



Enalapril:β-CD complex: Stability enhancement in solid state

Ariana Zoppi^a, Claudia Garnero^a, Yamila Garro Linck^b, Ana K. Chattah^b,
Gustavo A. Monti^b, Marcela R. Longhi^{a,*}

^a Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina

^b Facultad de Matemática, Astronomía y Física and IFEG (CONICET), Universidad Nacional de Córdoba, Córdoba, Argentina

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ABSTRACT

Complexation of enalapril maleate with β-cyclodextrin was used to overcome the known enalapril maleate–excipient interaction in solid state. The complex formation was characterized by ¹³C solid state nuclear magnetic resonance, X-ray powder diffraction and scanning electron microscopy. Additionally, thermogravimetric analysis (TGA) was used to evaluate the compatibility between magnesium stearate with enalapril maleate alone or in the complex form. Degradation of the drug at 40 °C/75% relative humidity in the presence of magnesium stearate was monitored by high performance liquid chromatography (HPLC). Enalapril maleate in the complex form was more stable than the drug alone, with the drug recovery after 6 month being: 95 ± 1% and 82.1 ± 0.5%, respectively. This satisfactory high stability of the enalapril:β-CD complex will be potentially useful for its application as solid pharmaceutical dosage products.

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1. Introduction

Most solid dosage forms are composed of one or more active pharmaceutical ingredients plus a variety of excipients (Kottke & Rudnic, 2002) whose concentrations for a formulation are selected based not only on their functionality, but also on their compatibility with the drug (Narang, Rao, & Raghavan, 2009). Whether or not solid–solid interactions are advantageous depending on their effect on the physical, chemical, microbiological and therapeutic properties of the formulation (Connors, Amidon, & Stella, 1986, chap. 6).

Enalapril maleate (Fig. 1(a)) is a prodrug widely used in the treatment of essential and renovascular hypertension, and is bioactivated by hydrolysis to enalaprilat (Fig. 1(b)), a potent angiotensin converting enzyme inhibitor (Ip & Brenner, 1987). It suffers degradation in the solid state as a consequence of its interaction with the different excipients commonly used in tablet formulation, such as microcrystalline cellulose, magnesium stearate (MGST) and Eudragit E. These interactions cause an important stability problem when considering its formulation (Al-Omari, Abdelah, Badwan, & Jaber, 2001; Cotton, Wu, & Vadas, 1987; Wang, Lin, Chen, & Cheng, 2004) with two major degradation products of enalapril maleate being enalaprilat and enalapril diketopiperazine (Fig. 1(c)), which are formed by hydrolysis of the ethyl ester moiety and by

intramolecular cyclization of the drug, respectively (Ip & Brenner, 1987; Lima, dos Santos, & Lima, 2008; Lin, Wang, Chen, & Hu, 2002; Pérez, Eichhorn, & Barceló, 2007; Simoncic et al., 2007).

The stability of drugs in solid state can be enhanced by complex formation with cyclodextrins (CDs) (Loftsson, 1995; Loftsson & Brewster, 1996; Narang et al., 2009; Szente & Budapest, 1993). These macromolecules are known to form noncovalent inclusion complexes with some drugs. This complexation improves their physico-chemical properties. For this reason, these excipients are widely used in pharmaceutical industry (Brewster & Loftsson, 2007; Fromming & Szejtli, 1994).

We have previously reported the preparation and physico-chemical characterization of enalapril maleate complex with β-cyclodextrin (β-CD, Fig. 1(d and e)) (Zoppi, Quevedo, & Longhi, 2008), we demonstrated its enhanced stability in solution. Additionally, preliminary results obtained by differential scanning calorimetry and thermogravimetric analysis suggest that complexation with β-CD can enhance drug stability in the solid state. However, no detailed description of this complex in the solid state is available. Therefore, in the present study, we employed ¹³C solid state nuclear magnetic resonance (SSNMR), scanning electron microscopy (SEM) and X-ray powder diffraction (XRPD), in order to complete the characterization of enalapril maleate:β-CD complex in the solid state. Additionally, TGA was used to evaluate the compatibility between enalapril maleate either alone or in the complex form with MGST. The effect of complexation on the stability of enalapril maleate in the presence of MGST was monitored by high performance liquid chromatography (HPLC).

* Corresponding author. Tel.: +54 351 433 4163; fax: +54 351 433 4163x115.
E-mail address: mrlicor@fcq.unc.edu.ar (M.R. Longhi).

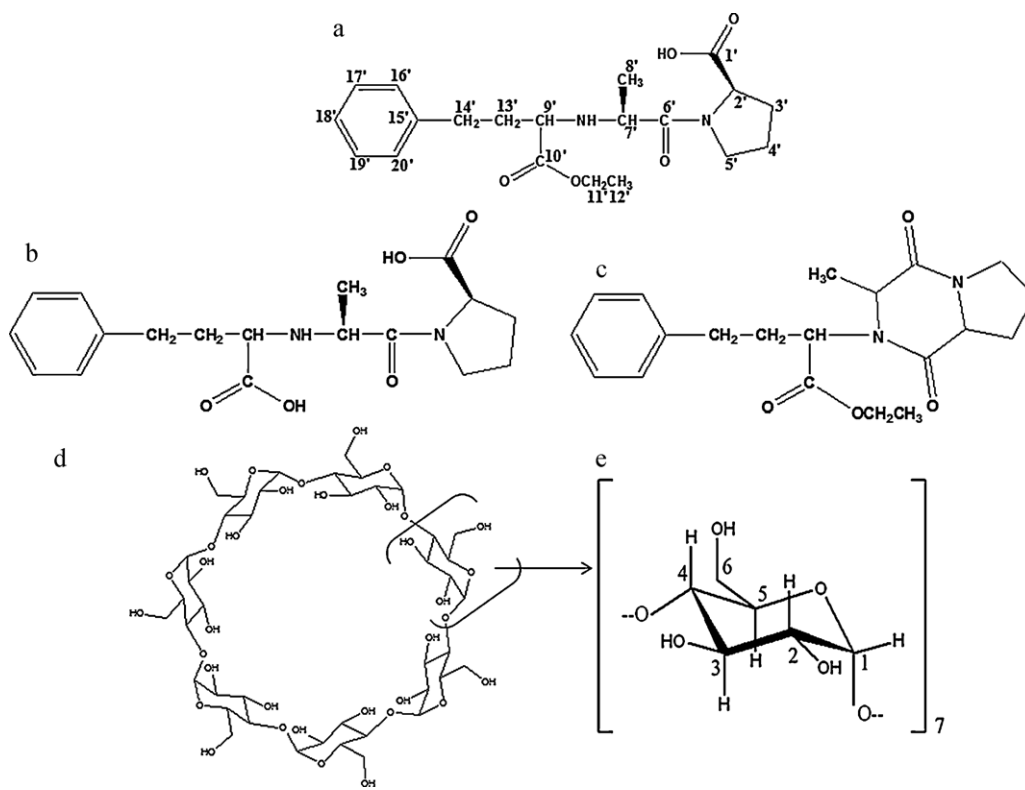


Fig. 1. Molecular structure. (a) Enalapril and carbon atoms numbering scheme, (b) enalaprilat (c) enalapril diketopiperazine, (d) molecular structure of β -CD and (e) carbon atoms numbering scheme of β -CD.

2. Materials and methods

2.1. Materials

Enalapril maleate was purchased from Sigma and β -CD (MW=1135) was kindly supplied by Roquette (Lestrem, France). All other materials and solvents were of analytical reagent grade. A Milli-Q Water Purification System (Millipore, Bedford, MA, USA) was employed to produce the water used in these studies.

2.2. Sample preparation

The preparation of enalapril maleate: β -CD complex and enalapril maleate: β -CD physical mixture was previously reported (Zoppi et al., 2008), and is summarised as follows:

Complex: A solution of enalapril maleate and β -CD (1:1 molar ratio) was prepared in distilled water, and the pH was adjusted to 7.0 by adding 1 N NaOH. The solutions were frozen at -40°C before freeze-drying started (Freeze Dri 4.5 Labconco Corp., Kansas City, MI).

Physical mixture: Physical mixture was prepared by mixing uniformly in a mortar the corresponding components with 1:1 molar ratio.

Physical mixtures of pure enalapril maleate with MGST (system A), enalapril maleate: β -CD physical mixture with MGST (system B), and enalapril maleate: β -CD complex (system C) with MGST at a molar ratio 1:1 or 1:1:1, respectively, were admixed together with an agate mortar and pestle for 5 min to obtain a homogeneous blend at room temperature.

2.3. Solid state nuclear magnetic resonance experiments

High resolution solid state ^{13}C cross polarization/magic angle spinning (CP/MAS) spectra for enalapril maleate, β -CD, the enalapril maleate: β -CD physical mixtures (with and without lyophilization) and the complex were recorded using a CP/MAS sequence with proton decoupling during acquisition. All solid state NMR experiments were performed at room temperature in a Bruker Avance II spectrometer operating at 300.13 MHz for proton and equipped with a 4 mm MAS probe. The operating frequency for carbons was 75.46 MHz. Adamantane was used as an external reference for the ^{13}C spectra and for setting the Hartmann–Hahn matching condition in the cross-polarization experiments with the spinning rate being 10 kHz. For all the samples, 2024 scans were recorded in order to obtain an adequate signal to noise ratio. The recycling time was 5 s and the contact time during CP was 2 ms. A two-pulse phase modulation (TPPM) sequence was used for decoupling during acquisition, with a proton field $H_{1\text{H}}$ satisfying $\omega_{1\text{H}}/2\pi = \gamma_{\text{H}}H_{1\text{H}}/2\pi = 60\text{ kHz}$ (Bennet, Rienstra, Auger, Lakshmi, & Griffin, 1995). Quaternary carbon edition spectra were recorded for all the samples. These spectra were acquired with the non-quaternary suppression (NQS) sequence, where the ^1H and ^{13}C radio frequency fields are removed during 40 μs after CP and before the acquisition. This delay allows the carbon magnetization to decay because of the ^1H – ^{13}C dipolar coupling, which results in spectra where CH and CH_2 are substantially removed (Harris, 1994).

2.4. Scanning electron microscopy studies

Microscopic morphological structures of the raw materials, the physical mixture and the complex were investigated and photographed using a scanning electron microscope LEO Model EVO 40XVP. The samples were fixed on a brass stub using double-sided

aluminium tape and then made electrically conductive by employing gold coating in vacuum by a sputter coater PELCO Model 3. The magnification selected was sufficient for appreciating in detail the general morphology of the samples under study.

2.5. X-ray powder diffraction studies

The X-ray powder diffraction patterns were recorded on a Rigaku Miniflex 2000 X-ray diffractometer, using Ni-filtered Cu-K α radiation at 30 kW 15 mA and a scan rate of 0.05°/min. The diffractograms were recorded from 5° to 40° (2 θ). Data were obtained using Standard Measurement software.

2.6. Thermogravimetric analysis

The TGA curves of the different samples were recorded on a TG TA 2950, applying a heating rate of 10°C min⁻¹. The thermal behavior was studied at a temperature range of 25–350°C, by heating 1–3 mg of samples in aluminium-crippled pans under nitrogen gas flow. Data were obtained and processed using the TA Instruments Universal Analysis 2000 software.

2.7. Accelerated stability studies

In order to investigate the effect of complexation on the stability of enalapril maleate in the presence of magnesium stearate under accelerated storage conditions, solid binary and ternary mixtures were stored (in open or closed containers) for 6 months at 40°C/75% RH and exposed to daylight, with samples of 20–25 mg being weighed before storage. To perform quantitative analysis, the samples were previously dissolved in water and filtered through a 0.45 μ m membrane (Millipore, USA) in order to remove the insoluble material. Each test was repeated at least three times. Quantization of enalapril maleate was carried out by applying the HPLC stability indicating method previously reported (Zoppi et al., 2008).

3. Results and discussion

3.1. Solid state nuclear magnetic resonance

Solid state ¹³C CP MAS NMR spectra for pure enalapril maleate and β -CD, the physical mixtures, and the complex are shown in Fig. 2. The assignments for enalapril maleate (see carbon numbering in Fig. 1(a)) were made by comparing it with solution NMR spectra previously reported (Ip & Brenner, 1987). The β -CD peaks (see carbon numbering in Fig. 1(e)) were assigned according to a previous work (Koontz, Marcy, O'keefe, & Duncan, 2009). NQS spectra were also used to assign quaternary carbons and methyl groups.

β -CD exhibits a complex spectrum (Fig. 2(b)) with multiple sharp resonances for each type of carbon atom. This fact has been previously correlated with distinct values of dihedral angles of the glycosidic $\alpha(1 \rightarrow 4)$ bond for carbons 1 and 4, and with torsion angles describing the orientation of the hydroxyl groups (Braga, Gonçalves, Herdtweck, & Teixeira-Dias, 2003; Gao et al., 2006). Fig. 2(c) shows that the characteristic resonances of enalapril maleate and β -CD are clearly distinguishable in the spectrum of the physical mixture. In the spectrum of the complex (Fig. 2(d)), the resonances of the C1–C6 host atoms appear mainly as single broad peaks. The broadening of these peaks indicates that the complex is a new solid and amorphous form. The resonance changes could also be due to the complexation that induced the ring of β -CD to adopt a less distorted conformation with each glucose unit in a similar environment. Moreover, the shift to higher frequencies observed for C(1), C(6) and C(10) atoms of enalapril maleate

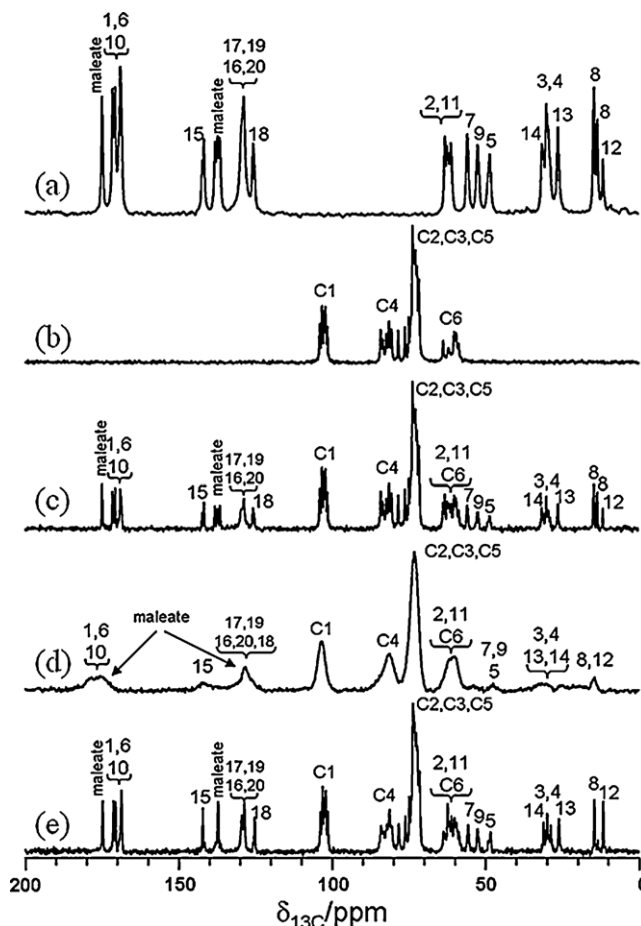


Fig. 2. Solid state ¹³C CP MAS NMR spectra of: (a) pure enalapril maleate, (b) pure β -CD, (c) enalapril maleate: β -CD physical mixture, (d) enalapril maleate: β -CD inclusion complex, (e) enalapril maleate: β -CD physical mixture after lyophilization of the pure components.

demonstrates a deshielding effect, which may be explained by the presence of hydrogen bond interactions between the carbonyl groups of enalapril maleate and the hydroxyl groups of β -CD. This behavior is in agreement with the results obtained from a study of enalapril maleate: β -CD complex in solution (Zoppi et al., 2008).

Additionally, to study the effect of the lyophilization process on each spectrum of both pure materials, enalapril maleate and β -CD were lyophilized separately under the same conditions used for lyophilizing the binary system enalapril maleate: β -CD complex, before mixing them together. Fig. 2(e) shows the spectrum of the physical mixture obtained as described above, which is comparable to that obtained without prior lyophilization of the individual compounds. These results indicate that the lyophilization process did not introduce changes in the molecular environment of either the drug or the macromolecule.

3.2. Scanning electron microscopy studies

Fig. 3 shows the SEM images of enalapril maleate, β -CD, physical mixture and complex. It can be seen that enalapril maleate is present as rectangular crystals of irregular sizes. In the case of β -CD particles, they have an irregular shape with cracks on their surfaces, while the microphotograph shows the adherence of smaller particles to the surface of larger particles.

The SEM image of the physical mixture reveals particles of β -CD embedded with enalapril maleate particles and morphology comparable to that of the pure compounds. In contrast, the SEM image

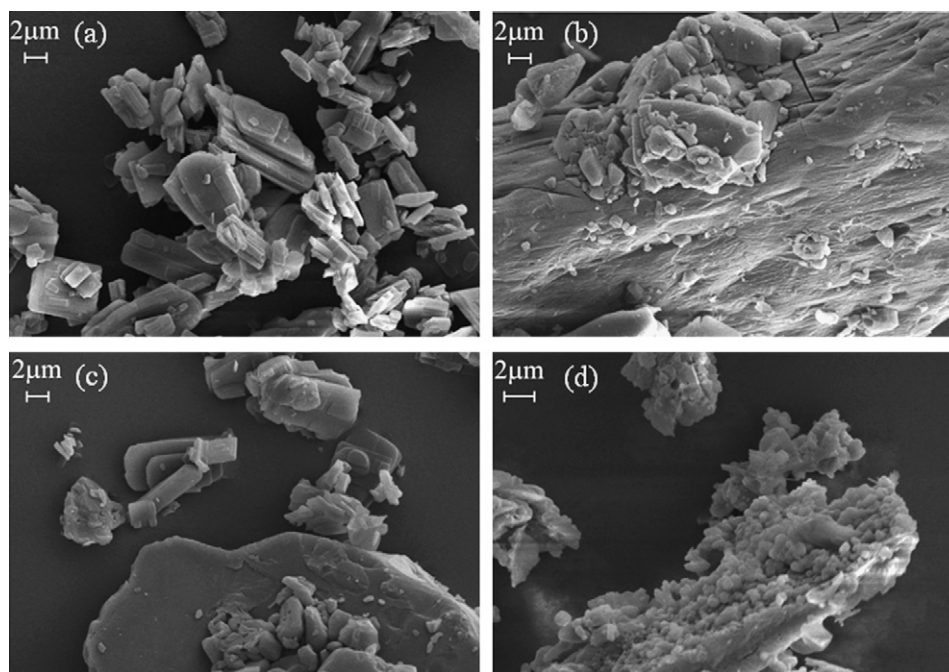


Fig. 3. Scanning electron microphotographs (a) pure enalapril maleate, (b) pure β -CD, (c) enalapril maleate: β -CD physical mixture, (d) enalapril maleate: β -CD inclusion complex.

for the complex shows changes in the morphology of the particles. Then, the original morphology of the raw materials disappears, with it being impossible to differentiate between the two components. This solid shows small size particles with a tendency to aggregation, suggesting the existence of an amorphous product. This drastic change in the particle shape and aspect could indicate the presence of a new solid phase.

3.3. X-ray powder diffraction studies

X-ray diffraction analysis was performed to examine crystallinity of the samples. The X-ray powder diffraction patterns of the pure components, physical mixture and the complex are shown in Fig. 4. The presence of intense peaks in the PXRD of enalapril maleate (2θ : 5.1° , 8.1° , 10.1° , 15.4° , 20.2° , 24.5° and 30.8°), and β -CD (2θ : 9.3° , 10.9° , 12.8° , 19.8° , 22.8° , 24.5° and 30.8°), indicated that these compounds exist in a crystalline form. In the case of physical mixture, the diffraction pattern was simply the superposition of the two patterns of the crystalline enalapril maleate and β -CD. The sharp peaks of the pattern indicate the retention of the crystalline structure of the drug in the physical mixture. The fact that the principal peaks corresponding to enalapril maleate and β -CD can be observed, although at a lower intensity due to the dilution of the raw materials, indicates that there is no interaction between the components. On the other hand, complete drug amorphization was observed in the enalapril maleate: β -CD system prepared by freeze dried, showing its diffractogram a typical halo pattern. These results confirm that enalapril maleate is no longer present as a crystalline material and that the complex is amorphous, this phenomenon may be attributed to the inclusion of enalapril maleate inside the β -CD cavity. These findings were also in accordance with the above results from the ^{13}C SSNMR and SEM studies, providing evidence that the complex is a new amorphous form.

3.4. Thermogravimetric analysis

Study of drug–excipient compatibility is an important process in the development of a stable solid dosage form. Thermal analysis

(differential scanning calorimetry, differential thermal analysis and thermogravimetric analysis) of pharmaceuticals are routinely used as a screening method for drug–excipient interactions, because calorimetric changes and weight changes caused by chemical and physical degradation of pharmaceuticals can be readily detected (Yoshioka & Stella, 2002). The thermogravimetric analysis (TGA) for compatibility evaluation involves recording the thermograms of the individual excipient, the drug, and their physical mixture. If the curve obtained for the physical mixture is the sum of the individual components curves it can be concluded that there are not

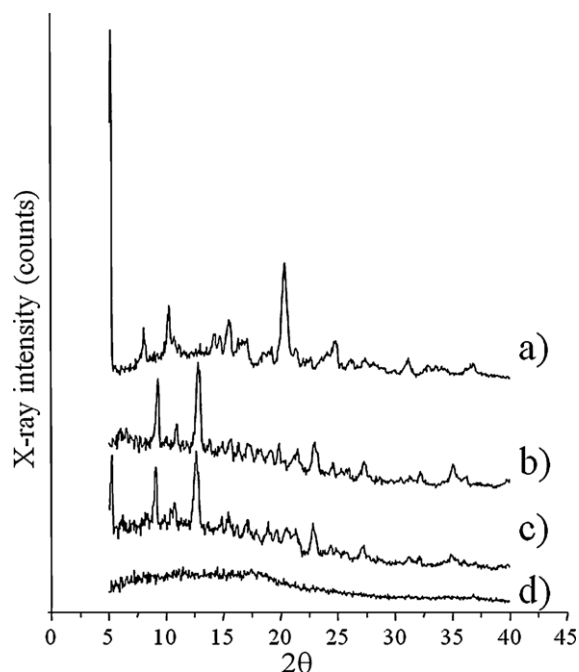


Fig. 4. X-ray diffractograms (a) pure enalapril maleate, (b) pure β -CD, (c) enalapril maleate: β -CD physical mixture, (d) enalapril maleate: β -CD inclusion complex.

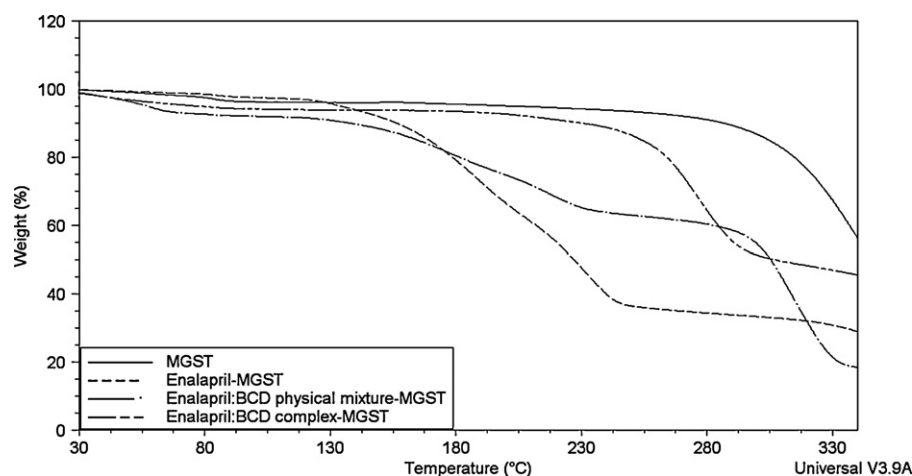


Fig. 5. Thermogravimetric analysis curves of: pure MGST (—), system A (---), system B (· · ·) and system C (— · —).

incompatibility, while an interaction is identified as changes in the physical mixture curve.

MGST compatibility with pure enalapril maleate (system A) as well as with the binary systems containing β -CD, physical mixture (B) and complex (C), was studied by TGA. The curves for systems A–C as well as for pure MGST are shown in Fig. 5, while curves of the materials without MGST are in a previous work (Zoppi et al., 2008).

MGST showed two thermal events: one due to dehydration (a weight loss of 4% in the range of 30–100 °C) and a second event related to its decomposition starting at 250 °C. It is known that enalapril maleate presents two steps of weight loss between 130 and 280 °C (Lin et al., 2002), with the first one corresponding to a complex reaction that includes the formation of enalapril diketopiperazine.

By assessing the formulation compatibility using physical mixtures (Fig. 5), changes can be seen in the decomposition behavior of the drug, TGA curves of systems A and B, show an onset temperature for decomposition of enalapril maleate significantly lower than that in the absence of MGST (114 °C and 130 °C, respectively). This fact demonstrates an incompatibility between enalapril maleate and MGST in systems A and B. In contrast, for system C, the weight loss starts at 155 °C and becomes severe from 185 to 310 °C, showing compatibility between enalapril maleate in the complex form and MGST.

3.5. Accelerated stability studies

To confirm the results obtained by TGA, drug degradation was investigated under accelerated storage conditions using an HPLC stability-indicating method. This experiment demonstrated that the amount of enalapril in the different solid systems decreased with time, when stored at 40 °C and 75% RH (Table 1), with the drug being significantly more stable when stored protected from moisture. For example, enalapril in system A showed less degradation in closed containers ($82.1 \pm 0.5\%$) than in open ones ($36 \pm 3\%$) when stored at 40 °C and 75% RH for 6 months.

On the other hand, in system C, the recovery of enalapril increased to $95 \pm 1\%$ and $64 \pm 6\%$ in closed and open containers, respectively. Finally, the recovery of enalapril in system B was higher than in system A but lower than in C. These results indicate that the presence of β -CD has a protective effect on the stability of enalapril maleate. This fact is more significant in the complex than when there is a simple physical mixture of components. Additionally, it was observed that the major products formed from systems

Table 1

Recovery percentages obtained for enalapril in the systems studied (40 °C and 75% RH for 6 months).

	% recovery
Sealed containers	
System A	82.1 ± 0.5
System B	91.8 ± 0.8
System C	95 ± 1
Open containers	
System A	36 ± 3
System B	56 ± 3
System C	64 ± 6

A and B were enalaprilat and enalapril diketopiperazine, while in system C the only degradation product formed was enalaprilat. This demonstrates that the enalapril maleate cyclization reaction that gave rise to enalapril diketopiperazine formation was prevented by complex formation.

4. Conclusion

Enalapril: β -cyclodextrin complex has been characterized in the solid state using the ^{13}C NMR technique, SEM and X-ray diffraction. From these experiments, we can postulate that the complex is a new solid amorphous form that is different from its precursors.

Our study confirms that the formation of enalapril maleate: β -CD complex prevents a significant drug:MGST interaction. Additionally, we can conclude that the complexation allowed the inhibition of the formation of the degradation product enalapril diketopiperazine. This satisfactory high stability of the ENA: β -CD complex will be potentially useful for its application as solid pharmaceutical dosage products.

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References

- Al-Omari, M. M., Abdelah, M. K., Badwan, A. A., & Jaber, A. M. Y. (2001). Effect of the drug-matrix on the stability of enalapril maleate in tablet formulations. *Journal of Pharmaceutical and Biomedical Analysis*, 25, 893–902.
- Bennet, A. E., Rienstra, C. M., Auger, M., Lakshmi, K. V., & Griffin, R. G. (1995). Heteronuclear decoupling in rotating solids. *The Journal of Chemical Physics*, 103, 6951–6958.
- Braga, S. S., Gonçalves, I. S., Herdtweck, E., & Teixeira-Dias, J. J. C. (2003). Solid state inclusion compound of S-ibuprofen in β -cyclodextrin: structure and characterization. *New Journal of Chemistry*, 27, 597–601.
- Brewster, M. E., & Loftsson, T. (2007). Cyclodextrins as pharmaceutical solubilizers. *Advanced Drug Delivery Reviews*, 59, 645–666.
- Connors, K. A., Amidon, G. L., & Stella, V. J. (1986). *Chemical stability of pharmaceuticals* (2nd ed.). New York: John Wiley & Sons.
- Cotton, M. L., Wu, D. W., & Vadas, E. B. (1987). Drug–excipient interaction study of enalapril maleate using thermal analysis and scanning electron microscopy. *International Journal of Pharmaceutics*, 40, 129–142.
- Fromming, K. H., & Szejtli, J. (1994). *Cyclodextrins in pharmacy*. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Gao, Y., Zhao, X., Dong, B., Zheng, L., Li, N., & Zhang, S. (2006). Inclusion complexes of β -cyclodextrin with ionic liquid surfactants. *Journal of Physical Chemistry B*, 110, 8576–8581.
- Harris, R. K. (1994). *Nuclear magnetic resonance spectroscopy*. London: Longman Scientific and Technical.
- Ip, D. P., & Brenner, G. S. (1987). Enalapril. In K. Florey (Ed.), *Analytical profiles of drug substances* (pp. 207–243). New York: Academic Press.
- Koontz, J. L., Marcy, J. E., O'keefe, S. F., & Duncan, S. E. (2009). Cyclodextrin inclusion complex formation and solid-state characterization of the natural antioxidants α -tocopherol and quercetin. *Journal of Agricultural and Food Chemistry*, 57, 1162–1171.
- Kottke, M. J., & Rudnic, E. M. (2002). Tablet dosage form. In G. S. Banker, & C. T. Rhodes (Eds.), *Modern pharmaceutics* (pp. 287–380). New York: Marcel Dekker.
- Lima, D. M., dos Santos, L. D., & Lima, E. M. (2008). Stability and in vitro release profile of enalapril maleate from different commercially available tablets: possible therapeutic implications. *Journal of Pharmaceutical and Biomedical Analysis*, 47, 934–937.
- Lin, S. Y., Wang, S. L., Chen, T. F., & Hu, T. C. (2002). Intramolecular cyclization of diketopiperazine formation in solid state enalapril maleate studied by thermal FT-IR microscopic system. *European Journal of Pharmaceutics and Biopharmaceutics*, 54, 249–254.
- Loftsson, T. (1995). *Drug Stability*, 1, 22–33.
- Loftsson, T., & Brewster, M. E. (1996). Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. *Journal of Pharmaceutical Sciences*, 85, 1017–1025.
- Narang, A. S., Rao, V. M., & Raghavan, K. S. (2009). Excipient compatibility. In Y. Qiu, Y. Chen, G. G. Z. Zhang, L. Liu, & W. Porter (Eds.), *Developing solid oral dosage forms: pharmaceutical theory and practice* (pp. 125–146). New York: Academic Press.
- Pérez, S., Eichhorn, P., & Barceló, D. (2007). Structural characterization of photodegradation products of enalapril and its metabolite enalaprilat obtained under simulated environmental conditions by hybrid quadrupole-linear ion trap-MS and quadrupole-time-of-flight-MS. *Analytical Chemistry*, 79, 8293–8300.
- Simoncic, Z., Zupancic, P., Roskar, R., Gartner, A., Kogej, K., & Kmetec, V. (2007). Use of microcalorimetry in determination of stability of enalapril maleate and enalapril maleate tablet formulations. *International Journal of Pharmaceutics*, 342, 145–151.
- Szente, L., & Budapest, H. (1993). Cyclodextrins: structure, application, stability and their influence on the stability of active substances. In W. Grimm, & K. Krummen (Eds.), *Stability testing in the EC, Japan and the USA* Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH, (pp. 225–244).
- Wang, S. L., Lin, S. Y., Chen, T. F., & Cheng, W. T. (2004). Eudragit E accelerated the diketopiperazine formation of enalapril maleate determined by thermal FTIR microspectroscopic technique. *Pharmaceutical Research*, 21, 2127–2132.
- Yoshioka, S., & Stella, V. (2002). Stability of dosage forms. In *Stability of drugs and solid dosage forms* (pp. 151–186). USA: Kluwer Academic Publishers.
- Zoppi, A., Quevedo, M. A., & Longhi, M. R. (2008). Specific binding capacity of β -cyclodextrin with cis and trans enalapril: Physicochemical characterization and structural studies by molecular modeling. *Bioorganic and Medicinal Chemistry*, 16, 8403–8412.