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Enhanced intestinal permeability and oral bioavailability of enalapril maleate upon complexation with the cationic polymethacrylate Eudragit E100

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ABSTRACT

The low bioavailability of enalapril maleate associated to its instability in solid state motivated the development of a polyelectrolyte-drug complex between enalapril maleate and the cationic polymethacrylate Eudragit E100. The solid complexes were characterized by DSC–TG, FT-IR and X-ray diffraction. Their aqueous dispersions were evaluated for drug delivery in bicompartimental Franz cells and electrokinetic potentials. Stability in solid state was also evaluated using an HPLC–UV stability indicating method. Absorption of enalapril maleate was assessed thorough the rat everted gut sac model. In addition, urinary recovery after oral administration in rats was used as an indicator of systemic exposition. The solid materials are stable amorphous solids in which both moieties of enalapril maleate are ionically bonded to the polymer. Their aqueous dispersions exhibited controlled release over more than 7 h in physiologic saline solution, being ionic exchange the fundamental mechanism that modified the extent and rate of drug release. Intestinal permeation of enalapril maleate was 1.7 times higher in the presence of the cationic polymer. This increase can be related with the capacity to adhere the mucosa due to the positive zeta potential of the complexes. As a consequence bioavailability was significantly improved (1.39 times) after oral administration of the complexes. In addition, no signs of chemical decomposition were observed after a 14 months period. The results indicated that the products are new chemical entities that improve unfavorable properties of a useful drug.

1. Introduction

Polyelectrolytes (PE) under the form of ionic exchange resins (insoluble PE) or dispersible hydrophilic polymers (soluble PE) have been largely used in pharmaceutical formulations (Anand et al., 2001; Guo et al., 1998; Heller, 1995; Jantzen and Robinson, 1996). Examples of such polymers include DNA (Van de Wetering et al., 1998; Wang et al., 2011), proteins (Kratz, 2008), carbomer (Parojčić et al., 2004), alginic acid (Tønnesen and Karlsen, 2002), hyaluronic acid (Dollo et al., 2004), carrageenan (Pavli et al., 2011), certain derivatives of cellulose polymers (Gallo et al., 2013; Ramírez Rigo et al., 2004), chitosan and other cationic polymethacrylates (Hamman, 2010; Kojima et al., 2012).

The ionic interaction between PE and acid or basic drugs (D) is a valuable resource to obtain new materials with physicochemical, pharmaceutical and biopharmaceutical properties different from those of their precursors. The ionic (PE–D) complexes can be obtained in a wide variety of qualitative and quantitative compositions as aqueous dispersions or in solid state. According to the properties of the newly formed chemical entity, the PE–D interaction can improve the drug stability in solution (Esteban et al., 2009; Jimenez-Kairuz et al., 2004), the permeability through biological membranes (Bonferoni et al., 2008), the apparent solubility in the vehicle (Dai et al., 2007) or modulate the release of drugs (Guzmán et al., 2012; Jimenez-Kairuz et al., 2005; Ramírez Rigo et al., 2006, 2009). Therefore these products are useful to develop drug delivery systems (Bermúdez et al., 2008; Kindermann et al., 2011; Vilches et al., 2002). Drugs recognized as safe and effective, but with some unfavorable physicochemical or biopharmaceutical properties, are good candidates to be loaded on a PE.

Within this framework, the maleate salt of Enalapril (EnM) was selected to develop PE–D complexes. EnM is the first choice in the

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treatment of hypertension and congestive heart failure. It is a prodrug that is administered in daily oral doses of 5–20 mg, being the maximum tolerated dose 40 mg per day (Clinical Pharmacology, 2011). Approximately 55–75% of the dose of EnM is rapidly absorbed through the digestive tract in healthy individuals and hypertensive patients (Tabacova and Kimmel, 2001). According to the biopharmaceutical classification system, EnM was provisionally classified as a class III drug because of its high solubility relative to the dose and low intestinal permeability (Pretorius and Bouic, 2009).

Transdermic formulations have been developed as a strategy to increase the bioavailability of EnM (Li and Nguyen, 2003) although the oral route is the preferred method of administration (Parente Dueña et al., 1999).

This prodrug is metabolized mainly in the liver where it is hydrolyzed by esterases to enalaprilat, its active metabolite. The therapeutic effects appear between 1 and 2 h after a single oral dose of enalapril, and they persist for 12–24 h. Excretion of both enalapril and enalaprilat is primarily renal and 61% of the dose (43% enalaprilat, 18% enalapril) is recovered in urine (Tabacova and Kimmel, 2001).

Another important aspect to consider is that EnM salt exhibits marked problems of compatibility (stability) with excipients generally used in solid formulations like microcrystalline cellulose, magnesium stearate, calcium phosphates, starch, sodium starch glycolate, crospovidone and croscarmellose sodium, a feature that is relevant in formulation stages (Bharate et al., 2010; Marcal Lima et al., 2008; Patel and Davila, 2005; Rezende et al., 2008). Moreover, water content and temperature have a large influence on drug stability. The degradation of EnM leads through two main degradation products, enalaprilat and diketopiperazine, which are formed by hydrolysis of the ethyl ester moiety and by intramolecular cyclization of the drug, respectively (Simončič et al., 2007). The use of excipients that have proved to be compatible with EnM (Novartis, 2005; Rezende et al., 2008) or the formation of cyclodextrin inclusion complexes (Zoppi et al., 2008) are pharmaceutical strategies used to increase the stability of EnM in solid formulations.

The acidic and basic groups which are present in the structure of enalapril (En) (Fig. 1a) enable it to interact with anionic and cationic PE. However, when it is salified with the maleate counterion (M), a cationic PE would have the ability to ionically interact with both moieties of the salt. On this basis, the cationic PE Eudragit® E100 (EU) was selected to design a complex for oral administration.

The EU is a copolymer based on dimethylaminoethylmethacrylate and neutral methacrylic esters (Fig. 1b). It is soluble in aqueous media up to approximately pH 5 and it is mainly used in pharmaceutical technology as a tablet coating excipient. After oral ingestion, EU was not absorbed and was excreted unchanged with the feces. Daily permissible intake limits for polymethacrylate derivatives are 2–20 mg/kg body weight (Rowe et al., 2006).

Cationic polymethacrylate-acid drug complexes have been obtained with different monofunctional poorly water-soluble drugs in order to improve their apparent solubility (Baena et al., 2011; Guzmán et al., 2012; Kindermann et al., 2011, 2012; Quinteros et al., 2008). In addition, a study of the stability of films obtained by solvent evaporation on an aluminum support of ethanolic dispersions of mixtures of EnM and EU at different weight ratios of 1:1, 1:2, 1:3 has been reported. The stability of the drug in these films depended on the proportion of EU present in the mixture, indicating an inverse relationship between the amount of EU and the appearance of the degradation product (Wang et al., 2004). This point will be further addressed in this report.

Although there have been some interesting contributions regarding this subject, no product has been developed from EnM and PE that improves the unfavorable properties described for this drug (low bioavailability and low chemical stability). With this motivation, the aim of this article was to describe the preparation and the physicochemical, pharmaceutical and biopharmaceutical characterization of a new particulate material based on a $(EU–EnM)_x$ complex.

2. Materials and methods

2.1. Materials

The following materials were used as received from the supplier: EU (poly (butyl methacrylate-co-(2-dimethyl aminoethyl) methacrylate-co-methyl methacrylate) 1:2:1) (Pharmaceutical Grade, Rohm, Germany), EnM (Pharmaceutical Grade, Parafarm, Argentina, melting point: 147.4–149.6 °C), maleic acid (PA grade, Aldrich, USA, melting point: 130.0–131.4 °C), HCl and NaOH solutions (1 M, Anedra, Argentina), methanol, acetonitrile, water (HPLC grade, Sintorgan, Argentina). The EnM and enalaprilat HPLC standards were kindly provided by Roemmers Laboratory (Argentina). All other reagents and solvents were *pro-analysi* grade.

2.2. Preparation of $(EU–EnM)_x$ complexes in solid state

The subscript × indicates the percentage of amino groups of EU neutralized by the $–COO^-$ group of enalapril.

EU was milled in a mortar and sieved through 40 and 70 mesh sieves (ASTM, Zonytest, Argentina). The equivalents of amino groups per gram of EU (3.10×10^{-3}) were assayed by direct acid–base titration.

A series of complexes were prepared in a mortar by dissolving 3.5 g of EU and the appropriate amount of EnM in 10 mL of ethanol (96°). During mixing, spontaneous evaporation at room tempera-

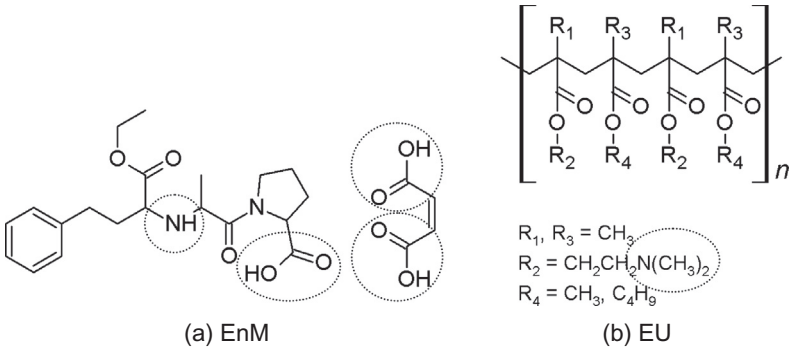

Fig. 1. Structural formula of (a) EnM and (b) EU showing their acidic and basic funtional groups.

ture occurred. The solid products deposited on the walls were taken off using a spatula and were placed under vacuum until constant weight. The rubber solids obtained were cooled in the refrigerator in a hermetic container to improve their processability, milled in a mortar and sieved through a 30 mesh sieve (ASTM, Zonytest, Argentina) to obtain particles smaller than 600 μm. The products packaged in amber bottles were stored at temperature below 15 °C. This series was named $(EU–EnM)_x$ in which x = 15%, 30% and 50%.

The physical mixture of EU and EnM with a composition equivalent to the $(EU–EnM)_{30}$ complex was prepared for comparative purposes.

2.3. Characterization of solid products

Solid $(EU–EnM)_x$ products were analyzed by different techniques together with pure EU and EnM, their physical mixtures and maleic acid.

2.3.1. FT-Infrared spectroscopy (FT-IR)

FT-IR analysis was performed with 150 mg potassium bromide disks containing 1% of the pure EU, maleic acid and EnM and 1.5% of the samples of $(EU–EnM)_x$ and the physical mixture of EU and EnM (Nicolet 5SXC FT-IR Spectrometer, Thermo Scientific, USA). The spectra were obtained (30 scans, 4 cm^{-1}) and processed using the EZ OMNIC ESP v.5.1 software. Samples and potassium bromide were dried under vacuum or at 120 °C respectively until constant weight prior to testing.

2.3.2. Thermal analysis

Differential scanning calorimetry (DSC), thermogravimetric analysis (TG) and derived termogravimetry (DTG) analysis were performed using the Modulated-DSC 2920, TA 2950 and the Universal Analysis-NT software version 2.5 (TA-Instruments, USA). To determine DSC curves, the temperature axis and the cell constant were previously calibrated with indium. The samples (2–4 mg) were heated at 10 °C/min over a temperature range of 25–160 °C in aluminum pans with a pine-hole under nitrogen atmosphere flowing at 60 mL/min. However, when glass transition temperature was explored, a first cycle of heating (from 25 to 100 °C) and cooling (from 100 to 10 °C) was run to eliminate sorbed water in the sample and then a second cycle of heating (from 10 to 160 °C) was performed following the conditions previously described. Results were expressed as onset, maximum and ΔH for the melting events and change in specific heat between the glassy state and the rubbery state (ΔCp) and half Cp extrapolated (T_g) for the glass transition events. On the other hand, TG curves were obtained by testing the samples (1–2 mg) over a temperature range of 25–300 °C in open aluminum pans under the same general conditions used in DSC determinations.

2.3.3. Powder X-ray diffraction (PXRD)

Diffraction patterns of powders were recorded using a Rigaku Geigerflek (DMAX-C) X-ray diffraction system (Rigaku Corporation, Japan). The anode X-ray tube was operated at 35 kV and 15 mA. Measurements were taken from 3° to 37° on the 2θ scale at a step size of 4°/min at 15 °C.

2.3.4. Isotherm of water vapor sorption

Sorption of water as a function of relative humidity (RH) at constant temperature and equilibrium conditions were determined according to USP 34 (2011). Samples of 100 mg were stored at 15 °C in closed recipients containing appropriated saline-saturated solutions of potassium hydroxide, calcium chloride, sodium bromide, and potassium bromide to get, respectively, 9, 31, 58, and 84%RH (Wade, 1980). Experiments were performed in triplicate.

2.4. Chemical and physical stability evaluation

The $(EU–EnM)_{30}$ complex, used as a model, was aged for 14 months in well closed amber glass containers at room temperature (25 °C) and ambient humidity avoiding its exposition to daylight. To perform quantitative analysis, samples were previously dissolved in water and then evaluated by HPLC–UV using a stability indicating method described in Section 2.8.1. The concentration of EnM was calculated using a calibration curve. For physical characterization DSC and PXRD measurements were done as it is defined in Sections 2.3.2 and 2.3.3.

2.5. Preparation of $(EU–EnM)_{30}$ aqueous dispersions

The dispersions were obtained by adding water or isosmotic buffer to the solid complexes and homogenizing the system assisted by ultrasonic vibration. The isosmotic phosphate buffer pH 6.8 with glucose (33 mM monobasic sodium phosphate, 38 mM dibasic sodium phosphate, 147 mM glucose) was prepared according to Wade (1980) and had an osmotic pressure of 300 ± 1 mOsm/kg (Osmomat 030-D, Gonotec, Germany).

2.6. Characterization of the complex in dispersion

2.6.1. Measurements of pH and electrokinetic potential

The pH determination of 0.022, 0.025 and 0.032%w/v of $(EU–EnM)_{15}$, $(EU–EnM)_{30}$ and $(EU–EnM)_{50}$ dispersions prepared in water were performed using a Hanna pH-meter (HI 9321, Hanna Instruments, USA) with Ag/AgCl glass electrode at 25 °C. The electrokinetic potentials of these dispersions were measured using a zeta potential analyzer (Brookhaven, USA). Ten determinations per sample were performed and the zeta potential values were calculated by Smoluchowski's equation from the electrophoretic mobility of the dispersions at 25 °C.

2.6.2. Drug delivery from aqueous solutions

Experiments were conducted under *sink* conditions in a modified Franz diffusion assembly at 37 ± 1 °C. A semi-permeable acetate cellulose membrane (Sigma® 12.000) was placed between the donor and the receptor compartments. Four mL of $(EU–EnM)_{30}$ aqueous dispersion (0.584% w/v) were placed in the upper compartment while the receptor compartment was filled with 76 mL of either water or 0.9% NaCl solution, and stirred at 200 rpm with Teflon-coated magnetic stirring bar. At selected times; 1.5 mL aliquots were withdrawn and replaced by the same volume of receptor medium. Data were corrected for dilution. En and M concentrations were determined by HPLC–UV. The assays were done in triplicate and the results were expressed as percentage of En or M released vs. time. The donor and receptor fluids were subjected to pH measurement before and after testing.

2.6.3. Everted rat intestine permeation

EnM and $(EU–EnM)_{30}$ powders were appropriately dissolved in the isosmotic buffer pH 6.8 to be used as mucosal solutions of 0.8×10^{-2} and 25×10^{-2}% w/v respectively which contained equivalent quantity of EnM. The drug concentration was fixed considering an oral dose of 20 mg dissolved in 250 mL of an isosmotic buffer (Section 2.5) that was selected due to its simple composition. At this concentrations no significant changes of pH or osmolarity (both necessary to maintain the viability of the intestine tissues) were observed for EnM or $(EU–EnM)_{30}$ solutions from the drug free buffer used. Male CR Wistar albino rats (Bioterio of the Faculty of Chemistry, National University of Cordoba) weighing between 250 and 300 g were used. Food was removed about 12 h prior to trial, but water was allowed *ad libitum*. The rats were sacrificed by CO_2 inhalation (ARAC guideline, 2010) and the entire

small intestine was immediately removed via a midline incision of the abdomen and placed into the buffer solution at 5 °C. After discarding the first 15 cm of the proximal end, the intestine was rinsed with 20–30 mL of buffer pH 6.8, then sleeved onto a glass rod and everted according to the method of Crane and Wilson (1958) adapted by Vilches et al. (2002) for the study of (PE–D) systems. Four 10 cm segments were measured after stretching the entire intestine with a 5 g weight. Each everted intestinal segment was ligated at the distal end, mounted onto a glass cannula and ligated at the proximal end so that 10 cm of the intestine were available for absorption. The entire device was placed at 37 °C into a flask containing 70 mL of mucosal solution. Air was constantly bubbled through the mucosal solution at a rate of 3 bubbles per s. One mL of buffer solution (drug free) was then placed into the serosal compartment. This compartment was sampled after 15 min once and then every 10 min for 85 min. The entire serosal volume was removed at each sampling time and rinsed with 1 mL of buffer then another 1 mL of buffer 6.8 was placed into the serosal compartment to be withdrawn at the next sampling interval. Serosal concentrations of En were analyzed by HPLC–UV (described in Section 2.8.1). Cumulative amounts of drug transferred per unit concentration of drug in the mucosal solution were plotted for each intestinal segment as a function of time. The experiments were conducted in quadruplicate. Permeability flux coefficient (k_U) was obtained from the slope of the straight line applying minimum square method to the experimental points.

For comparative purpose, non-everted segments were used to evaluate drug permeability from the serosal to the mucosal side of the membrane in the same conditions described previously.

This methodology was evaluated and approved by the Ethics Committee of the Faculty of Chemistry, UNC.

2.7. Determination of oral bioavailability

The oral bioavailability of enalapril in rats was determined in urine. The experimental design was completely randomized. Male Wistar rats (Bioterio of the Pharmacological Research Institute, UBA-CONICET) weighing between 250 and 300 g were housed in metabolic cages at standardized temperature with food and water *ad libitum*. Aqueous solutions of $(EU–EnM)_{30}$ or EnM salt, both containing 15 mg of En/kg, were administered by a gastric cannula to the test ($n = 5$) or reference group ($n = 5$) respectively. The urine samples were collected during 36 h. A 600 μL aliquot of each homogenized sample was acidified with a 100 μL aliquot of 0.5 M PO_4H_3 solution and then a 3 mL aliquot of ethyl acetate was added. After mixing for 10 min, the organic phase was separated from the aqueous phase and evaporated under a stream of nitrogen gas. The residue was reconstituted in mobile phase (described in Section 2.8.2) and the processed sample was ready to be assayed. The quantification of enalaprilat was performed by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS–MS) and was expressed as percentage of enalaprilat moles recovered in urine referred to the EnM moles administered. The results were analyzed by the two-tailed t test. The study protocol was approved by the Animal Ethics Committee of the School of Pharmacy and Biochemistry, UBA.

2.8. Quantification of En, M and enalaprilat

2.8.1. Stability indicating HPLC–UV method

An isocratic technique described by Argentine Pharmacopoeia 7th Ed (2003) was used for drug assay in aqueous samples because it was reported that it can satisfactorily resolve En, its degradation products and maleic acid peaks (Zoppi et al., 2008). The analysis was performed in an Agilent S1100 equipment (Agilent Technologies, Germany) with UV detection at 215 nm, using a C8 column with precolumn (5 μm, 150 × 4.6 mm, Luna, Phenomenex, USA) thermostated at 50 °C. The mobile phase was an acetonitrile: pH 2.2 potassium phosphate buffer (10 mM) 32:68 mixture (v/v) at a flow rate of 2.0 mL/min. The volume of injection was 50 μL. Under these conditions, the retention times for M and En were 1.0 and 1.6 respectively.

2.8.2. HPLC–MS–MS method

The concentrations of enalaprilat in urine samples were analyzed using a sensitive isocratic technique reported by Gu et al. (2004). A Shimadzu LCMS 2020 equipment (Shimadzu Corporation, Japan) with a C18 column and precolumn (5 μm, 150 × 4.6 mm, Gemini, Phenomenex, USA) thermostated to 30 °C was used. The mobile phase, methanol:water:formic acid (65:34:1 v/v/v), was run at a flow rate of 0.8 mL/min. The volume of injection was 10 μL. The method of external standard was used. The response was linear over a concentration range of 1 ng/mL and 1 μg/mL. The intra-day precision was <5.2%. The mass spectrometer was used in the positive ion detection mode. The following parameters were fixed: temperature of the vaporizer, 400 °C; needle voltage, 5000 V and declustering potential, 60 V. Nitrogen was used as the nebulizing turbo spray and curtain gas at 55, 35 and 10 psi, respectively. The measurement was performed monitoring the transition m/z 349 → m/z 206 with a dwell time of 200 ms, flowing nitrogen at 4 Pa and using collision energy of 26 eV.

3. Results and discussion

The procedure used to obtain $(EU–EnM)_x$ complexes in solid state takes advantage of the particular properties of the EnM salt, given that En has an acidic group (pK_a 2.97) and a basic group (pK_a 5.35), and the counterion (maleate) has two acidic groups (pK_{a1} 1.88, pK_{a2} 6.22) (Ip and Brenner, 1987). Therefore, both components of the salt have the ability to generate an acid–base interaction with the EU (strong polybase of $pK_a \sim 7.7$; Kindermann et al., 2011; Van de Wetering et al., 1998) according to following equations:

$$EU + R\text{-}HN\text{-}R\text{-}COOH \leftrightarrows EUH^+ + R\text{-}HN\text{-}R\text{-}COO^- \leftrightarrows (EUH^+R\text{-}HN\text{-}R\text{-}COO^-) \quad (1)$$

$$EU + HOOC\text{-}R\text{-}COOH \leftrightarrows EUH^+ + HOOC\text{-}R\text{-}COO^- \leftrightarrows (EUH^+HOOC\text{-}R\text{-}COO^-) \quad (2)$$

where R-HN-R-COOH and HOOC-R-COOH represent En and M, respectively and (EUH^+R-HN-R-COO^-) and (EUH^+HOOC-R-COO^-) represent ionic pairs.

It has been previously reported that the interaction between EU and anionic drugs yields a high proportion of counterionic condensation (Guzmán et al., 2012; Quinteros et al., 2008, 2012). The presence of non-electrolytes does not affect the equilibrium of ion-pair formation, whereas the addition of ions to the system generates ion exchanges and regrouping of charges causing a partial dissociation of the ion pairs (Jimenez-Kairuz, 2004; Kindermann et al., 2011, 2012; Quinteros et al., 2011).

3.1. Preparation and characterization of complexes in solid state

3.1.1. Preparation

The series of solid $(EU–EnM)_x$ complexes were obtained using ethanol 96% as a solvent. The advantage of preparing the complexes in this solvent is that both the cationic polymer and EnM are soluble and consequently the reaction is fast. Besides, it is paralleled with the evaporation of ethanol which conduces to the solid product. The series of solid $(EU–EnM)_x$ obtained as particulate

materials exhibit some degree of cohesiveness at room temperature, but when they were stored below 15 °C no agglomeration was observed. The complexes in contact with water or pH 6.8 buffer produced clear stable colloidal dispersions that were used in release, permeability and bioavailability assays.

3.1.2. FT-IR spectroscopy

In the FT-IR analysis, the indicative bands of the acid–base interaction were investigated for changes arising as a result of the association between EU and EnM. The appearance of new bands was also investigated. The individual spectra are provided as supplementary material.

The FT-IR spectra of the solid complexes showed some remarkable differences when they were compared to that of the EnM and EU:EnM physical mixture. There are some significant changes that account for an ionic interaction between EU and EnM.

As shown in Fig. 2, the absorption bands of the nonprotonated dimethylamine groups appear at 2770 and 2822 cm^{-1} in EU and the physical mixture. In the complexes, these bands are significantly reduced indicating that the polyelectrolyte is partially neutralized by the drug. This reduction is related to the neutralization percentages of the samples.

In EnM, a band at 1750 cm^{-1}, ascribed to the C=O st of the carboxylic group is observed (Ip and Brenner, 1987). This band is clearly observed in the physical mixture but disappears in all the complexes indicating the complete reaction of the acidic groups of EnM with EU, which only display the characteristic band at 1731 cm^{-1} ascribed to the keto group of EU ester (Menjoge and Kulkarni, 2007). Interestingly, the band at 1573 cm^{-1} ascribed to the monohydrogen maleate carboxyl stretch of EnM (Ip and Brenner, 1987; Lin et al., 2002) is significantly reduced and widen in the complexes showing some degree of participation of these groups in the interaction with EU.

In addition, the band corresponding to the out-of-plane OH vibration of the COOH group could be observed at 903 cm^{-1} in EnM and at 904 cm^{-1} in the physical mixture of EU and EnM, whereas in the complexes, this signal disappear.

These results may indicate that one carboxylic group of M interacts with the amino group of En while the other carboxylic group of M and/or the carboxylic group of En interact with dimethylamine groups of EU as Wang et al. (2004) previously proposed for films.

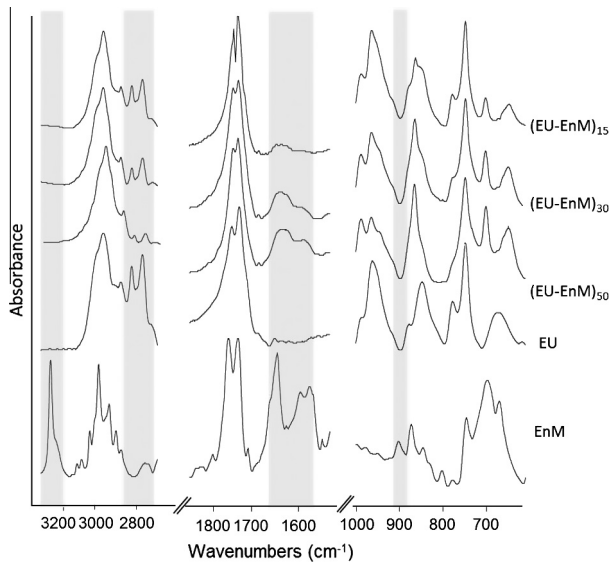

Fig. 2. Representative FT-IR spectra of EnM, EU and $(EU–EnM)_x$ complexes.

The absence of unreacted EnM in the complexes also suggests that the preparation method allows a complete reaction between EU and EnM.

The remarkable differences observed between the spectra of the complex and the raw materials or the physical mixture provided evidence of ionic interaction between EU and EnM in the solid complexes. Notice that, the spectra of the complexes are quite similar to that of EU and the characteristic bands of EnM, which are clearly observed in the physical mixture, are rather week. In this context, the sharp band at 3208 cm^{-1} observed in EnM and the physical mixture spectra, previously assigned by Lin et al. (2002) to the stretching vibration of N—H, disappeared completely in the complexes.

The spectra of the complexes also show a broad band at 1632 cm^{-1} with a shoulder at 1585 cm^{-1}. This band can be associated with the carbonyl stretching of the ternary amide of EnM (observed at 1649 cm^{-1} in the pure EnM and the physical mixture spectra) which can also be overlapped with the antisymmetric vibration of carboxylate groups of EnM. The intensity of this band increased with the proportion of EnM in the complex.

3.1.3. Thermal analysis (DSC–TG)

Table 1 reports the events observed in the DSC profiles of the materials under study. EnM presented a sharp endothermic peak at 148 °C corresponding to the melting point accompanied by decomposition of the drug previously reported by Ip and Brenner (1987). This endotherm was also present in the physical mixture profile as a broader peak at 143 °C but disappears in the complexes thermogram. The lack of the melting endotherm during heating was indirect evidence that unreacted drug was absent in the complexes.

Moreover, the T_g of the $(EU–EnM)_x$ complexes were also determined because this parameter was useful to assess the drug distribution in the solid samples (Qi et al., 2008).

The pure polyelectrolyte glass transition behavior was studied previously and we measured the T_g value of the particulate material obtaining a half *Cp* temperature of 46 °C that was in agreement with the data reported by Sauer et al. (2005).

For $(EU–EnM)_x$ complexes, this transition was present at lower temperatures. The T_g determined was between 29.5 and 31.3 °C (Table 1), values close to room temperature which explains the deformation and agglomeration observed by visual inspection when the complexes were stored at 25 °C. Clearly, the polymer had been plasticized by the drug incorporated implying molecular dispersion of EnM in the system studied. It is postulated that in the solid state, the interactions between the polymeric chains will be lower due to the EnM present in the structure. Thus less energy is necessary to achieve the glass-rubber transition (Kalogeras, 2011; Wu and McGinity, 1999).

On the other hand, the TG/DTA curves were analyzed to determine water content in the sample and temperature of decomposition.

The content of sorbed water in $(EU–EnM)_{30}$ complex was determined from the weight loss observed in the TG curves between 30 and 120 °C. The values were in the range of 2.24–2.83% w/w and indicated that the method of drying was adequate to obtain particulate materials with water content similar to the pure EU (~5% w/w, Petereit and Weisbrod, 1999).

In addition, DTA curves (Fig. 3) show that EnM is thermally stable up to 140 °C since it exhibits a mass loss with a maximum peak at 159 °C. According to Lin et al. (2002), thermal decomposition of EnM begins with the loss of a molecule of maleic acid along with a molecule of water that is eliminated in an intramolecular cyclization reaction forming enalapril diketopiperazine.

When EnM is loaded on EU in the $(EU–EnM)_{30}$, the compound is stable up to 160 °C and the maximum decomposition temperature

Table 1
DSC thermal events of $(EU–EnM)_x$ powders, pure EU, pure EnM and the physical mixture of EU and EnM.

Samples	Thermal events of materials				
	Vitreous transition		Endotherm of melting		
	ΔCp (J/g °C)	T_g (°C)	ΔH_m (J/g)	T_{onset} (°C)	T_{peak} (°C)
EU	0.13	45.6	–	–	–
EnM	–	–	124.2	146.8	148.7
$(EU–EnM)_{15}$	0.19	31.3	–	–	–
$(EU–EnM)_{30}$	0.18	30.2	–	–	–
$(EU–EnM)_{50}$	0.23	29.5	–	–	–
EU:EnM physical mixture	0.17	47.6	22.3	128.85	143.7

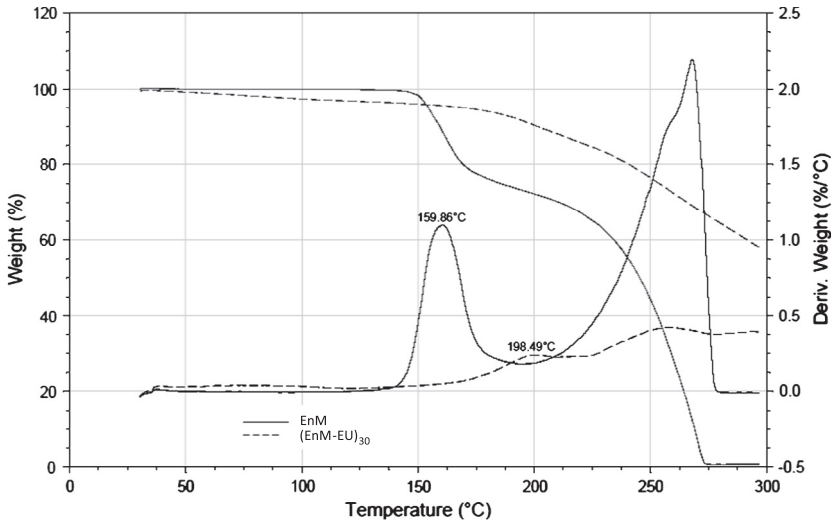

Fig. 3. TG/DTG curves of EnM and $(EU–EnM)_{30}$.

is 198 °C; in other words, the rise of the degradation temperature of En reveals a higher thermal stability when it is part of the complex.

3.1.4. X-ray powder diffraction

EnM is a crystalline solid that presents characteristic peaks at 5.3°, 8.1°, 10.5°, 15.7°, 21.5°, 24.9° and 31.6° (Fig. 4). The diffraction profile of EU was characteristic of an amorphous compound. The two low-intensity peaks at 19.2° and 31.2° 2θ indicate an ordered structure immersed in an amorphous matrix. This behavior was previously related to some crystalline points in the samples (Guzmán et al., 2012; Quinteros et al., 2008). The diffraction pattern of the binary physical mixture was the sum of the patterns presented by the pure components.

In line with the ionic interaction observed by FT-IR and DSC results, no reflections of crystalline EnM were present in the complexes, which showed characteristic profiles of amorphous compounds, similar to EU. The disappearance of the peaks of EnM suggests that it exists in an amorphous form and a complex was formed between EU and EnM. These results are in line with those previously reported by Guzmán et al. (2012) and Quinteros et al. (2008). Notice that the low reflections at 19.2° and 31.2° 2θ appear in the complexes in a region where crystalline EnM has no signals. Then, they are not attributable to EnM.

From FT-IR, DSC–TG, PXRD it can be concluded that the complexes are stable amorphous solids in which both moieties of EnM are ionically bonded to the EU. For subsequent studies, $(EU–EnM)_{30}$ was selected as the model complex.

3.1.5. Moisture sorption study

Both EU and EnM were reported as non-hygroscopic powders (Petereit and Weisbrod, 1999; Simončič et al., 2007).

The complex $(EU–EnM)_{30}$ exhibited a typical water sorption isotherm of an amorphous material presenting a low weight increase that reached 12% at the highest RH used (Fig. 5). Low hygroscopicity values are desired in order to avoid stability problems in the solid product.

3.1.6. Stability

The crystalline EnM stored at room temperature in amber glass containers is a stable solid (Ip and Brenner, 1987). However, the drug presents stability problems when it is formulated with some excipients.

As it was previously mentioned, the stability of EnM in films of EU, studied by thermal FT-IR microspectroscopic technique, was previously reported by Wang et al. (2004). The authors proposed a mechanism of stabilization of EnM by EU when the polyelectrolyte was in high proportion. They also reported compatibility problems between them in mixtures in which the acidic functional groups of enalapril and maleic acid were present in excess with regard to EU basic groups. However, previous studies evaluated neither a particulate complex nor the effect of normal storage conditions on the stability of material.

In this line, the $(EU–EnM)_x$ complexes developed were prepared using proportions of each component in the range proposed as chemically stable. The $(EU–EnM)_{30}$, used as a model, was aged for 14 months at room temperature and was evaluated by HPLC–UV using a stability-indicating method described in Section 2.8.1.

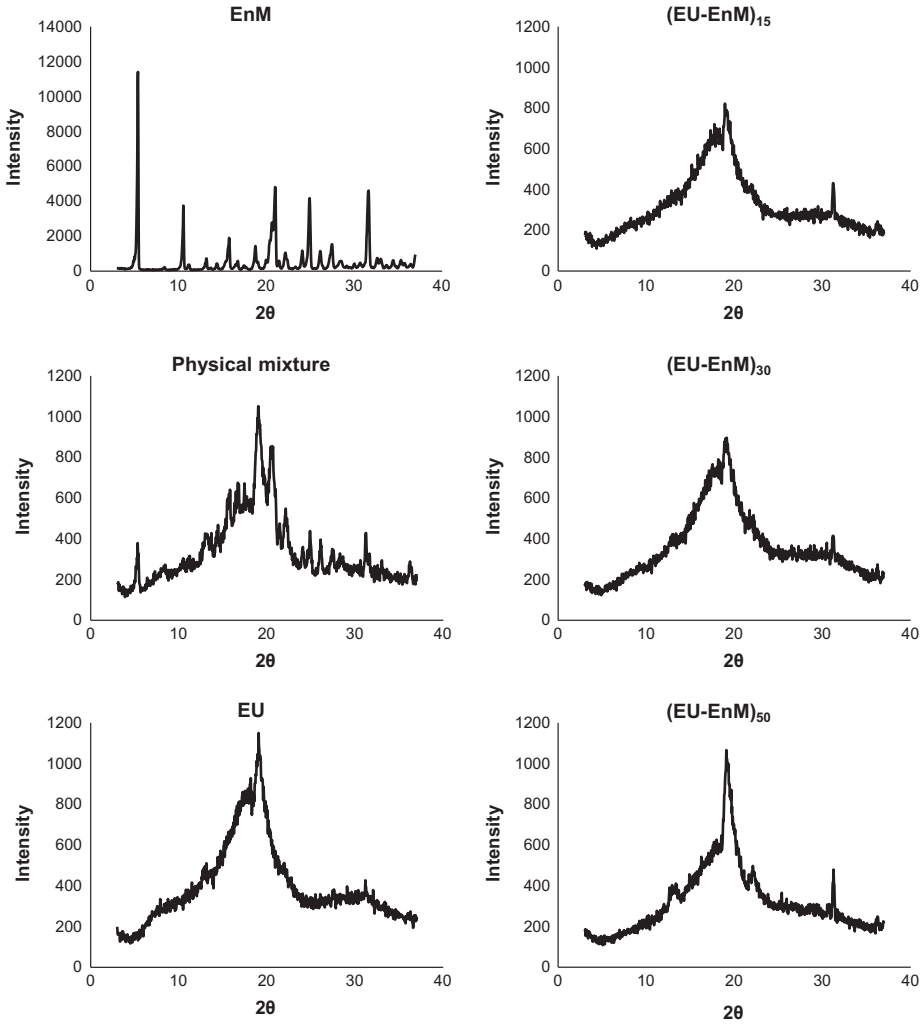

Fig. 4. X-ray powder diffraction pattern of the samples.

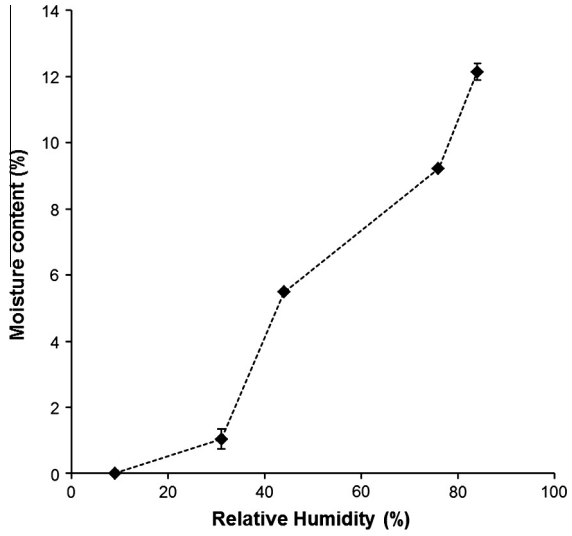

Fig. 5. Equilibrium moisture content of $(EU–EnM)_{30}$ at 15 °C.

The stability of EnM in the aged solid $(EU–EnM)_{30}$ complex was confirmed. The chromatograms showed the peaks corresponding to En and M and no other peaks that could be attributed to the degradation products (enalaprilat and diketopiperazine) were observed.

The content of the EnM in the complex after aging was the 100% of the drug loaded in EU during the process of preparation of the materials.

This behavior was related to a reduction of the attacking ability of the nitrogen atom in the amine group of the drug to its carboxylic group due to the ionic interaction between this carboxylic group of the drug and the amino groups of the polylectrolyte used as carrier (Wang et al., 2004).

These findings are in line with other reports of stabilization of drugs like pilocarpine and azithromycin by polyelectrolyte complexation (Esteban et al., 2009; Jimenez-Kairuz et al., 2004).

In addition, aged samples of the complexes showed no melting endotherm and no reflection peaks when they were subjected to DSC and PXRD evaluation. This behavior indicates that the amorphous structure of EnM was maintained during storage proving their physical stability.

3.2. Properties of the complexes in dispersion

Table 2 reports the pH and electrokinetic potentials of the translucent $(EU–EnM)_x$ dispersions prepared in distilled water. The pH value decreased with the proportion of EnM in the sample due to the acid–base neutralization of the cationic groups of EU. In addi-

Table 2
pH and electrokinetic potential of the $(EU–EnM)_x$ dispersions.

Sample	Properties of complexes dispersed in water	
	pH	Electrokinetic potential (mV)
$(EU–EnM)_{15}$	5.72	31.9 ± 5.0
$(EU–EnM)_{30}$	5.28	28.0 ± 3.8
$(EU–EnM)_{50}$	4.58	27.8 ± 4.9

tion, the high and positive electrokinetic potentials indicated significant electrostatic repulsions between the colloidal particles that correlate with observed physical stability (Ishikawa et al., 2005).

3.2.1. Release of EnM in Franz cells

Kinetic measurements in Franz cells provide valuable information about the main mechanism of drug release from dispersions of PE–D complexes. This methodology has been successfully applied to complexes of monobasic (Ardusso et al., 2010; Jimenez-Kairuz et al., 2002, 2003, 2004) and monoacid (Guzmán et al., 2012; Quinteros et al., 2008, 2011, 2012) drugs as well as drugs having two basic (Esteban et al., 2009) or a basic and an acid group (Vilches et al., 2002). In line with the cumulative information available, the *in vitro* release of En and M from an aqueous dispersion of $(EU–EnM)_{30}$ was evaluated using Franz cells with water and NaCl solution (0.9% w/v) as receptor media. The release profiles and the pH registered in the experiments are presented in Fig. 6a and b and Table 3 respectively. As the receptor compartment was filled with water, the release rate of both En and M from $(EU–EnM)_{30}$ was very slow. As expected, the drug release was accompanied by a slight rise in the pH (5.28–5.78 in 7 h of experiment). This pH is very close to that of $(EU–EnM)_{15}$ (see Table 2). Such a behavior is related to the association of both moieties of the drug with the cationic PE through ion pair complexation.

Generically, with cationic PE, the electrostatic attraction of the macroion prevents the diffusion of anionic species from the complexes. However, neutral species are able to diffuse freely and therefore the release rate essentially depends on its concentration.

Although in this case R-HN-R-COOH is accompanied by the dipolar ion $R\text{-}^{+}H_2N\text{-}R\text{-}COO^{-}$, being the experimental pHs higher than the isoelectric pH of En (4.16), both species are accompanied by the anionic one $R\text{-}HN\text{-}R\text{-}COO^{-}$. As a consequence the release rate towards water remained slow. From this point of view, the degree of ionization of the M moiety is even higher, giving rise to a slower release rate than that of En.

On the other hand, when the receptor medium was filled with NaCl solution, the release profiles showed that $(EU–EnM)_{30}$ released both En and M at a higher rate, which was also accompanied by a wider shift of pH. The diffusion of the salt towards the donor compartment generates the ion exchange with Cl^{-} depicted in Eq. (3) and the interaction of Na^{+} with the anionic species that yields salts able to freely diffuse. These results indicate that under such conditions, the ionic interaction is the mechanism that controls the release.

$$(EUH^{+}R\text{-}HN\text{-}R\text{-}COO^{-}) + NaCl \leftrightarrows (EUH^{+}Cl^{-}) + R\text{-}HN\text{-}R\text{-}COO^{-} + Na^{+} \quad (3)$$

3.2.2. Permeability in everted rat intestinal sac

The transport of EnM through intestinal membranes in rats or Caco-2 cells seems to be a combination of passive and active processes involving a peptide carrier-mediated transport system (Friedman and Amidon, 1989a,b; Morrison et al., 1996; Pretorius and Bouic, 2009; Swaan et al., 1995). The expression of these influx transporters was constant along the small intestine (Erickson et al., 1995) therefore permeability changes through the segments selected was not expected. Moreover, efflux transport was not reported for this drug.

The permeability of En in everted rat intestinal sac from solutions of EnM free salt and of the $(EU–EnM)_{30}$ complex of equivalent concentration in isosmotic phosphate buffer of pH 6.8 was examined. Notice that at this pH the complex dispersion remained unaltered, which is indirect evidence of a high degree of counterionic condensation present at that pH due to the loaded EnM (see Eqs. (1) and (2)).

In these experiments, an increase in the amount and rate of En permeated into the serosal solution (SS) was observed when the complex was placed in the mucosal solution (Fig. 7). The ratio between their k_U is 1.7 revealing an interesting permeability enhancing effect produced by the complexation of EnM with EU.

The enhancing mechanism of PE in drug permeability is generally attributed to the increase of the contact time of the drug with the surface of the mucous membrane due to the mucoadhesiveness of the macromolecule (Harris and Robinson, 1990), the increase in activity of intestinal transporters (Nozawa et al., 2003) or the modification of the membrane intercellular spaces (Aungst, 2000; Cano-Cebrián et al., 2005; Thanou et al., 2001).

The positive electrokinetic potential of the complexes would be related to their capacity of adhesion to the intestinal mucosa due to the ionic interaction between positively charged amino groups in EU and negatively charged mucus gel layer. Similar results were observed for chitosan and their quaternary analogs (Junginger, 2009). In fact, it was reported that the poly (2-dimethylamino) ethyl methacrylate with varying molecular weights was able to introduce DNA into model cells (Van de Wetering et al., 1997). In

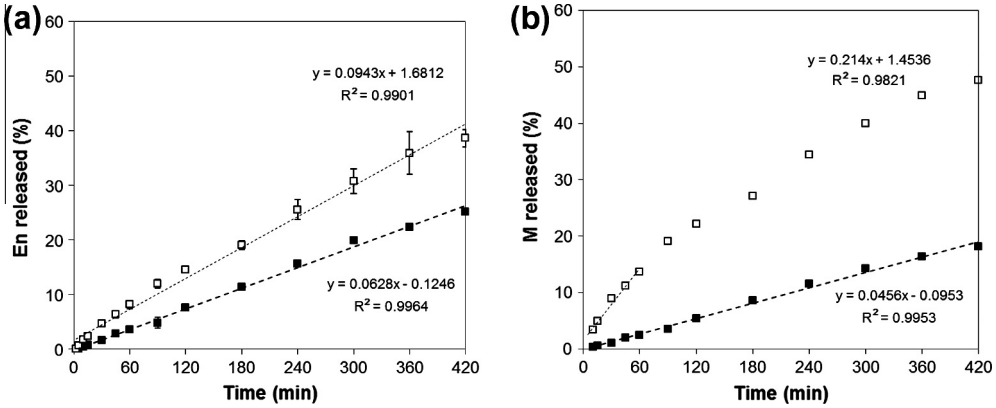

Fig. 6. Release of En (a) and M (b) from $(EU–EnM)_{30}$ aqueous dispersion towards distilled water (full square) and 0.9% NaCl solution (empty square).

Table 3
Values of pH registered in the drug release experiments.

Donor fluid			Acceptor fluid		
Sample	pH (t = 0)	pH (t = 7 h)	Composition	pH (t = 0)	pH (t = 7 h)
$(EU–EnM)_{30}$	5.28	5.78	Water	6.05	6.27
		6.30	NaCl 0.9% aqueous solution	5.56	6.02

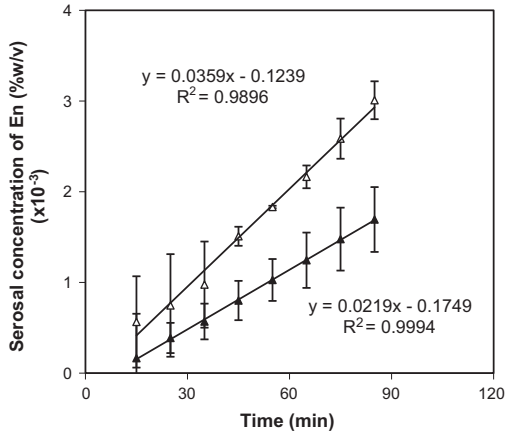

Fig. 7. Everted intestinal sac permeation of En from pH 6.8 isotonic solutions of EnM (▲) or $(EU–EnM)_{30}$ (△).

Table 4
Enalaprilat percentage recovered in rat urine after oral administration of EnM solution and $(EU–EnM)_{30}$ dispersion to the reference and test groups respectively. The urine samples were collected for 36 h.

Enalaprilat recovered in rat urine			
Reference group		Test group	
Animal	Enalaprilat percentage	Animal	Enalaprilat percentage
1	36	6	49
2	31	7	41
3	38	8	50
4	35	9	45
5	28	10	48
Mean ± SD	33.60 ± 4.04	Mean ± SD	46.60 ± 3.65

this context, EnM loaded on EU would benefit from the permeability enhancing effect of the complex.

However, a certain influence of the PE on the active transport mechanism of EnM is not ruled out. In that respect, preliminary permeability studies determined from the serosal to the mucosal side of the membrane showed no quantificable concentrations of enalapril in the mucosal solution when this drug is alone or combined with EU in the serosal solution. In other words, enalapril needed the membrane transporters to permeate in detectable concentrations in both systems.

EU is a basic PE and at pH = 6.8 some fraction of the polymer is still protonated. It has been reported that the acidic PE Eudragit L100-55 enhances the activity of the peptide transporter PEPT1 due to its proton donor capability (Nozawa et al., 2003). However, the concentrations of PE in which this mechanism was observed to occur (10–20%) are far higher than those used in the permeability experiment. In fact, the PE concentrations used in the experiment has a negligible effect on the pH of the donor solution. Therefore, more studies would be necessary to clarify the transport mechanism.

3.2.3. Bioavailability of enalapril in rats

The amount of the active metabolite enalaprilat eliminated in urine in a period of 36 h was selected as the descriptor of the amount of drug absorbed.

Table 4 presents the percentage of enalaprilat recovered in urine in two groups of rats: Test and reference following oral administration of $(EU–EnM)_{30}$ complex and of EnM solutions, respectively. The main value of the enalaprilat recovered in the test group was 1.39 times higher than that of the reference. The increase in bioavailability of EnM in the test group is statistically very significant ($p < 0.01$) according to the statistical analysis (t test) performed.

These results would be explained in terms of the increase in permeability of the drug through the gastrointestinal membranes when is loaded on EU as previously predicted by *in vitro* experiments.

4. Conclusions

The procedure to prepare an ionic complex between EU and EnM yields chemically stable particulate solid products that are easily dispersed in aqueous media.

The complex $(EU–EnM)_{30}$ exhibits higher permeability than an equivalent solution of EnM in everted rat intestine. The increase of *in vitro* permeability was correlated with the increase of the rat oral bioavailability observed through urine recovery.

The complex $(EU–EnM)_{30}$, therefore, appears to be a good candidate to design solid oral formulations of EnM that would exhibit improved biopharmaceutical performance.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejps.2014.01.001.

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