125 (±0.00156) and 0.0240 (±0.0069) $\mu g\ mL^{-1}$ after 20 and 60 min, respectively, whereas animals that received microparticles containing felodipine showed respective mean plasmatic levels of 0.0651 (±0.0120) and 0.0369 (±0.0145) $\mu g\ mL^{-1}$. Our data suggest that the incorporation into microparticles significantly enhanced the release of felodipine, improving its absorption in rats. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:1518–1523, 2012

Keywords: bioavailability; Eudragit® E; felodipine; microencapsulation; microparticles; oral drug delivery; PHB; solubility

INTRODUCTION

Felodipine is a calcium channel blocker used to treat systemic arterial hypertension based on its high vascular selectivity. This drug is classified according to the Biopharmaceutics Classification System as class II, indicating that it has very low water solubility and high permeability. The oral bioavailability of this drug is limited and, thus, the enhancement of its dissolution rate would be a useful achievement.[1]

The enhancement of the dissolution of drugs with poor water solubility has been one of the main targets of drug development during the last decade and several pharmaceutical technologies have been investigated to this end. Several strategies have been employed to improve the solubility of felodipine and increase its dissolution rate in aqueous media (including the preparation of solid dispersions with hydroxypropylmethylcellulose (HPMC),[1,2] polyvinylpyrrolidone, polyethyleneglycol,[3] and HPMC acetate succinate[4]), the drug micronization,[5] and its cogrinding with excipients.[6]

The microencapsulation and nanoencapsulation of drugs have been widely used in pharmaceuticals in recent decades. However, the use of this technique in order to increase the dissolution and bioavailability of drugs is still a little explored area and only a few studies have focused on this aspect, for instance, the preparation of poly(lactide-co-glycolide) nanoparticles to improve the oral bioavailability of curcumin,[7] lipid nanoparticles to enhance the dissolution rate of simvastatin,[8] enteric microparticles to enhance the oral bioavailability of poorly soluble basic drugs,[9] and hyaluronic microspheres to improve the oral bioavailability of cyclosporin A.[10]

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The incorporation of felodipine into polymeric microspheres has not yet been studied for this purpose. Solid microspheres offer advantages because they can be used to prepare solid pharmaceutical forms that are technologically easy to manufacture and can be easily administered to the patient.

The aim of this study was to evaluate a new approach to enhance the dissolution rate and the oral bioavailability of felodipine on the basis of its encapsulation into polymeric microparticles. The polymethacrylate Eudragit® E and polyester poly(3-hydroxybutyrate) (PHB), widely used in the pharmaceutical field, were used as polymeric carriers. The morphological and physicochemical properties of microparticles were characterized by scanning electron microscopy (SEM), differential thermal analysis (DTA), and X-ray powder diffraction (XRD). The felodipine dissolution rate and oral bioavailability in rats were also evaluated.

MATERIALS AND METHODS

Materials

Poly(3-hydroxybutyrate) (molecular weight (M_n)= 312,800 g mol^{-1} and polydispersity degree of 1.23, determined by gel-permeation chromatography) was kindly supplied by PHB Industrial S.A. (Serrana, São Paulo, Brazil) and Eudragit® E 100 (Röhm Pharma Polymers) was supplied by Almapal S.A. (São Paulo, Brazil). Felodipine was purchased from Henrifarma Produtos Químicos e Farmacêuticos (São Paulo, Brazil) and poly(vinyl alcohol) (M_n = 92,000 g mol^{-1}, according to the information supplied by the manufacturer) was from Vetec Química (Rio de Janeiro, Brazil). Dichloromethane, ethanol, acetonitrile, and acetic acid were acquired from Biotec Reagentes Analíticos (Pinhais, Paraná, Brazil). All chemicals were used without further purification.

Preparation of the Microparticles

The microparticles were prepared by oil-in-water emulsion–solvent evaporation technique. The polymer(s) (500 mg of Eudragit® E or 250 mg of PHB and 250 mg of Eudragit® E) and felodipine (200 mg) were dissolved in 10 mL of dichloromethane (internal phase) and then emulsified in 200 mL of an aqueous phase containing 0.15% (w/v) of poly(vinyl alcohol) as a stabilizer (external phase). The resulting emulsion was stirred at 700 rpm at room temperature for 24 h until the evaporation of the organic solvent. The microparticles were washed with distilled water, centrifuged, dried, and stored under vacuum at room temperature.

Determination of the Drug Content and Loading Efficiency

To determine the drug content, 10 mg of microparticles were accurately weighed and dissolved in 10 mL of ethanol. The solution was diluted at a concentration of 10 mg L^{-1} of felodipine and its absorbance was determined by ultraviolet (UV)–visible spectrophotometry (Shimadzu 1601 PC spectrophotometer; Shimadzu, Kyoto, Japan) at 364 nm. The analytical method was validated according to the following characteristics: linearity, precision, accuracy, and specificity. The loading efficiency (LE) was obtained using Eq. 1 and the results were expressed considering the drug entrapped into the microparticles and the nonencapsulated drug crystals:

$$LE\% = \frac{\text{drug found in microparticle (mg)}}{\text{drug initially added to the formulation (mg)}} \times 100 \quad (1)$$

Scanning Electron Microscopy

The morphology of the microparticles was examined in a Zeiss DSM 940A scanning electron microscope (Zeiss, Oberkochen, Germany). The arithmetic mean diameter of at least 100 particles was measured on micrographs obtained by SEM.

Differential Thermal Analysis

Differential thermal analysis curves were obtained using a Netzch STA 449C differential scanning calorimeter (Netzch, Selb, Germany) by heating from 25°C to 200°C at 10°C min^{-1} in a nitrogen atmosphere (50 mL min^{-1}).

XRD Analysis

Diffractograms were recorded from 5° to 50° (2θ) at a scanning speed of 2° min^{-1} on an X-ray powder diffractometer (Shimadzu XRD-6000; Shimadzu). Cu Kα radiation was used as the X-ray source and the equipment was operated at a voltage of 40 kV and a current of 30 mA.

In Vitro Drug Release

Hard gelatin capsules were filled with felodipine (5 mg) or felodipine microparticles (equivalent to 5 mg of the drug). Dissolution tests were performed using the basket apparatus at 100 rpm and 900 mL of HCl (0.1 N, pH 1.2) containing 1% (w/v) of sodium lauryl sulfate (sink conditions) at 37 °C. At predetermined time intervals (5, 10, 15, 20, 40, and 60 min), a 10 mL sample of the medium was taken, centrifuged, and the drug concentration in the solution was determined using a Merck–Hitachi LaChrom D7000 liquid chromatograph (Merck, Darmstadt, Germany), a

LiChrospher® RP-18 column (5 μm) (Merck, Darmstadt, Germany), acetic acid–acetonitrile (95:5) as the mobile phase, and UV detection at 364 nm. An equal volume of fresh dissolution medium was transferred to the vessel after sample withdrawal. Experiments were carried out in triplicate.

Preliminary Investigation into Drug Absorption

Male Wistar rats ($n = 6$ for each group) weighing 280–320 g were given 0.5% carboxymethylcellulose [CMC; 0.1 mL/100 g, orally (p.o.)], felodipine (30 mg/Kg, p.o.), or Eudragit® E–PHB felodipine microparticles (30 mg/Kg, p.o.). At periods of 20 and 60 min after the administration, 5 mL of blood was collected in previously heparinized tubes. The blood was analyzed by high-performance liquid chromatography in order to quantify the felodipine. This analysis was conducted with a LiChrospher® RP-18 column (5 μm) employing acetic acid–acetonitrile (95:5) as the mobile phase and the detection was performed in the UV region at 364 nm. Data are presented as mean ± standard error of the mean and were submitted to one-way analysis of variance (ANOVA) followed by post-hoc Bonferroni test to determine the statistical difference between the groups, which was considered significant when p values were lower than 0.05 (95% confidence interval). All procedures involving experimental animals were carried out according to the official ethics guidelines for tests involving animals and approved by the Ethics Committee of Research of the University of Joinville (Joinville, Santa Catarina, Brazil).

RESULTS AND DISCUSSION

Microparticle Morphology and LE

The emulsion–solvent evaporation technique consisted of the emulsification of an organic solvent solution containing the polymers and the drug in an aqueous phase. The diffusion of the organic solvent to the aqueous phase and its later evaporation at the air–water interface lead to the formation of microparticles.

Eudragit® E microparticles containing felodipine (Figs. 1a and 1b) presented an irregular morphology, brittle aspect, and little cluster formation. With the aim of improving the morphological characteristics and flow properties of the particles, PHB was added to the formulation, leading to the obtainment of spherical particles (Figs. 1c and 1d), with a mean diameter of 64 ± 10 μm. The external surface of the microparticles was rough and porous, which is a common characteristic of PHB microspheres, as described in the literature.[11]

The loading efficiencies of felodipine were 82.6% and 103.9% for Eudragit® E and Eudragit® E–PHB microparticles, respectively, indicating that the process conditions used to prepare the microparticles were efficient in terms of obtaining high drug contents. Nonencapsulated drug crystals can be seen in the SEM micrographs of both formulations, which were not removed during the washing step of the microparticle formation process, as indicated in Figure 1.

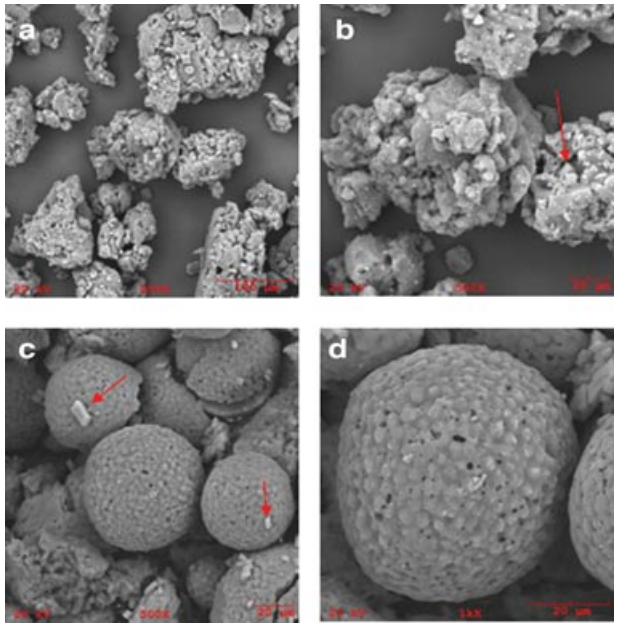

Figure 1. Scanning electron micrographs of (a and b) Eudragit® E microparticles and (c and d) Eudragit® E–PHB microparticles. The arrows show the nonencapsulated felodipine crystals.

XRD and DTA

The solid-state characteristics of felodipine before and after its incorporation into the microparticles were investigated using XRD and DTA. The results are presented in Figures 2 and 3, respectively.

Felodipine as a raw material is crystalline, as demonstrated by sharp and intense diffraction peaks at 2θ of 10.3° and 23.3°, as reported in the literature.[12] In the XRD patterns of the Eudragit® E and Eudragit® E–PHB microparticles, the peaks of felodipine can also be observed, but with less intensity. This result may be associated with the presence of a low quantity of felodipine crystals that were not encapsulated, as observed in the SEM analysis (Fig. 1).

In the diffractogram of the microparticles containing PHB, two peaks can be observed at 2θ of 13.5° and 16.9°, corresponding to the semicrystalline pattern of this polymer.[13] However, no peaks were observed in the XRD pattern of the Eudragit® E microparticles, indicating the amorphous state of the polymer after the microencapsulation process.

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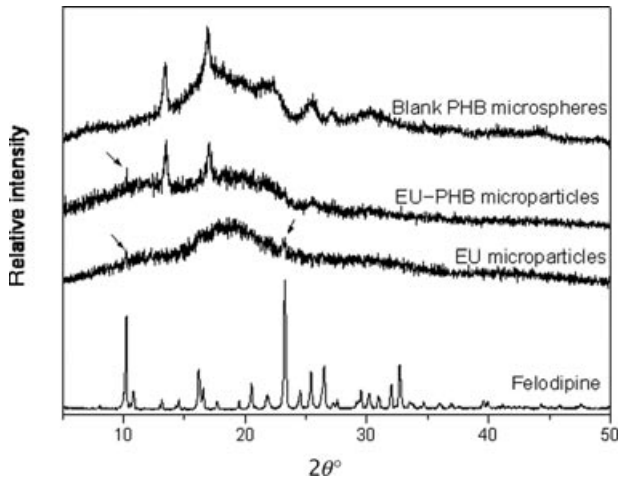

Figure 2. X-ray diffraction patterns of blank microspheres (PHB), Eudragit® E (EU)–PHB and Eudragit® E (EU) microparticles, and pure felodipine.

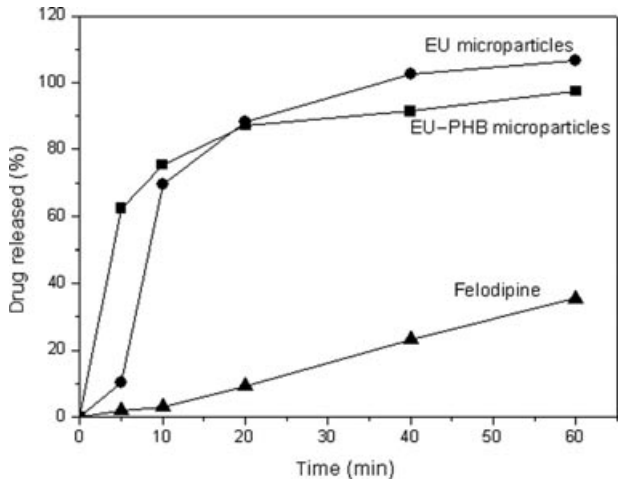

Figure 4. Release profiles of pure felodipine and Eudragit® E (EU)–PHB and Eudragit® E (EU) microparticles.

The DTA curve for pure felodipine (Fig. 3) showed an endothermic melting peak at 150.9°C, whereas no such peak was observed in the curves of the microparticles, suggesting that felodipine was molecularly dispersed in the polymeric matrix in an amorphous form. Other peaks at 155.9°C and 168.8°C were observed in the DTA curve for the Eudragit® E–PHB microparticles corresponding to PHB melting peaks, as described in the literature[13,14] and observed in the DTA curve for the blank microspheres prepared only with PHB.

The XRD and DTA results suggest that the total amorphization of the felodipine did not occur, but a reduction in crystallinity was detected after its incorporation into the microparticles.

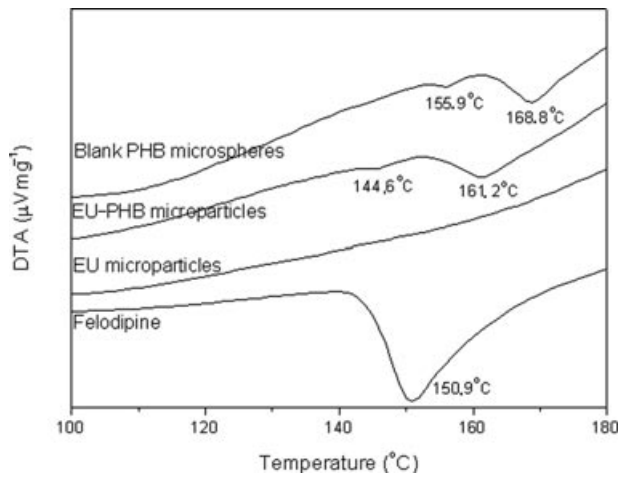

Figure 3. Differential thermal analysis (DTA) curves of blank PHB microspheres, Eudragit® E (EU)–PHB and Eudragit® E (EU) microparticles, and pure felodipine.

In Vitro Dissolution Study

In order to assess whether the goal of improving the dissolution rate of felodipine microparticles was reached, *in vitro* dissolution profiles were compared with that of pure drug (Fig. 4).

The dissolution rate of the pure felodipine was very low. As can be seen in Figure 4, only 2.9% was dissolved in the first 10 min, whereas 69.3% and 75.4% of the drug present in the Eudragit® E and Eudragit® E–PBH microparticles, respectively, dissolved within the same time period. Similar results were observed after 60 min with 35.5%, 103.6%, and 97.4% of felodipine dissolved for the pure drug, Eudragit® E, and Eudragit® E–PHB microparticles, respectively. The area under the curve (AUC) of the dissolution profiles was used to compare the drug release profiles. ANOVA showed that there was no statistical difference between the AUC of the two microparticle formulations ($p > 0.05$), indicating that both improved the dissolution of felodipine.

The enhancement of the drug dissolution rate can be ascribed to several factors: (i) the improvement of wetting and solubilization by a hydrophilic carrier, (ii) the reduction of the drug particle size, (iii) the reduction of the aggregation of particles, and (iv) the transformation of the solid state of the drug from a crystalline to an amorphous form.[1,15,16] In this study, it is considered that the enhancement of the felodipine dissolution after its incorporation into the microparticles was mainly associated with the amorphization of the drug and with the use of Eudragit® E as a polymeric carrier. Also, the amorphization of Eudragit® E microparticles was assumed to be greater than that of Eudragit® E–PHB because PHB maintained its semicrystalline pattern after the microencapsulation, as shown in Figures 2 and 3.

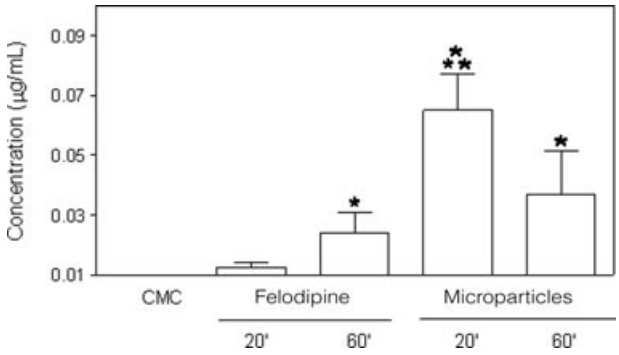

Figure 5. Felodipine blood concentrations 20 and 60 min after administration of felodipine and felodipine microparticles.

X-ray powder diffraction patterns and DTA curves of the microparticles (Figs. 2 and 3) show a reduction in the crystallinity of felodipine. The presence of an amorphous form of a drug in polymeric carriers may result in improved solubility and dissolution rates when compared with crystalline material. Drugs molecularly dispersed in polymeric carriers may achieve the highest levels of particle size reduction and surface area enhancement, which result in improved dissolution rates.[1]

Furthermore, felodipine solubility may be increased by the use of Eudragit® E as a carrier, which has high solubility in acid medium (pH > 5).[17] The rapid solubilization of this polymer in the dissolution medium may have contributed to the solubilization of felodipine. It has been demonstrated that the use of hydrophilic carriers such as polyvinylpyrrolidone and polyethyleneglycol enhances the dissolution rate of felodipine.[3]

Preliminary Investigation into Drug Absorption

Our data show that 20 and 60 min after administration of felodipine alone produced blood levels of 0.0125 (±0.0016) and 0.024 (±0.0069) μg mL^{-1}, respectively, whereas the corresponding values for felodipine associated with Eudragit® E–PHB microparticles were 0.0648 (±0.012) and 0.0367 (±0.0145) μg mL^{-1} (Fig. 5). Thus, in the latter case, there were fivefold and 1.5-fold increases in the concentration of felodipine, respectively, in comparison with the administration of felodipine alone. These results verify that the microparticles improved the absorption of felodipine significantly ($p < 0.01$). Animals that were given CMC had no detectable levels of felodipine.

CONCLUSIONS

The dissolution rates of felodipine incorporated into Eudragit® E and Eudragit® E–PHB microparticles were faster than that of the pure drug, suggesting that these are suitable systems for the enhancement of the aqueous solubility of this drug. The microparticles promoted a significant increase in the felodipine release because of its improved solubility in organic fluids. This increase might reduce the time necessary for the effect to begin and the antihypertensive effect could also potentially be enhanced, allowing the use of lower doses and thus minimizing potential side effects. Furthermore, the development of formulations for use in emergencies might be possible because a faster release of the felodipine occurs.

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