International Journal of Pharmaceutics 404 (2011) 116–123

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Supramolecular interactions between losartan and hydroxypropyl-β-CD: ESI mass-spectrometry, NMR techniques, phase solubility, isothermal titration calorimetry and anti-hypertensive studies

Washington X. de Paula [a], Ângelo M.L. Denadai [b], Marcelo M. Santoro [c], Aline N.G. Braga [d], Robson A.S. Santos [d], Rubén D. Sinisterra [a,*]

[a] Laboratório de Encapsulamento Molecular e Biomateriais (LEMB), Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais (UFMG), Av. Antônio Carlos 6627, Belo Horizonte 31270-901, MG, Brazil
[b] Centro Federal de Educação Tecnológica de Minas Gerais (CEFET-MG), Campus VII, Timóteo 35183-006, MG, Brazil
[c] Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas (ICB), Universidade Federal de Minas Gerais (UFMG), Belo Horizonte 31270-901, MG, Brazil
[d] Departamento de Fisiologia-Biofísica, Instituto de Ciências Biológicas (ICB), Universidade Federal de Minas Gerais (UFMG), Belo Horizonte 31270-901, MG, Brazil

ARTICLE INFO

Article history:
Received 3 March 2010
Received in revised form 3 November 2010
Accepted 10 November 2010
Available online 17 November 2010

Keywords:
Losartan
Angiotensin II (AT_1)
Receptor antagonist
Inclusion compounds
Cyclodextrins

ABSTRACT

In this work, low soluble supramolecular complex between the losartan potassium (Los) and hydroxypropil-β-cyclodextrin (HPβCD) were characterized throughout phase-solubility, NMR techniques (^{1}H and 2D-ROESY) and isothermal titration calorimetry (ITC) in order to attain physical–chemical knowledge of the system. In addition, the hypertensive effect of composition Los/HPβCD was evaluated aiming to obtain a more efficient oral pharmaceutical composition. ESI mass spectrometry and ITC blank experiment demonstrate the presence of Los clusters at 30 mM pure solution. Phase-solubility experiments showed a "*Bs*" type system, due to the formation of a less soluble complex than pure Los. NMR demonstrated the short distance interactions between the Los and the cyclodextrin, where several possibilities of interactions were observed. ITC data suggest an average 1:1 stoichiometry of Los and the cyclodextrin. The complex demonstrated efficiency in hypertension control, presenting antagonist action on the pressure effect of angiotensin II within 30 h, as compared to Los alone, 6 h, indicating that inclusion of Los in HPβCD enhanced the extent and duration of its antagonistic action. In this work, a model of interaction between Los and HPβCD was proposed based on dissociation of self-assembled Los followed by complexation with HPβCD.

1. Introduction

Losartan potassium (Los – Fig. 1) is a higher soluble orally active, non-peptide drug used in the treatment of hypertension and related disorders (Kaplan, 1999; Lambot et al., 2001; Mcintyre et al., 1997; Oparil et al., 2001). It has been demonstrated to have a greater effect than previous peptide receptor antagonists and angiotensin converting enzyme (ACE) inhibitors because of its enhanced specificity, selectivity, and tolerability. Los is metabolized in the body, forming a pharmacologically active carboxylic acid metabolite EXP-3174 that presents low bio-availability of 33% (19–62%) with an approximate one-hour post-administration plasma peak concentration and a mean half-life of 2 h (Mcintyre et al., 1997).

Thus we hypothesized that if sequestering a highly water soluble drug, such as Los, through supramolecular non-covalent host–guest complexes formation using modified cyclodextrin, such as hydroxypropil-β-cyclodextrin (HPβCD), we can obtain lower water Los solubility and a more efficient angiotensin II AT1 receptor antagonist oral pharmaceutical composition.

This host–guest strategy is well-established in literature where cyclodextrins complexes with hydrophobic guests in order to increase water solubility and bioavailability (De Sousa et al., 2008; Denadai et al., 2006a, 2007a,b; Irie and Uekama, 1997; Loftsson and Brewster, 1996; Lula et al., 2007; Sousa et al., 2008; Uekama et al., 1998). However, works using this strategy when water-soluble molecules as Los are used as guest molecules are scarcely found in literature.

Cyclodextrins are very important for supramolecular chemistry, since they form non-covalent complexes with a wide range of host molecules, which can be used as models for studying weak interaction. Their cavity offers a suitable hydrophobic environment to the guest molecule (Fig. 2), allowing the formation

* Corresponding author at: Departamento de Química, Universidade Federal de Minas Gerais. CEP: 31270-901Avenida Pres.,Antônio Carlos 6627, Belo Horizonte, Minas Gerais, Brazil. Tel.: +55 31 34995778; fax: +55 31 3499 5700.
E-mail address: sinisterra@ufmg.br (R.D. Sinisterra).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.11.008

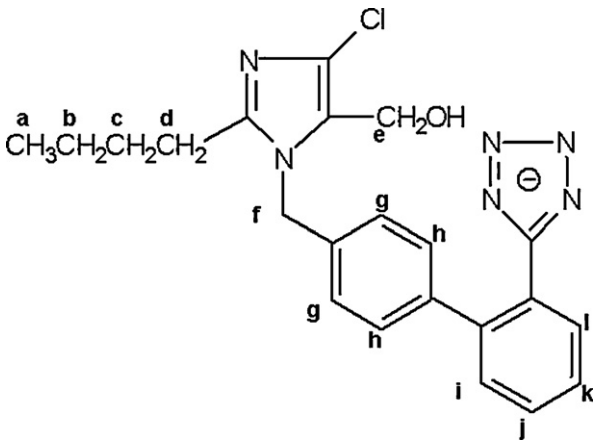

Fig. 1. Losartan potassium structure.

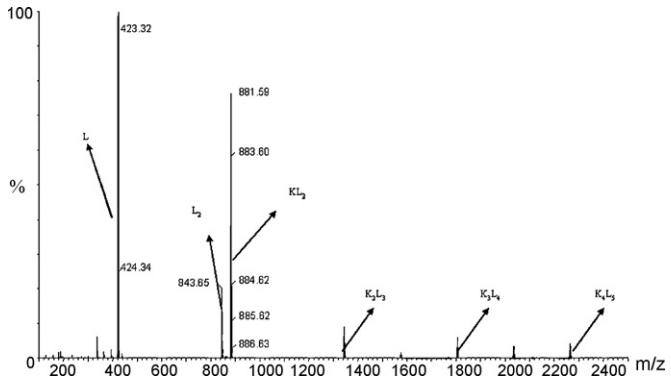

Fig. 3. Electrospray mass spectroscopy of Losartan potassium 5 mM.

of inclusion compounds. Hence cyclodextrins are used for the solubilization and encapsulation of drugs, perfumes and flavorings forming supramolecular structures (De Sousa et al., 2008; Denadai et al., 2006a, 2007a,b; Irie and Uekama, 1997; Loftsson and Brewster, 1996; Sousa et al., 2008).

In this work, the supramolecular complexes between the Los and HPβCD were characterized throughout electrospray mass spectrometry, phase-solubility, NMR techniques (^{1}H and 2D-ROESY) and isothermal titration calorimetry (ITC) in order to look further into the physical–chemical characteristics of the system. Furthermore, in vivo anti-hypertensive tests were carried out aiming to assess higher efficient Los oral pharmaceutical composition.

2. Materials and methods

2.1. Reagents

Los was purchased from Galena Química e Farmacêutica Ltda, Brazil; and HPβCD (substitution degree 5–8 and $M_W \approx 1400$ g/mol) was obtained from Cerestar Company, USA. All the other materials and solvents were of analytical reagent grade and used as received.

2.2. Inclusion compound preparation

For NMR and ESI-MS analysis and anti-hypertensive evaluation, a 1:1 Los/HPβCD inclusion compound was prepared by freeze-dry method. In briefly, the Los salt and HPβCD were dissolved in milli-Q water at 1:1 molar ratio. This mixture was submitted to stirring during 48 h. Next, the solution was freeze-dried by 48 h.

2.3. ESI-MS measurements

Mass spectrometry experiments were performed using an ESI Micromass Q-TOF instrument in order to check the previous aggregation state of the Los and the stoichiometry of the complex in solution. The data were collected from a 5 mM sample of Los and Los/HPβCD 1:1 aqueous solution by using the electrospray ionization mode. The standard conditions employed were: vaporization temperature of 120 °C, capillary voltage of 2000 V, sample cone 40 V, extraction cone 2.0 V.

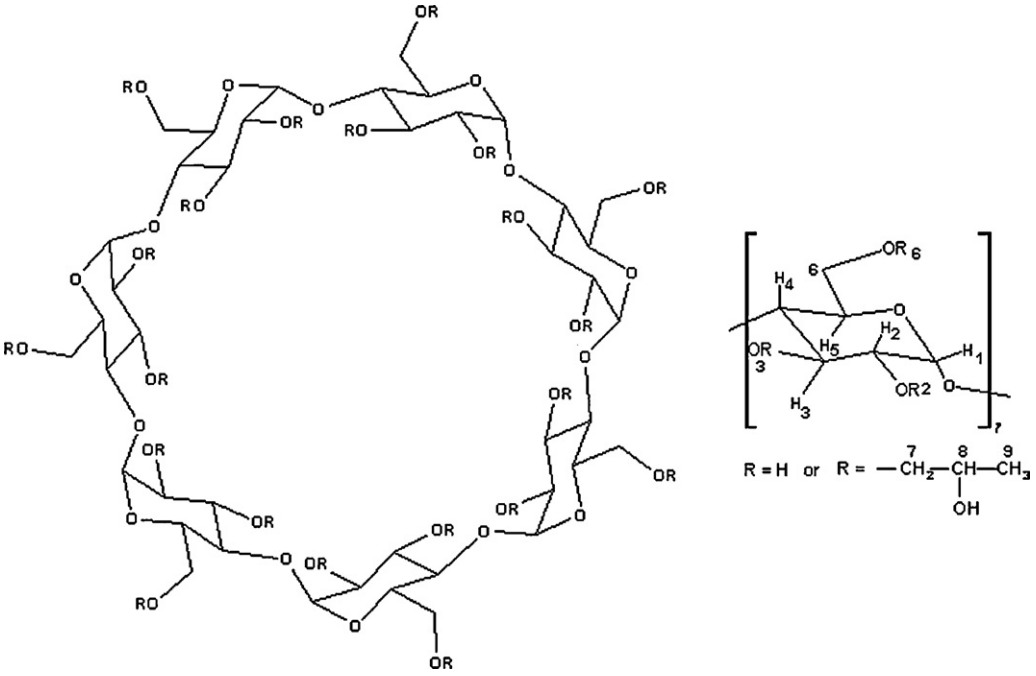

Fig. 2. Hydroxipropil-β-cyclodextrin structure.

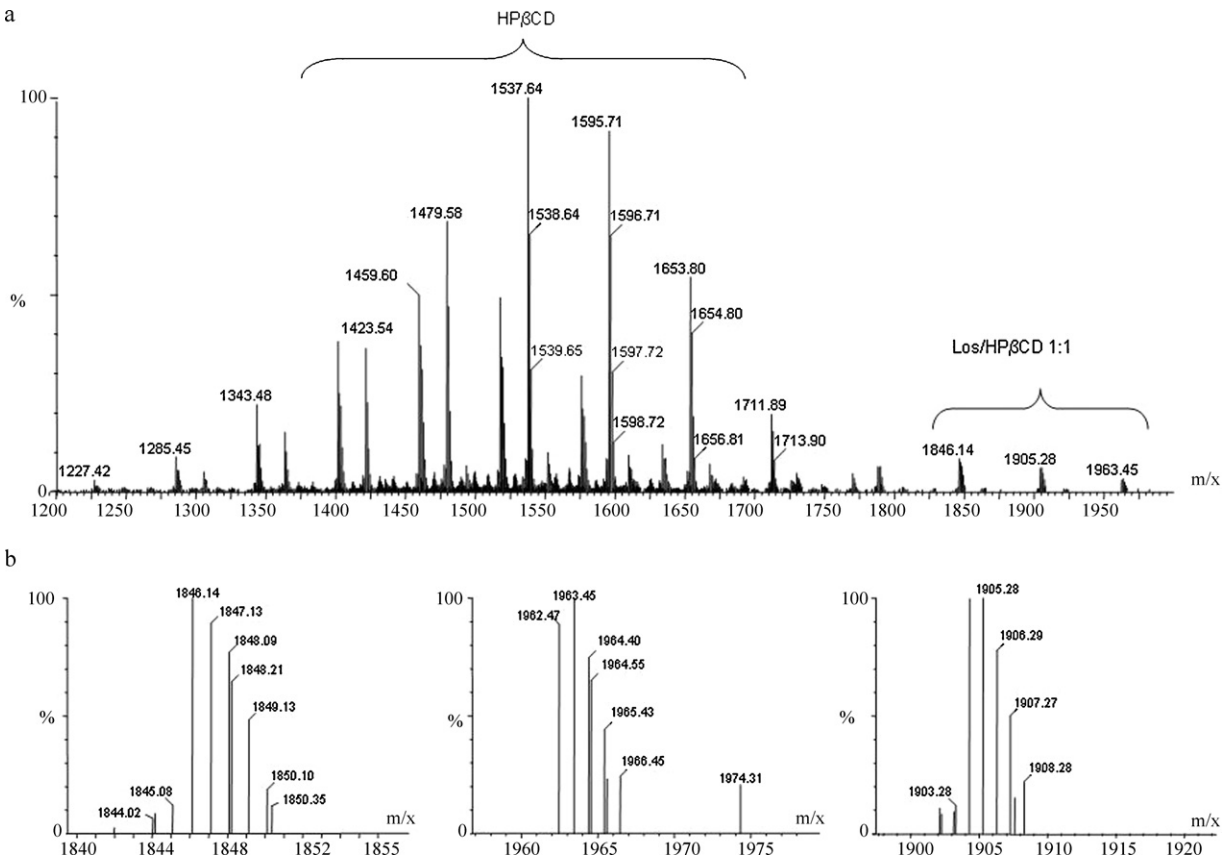

Fig. 4. (a) Electrospray mass spectrum of 5 mM Los/HPβCD 1:1 water solution. (b) Expansion of the region between 1800 and 2000, referent to inclusion compounds.

2.4. NMR experiments

^{1}H NMR chemical shifts (δ) and 2D ^{1}H-^{1}H-ROESY experiments were obtained using a Brucker DPX-400 Avance (400 MHz) spectrometer, at 300 K.

The solutions used were 2.0 mM of Los and 2.0 mM (1:1) of Los/HPβCD, both dissolved in D_2O (Cambridge Isotope Laboratories, Inc – 99.9% of isotopic purity). The HOD signal at δ = 4.80 was used as reference. No solid formation was observed in the solution during analysis. The 2D-ROESY experiments were recorded at spin lock of 600 ms, which was previously calculated throughout the inversion-recovery sequence (Rahman, 1989; Werner, 1994).

The sample for this experiment was prepared by utilizing the freezing-drying method in the molar ratio of 1:1 Los/HPβCD. In this method, the aqueous solution, which contains the dissolved materials (Los and HPβCD), was stirred for 2 h. After, the solution was frozen in liquid nitrogen and freeze-dried for 24 h before dissolution in D_2O.

2.5. Solubility studies

The phase-solubility diagrams were made according to the Higuchi and Connors method (Higuchi and Connors, 1969). For this purpose, aqueous solutions of HPβCD with concentrations of 0–2.6 mM with a known concentration of Los at 30 mM were prepared. These were placed in a thermostatic bath at 298 K by 48 h. The samples were centrifuged at 15 rpm for 10 min and filtered with an ultra-filtration membrane of 0.22 μm from Millipore®. Quantification was performed in triplicate on a UV–vis equipment HP-8453 at 248 nm and a one-centimeter cell.

2.6. Microcalorimetric measurements

Calorimetric titrations were carried out in duplicate with a VP-ITC Microcalorimeter (Microcal Company, Northampton, MA, USA) at 298.15; 303.15 and 308.15 K next the electrical and chemical calibration.

Each titration experiment consisted of 41 successive injections of Los aqueous solution (100 mM) into the reaction cell charged with 1.6 mL of HPβCD aqueous solution (4.0 mM), with time intervals of 540 s. The first injection of 1.0 μL was discarded to eliminate diffusion effects of material from syringe to cell calorimetric. The subsequent injections were used at constant volume of 5.0 μL of Los. The time of injection was 2.0 s.

The HPβCD concentration in the calorimeter cell varied from 4.0 to 3.6 mM and the concentration of the Los from 0.0 to 11.1 mM. The raw data were analyzed using the "one site model" set forth by Microcal Origin 5.0 for ITC after the subtraction of the blank experiment (dilution of Los in water).

The enthalpy values obtained at each temperature were plotted against temperature values in order to determinate the heat capacity at pressure constant $\Delta C^\circ{}_p$, according to Eq. (1), where the angular coefficient of the curve $\partial\Delta H^\circ/\partial T$, was obtained by linear regression by using of Microcal Origin 5.0.

$$\Delta C^\circ{}_p = \left(\frac{\partial \Delta H^\circ}{\partial T}\right)_p \quad (1)$$

2.7. Dissolution tests

The dissolution tests were performed in an incubator model Q120A3 Qualitas according to the method described by Oskan et al. (2000). Initially were weighed in triplicate 100 mg of Los

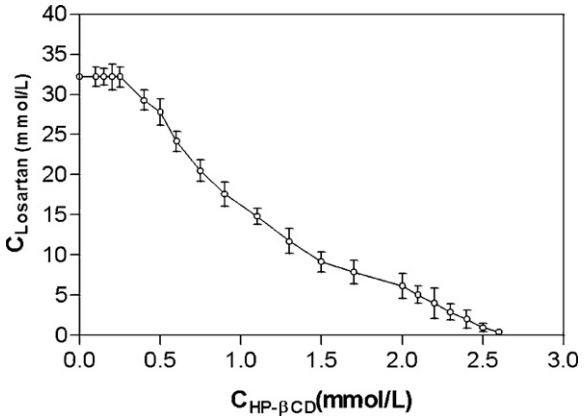

Fig. 5. Solubility diagram of [Losartan] vs. [HPβCD] at 25 °C.

and 300 mg of the freeze dried Los/HPβCD, after being passed by a separation particle size of 50, 100 and 200 mesh. They were then transferred to tubes eppendorfs and added 2.0 mL of phosphate buffer pH 7.4 and undergoing agitation 110 rpm at 37 °C for 5 min. Then 1.0 mL was collected from each tube and added more 1.0 mL of buffer. This procedure was performed at various time intervals (0.25–24 h) after the beginning of the experiment, reaching 48 h. The solutions collected from each tube at different time intervals were stored for quantification on a UV–vis equipment HP-8453 at 248 nm and a one-centimeter cell.

2.8. Antagonist action of Los and Los/HPβCD complex on the pressor effect of angiotensin II in Wistar rats

The antagonist action of Los and Los/HPβCD complex on angiotensin pressor effect was evaluated in Wistar rats (male, weighting 300–350 g) obtained from "CEBIO – Centro de Bioterismo de Ciências Biológicas–UFMG".

Before and after surgery, the animals were kept in a temperature-controlled room using a 14/10-light/dark cycle. One day before the experiment, a polyethylene catheter (PE-10 connected to PE-50) was inserted into the abdominal aorta through the femoral artery for blood pressure measurements. For intravenous injections and infusions, polyethylene cannulas were implanted into the veins. The cannulas, closed by a metallic pin and filled with isotonic saline, were driven subcutaneously to the interdescapular region of the back of the animals. After recovery from anesthesia, the rats were kept in individual cages with free access to water and chow until the end of the experiment. A data acquisition system (Biopac, USA) was used to measure systolic, diastolic and mean pressure. In experiments, Los/HPβCD solution was prepared by directly dissolution of complex in mili-Q water, which was followed by further dilutions. Los and Los/HPβCD were administered by oral route, and the hypertensive effect produced by i.v. infusion of angiotensin II (Ang II) (0.10 mL 20 ng *in bolus*). The pressure data were collected 2, 6, 12, 24, 48 and 72 h after Los or Los/HPβCD complex administration. All experimental protocols were performed in compliance with the guidelines on laboratory animals from our institute and approved by local authorities.

The basal values mean arterial pressure were (98 ± 0.5) mmHg. Comparisons between the control period and the experimental and recovery periods were made by one-way ANOVA, followed by the Dunnet's test. For this, the control values were obtained by averaging all values obtained in the last 3 days of the pre-infusion period. Comparison between changes in mean arterial pressure produced by Ang II infusion was made using *t*-test. A value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. ESI-MS measurements

Los is an amphiphilic substance and shows an intense and endothermic heat of dilution (see below ITC data) suggesting disaggregating phenomenon upon dilution. Hence, in order to check the possibility of self-assembly of the Los under the experimental conditions, ESI mass-spectrometry measurements were used once the ESI experiments were able to evaluate weak non-covalent complexes (Fig. 3) (Rekharsky et al., 2002; Toma et al., 2004).

The data presented in Fig. 3 show the presence of homologue series of small $K_{n-1}Los_n$ clusters, not excluding necessarily the presence of high order self-assembly complexes in water solutions, as described in the literature for other amphiphilic systems (Rekharsky et al., 2002; Toma et al., 2004).

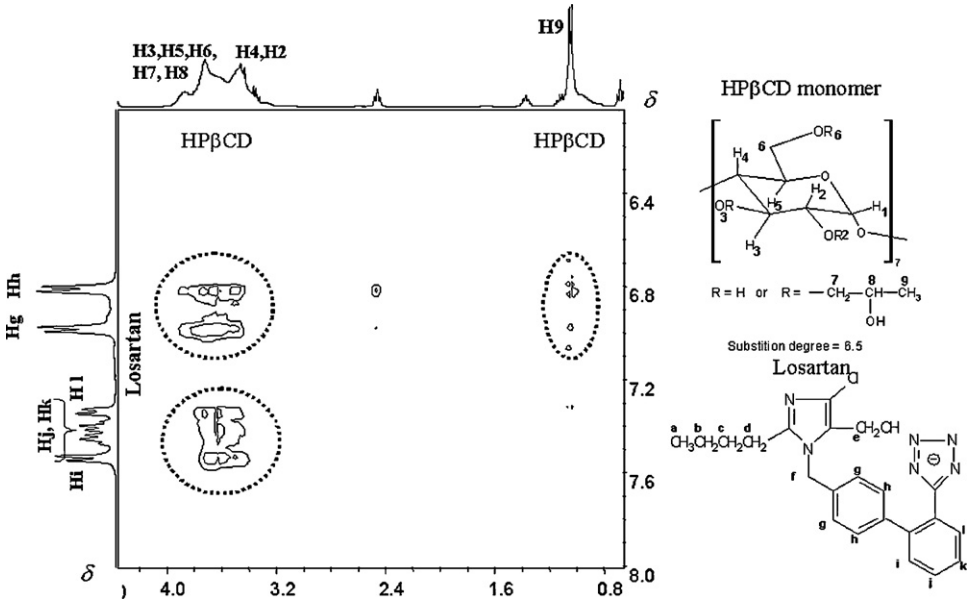

Fig. 6. 2D-ROESY spectrum of the inclusion compound between Losartan and HPβCD.

ESI mass-spectroscopy was also used to assess qualitatively the species present in the Los/HPβCD solution at a 1:1 molar ratio (Fig. 4). The ESI technique is not precise enough to monitor weak non-covalent interactions because this type of experiment may disturb the chemical equilibrium of the system due to the drastic conditions (high electrical field and high temperature).

Fig. 4 shows parts of the ESI experiment where the m/z distribution of peaks between 1285.45 and 1713.90, with maximum at 1537.64, is observed. Such distribution is due the fact that HPβCD sample is a mixture of several cyclodextrins with different substitution degree. At m/z values localized between 1800 and 2000 is observed three peaks, 1846.13, 1905.28 and 1963.45, relating to three types of inclusion compounds with stoichiometry 1:1. The correspondent peaks of free cyclodextrins are those localized at m/z 1423.54, 1479.58 and 1537.64 (considering the m/z of Los^- anion of 423.32).

3.2. Phase solubility studies

Thermodynamic solubility experiments allow to demonstrate the hydrotrope effect on the solubility of a substance and evaluates the interactions between the species (Higuchi and Connors, 1969).

Fig. 5 shows the solubility of Los against HPβCD concentration where an initial Los constant concentration of up to 0.25 mM of cyclodextrin is observed. After achieving this value, a strong reduction of Los solubility upon increase of HPβCD concentration is observed. This diagram corresponds to the formation of a complex less soluble than the pure substrate, named Higuchi's B_s type (Higuchi and Connors, 1969).

Los and HPβCD are very soluble molecules presenting high aqueous solubility at 298 K (both species have solubility greater than 300 mM). However, as demonstrated in Fig. 5, a lower soluble than pure Los species is formed upon complexation, showing stronger host–guest interaction than host–solvent and guest–solvent.

The Los/HPβCD complex is a very small and quasi invisible precipitate which was separated from solution only by centrifugation and ultra-filtration. According to the solubility data, the precipitate formation is dependent on the molar ratio and it starts to form above 0.25 mM of HPβCD.

The Los solubility reduction after the HPβCD interaction could be explained by the ESI results for pure Los. The ESI results showed the presence of Los self-assemblies, which could be an intrinsic solubilization mechanism of the drug. Thus, complexation of Los with HPβCD could break down the self-assemblies, leading to reduction of Los solubility.

3.3. NMR experiments

NMR experiments allowed an investigation of the short distance interaction of supramolecular complexes between Los and HPβCD. ^{1}H NMR chemical shift changes are related to the disturbance caused by unpaired C_1-O-C_4 electrons of the HPβCD cavity on electronic density of the Los hydrogen's (De Sousa et al., 2008; Denadai et al., 2007b; Loftsson et al., 1993; Schneider et al., 1998; Sousa et al., 2008). Changes in chemical environment and conformation upon break down of self-assembly also contribute to the changes in chemical shift. The Table 1 shows the chemical shift of ^{1}H NMR values for free and complexated Los.

Possible architectures of the Los complexes with cyclodextrins were evaluated by the 2D-ROESY experiments, which are able to detect dipolar coupling in solution through space upon 5 Å distance (Denadai et al., 2007a,b; Lula et al., 2007; Rahman, 1989; Schneider et al., 1998; Werner, 1994).

Cross peaks correlations were found between aromatic Los hydrogens (region at $\delta \approx 6.5$–7.6) with H2, H3, H4, H5 and H6 HPβCD hydrogens. There were also cross peaks found between H12, H13 and H16 of the Los with H9 hydrogens of HPβCD hydroxypropyl group. These data are indicating correlations of aromatic and aliphatic moieties of Los with external hydrogens H9 of HPβCD (Fig. 6).

These results suggest very complex supramolecular architecture between Los and HPβCD which could be explained in terms of three plausible hypotheses:

(1) dynamic equilibrium between the species, where several complexes stabilized by different non-covalent interactions can exist within the time scale of NMR detection,
(2) conformational equilibrium of Los molecule, which could let several kinds of interactions with cyclodextrins within the time scale of NMR detection,
(3) self-assembly of inclusion compounds, where the complex will be packaged in different way, stabilized by hydrogen bonds.

Table 1
^{1}H NMR (at 400 MHz) chemical shifts of Los and Los/HPβCD in D_2O at 27 °C.

Hydrogen	δ_{Los}	$\delta_{Los/HP\beta CD}$	$\Delta\delta$[a]
H6	2.49	2.51	+0.02
H7	1.48	1.40	−0.08
H8	1.25	1.20	−0.05
H9	0.81	0.79	−0.02
H10	5.21	5.24	+0.03
H12	6.90	6.97	+0.05
H13	7.10	7.16	+0.04
H16	7.29	7.28	−0.01
H17	7.34	7.36	+0.02
H18	7.36	7.37	+0.01
H19	7.52	7.54	+0.02
H22	4.31	4.30	−0.01

[a] $\Delta\delta = \delta_{Los} - \delta_{Los/HP\beta CD}$.

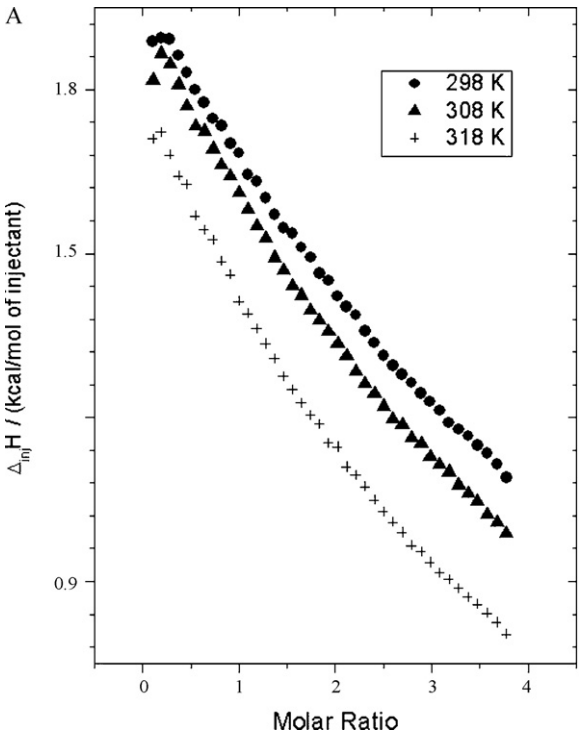

Fig. 7. Dilution curves obtained from 41 successive 5 μL injections of an aqueous solution of 100 mM Losartan potassium into a 1.4 mL cell containing water at 298, 308 and 318 K.

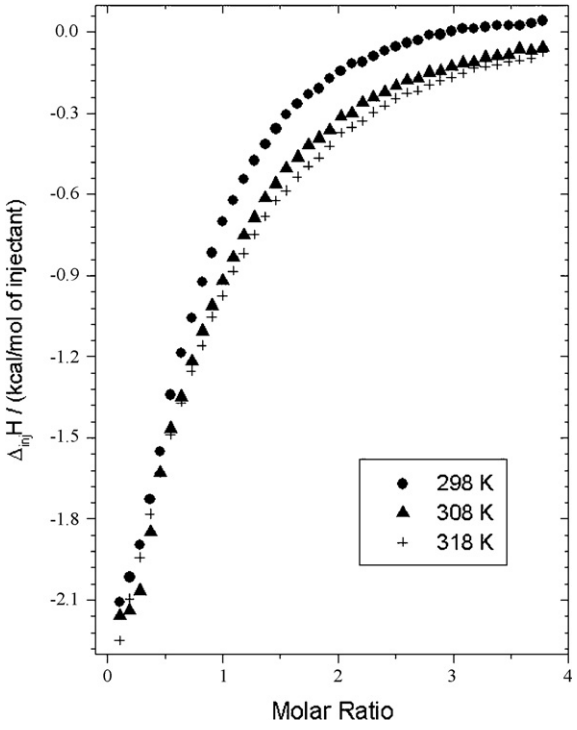

Fig. 8. Titration curves obtained from 41 successive 5 μL injections of an aqueous solution of 100 mM Losartan potassium into a 1.4 mL cell initially containing 4 mM HPβCD at 298, 308 and 318 K, after subtracted from blank data at each temperature.

3.4. Microcalorimetric measurements

In order to know the average stoichiometry of the complex in solution as well as its thermodynamic parameters, isothermal titrations calorimetry (ITC) of Los 100 mM into the 4 mM HPβCD solution, at 298, 308 and 318 K were performed (Figs. 7 and 8). ITC experiment allows for the simultaneous determination of the enthalpy ΔH°, equilibrium constant K_{eq} and stoichiometry N from a single titration curve, using the least square non-linear adjustment (MicroCal, 1998a,b; Turnbull and Daranas, 2003). Through the use of classical thermodynamic equations (Levine, 1995), is possible calculate the changes on free energy ΔG°, entropy energy $T\Delta S^{\circ}$ and heat capacity at pressure constant $\Delta C^{\circ}{}_{p}$.

For all temperatures, the sigmoid adjust showed an equivalence point at molar ratio of approximately 0.8 (Table 2). These data and the relative size between the molecules suggested a 1:1 minimal stoichiometry not necessarily excluding the existence of higher order complex.

It is well accepted in the literature that the cyclodextrin system there is a distribution of equilibrium species, named $Los_n/HP\beta CD_m$, wich has an average stoichiometry (Denadai et al., 2007b; Loftsson and Brewster, 1996; Sousa et al., 2008). Thus, the equilibrium constant calculated on the basis of ITC experiment is a global equilibrium constant $K_{eq,g}$, referring to some process which leads to the formation of a $Los_n/HP\beta CD_m$ (Eqs. (2) and (3)).

$$nS + mCD \overset{K_{eq,g}}{\rightleftharpoons} S_mCD_n \quad (2)$$

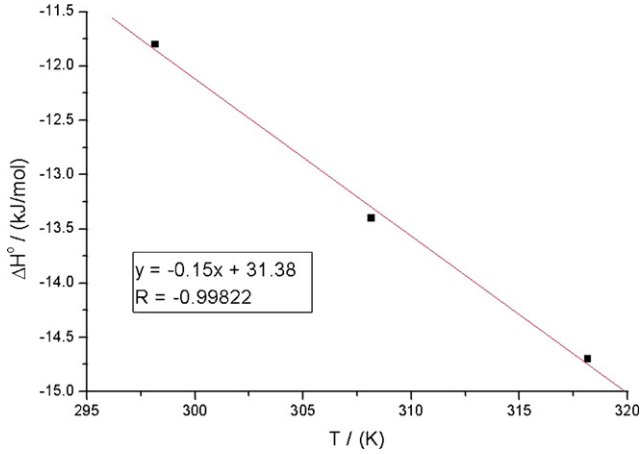

Fig. 9. $\Delta C^{\circ}{}_{p}$ calculus from Eq. (1).

$$K_{eq,g} = \frac{[S_mCD_n]}{[S]^n[CD]^m} \quad (3)$$

As depicted in Fig. 7, the Los dilution experiment showed endothermic signals in an overall range of analyzed concentration, indicating that dilution is entropy-driven.

Considering that ESI mass spectroscopy experiments showed the presence of clusters at 30 mM solutions, the endothermic dilution of Los could be understood in terms of disaggregation of self-assemblies, which requires energy to separate monomers from the clusters.

Analyzing the data from Table 2, one can observe that the overall interaction between Los and HPβCD is exothermic and accompanied by entropy increase. However, the global equilibrium constant is relatively low and similar to other cyclodextrins/guest systems (Rekharsky and Inoue, 1998).

Enthalpy changes may well be attributed to the binding of enthalpy-rich water molecules released from the cyclodextrin cavity together with bulk water molecules (De Sousa et al., 2008; Denadai et al., 2006b, 2007a,b; Loftsson and Brewster, 1996; Rekharsky and Inoue, 1998; Sousa et al., 2008), by the formation of cooperative Van der Waals interactions between guest and the cyclodextrin cavity through the hydrophobic Los moiety and by the formation of ion-dipole interaction between tetrazolic ring and cyclodextrin OH groups.

By performing ITC experiments, positive entropy changes were found, although the Los/HPβCD complex had been precipitated. For an increase of entropy in the precipitation process, high dessolvation of molecules is needed to compensate the formation of solid-state phase, and the water molecules must gain translational and rotational freedom degree (Rekharsky et al., 2002).

The deepest understanding of the host–guest interactions was achieved by considering the ΔC_p changes during the process, calculated by Eq. (1) through linear regression in a "ΔH° versus T" graphic (Fig. 9).

It is well known that ΔC_p data is related to changes in hydrophobic interactions during binding process. According to the literature (Rekharsky and Inoue, 1998), if ΔC_p is negative (−0.15 kJ/mol K for the Los/HPβCD system), hydrophobic bonds are formed as con-

Table 2
Thermodynamic parameters obtained by ITC experiments.

T/K	N	K	ΔH°/kJ mol^{-1}	ΔG°/kJ mol^{-1}	$T\Delta S^{\circ}$/kJ mol^{-1}	ΔC_p/kJ mol^{-1}K^{-1}
298.15	0.74	1147.0	−11.8	−17.5	5.8	
308.15	0.79	739.2	−13.4	−16.9	3.5	−0.15
318.15	0.83	496.8	−14.7	−16.4	1.7	

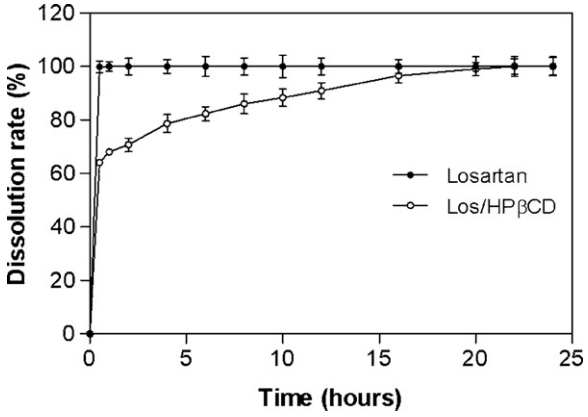

Fig. 10. Dissolution profile of Losartan and Los/HPβCD complex. Mean ± SE (vertical bar), $n = 3$.

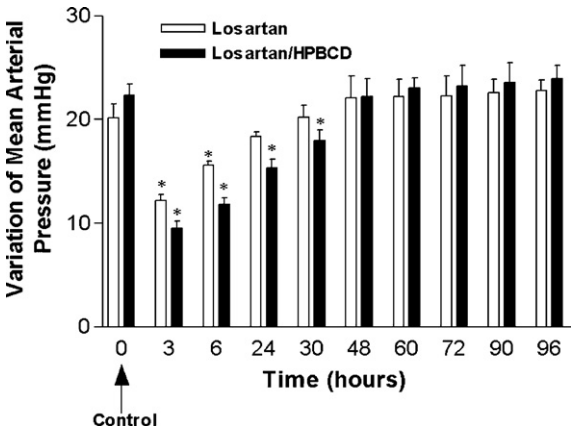

Fig. 11. Effect of Losartan and Los/HPβCD complex administered by oral route at 0.7 mg/kg to Wistar rats, on the mean arterial pressure changes induced by Ang II (20 ng i.v.). $^*p < 0.05$ compared with the average of the 3 last day of the period before infusion (98 ± 0.5 mmHg) (ANOVA followed by Dunnett's test). Mean ± SE (vertical bar), $n = 4$.

sequence of breakdown water clatrates around apolar molecules, corroborating the dessolvation hypothesis mentioned above.

Thus, in this work it was suggested that the interaction between Los and HPβCD involves an initial disaggregation of Los self-assemblies, followed by complexation with cyclodextrins. The formed complex presents low solubility and it is stabilized by favorable interactions such as Van der Waals, hydrogen bonding and ion-dipole interactions. The interaction is increased by the release of the enthalpy-rich water molecules from cyclodextrins cavity and hydration shells of cyclodextrins and Los, which gain rotational and translational freedom degree in order to overcome the entropy reduction due to precipitation.

3.5. Dissolution tests

Fig. 10 shows the in vitro dissolution profiles for Losartan and Los/HPβCD. The curve of Losartan showed a rapid dissolution, with approximately 100% dissolved in the first minutes, as compared to 65% dissolution of Los/HPβCD at the same time interval. Moreover, the dissolution of the complex was observed until 20 h after the start of testing, confirming that inclusion leads to a decrease in solubility of Losartan.

3.6. Antagonist action of Los and Los/HPβCD complex on the pressor effect of angiotensin II in Wistar rats

Fig. 11 shows the antihypertensive effect of Los and Los/HPβCD in Wistar rats. The Los/HPβCD complex, when administered orally as a single dose of 0.7 mg/kg, blocked in approximately 75% ($p < 0.01$, $n = 4$) the pressor effect of Ang-II for approximately 30 h. In contrast, the free Los given at the same dose blocked the effect of Ang II for a period of only 6 h ($p < 0.01$, $n = 4$). The increase in pressure due to angiotensin II administration in the control period was 20.2 mmHg and 22.3 mmHg for Los and Los/HPβCD, respectively. After 3 h, 13 mmHg and 9 mmHg, reaching around a variation of 20.5 mmHg and 17.1 mmHg after 30 h.

These data indicate that inclusion compound Los/HPβCD enhanced the extent and duration of its Los antagonist action. The higher Los bioavailability upon inclusion could be due to the lower Los solubility and solubilization rate as verified by phase solubility studies and dissolution tests.

To the extent of our knowledge, such an approach to alter the pharmacological profile of highly water-soluble drug when administered orally has not yet been explored. Thus, the formulation using the host–guest strategy could be improved by increasing the efficacy and reducing the dose or spacing with each dose intake.

4. Conclusion

A model based on dissociation of the Losartan potassium clusters, followed by complexation with HPβCD forming a lower soluble 1:1 complex than their precursor has been proposed. This approach offers an alternative means of improving the bioavailability of water-soluble drugs and represents a significant step towards the development of sustained release of oral dosage forms. The Los formulation accompanied with this strategy could be useful in reducing costs and increasing compliance with the antihypertensive treatment.

Acknowledgments

The authors acknowledge financial support by Brazilian agencies FAPEMIG, CAPES and CNPq and Biolab-Sanus pharmaceutical company for the technology transfer contract assigned with the UFMG to scale up this technology.

References

De Sousa, F.B., Denadai, A.M., Lula, I.S., Lopes, J.F., Dos Santos, H.F., De Almeida, W.B., Sinisterra, R.D., 2008. Supramolecular complex of fluoxetine with beta-cyclodextrin: an experimental theoretical study. Int. J. Pharm. 353, 160–169.

Denadai, A.M., Ianzer, D., Alcantara, A.F., Santoro, M.M., Santos, C.F., Lula, I.S., de Camargo, A.C., Faljoni-Alario, A., dos Santos, R.A., Sinisterra, R.D., 2007a. Novel pharmaceutical composition of bradykinin potentiating penta peptide with beta-cyclodextrin: physical–chemical characterization and anti-hypertensive evaluation. Int. J. Pharm. 336, 90–98.

Denadai, A.M., Teixeira, K.I., Santoro, M.M., Pimenta, A.M., Cortes, M.E., Sinisterra, R.D., 2007b. Supramolecular self-assembly of beta-cyclodextrin: an effective carrier of the antimicrobial agent chlorhexidine. Carbohydr. Res. 342, 2286–2296.

Denadai, A.M.L., Santoro, M.M., Da Silva, L.H., Viana, A.T., Santos, R.A.S., Sinisterra, R.D., 2006a. Self-assembly characterization of the beta-cyclodextrin and hydrochlorothiazide system: NMR, phase solubility, ITC and QELS. J. Incl. Phenom. Macro. 55, 41–49.

Denadai, A.M.L., Santoro, M.M., Lopes, M.T.P., Chenna, A., de Sousa, F.B., Avelar, G.M., Gomes, M.R.T., Guzman, F., Salas, C.E., Sinisterra, R.D., 2006b. A supramolecular complex between proteinases and beta-cyclodextrin that preserves enzymatic activity – physicochemical characterization. Biodrugs 20, 283–291.

Higuchi, T., Connors, K., 1969. Phase-Solubility Techniques. Advances in Analytical Chemistry and Instrumentation, 4, pp. 117–212.

Irie, T., Uekama, K., 1997. Pharmaceutical applications of cyclodextrins 3. Toxicological issues and safety evaluation. J. Pharm. Sci. 86, 147–162.

Kaplan, N.M., 1999. Angiotensin II receptor antagonists in the treatment of hypertension. Am. Fam. Physician 60, 1185–1190.

Lambot, M.A., Vermeylen, D., Noel, J.C., 2001. Angiotensin-II-receptor inhibitors in pregnancy. Lancet 357, 1619–1620.

Levine, I.N., 1995. Physical Chemistry, fourth edition. McGRAW-HILL INC., New York.

Loftsson, T., Brewster, M.E., 1996. Pharmaceutical applications of cyclodextrins 1. Drug solubilization and stabilization. J. Pharm. Sci. 85, 1017–1025.

Loftsson, T., Olafsdottir, B.J., Frioriksdottir, H., Jonsdottir, S., 1993. Cyclodextrin complexation of Nsaids – physicochemical characteristics. Eur. J. Pharm. Sci. 1, 95–101.

Lula, I., Denadai, A.L., Resende, J.M., de Sousa, F.B., de Lima, G.F., Pilo-Veloso, D., Heine, T., Duarte, H.A., Santos, R.A., Sinisterra, R.D., 2007. Study of angiotensin-(1-7) vasoactive peptide and its beta-cyclodextrin inclusion complexes: complete sequence-specific NMR assignments and structural studies. Peptides 28, 2199–2210.

Mcintyre, M., Caffe, S.E., Michalak, R.A., Ried, J.L., 1997. Losartan, an orally active angiotensin (AT1) receptor antagonist: a review of its efficacy and safety in essential hypertension. Pharmacol. Ther. 74, 181–194.

MicroCal, 1998a. ITC data analysis in origin^{r} tutorial guide. In: MicroCal (Ed.), The Calorimetry Experts, 5.0 ed. MicroCal, www. microcalorimetry. com.

MicroCal, 1998b. VP-ITC microcalorimeter. User's manual. In: The Calorimetry Experts. MicroCal, www. microcalorimetry. com.

Oparil, S., Aurup, P., Snavely, D., Goldberg, A., 2001. Efficacy and safety of losartan/hydrochlorothiazide in patients with severe hypertension. Am. J. Cardiol. 87, 721–726.

Oskan, Y., Atay, T., Dikmen, N., Isimer, A., 2000. Improvement of water solubility and in vitro dissolution rate of glicazide by complexation with β-cyclodextrin. Pharm. Acta Helv. 74, 365–370.

Rahman, A., 1989. One and Two Dimensional NMR Spectroscopy. Elsevier, New York.

Rekharsky, M., Inoue, Y., Tobey, S., Metzger, A., Anslyn, E., 2002. Ion-pairing molecular recognition in water: aggregation at low concentrations that is entropy-driven. J. Am. Chem. Soc. 124, 14959–14967.

Rekharsky, M.V., Inoue, Y., 1998. Complexation thermodynamics of cyclodextrins. Chem. Rev. 98, 1875–1917.

Schneider, H.J., Hacket, F., Rudiger, V., Ikeda, H., 1998. NMR studies of cyclodextrins and cyclodextrin complexes. Chem. Rev. 98, 1755–1785.

Sousa, F.B.d., Denadai, Â.M.L., Lula, I.S.e., Nascimento, C.S., Neto, N.S.G.F., Lima, A.C., Almeida, W.B.d., Sinisterra, R.D., 2008. Supramolecular self-assembly of cyclodextrin and higher water soluble guest: thermodynamics and topological studies. J. Am. Chem. Soc. 130, 8426–8436.

Toma, S.H., Uemi, M., Nikolaou, S., Tomazela, D.M., Eberlin, M.N., Toma, H.E., 2004. {trans -1,4-bis[(4-pyridyl)ethenyl]benzene}(2,2′-bipyridine)ruthenium(II) complexes and their supramolecular assemblies with beta-cyclodextrin. Inorg. Chem. 43, 3521–3527.

Turnbull, W.B., Daranas, A.H., 2003. On the value of c: can low affinity systems be studied by isothermal titration calorimetry? J. Am. Chem. Soc. 125, 14859–14866.

Uekama, K., Hirayama, F., Irie, T., 1998. Cyclodextrin drug carrier systems. Chem. Rev. 98, 2045–2076.

Werner, M.H., 1994. Advance User's Guide, Brucker. Spectrospin AG, Fallanden, 940712.