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Self-assembly Characterization of the β-Cyclodextrin and Hydrochlorothiazide System: NMR, Phase Solubility, ITC and QELS

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Abstract

Supramolecular self-assembly formed by β-cyclodextrin (β-CD) and hydrochlorothiazide (Htz) was characterized in two aspects: (1) Short distance host–guest interactions, by NMR techniques such as ^{1}H chemical shift, longitudinal relaxation times – T_1, 2D ^{1}H–^{1}H nuclear Overhauser spectroscopy, and thermodynamic solubility experiments in which the interaction thermodynamic parameters were calculated for host–guest interactions and (2) Self-assembly of inclusion complex, which was observed in excess of CD by isothermal titration calorimetry and quasi-elastic light scattering. In this work, the self-assembly of Htz and β-CD occurred above the concentration of $\approx 10^{-4}$ mM. This phenomenon was verified when excess of β-CD was used in the presence of the higher hydrogen bonding formation molecule as Htz. At lower CD molar ratio the inclusion compound formation is the preferential phenomenon. Thus the Htz seems act as a seeding molecule capable of inducing the self-assembly phenomena, through hydrogen bonding formation between the inclusion compounds.

Abbreviations: β-CD: β-cyclodextrin; Htz: hydrochlorothiazide; δ: chemical shift; Δδ: change in chemical shift; $\Delta_{int}X^{\circ}$: Thermodynamic property of interaction (G, H, S); $\Delta_{\beta\text{-CD}}H^{\circ}$: Partial molar enthalpy of β-CD; I_s: Relative light scatter

Introduction

Supramolecular self-assembled species may be achieved by means of the association of two or more molecular entities through weak interaction such as electrostatic forces, hydrogen bonding, hydrophobic effect, or van der Waals interactions. As examples of these species, there is a range of synthetic and biologic complexes such as micelles, vesicles, membranes, bi-layers, enzymatic-complex and many kinds of host–guest complexes [1–3].

Among synthetic supramolecular systems, cyclodextrin (CD) complexes are very well known for their vast applications in food, pharmaceutical, and biological areas, because of their ability to form inclusion compounds with several guest molecules, which affords a controlled-sustained release profile [4–6].

Considering that the complexation phenomena changes the properties of complexated species if compared with the free form, these interactions have been broadly studied by several physical–chemical techniques in both solution (equilibrium solubility [7, 8], UV-Vis [9, 10], NMR [11, 12], isothermic titration calorimetry [13–16], liquid chromatography [17]) and solid state (IR [18] and Raman spectroscopy [19], differential scanning calorimetry [18], thermogravimetric analysis [9, 18]).

A key parameter of these kinds of complexes is their stoichiometry. However, the experimental determination of stoichiometry is not an easy task and present some complexation requirements such as high change of molar absorptivity of the guest (UV–Vis titrations [9, 20]), high change of chemical shift of the guest or CD (NMR titrations [11, 21]), high equilibrium constant (isothermic titration calorimetry [13–16, 22]), since it is necessary to differentiate free species from the complexes.

In general, these host–guest complexes are described in terms of simple stoichiometry such as 1:1, 1:2, 2:1, although it is accepted that in CD complexes solutions there is a distribution of several stoichiometries species and when there is no information about the stoichiometry, the 1:1 stoichiometry is commonly assumed [5, 6, 12, 23–26].

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These fixed stoichiometry considerations are made based on the relative size of the hosts and the cavity of CDs among others. However, it is important to remember that CD has an amphiphilic behavior, i. e., has a hydrophobic cavity and hydrophilic moiety on the outer region provided by primary and secondary hydroxyls. When complexated with guest molecules, at different molar ratios, it can show new possibilities of interaction with other substances in solution and in solid state.

In spite of the versatile host–guest systems, there are few reports in literature about the formation of self-assembly with more complex architecture [5, 27–30], and such possibilities have been little explored.

Thus, the goal of this work was to study the formation of complexes by β-CD and an higher hydrogen bonding model molecule, Htz (common diuretic antihypertensive drug, of the thiazide family with amine and sulfamide functional groups [31, 32]). Here, two aspects were evaluated in the system:

1. Short distance host–guest interactions, which were characterized by NMR techniques such as ^{1}H chemical shift, longitudinal relaxation times – T_1, 2D ^{1}H–^{1}H nuclear Overhauser spectroscopy, and thermodynamic solubility experiments in which the host–guest thermodynamic parameters were calculated.
2. Self-assembly of host–guest complex, which was observed in excess of CD by isothermal titration calorimetry (ITC) and quasi-elastic light scatter.

Experimental

Materials

All the reagents used were of analytical grade (β-CD was purchased from CERESTAR®, USA and Htz from GALENA Pharmaceutical Laboratory, Brazil. Purity was confirmed by elementary analysis CHN and by ^{1}H-NMR spectra, which did not reveal any impurities. β-CD NMR ^{1}H spectrum was attributed according to reference [11].

Nuclear magnetic resonance

NMR spectra and T_1 measures were obtained in triplicate and 2D-NOESY in duplicate, at 298.15 K on a Brucker DPX 200 (200 MHz) spectrometer with the Bruker software package XWIN-NMR. The solutions used were 2.0×10^{-3} mol.L^{-1} of Htz and 2.0×10^{-3} mol.L^{-1} (1:1) of Htz/β-CD, both dissolved in D_2O (Cambridge Isotope Laboratories, Inc – 99.9% of isotopic purity). The water signal at $\delta = 4.80$ was used as reference. T_1 experiments and mixing time (300 ms) in NOESY experiments were both calculated by using the inversion-recovery sequence (90- τ-180) [33, 34]. The mixing time parameter is the time interval given during the pulse sequences, while the evolutions of the dipolar coupling through space, among hydrogen nuclei within a distance of up to 5 Å occurs [33–35].

Thermodynamics solubility

Saturated Htz aqueous solutions were prepared in several concentrations of β-CD from 0.0 to 12.3×10^{-3} mol.L^{-1}. β-CD concentration was increased in steps of 8.8×10^{-4} mol.L^{-1}. Solutions were left in rest at 293.0, 298.0 and 303.0 $\pm$ 0.1 K in a thermostatic bath for 48 h to reach thermodynamic equilibrium. Next the solutions were filtered. The Beer–Lambert law dilution factor used was 1/100. Quantification was performed in triplicate on an UV–Vis equipment HP-8453 at 226 nm and cell with 1 cm.

Isothermal titration calorimetry

The isothermal titration calorimetry was accomplished in duplicate in a Microcal Microcalorimeter VP-ITC. The solutions were prepared by dissolution of the chemicals in milli-Q water. The concentrations were verified by UV–Vis spectroscopy as discussed above. Titration was accomplished by injecting solutions of 5 μL of CD (12.3×10^{-3} mol.L^{-1}) into solutions of Htz (2.00×10^{-3}, 0.67×10^{-3} and 0.20×10^{3} mol.L^{-1}) after the electrical and chemical calibration of the calorimeter. The initial 1 μL injection was discarded from each dataset in order to eliminate the effect of titrant diffusion across the syringe tip during the pre-equilibration process. The initial cell volume was 1.6 mL. The concentration correction as well as the integration of the heat flow peaks involved in partial molar enthalpy of βCD – (dQ/d[β-CD] = $\Delta_{\beta\text{-CD}}H^{\circ}$), were made with software Microcal Origin 5.0 for ITC.

Static light scattering

The relative intensities of *static light scattering* was made with a Light Scatter photometer constituted of a Helium/Neonium laser λ = 632 Å (Spectra-Physics, model 127), a photomultiplier cell as a detector positioned in a goniometer BI-200SM (Brook Haven Inst. Co.) at 90° to the sample. Data acquisition was made by a computer with a correlation plate BI9000-AT (Brook Haven Inst. Co.). The solutions were prepared by dissolving the chemicals in Milli-Q water. They presented a constant Htz concentration of 0.67×10^{-3} mol.L^{-1} and several gradative concentrations of CD ranging from 0.0 to 4.1×10^{-3} mol.L^{-1}.

Results and discussion

Mode of host–guest interaction – NMR and solubility studies

NMR results

The 1D and 2D NMR experiments were performed to characterize the complex at molecular level (host–guest interactions). Figure 1a and 1b show the ^{1}H-NMR spectra of Htz/D_2O and Htz/ β-CD/D_2O.

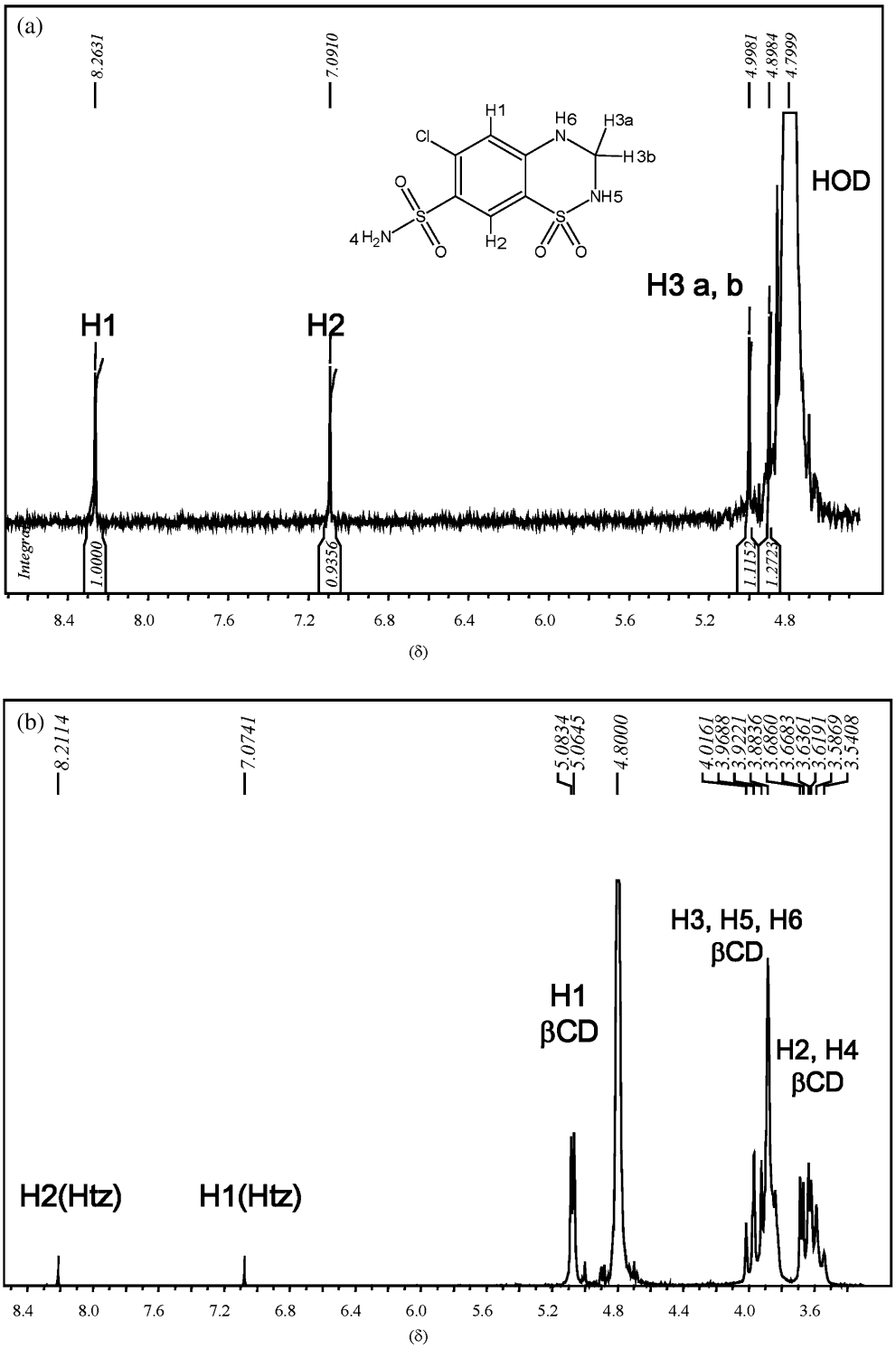

Figure 1. ^{1}H-NMR 200 MHz of (a) pure Htz, and (b) Htz/β-CD (molar ratio 1:1), both in D_2O solvent.

Table 1 lists the chemical shift values for Htz hydrogens 1 and 2 in the Htz/D_2O and Htz/β-CD/D_2O systems, in which changes in chemical shifts in presence of β-CD were observed. The H3 Htz chemical shift at $\delta \approx 4.99$ was not analyzed in this work because this is quite near the strong HOD peak. It is well known that chemical shift supplies information about the magnetic and chemical environment at the nucleus. Thus, the change in chemical shift when comparing Htz/D_2O and Htz/β-CD/D_2O systems was attributed to the disturbance caused by unpaired oxygen electrons from the CD cavity (C1–O–C4), as well related and reviewed in the literature [11, 36].

Relaxation time measurements were made aiming to monitor the molecular mobility of Htz in the absence and in the presence of β-CD.

For organic molecules whose relaxation process occurs principally by dipolar relaxation mechanism, the

Table 1. [1]H-chemical shifts (200 MHz) of H1 and H2 signals for Htz/D_2O and Htz/β-CD/D_2O systems

Pure Htz hydrogens δ^a	Htz/CI/Hydrogens δ^a	$\Delta\delta$	Maximal standard deviation[a]
H1: 7.098 ± 0.007	7.077 ± 0.005	−0.059	0.005
H2: 8.269 ± 0.006	8.21 ± 0.01	−0.021	0.01

[a]^{1}H-NMR experiments were performed in triplicate. The maximal standard deviation (S. D.) refers to the largest value between the two standard deviations of Htz/D_2O and Htz/β-CD/D_2O.

longitudinal relaxation time constants are either inversely proportional to the molecular correlation time – τ_c or directly proportional to the rotational diffusion coefficient – D_{rot} [33, 34], according to Equation (1):

$$T_1(DD)^* \propto \frac{1}{\tau_c} \propto D_{rot} \quad (1)$$

Smaller T_1 values of Htz it was observed upon complexation with β-CD if compared with pure Htz (Table 2), showing that complexation leads the reduction of the mobility of Htz due the formation of a new specie with a new size and symmetry, and consequently, a new molecular dynamics.

2D-NOESY experiments allowed to observe the 5 Å spatial proximity limit among the functional groups of Htz and β-CD [11, 33–35]. Figure 2 shows the expansion contour map of the NOESY experiment of Htz/β-CD/D_2O system. Through this experiment, it was observed that there is a correlation between Htz H1 and H2 aromatic hydrogens and H3, H5, inside the β-CD cavity demonstrating penetration of Htz into the β-CD cavity.

Thermodynamic solubility results

Thermodynamic solubility experiments allow to demonstrate quantitatively the hydrotrope effect [37, 38] on the solubility of a substance and evaluate the interactions between the species through an apparent equilibrium constant, considering previously fixed stoichiometry.

Although there are constraints introduced by suppositions about the stoichiometry and the thermodynamic control of the composition of the liquid phase by a solid phase [37], this is an interesting experiment widely used in the analysis of complexation phenomena and it allows the analysis of the complexation phenomena in terms of host–guest interaction.

Figure 3 shows the solubility of Htz against β-CD concentration. This diagram is a linear relation corresponding to the formation of complexes more soluble than the pure substrate and of first-order in term of CD stoichiometry, Higuchi's A_L type [37].

Using the stoichiometry value of Htz1: β-CD(1:1), the equilibrium constant was calculated by the classical equation of Higuchi and Connors [37]:

Table 2. T_1values for H1 and H2 Htz hydrogens in Htz/D_2O and Htz/ β-CD/D_2O systems

Hydrogen	T_1/s (Htz/D_2O)	T_1/s (Htz/ β-CD/D_20)	ΔT_1/s
H1	5.8 ± 0.7	0.90 ± 0.02	−4.94
H2	12.3 ± 2.0	1.11 ± 0.05	−11.14

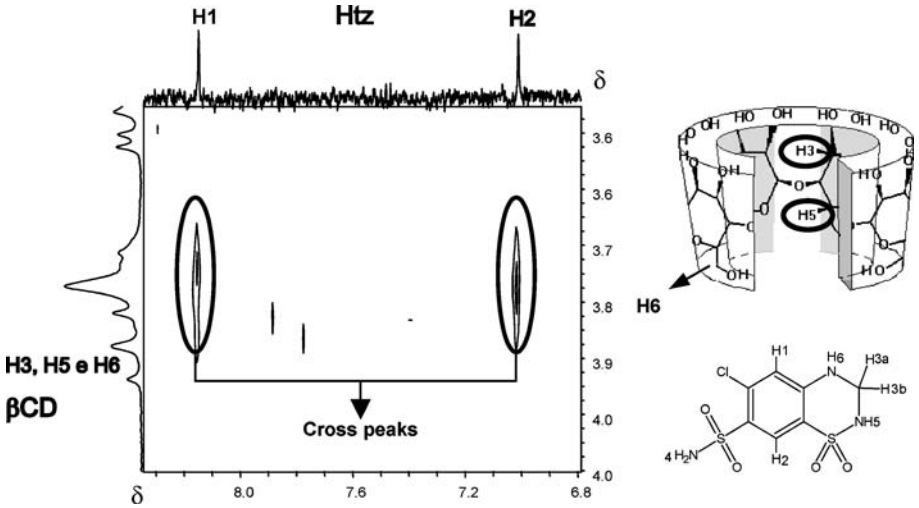

Figure 2. Expansion of the contour map RMN NOESY (200 MHz, D8 = 300 ms) of the Htz/β-CD/D_2O system.

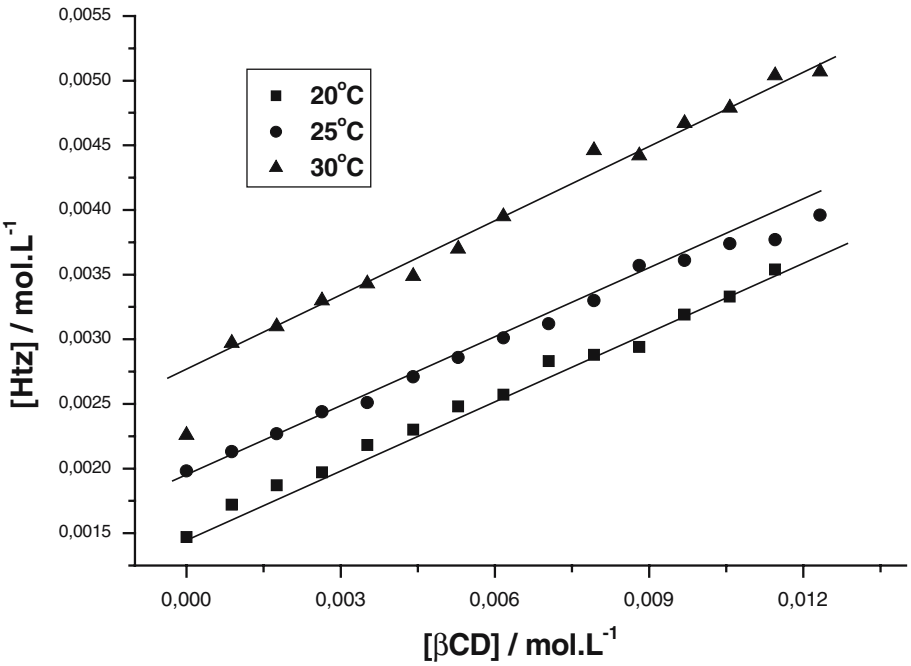

Figure 3. Solubility experiments of [Htz] vs. [β-CD] at 293.15, 298.15 e 303.15 ± 0.1 K.

$$\text{Angular Coefficient} = \frac{mKS_0^m}{1 + KS_0^m}$$

where K is the association constant, S_0 the substrate solubility in the absence of hydrotrope, and m the stoichiometric coefficient in terms of the substrate. The angular coefficient was determined by linear fitting.

With K values, it was calculated the thermodynamics partial molar interaction energies ($\Delta_{int}G^\circ$, $\Delta_{int}H^\circ$ and $T\Delta_{int}S^\circ$) between the species at 293.15, 298.15 and 303.15 ± 0.1 K with the use of Equations (3)–(5). The molar partial interaction enthalpy of the species was assumed not to vary at the temperature ratio studied to allow the use of Van't Hoff equation (Equation 4) [39].

$$\Delta_{int}G^\circ = -RT \ln K \quad (3)$$

$$\frac{\partial \ln K}{\partial(1/T)} = -\frac{\Delta_{int}H^\circ}{R} \quad (4)$$

$$\Delta_{int}G^\circ = \Delta_{int}H^\circ - T\Delta_{int}S^\circ \quad (5)$$

Table 3 shows the thermodynamic interaction values calculated for Htz and β-CD at the stoichiometry assumed (1:1), which are similar to previous data reported in the literature [40a and 40b].

The single fact that the equilibrium constants decrease with the temperature is shows that process is exothermic [39].

By analysis of the numerical data, independently of the stoichiometry assumed, in all cases it was seen that the association process is enthalpy driven, and it needs to be sufficiently large to overcome the entropy lost in the process. Thus, the host–guest specific interaction is certainly an important contribution to the total interaction process.

Host–guest interaction have generated some discomfort in the literature since there is a difficult in explaining an "enthalpic hydrophobic interaction". The explanation given in the literature is that the enthalpy driven interaction is due to release of enthalpy-rich water molecules from the CD cavity [13, 14, 25]. These water molecules present in the CD cavity do not satisfy their total hydrogen bond potential formation between itself and the C_1–O–C_4 oxygen, due to the ring tension. In addition, the interior of the cavity has hydrophobic hydrogens H3 and H5 that do not contribute with strong interaction energy. The inclusion compounds formation can be expressed by the follow chemical equilibrium equation:

$$S + \beta\text{-CD} \cdot n H_2O \rightleftharpoons S\text{-}\beta\text{-CD} + n H_2O$$

Table 3. Thermodynamic interaction parameters of Htz and β-CD calculated for a 1:1 stoichiometry

Temperature/K	$K_{1:1}$	$\Delta_{int}G^\circ_{1:1}$ (kJ mol^{-1})	$\Delta_{int}H^\circ_{1:1}$ (kJ mol^{-1})	$T\Delta_{int}S^\circ_{1:1}$ (kJ mol^{-1})
293.15	259.1	−13.5	–	−33.7
298.15	168.5	−12.7	−47.3	−34.6
303.15	140.4	−12.4	–	−34.8

The water molecules released during the complexation process can now make stronger hydrogen bonds with the lattice water molecules, which partially explain the enthalpic character of this process. Another contribution that arises is the dipolar interaction of the guest with the inner of the CD cavity, as demonstrated by Rekharsky and Ynoue [14], who found different Gibbs free energy of interaction as a function of the size of CD cavity for a same guest.

Referring now to the entropic term, it is considered as resulting from at last three aspects:

1. Reduction of the number of species in the reaction product (two species in the reagents and one specie in the product for stoichiometry 1:1)

$$S + \beta\text{-CD} \rightleftharpoons S\text{-}\beta\text{-CD}$$

2. Resolvation of the inclusion compound.
3. Lost of rotational and translational mobility of the species when the inclusion compound is formed. The rotational diffusion reduction was proven by the T_1-NMR experiments discussed above.

Mode of self-assembly interaction – ITC and QELS

Isothermal titration calorimetry

ITC is a powerful technique used to study the complexation thermodynamics in both biological and synthetic supramolecular systems [13–16, 22, 41].

The ITC curves for systems with very well-defined stoichiometries normally show a sigmoidal profile whose plateau difference and inflection are the enthalpy and stoichiometry of the process, respectively [22, 42].

The dilution experiment of β-CD (Figure 4) and Htz (data not shown) in water demonstrates heat patterns typical of dilution of simple weak enthalpic interacting substances [41], supporting the assumption that the species do not self-associate under the experimental condition employed in present work.

Figure 4 shows the ITC data of a β-CD titration in several concentrations of Htz (at 298.15 K). The curves did not have the sigmoidal profile, which hindered the determination of the thermodynamic parameters by non-linear regression [22, 40].

However, important information can be obtained from the data. The interaction between Htz and β-CD is an exothermic process, but as the interactions are concentration dependent, $\Delta_{\beta\text{-CD}}H^\circ$ does not assume constant values.

The expansion of the curve (Figure 5) demonstrates that initially there is an inflexion that apparently splits the curve into two ranges with different behavior as a function of CD concentration. These results led us to design the hypothesis that host–guest system has at least two kind of domain in function of CD concentration.

The first domain is characterized by high values of $\Delta_{\beta\text{-CD}}H^\circ$, attributed to the host–guest interaction, which occur due the formation of hydrogen bonds between Htz sulfonamide hydrogens and C_1–O–C_4 oxygens of CD's, additional van der Waals interaction with the aromatic groups of Htz and formation of hydrogen bond between released highly energetic water molecules from cavity of β-CD with lattice water molecules agreeing with the Loftson et al. observations [25] and thermodynamic data calculated by solubility experiment. If only these interactions occurred, it would be observed by the presence of a plateau in $\Delta_{\beta\text{-CD}}H^\circ$ curves

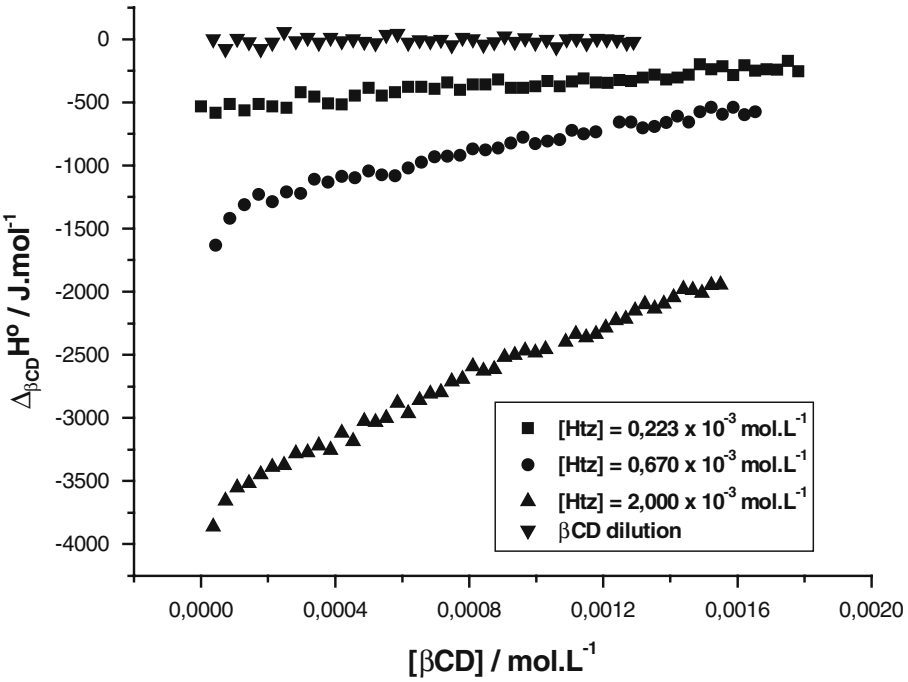

Figure 4. ITC experiments to β-CD 12.3 mM in (▼) water, (■) Htz 0.223×10^{-3} mol.L^{-1}, (●) Htz 0.67×10^{-3} mol.L^{-1} and (▲) Htz 2×10^{-3} mol.L^{-1}.

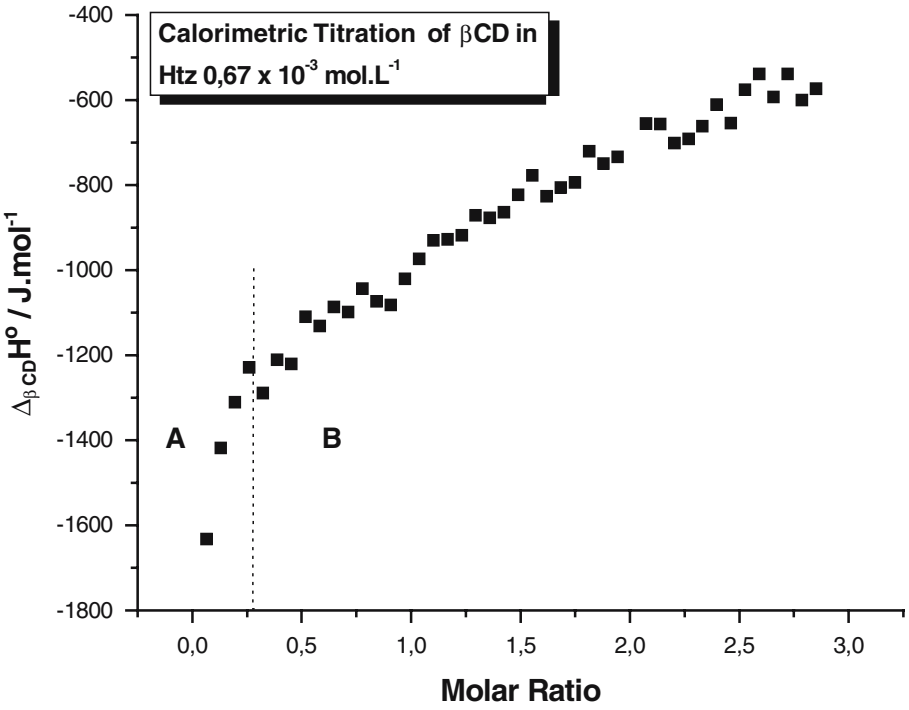

Figure 5. ITC experiments expansion to β-CD 12.3×10^{-3} mol.L^{-1} in (■) Htz 0.67×10^{-3} mol.L^{-1}.

even at such high concentrations. In this case, it is supposed that the inclusion compound is formed simultaneously to other processes.

At all Htz concentrations, the injection calorimetry curve profile never reached the value of dilution of β-CD, showing that even high concentrations of CDs, exothermic event always happen.

These results could be explained based on the physical–chemical characteristics of CD and its solvent interactions. First CD molecules are very rich in hydroxyl groups, and second it is thought that the inclusion compounds can associate through the hydrogen bonding, which is a characteristic exothermic process.

In the second domain of concentration, it is supposed that there is no free Htz species. As the concentration of β-CD is increased continuously by titration, the probability of multiple equilibrium is possible:

$$\beta\mathrm{CD} \cdot n\mathrm{H_2O} + \mathrm{Htz} \cdot m\mathrm{H_2O} \rightleftharpoons \beta\mathrm{CD} \cdot \mathrm{Htz} \cdot x\mathrm{H_2O} + [(n+m) - x]\mathrm{H_2O}$$

$$\beta\mathrm{CD} \cdot \mathrm{Htz} \cdot x\mathrm{H_2O} + w\beta\mathrm{CD} \cdot n\mathrm{H_2O} \rightleftharpoons \beta\mathrm{CD}_{(1+w)} \cdot \mathrm{Htz} \cdot y\mathrm{H_2O} + [(x+wn) - y]\mathrm{H_2O}$$

where y is larger than x and $\beta\mathrm{CD}_{(1+w)} \cdot \mathrm{Htz} \cdot y\mathrm{H_2O}$ can be some kind of tertiary supramolecular arrangement.

Complexes having stoichiometry larger than 1:1 must be driven by the increase enthalpy resulting of strong interactions or desolvation entropy. If the module of $\Delta_{\beta\text{-CD}}\mathrm{H}^\circ$ becomes smaller with the increase in concentration, the entropy must start to act to stabilize the system. May be in these situations, the reduction of entropy due to complexation and the loss of mobility will be balanced by the desolvation of outer water molecules of the interacting host–guest complexes.

Reckharsky et al. [41] have shown that the entropy is a driving force in the formation of high stoichiometry complexes, when the concentration of one of the components of the system is smaller, for citrate/tris-guanidinium system. His attribution was due to the desolvation phenomena during the sequential complexation.

In the β-CD/Htz system, subsequent complexation upon initial host–guest interaction could be occurring during the ITC experiment, when the concentration of β-CD is increasing and keeping the Htz concentration constant. In this complexation, the endothermic desolvation of outer water molecules of the complexes could be leading to a reduction of $\Delta_{\beta\text{-CD}}\mathrm{H}^\circ$ module.

In solubility experiment condition, there is no possibility for the self-assembly formation because there is a precipitate which confers an indefinite quantity of Htz, which prevents that the concentration of β-CD becomes larger than Htz concentration.

In the absence of Htz, self-assembly formation was not observed. These results suggest strongly that the Htz induces the aggregation phenomenon. Htz molecules must be contributing for the formation of these tertiary supramolecular arrangements thorough formation of hydrogen bonds with the amine groups, since as demonstrated by NOESY, the most probable interaction of Htz with β-CD is through the introduction of aromatic group into the cavity of CD.

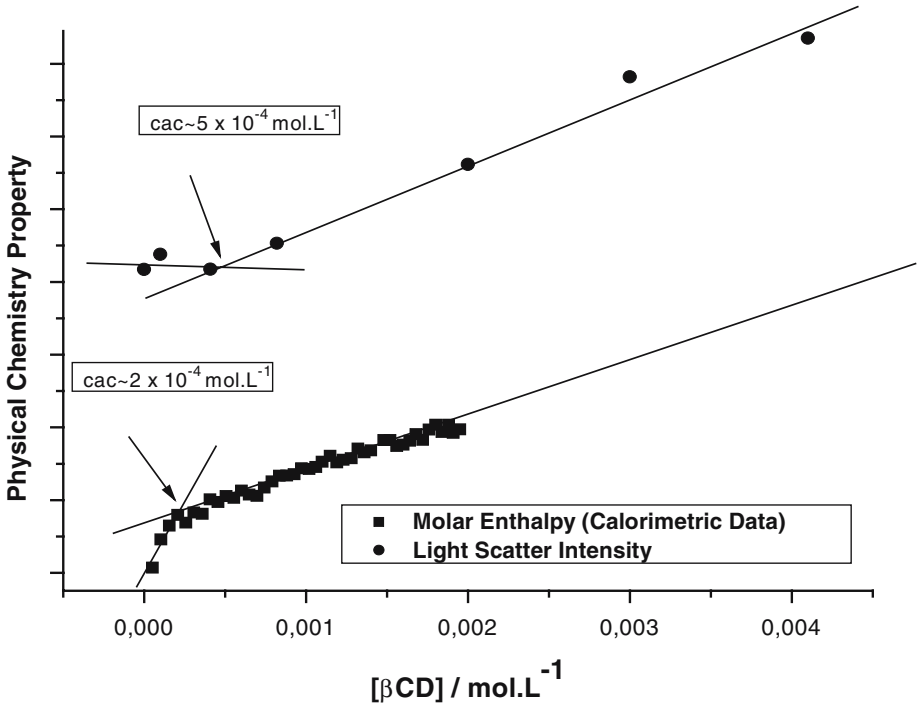

Figure 6. Physical–chemistry properties plotted against the β-CD concentration in presence of the 0.67×10^{-3} mol.L^{-1} of Htz.

Quasi-elastic light experiment

To confirm the self-assembly phenomenon hypothesis, quasi-elastic light experiment at 298.15 K was performed. This was done in conditions near to those of the calorimetric experiment for comparison sake.

The light scatter intensity I_s, of a polydisperse solution is given by the following equation [43]:

$$I_s = \frac{c}{\left[\frac{1}{M_w} + 2Bc + \cdots\right]\left[1 + \frac{16\pi^2\langle Rg^2\rangle \mathrm{sen}^2(\theta/2)}{2\lambda^2}\right]}$$

where c denotes the concentration, M_w is the weight average molecular weight, B is the second virial coefficient, Rg is the radius of gyration, θ is the scattering angle and λ is the wavelength.

In this work, it was assumed that the size of the particles was much smaller than the light wavelength. Thus, the second term of the denominator can be neglected. It was also assumed that the concentration range was very low and consequently, the scattering intensity was directly proportional to the concentration of the solutions with negligible interactions between particles ($B \rightarrow 0$) and angular coefficient directly proportional to the weight molecular.

$$I_s \propto \mathrm{M_w}[\beta\text{-CD}]$$

Variation in the relative light scattering intensity as a function of CD concentration and the partial molar enthalpy of β-CD by ITC experiment, in the presence of Htz (0.67 mM), it were plotted on Figure 6. Splitting on both curves into two ranges, at certain β-CD concentration range ($\approx 10^{-4}$ mM) it was observed. These results are suggesting two kind of domains: first domain due to host–guest inclusion compound and the second one to the self-assembly formation.

Conclusions

In this work, the self-assembly of Htz and β-CD was described and it occurred above the concentration of $\approx 10^{-4}$ mM. This phenomenon occurred when excess of β-CD was used in the presence of the higher bonding formation molecule as Htz. At lower CD molar ratio the inclusion compound formation is the preferential phenomenon. Thus the Htz seems act as a seeding molecule capable of inducing the self-assembly phenomena, through hydrogen bonding formation between the inclusion compounds.

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