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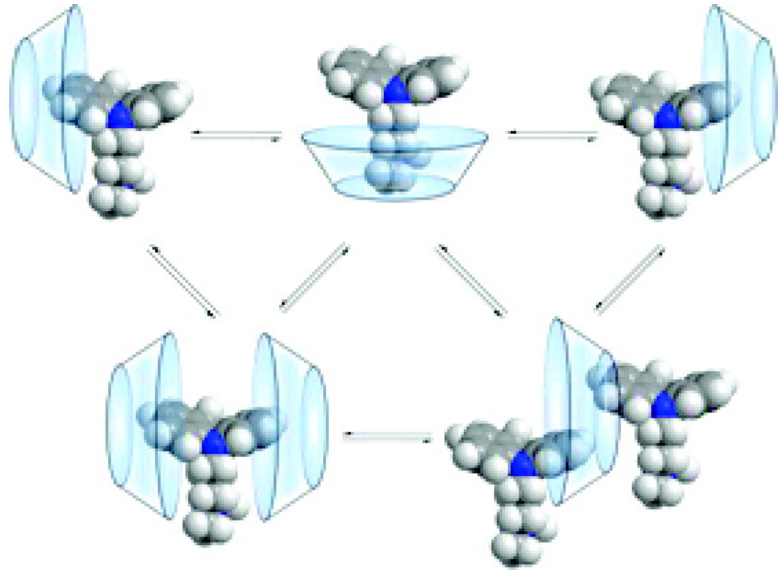

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Supramolecular Self-Assembly of Cyclodextrin and Higher Water Soluble Guest: Thermodynamics and Topological Studies

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Abstract: The supramolecular interactions between Imipramine hydrochloride (IMI), a tricyclic antidepressant, and β-cyclodextrin (βCD) have been investigated by experimental techniques and theoretical calculations. The association between these molecules might be lead to a host/guest compound, in which the physical chemistry properties of the guest molecule, such as high solubility, can be decreased. These new properties acquired by the inclusion phenomena are important to develop a strategy for pharmaceutical formulation. Nuclear magnetic resonance and horizontal attenuated total reflectance provided relevant information on the complex stoichiometries and the sites of interactions between the host and guest molecules. Stoichiometries of 1:2, 1:1, and 2:1 βCD/IMI have been detected in solution. Self-diffusion coefficient and dynamic light scattering analysis provided information on the self-aggregation of the complex. Also, isothermal titration calorimetry studies indicated the existence of equilibrium between different complexes in solution. In order to determine the preferred arrangement for the inclusion complex formed by the IMI molecule and βCD, theoretical calculations were performed. Of all proposed supramolecular structures, the 2:1 βCD/IMI complex was calculated to be the most energetically favorable, in both gas and aqueous phases. The calculations indicated that the intermolecular hydrogen bonds involving the hydroxyl groups of βCD play a major role in stabilizing the supramolecular 2:1 structure, corroborating experimental findings.

1. Introduction

Understanding supramolecular topology and stoichiometry is fundamental for the design and control of supramolecular systems.[1] The interactions that occur between molecules in order to assemble the supramolecular systems determine their properties, which can be quite different from those of the individual molecular species.[2] Among these properties, one can highlight changes in solubility, reactivity, volatilization, and also, when in the solid state, the microcrystalline structure.[3] In general, supramolecular systems can be generated by various interactions, which can involve simultaneous interaction between two or more moieties.[4] The main interactions observed in the supramolecular systems are electrostatic interactions, hydrogen bonding, van der Waals forces, and hydrophobic interactions.[5]

One of the most investigated classes of molecules, which possess the ability to form a supramolecular system, are cyclodextrins (CDs), naturally occurring water-soluble oligosaccharides.[6] Such molecules have a toroidal shape with a hydrophobic cavity, into which can be inserted a variety of organic and inorganic guest molecules, with formation of inclusion complexes (ICs).[3,7–9] The hydrophilic nature of the outer surface of the cyclic structure makes the cyclodextrins somewhat water-soluble.

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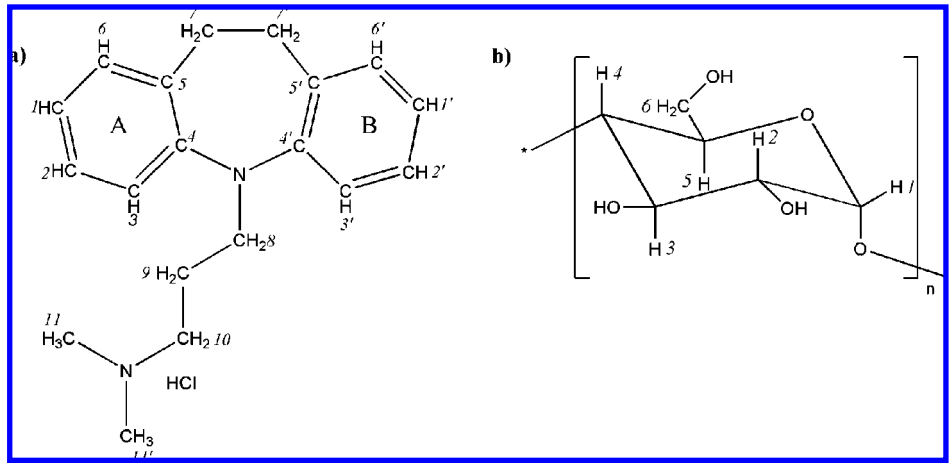

Figure 1. Structure of (a) Imipramine hydrochloride and (b) β-cyclodextrin monomer.

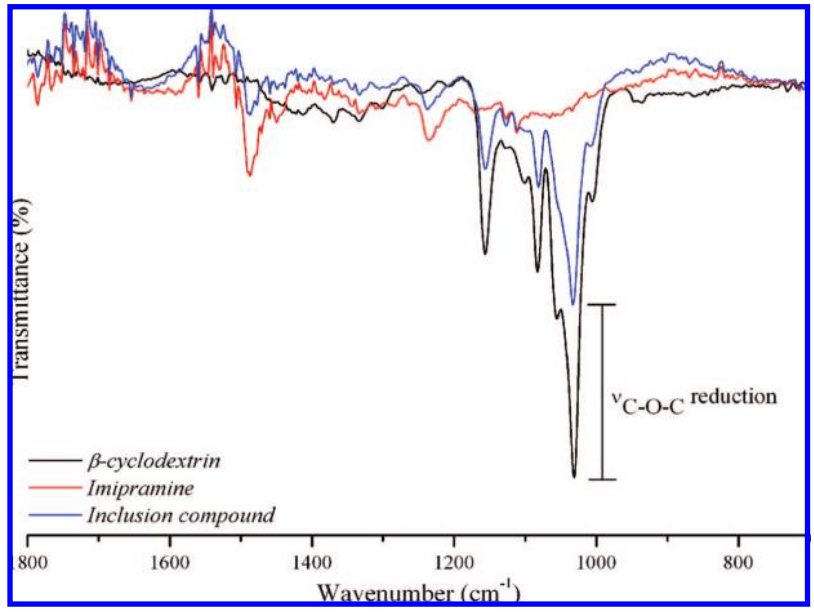

Figure 2. Comparison of the FTIR-HATR spectra of βCD 6.65, IMI 13.35 mM, and the respective IC, at 1600−800 cm^{-1} region.

CDs have been extensively used in several research areas. These compounds find important applications in particular in biochemistry and drug research, due to their multifunctional characteristics and bioadaptability. The study of drug inclusion with pharmaceutical activity is one of the most extensively researched fields. The main advantages in using CDs in pharmaceutical formulations include enhanced aqueous solubility, bioavailability, and stability.[10] In addition, from the viewpoint of pharmacotherapy optimization, drug release should be controlled in accordance with the therapeutics proposed and the pharmacological properties of active substances.[11]

Another significant research area is the self-assembly of inclusion complexes to give high molecular weight species.[12] Recently the self-aggregation of CDs themselves has also been postulated.[13,14] Self-aggregation could fundamentally effect the release of the guest molecule from the nanostructured matrix, and it could account for the sustained release profiles of several pharmaceutical compounds from IC formulations. Thus, it is crucial to understand the nature of these interactions, which lead to the formation of high molecular weight systems.

In this article we report on the complexation between β-cyclodextrin (βCD) and Imipramine (IMI), Figure 1, and their topologies and stoichiometry. IMI is a tricyclic antidepressant (TCA): it is only effective in 60−70% of cases and is in use today.[15] This class of drug is increasingly prescribed for multiple indications but is responsible for many pediatric poisoning fatalities.[16] In order to design a more advanced dosage form and to reduce the drawbacks from its administration, the association of IMI with βCD can be considered an alternative

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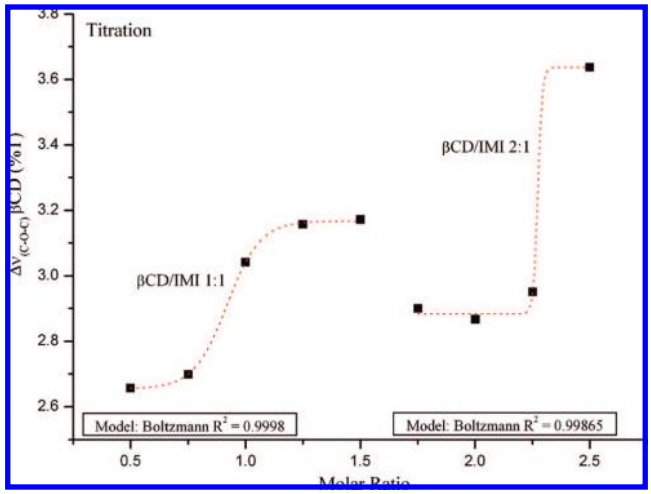


Figure 3. Titration process of IC, FTIR-HATR transmittance (at 1030 cm^{-1}) versus molar ratio (IMI/βCD).

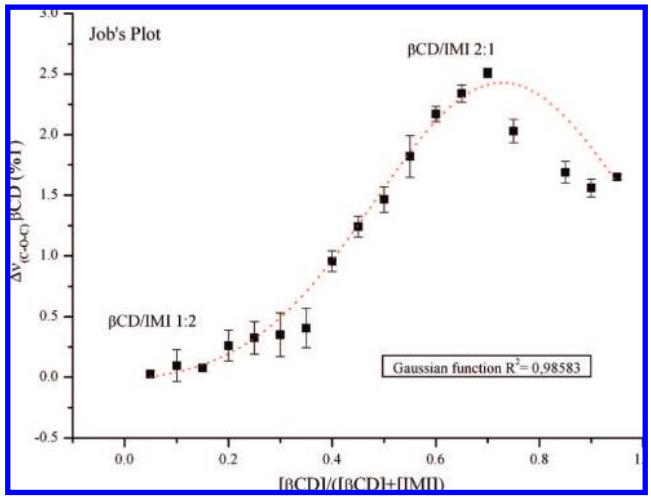
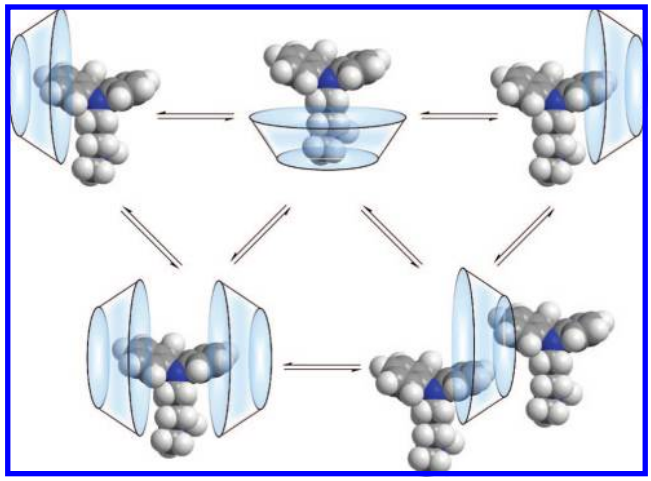


Figure 4. Continuous variation plot (Job plot) obtained from the βCD FTIR-HATR transmittance at 1030 cm^{-1}.

Scheme 1. Most probable supramolecular complexes (1:1; 2:1, and 1:2 βCD/IMI) which occur in solution



strategy for pharmaceutical formulation. It is also important to mention that the high solubility of IMI is not a desirable feature for a drug delivery system, since it reduces its biodisponibility.

Table 1. ^{1}H NMR (at 400 MHz, in D_2O) Chemical Shifts of IMI and ICs at 1:1 and 2:1 βCD/IMI at 27.0 °C

hydrogens	δ IMI	δ IC 1:1	$\Delta\delta$ IC 1:1[a]	δ IC 2:1	$\Delta\delta$ IC 2:1[b]
1/1′	6.92	7.03	−0.11	7.04	−0.12
2/2′	7.12	7.19	−0.07	7.19	−0.07
3/3′	7.04	7.16	−0.12	7.14	−0.10
6/6′	7.06	7.16	−0.10	7.14	−0.08
7/7′	2.99	3.23	−0.24	3.25	−0.26
8	3.64	3.97	−0.30	4.00	−0.36
9	1.78	2.06	−0.28	2.08	−0.30
10	2.88	3.15	−0.27	3.17	−0.29
11/11′	2.56	2.75	−0.19	2.77	−0.21

[a] $\Delta\delta = \delta$ IMI - δ 1:1 βCD/IMI. [b] $\Delta\delta = \delta$ IMI - δ 2:1 βCD/IMI.

Another focus of our work was the determination of whether the simple inclusion compounds were able to form high molecular weight aggregates. In order to investigate and characterize the βCD/IMI IC a variety of experimental techniques have been used: nuclear magnetic resonance techniques (^{1}H, spin−lattice relaxation times, nuclear Overhauser effect, and diffusion-ordered spectroscopy),[8,17] horizontal attenuated total reflectance,[18,19] dynamic light scattering, and also isothermal titration calorimetry.[17] In addition, all topologies and stoichiometries proposed for the ICs were also investigated by theoretical calculations carried out at semiempirical and density functional theory (DFT) levels of theory.

2. Experimental Section

Reagents and Inclusion Compounds Preparation. Imipramine hydrochloride (IMI) and β-cyclodextrin (βCD) were obtained from Galena, Minas Gerais (Brazil), and Xiamen Mchem, Xiamen (China), respectively. All ICs were prepared by the freeze-drying method using 0.5:1, 1:1, and 2:1 βCD/IMI molar ratios. An aqueous solution of the components, βCD and IMI, was stirred for 4 h to ensure that equilibrium had been reached and then were frozen in liquid nitrogen and lyophilized (Savant ModulyoD - Freeze-Dryer, Thermo Electron Corp., Waltham, MA, USA) for 48 h.

FTIR-HATR Spectroscopy. A Perkin-Elmer spectrophotometer model (Spectrum GX; Perkin-Elmer, Boston, MA, USA), with a KBr beam splitter, equipped with a Horizontal Attenuated Total Reflectance (HATR) accessory with a ZnSe cell was used to acquire all spectra. The spectra, 128 scans, were recorded in the range 4000−590 cm^{-1}, with a resolution of 4 cm^{-1}. The reference spectrum of pure water was subtracted from each spectrum obtained during the titration process. To reduce atmosphere effects, the sample detector was purged with N_2, before collecting the background and titration spectra. All spectra were recorded in triplicate at room temperature, 23.0 ± 1.0 °C.[18,19]

Two methodologies were used to investigate the βCD/IMI inclusion complex: (i) the Job (continuous variation) plot, using βCD ν_{COC} at 1030 cm^{-1}: the total molar concentration of the two components was kept constant (10.0 mM) but the molar fraction of βCD {[βCD]/([βCD] + [IMI])} varied from 0.05 to 0.95;[20] (ii) titration of βCD with IMI in which the concentrations of the species, βCD and IMI, varied from 12.0 to 6.0 mM and 2.7 to 15.0 mM, respectively. These two methodologies were used not only to investigate the vibrational spectral changes but also to estimate the existence of different stoichiometries in aqueous solution.

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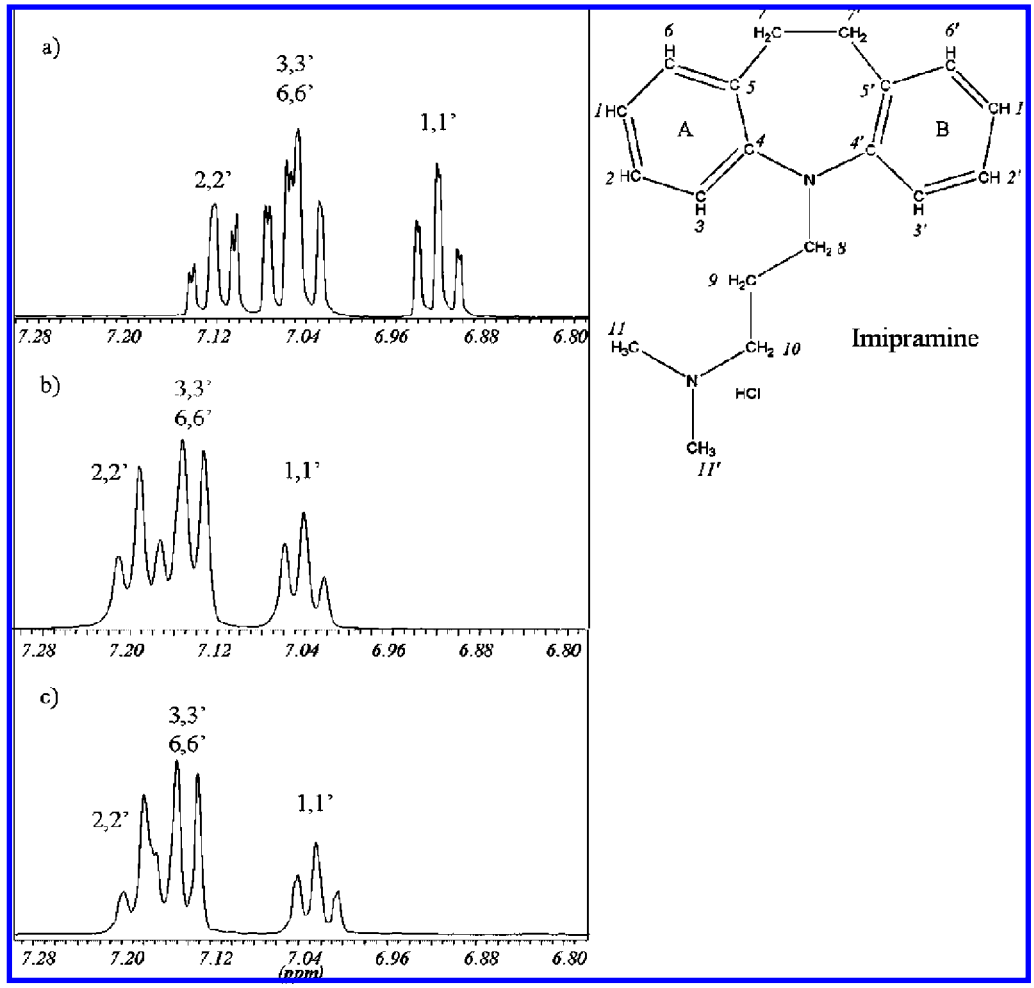

Figure 5. Expansion of ^{1}H NMR spectra (at 400 MHz in D_2O) at $\delta = 6.80-7.30$ to (a) pure IMI, (b) IC at 1:1 βCD/IMI molar ratio, and (c) 2:1 βCD/IMI molar ratio.

Table 2. T_1 Values to IMI, ICs at 1:1 and 2:1 βCD/IMI, and the ΔT_1/s for each system

hydrogens	T_1/s IMI	T_1/s IC 1:1	$\Delta T_1{}^a$/s	T_1/s IC 2:1	$\Delta T_1{}^b$/s
1/1′	1.76	1.27	0.49	1.10	0.66
2/2′	1.67	1.03	0.64	1.09	0.58
3/3′					
6/6′					
7/7′	0.62	0.43	0.19	0.38	0.24
8	0.37	0.24	0.13	0.22	0.15
9	0.50	0.36	0.14	0.32	0.18
10	0.64	0.45	0.19	0.40	0.24
11/11′	0.91	0.84	0.07	0.77	0.14

^{a} ΔT_1/s = T_1/s IMI − T_1/s IC 1:1. ^{b} ΔT_1/s = T_1/s IMI − T_1/s IC 2:1.

Nuclear Magnetic Resonance Spectroscopy. The ICs prepared by the freeze-drying method were dissolved in D_2O (Cambridge Isotope Laboratories, Inc. - 99.9% of isotopic purity) and used to investigate the inclusion process by nuclear magnetic resonance spectroscopy.

NMR spectra were recorded at 27.0 °C on a Bruker DRX 400 - AVANCE spectrometer (Bruker BioSpin, Rheinstetten, Germany) operating at 400 MHz, equipped with a 5 mm inverse probe with a z-gradient coil. ^{1}H NMR spectra were achieved using the WATERGATE technique for suppression of the residual water signal.[21,22] The nuclear Overhauser enhancement spectroscopy (2D NOESY) experiments (mixing time 500 ms) and spin−lattice relaxation times (T_1) were acquired using standard experiments from the spectrometer library.[23,24] Diffusion-ordered spectroscopy (DOSY) experiments were performed using the simulated echo with a bipolar gradient pulse pair sequence, modified with an introduction of a presaturation pulse for solvent signal suppression (STEBPGP1S).[25,26] The magnetic field pulse gradients duration (δ) and the diffusion times (D) were optimized for each sample in order to obtain a complete signals dephasing with the maximum gradient strength. A series of 16 STEBPGP1S spectra on 16 K data points were collected in each NMR experiment, and the δ and D values were 3 and 33 ms, respectively. The pulses gradients were incremented from 2 to 95% of the gradient strength in a linear ramp.

Dynamic Light Scattering. The dynamic light scattering (DLS) measurements were performed in a Zetasizer ZS Nano Series (Malvern Instruments Ltd., United Kingdom), using a polyethylene square cell to measure the average hydrodynamic diameter of pure IMI and the 1:1 βCD/IMI complex. The solutions were subjected to scattering by a monochromatic light (10 mW He−Ne laser,

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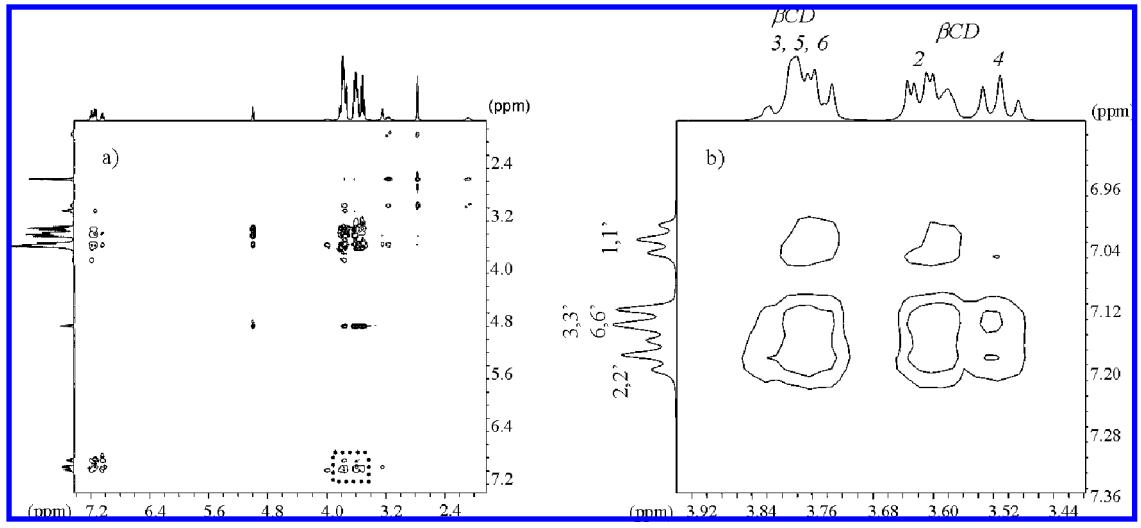

Figure 6. (a) NMR 2D NOESY contour map (at 400 MHz, mixing time 500 ms) in D_2O of βCD/IMI inclusion complex and (b) expansion of the NMR 2D NOESY contour map of the IMI aromatic region.

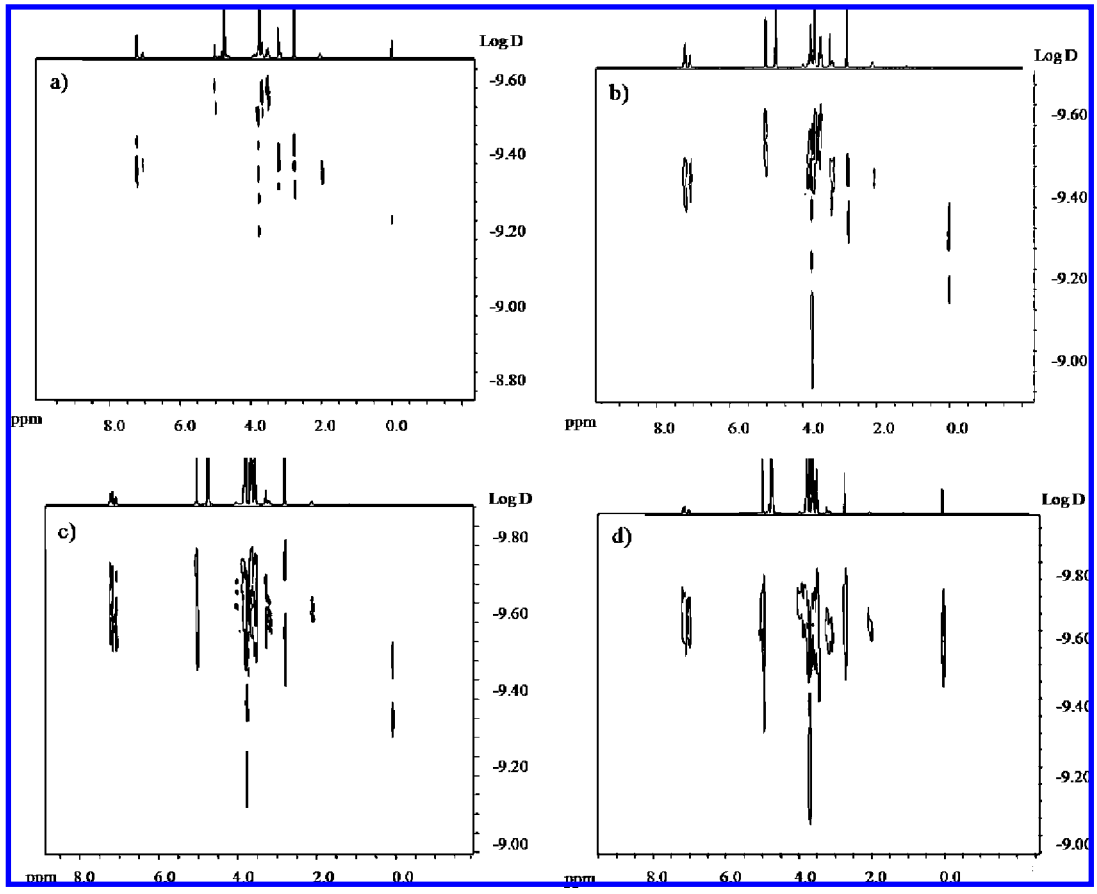

Figure 7. Representative DOSY spectra of different molar ratio ICs at (a) 0.5:1 βCD/IMI, (b) 1:1 βCD/IMI, (c) 1.5:1 βCD/IMI, and (d) 2:1 βCD/IMI.

wavelength 632.4 nm), with a scattering light angle of 90°. The experiment temperature was controlled at 25 °C.

The Milli-Q water was filtered, using a Durapore membrane filters (0.1 μm), before the solution preparation and DLS analysis. The hydrodynamic diameters are the average of five independent measurements, each one obtained as the mean of 20 counts.

Isothermal Titration Calorimetry. Calorimetric titrations were performed in duplicate with a VP-ITC Microcalorimeter (Microcal Company, Northampton, MA, USA) at 25 °C for electrical and chemical calibration. Each titration experiment consisted of 41 successive injections of an aqueous solution of IMI (10.0 mM) into the reaction cell charged with 1.5 mL of a βCD aqueous solution (0.5 mM), with time intervals of 300 s, which were sufficiently long for the signal to return to the baseline, in order to ensure that equilibrium had been reached. The first injection of 1 μL was discarded to eliminate diffusion effects of material from the syringe to the calorimetric cell. Subsequent injections of constant volume of 5 μL of IMI were made with an injection time of 2 s.

The βCD concentration in the calorimeter cell varied from 0.5 to 0.44 mM, and the concentration of IMI, from 0.0 to 1.17 mM.

The ITC data were analyzed by the software supplied with the calorimeter (Microcal Origin 5.0 for ITC).

The peaks produced during the titration were converted to heat output per injection by integration and correction for the cell volume and sample concentration. The dilution process of IMI was carried out in water to evaluate the heat of dilution of this molecule. In addition, the heat dilution curve was subtracted from the titration curve (IMI in βCD) to eliminate interactions between IMI and water. Binding enthalpies, stoichiometry, and binding constant were obtained by nonlinear fitting (Wiseman isotherm),[27] incorporated in the software, which assumes a single set of identical binding sites.

3. Computational Details

Both crystal structures of IMI and βCD, obtained from the Protein Data Bank (PDB)[28] and from our previous work,[29] respectively, were fully optimized at the DFT level using the BLYP[30] functional with the 6-31G(d,p)[31] basis set containing polarization functions on all atoms.

Three orientations were initially assumed for the 1:1 βCD/IMI supramolecular complexes: form A, when the IMI aromatic ring A is included in the βCD cavity; form B, when the B aromatic ring is included; and finally form C, in which the IMI aliphatic group is placed into the βCD cavity. The last structure has already been proposed in the literature.[32] The initial orientation proposed for the 2:1 βCD/IMI complexes had the IMI molecule placed between two βCDs molecules, with their aromatic rings A and B located inside the distinct βCDs cavities. The formation of a 1:2 βCD/IMI complex was also considered. In this case, the βCD was placed between two IMI molecules, with the A and B aromatic rings of different IMI molecules located inside the cavity of βCD for either a wider and narrower rim of βCD.

Semiempirical and DFT calculations were carried out to obtain reliable structures and energetic properties of the βCD/IMI complexes. The initial guess geometries for the distinct arrangements were fully optimized at the semiempirical PM3[33] level, which has been shown to give very reasonable geometrical parameters for CDs.[29] PM3 harmonic frequency calculations were also performed for the equilibrium structures, characterizing them as true minima on the potential energy surface (all frequencies are real). Afterward, the electronic plus nuclear repulsion energy contribution ($\Delta E_{\text{ele-nuc}}$) was evaluated as single point BLYP/6-31G(d,p)/PM3 calculations using the fully optimized PM3 geometries. This sequential methodology has been successfully used for CD hydrated species.[34,35]

Within the quantum mechanical formalism the solvent effect was considered using the polarized continuum model (IEF-PCM).[36] In the condensed phase, the presence of the solvent is replaced by its dielectric constant (for water $\varepsilon = 78.39$). The solute is placed in a cavity of suitable shape to enclose the whole molecule, which is immediately contemplated in the continuum dielectric. The PCM single-point calculations in solution were carried out at the BLYP/6-31 G(d,p)/PM3 level theory.

All theoretical calculations were carried out using the Gaussian 2003 quantum mechanical package.[37]

4. Results and Discussion

4.1. Experimental Results. 4.1.1. FTIR-HATR Spectroscopy. The utilization of the FTIR-HATR spectroscopy technique to investigate changes in the vibrational modes of host and guest molecule in aqueous solution was previously reported by our research group.[17] At this time, the FTIR-HATR spectroscopy approach was used to give support for evaluation of possible βCD/IMI stoichiometries in solution.

The FTIR-HATR spectra of both molecules, βCD and IMI, indicated linear dependences of intensities with concentration, as already reported, for the βCD/tetracycline complex.[17] However, the spectra obtained during the titration of IMI (30.0 mM) in βCD (12.0 mM) showed significant concentration/intensity deviations, mainly with the βCD ν_{COC} 1030 cm^{-1}. This could arise from the exchange of the high enthalpy intracavity water molecules with the guest molecule (IMI). The comparison among FTIR-HATR spectra of βCD (6.65 mM), IMI (13.35 mM), and the respective IC are depicted in Figure 2, to clarify the vibrational differences among them.

The ICs stoichiometries were obtained from the titration data, Figure 3. Since the transmittance, as a function of molar ratio, did not exhibit linearity, two sigmoid curves were obtained, with the inflections providing the ICs stoichiometries. The results plotted in Figure 3 suggest that two complexes are favored: a 1:1 and 2:1 βCD/IMI. This nonlinear profile of the complexation process might be attributed to the cooperation interactions on the host/guest systems, where the cooperative effect of several weak forces governs the association processes.[17,38]

A Job plot was also used to investigate the supramolecular complex stoichiometry, Figure 4. The existence of a 2:1 βCD/IMI complex was indicated from the Job plot, confirming the conclusion from the titration process. These results indicate that this supramolecular structure would be favored at higher concentration of βCD in solution. The Job curve suggests also the existence of a 1:2 βCD/IMI complex when βCD concentration is less than that of IMI. All FTIR-HATR experiments indicate equilibria between different complexes (1:2; 1:1, and 2:1 βCD/IMI) in aqueous solution. Scheme 1 summarizes the most probable supramolecular structures of the βCD/IMI complexes. Significantly, the FTIR-HATR results demonstrate that the 2:1 βCD/IMI complex is the most favored. To confirm these possibilities, and also propose the supramolecular topology, by the interactions sites of host and guest molecule, NMR techniques were used.

4.1.2. Nuclear Magnetic Resonance Spectroscopy. The chemical shifts of the IMI hydrogen's in the IC at 1:1 and 2:1 βCD/IMI are listed in Table 1. A change in the chemical shift values is observed on increasing the relative βCD concentration in solution. This observation is more evident with the aliphatic hydrogens of IMI, which are assumed to be closest of the βCD

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hydroxyl groups. However, this result might also arise from the inclusion of this aliphatic region within the βCD cavity. The hypothesis that these aliphatic hydrogens are close to the βCD hydroxyl group can only be considered, if the aromatic rings of the IMI molecule are interacting with the βCD cavities, forming a sandwich type structure. Figure 5 shows the change in the chemical shifts of the aromatic protons in the 1:1 and 2:1 βCD/IMI complexes. The proposed 2:1 βCD/IMI complex is in accordance with the results obtained by FTIR-HATR experiments (titration and Job plot).

To verify the mobility of the IMI hydrogens, T_1 measurements were carried out, and these data are presented in Table 2. For organic molecules the relaxation process occurs mainly by a dipolar relaxation mechanism, thus the T_1 is inversely proportional to the molecular correlation time (τ_c) or directly proportional to the rotational diffusion coefficient (D_{rot}), eq 1.[12] The data shown in Table 2 indicate an interaction of the IMI aromatic rings with the βCD cavity, since the major reductions in T_1 values were observed with the hydrogens (H1 and H2) of IMI. In addition, the smaller reduction in T_1 values of the aliphatic hydrogens indicate their greater mobility, and this result corroborates the ^{1}H NMR spectral data.

$$T_1(DD)\alpha\frac{1}{\tau_c}\alpha D_{rot} \quad (1)$$

Figure 6a shows the 2D NOESY contour map, in which the intermolecular cross peak between all IMI aromatic hydrogens with the internal and external protons of the βCD can be seen. These dipolar correlations are better observed in the contour expansion map, Figure 6b: the dipolar correlation indicates the inclusion of the IMI aromatic rings in the βCD cavity. Inclusion of both rings, in the 2:1 βCD/IMI complex, was indicated too by the FTIR-HATR experiments as the most stable arrangement. This arrangement allows the close approach of the hydroxyl βCD groups to the aliphatic IMI hydrogens, thereby resulting in the greater change in the chemical shifts presented in Table 1 and also the major T_1 reduction listed in Table 2. The nondipolar correlations between the IMI aliphatic hydrogen's with the βCD molecule indicate that this region of the IMI molecule is not included in the βCD cavity. In addition, the cross peak correlation between the IMI aromatic hydrogens with the external βCD hydrogen's (H2 and H4) might be explained by the formation of a large supramolecular structure, with a high molecular weight, as a the self-assembly of the supramolecular complex.

In order to gain a deeper insight into the self-assembly process, DOSY experiments were carried out. In addition, self-diffusion coefficients (D) of the free guest molecule and the ICs at different molar ratios (1:1; 2:1, and 3:1 βCD/IMI) were also determined. Figure 7 depicts the DOSY spectra of these supramolecular complexes, and one can observe that when the βCD concentration increases in solution, the IMI and βCD molecules tend to have the same D values. This observation can be best observed with the 3:1 βCD/IMI complex, in which both molecules have the same diffusion coefficient. This result indicates the tendency of the IMI molecule to form ICs with high concentrations of βCD, as in a larger aggregate.

In addition, an exponential decay fit could be adjusted to the D values for the pure IMI (2.5 mg/0.7 mL) and the respective ICs (0.5:1; 1:1; 2:1, and 3:1 βCD/IMI); see Figure 8. In all experiments, dioxane was used as a reference. Analysis of the D values ($D_{sample}/D_{dioxane}$) reveals that an increase in the βCD concentration makes the supramolecular system diffuse more slowly than the pure IMI molecule. This tendency might be associated with the interaction between host and guest molecules, and also to the possibility of the self-aggregation of this supramolecular complex formed in solution, in accordance with other systems referred to in the literature.[39] In order to determine the supramolecular complex size in solution, DLS measurements were carried out.

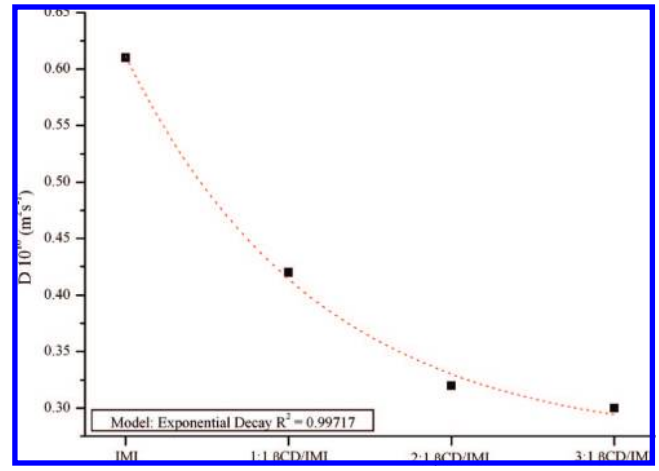

Figure 8. Self-diffusion coefficients tendency to the pure IMI and the ICs at different molar ratios.

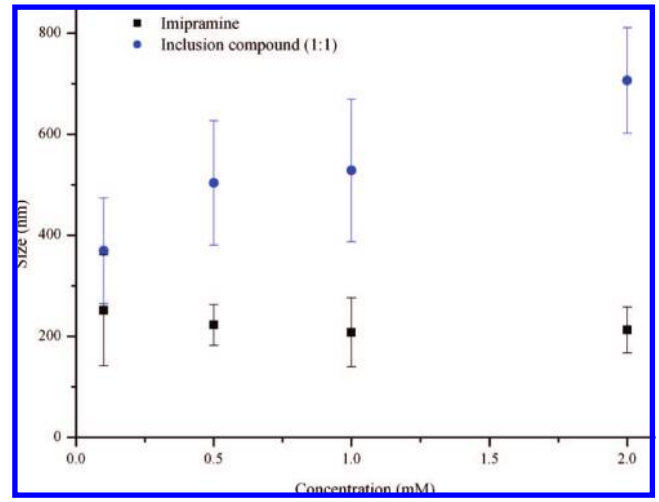

Figure 9. DLS measurements of pure IMI (at 0.1, 0.5, 1.0, and 2.0 mM) and of IC at 1:1 βCD/IMI (at 0.1, 0.5, 1.0, and 2.0 mM).

4.1.3. Dynamic Light Scattering. The DLS curves (size versus concentration) of the pure IMI and the 1:1 βCD/IMI complex are presented in Figure 9. The IMI at 0.1, 0.5, 1.0, and 2.0 mM do not exhibit a size variation, their size remaining near 220 nm. This result indicates that the IMI molecule has a tendency to aggregate in solution; this observation is in accordance with the results already reported in the literature.[32] However, the 1:1 βCD/IMI (0.1, 0.5, 1.0, and 2.0 mM) has a tendency to self-aggregate and this aggregation process makes the assembly larger, as a function of the concentration. This observation was also verified for other supramolecular systems studied by our research group.[7,12,40] The comparison between the two species (IMI and 1:1 complex) is in accordance with the results determined by the DOSY experiments, in which IMI presented a higher D value than that of the 1:1 complex, indicating the small size of the IMI assembly.

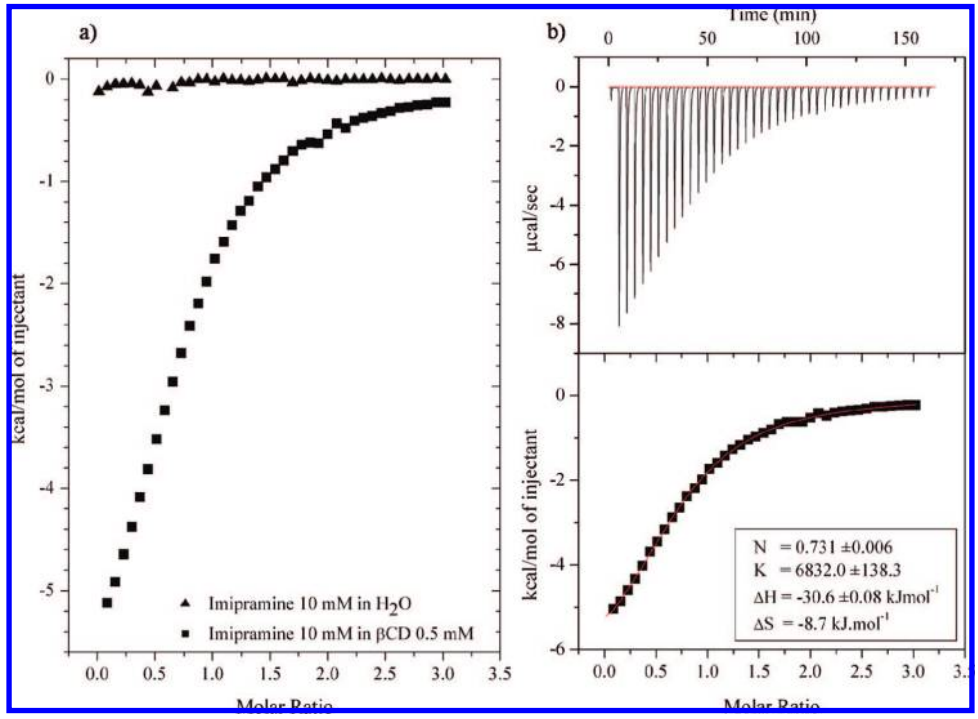

Figure 10. (a) ITC experiments for IMI, 10.0 mM (▲) in water and (■) in βCD 0.5 mM. (b) Final figure: (—) raw data, (■) subtracted curve of titration, (- - -) nonlinear regression.

4.1.4. Isothermal Titration Calorimetry. Another technique used to investigate the interaction between the host and guest molecules was ITC, which provides more information about the thermodynamic parameters of the complexation process. Figure 10a shows the IMI titration in water and its titration in βCD solution.

The enthalpy variation during the titration can be related to intermolecular association. In this case, the IMI titration curve in water reveals that these molecules tend to keep their size, since the enthalpy variation for this process is close to zero. This result is in accordance with the DLS experiment. However, in the presence of βCD, the IMI titration process assumes exothermic values demonstrating the host/guest interactions.

The sigmoidal profile obtained for the IMI in the βCD titration process allowed the fitting of the experimental data to a Wiseman isotherm, eq 2, in which the stoichiometry coefficient ($N = 0.73$), equilibrium constant ($K \approx 6832 \pm 138$), and the enthalpy ($\Delta H° = -30.6$ kJ·mol^{-1}) were calculated.[27]

$$\left(\frac{dQ}{d[IMI]_{tot}}\right)_P = \Delta H° V_o \left[\frac{1}{2} + \frac{1 - X_R - r}{2\sqrt{(1 + X_R - r^2) - 4X_R}}\right] \quad (2)$$

The equation relates the stepwise change in the heat of the system normalized with respect to IMI concentration added per injection $(dQ/d[IMI]_{tot})_P$, at constant pressure, to the absolute ratio of ligand to receptor concentration ($X_R = [IMI]_{tot}/[\beta CD]_{tot}$) at any point during the titration process. The $\Delta H°$, V_o, and r are, respectively, the molar enthalpy of binding, the effective volume of the solution in the titration cell, and a composition variable $1/[\beta CD]_{tot} \cdot K_{eq}$.

The N value suggests the formation of more than one complex in solution, and it was already verified by the spectroscopic techniques (FTIR-HATR and NMR experiments). From eqs 2, 3, and 4, the Gibbs energy ($\Delta G° = -21.9$ kJ·mol^{-1}) and the entropic contribution ($T\Delta S° = -8.7$ kJ·mol^{-1}) were determinated.[41]

$$\Delta G° = -RT \ln K \quad (3)$$

$$\Delta G° = \Delta H° - T\Delta S° \quad (4)$$

The ITC curve declivity for the IMI in βCD solution, Figure 10b, demonstrates a strong interaction between the species, when compared with other supramolecular systems related in the literature.[8,17,42] These strong intermolecular interactions could be associated with equilibria involving many different species in solution and also to the greater affinity of the βCD cavity for the IMI aromatic rings, what favors van der Waals interactions. In addition, the possibility of a hydrogen bond between the hydroxyl groups of two βCD molecules in the 2:1 βCD/IMI complex might contribute to the high equilibrium constant.

The enthalpy changes could be attributed to the binding of the high enthalpy intracavity water molecules released from the βCD cavity with the bulk water molecules,[3] as well as to the formation of cooperative interactions between host and guest molecules.

The entropic contribution was attributed to a balance of desolvation of guest molecules during the complexation, where water molecules must gain translational and rotational degrees of freedom, while a more rigid architecture and new conformation assumed by species upon complexation confer a greater

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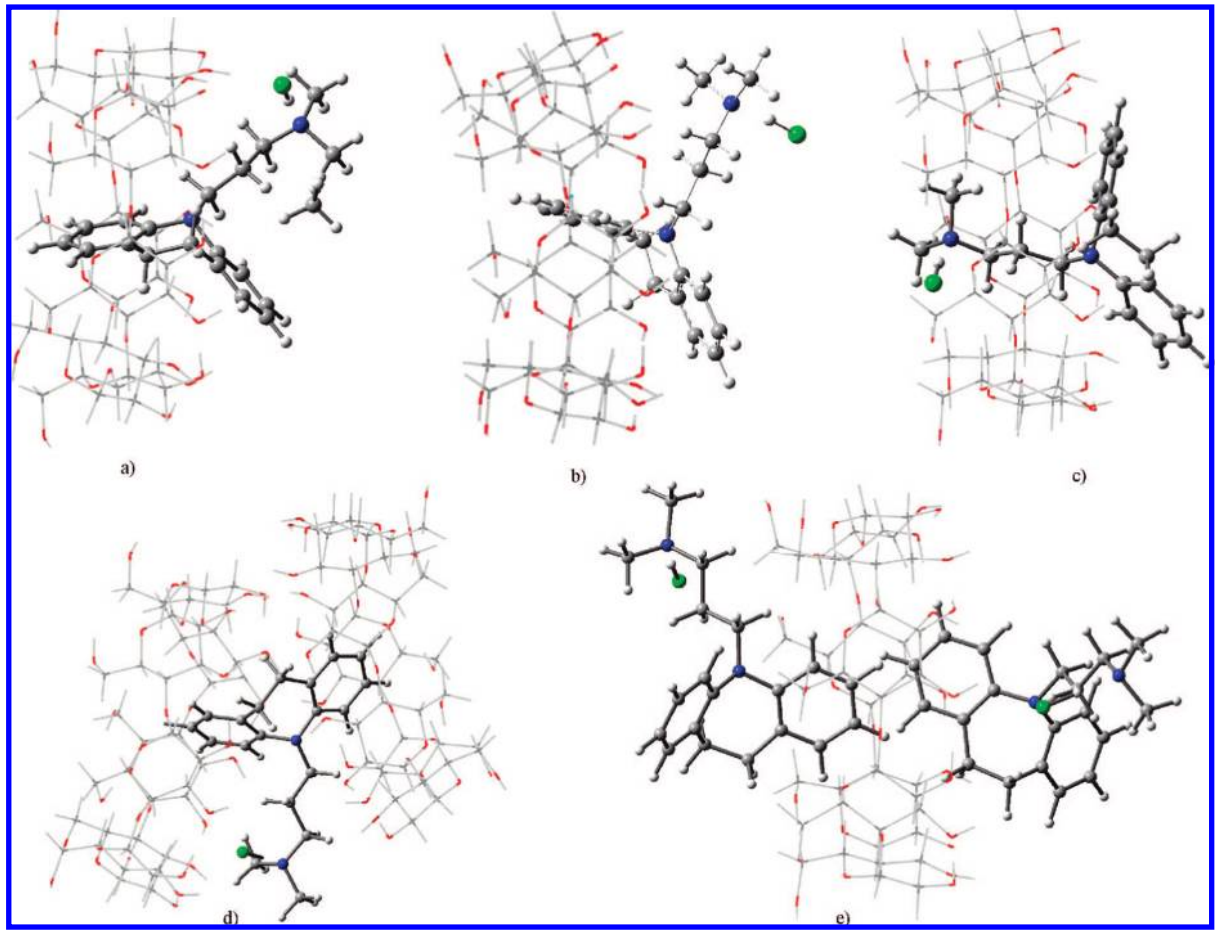

Figure 11. Equilibrium structures obtained through theoretical calculations at the PM3 level. (a) IC optimized geometry formed when the IMI aromatic ring A is included in the cavity, (b) when the B aromatic ring is included, (c) when the aliphatic group is included, (d) 2:1 βCD/IMI optimized geometry having the IMI molecule placed between two βCDs with their aromatic rings located inside the cavity, (e) 1:2 βCD/IMI optimized geometry having two IMI molecules, being the A and B aromatic ring of distinct IMI located inside the cavity.

molecular volume and the least rotational and translational degrees of freedom when compared to pure substances. This observation can be linked to both the DOSY and DLS results, which indicated the formation of a structure with high molecular weight, in other words the self-assembly of the supramolecular complex.

4.2. Computational Results. One of the strengths of computational chemistry is the ability to generate data, in which an experimentalist may gain insight and thus rationalize the behavior of determined physical and chemical phenomena. In most cases, such insights are not extremely accurate from a quantum mechanical viewpoint, due to the size of the systems investigated. However, an investigation on the molecular level can be very useful to predict experimental findings.

Theoretical calculations were performed to determine the preferred arrangement of the ICs, formed from IMI and βCD. Distinct modes of inclusion were proposed based on the orientations and ratios of the species involved in these complexes. Our main goal was an attempt to model the structural and energetic parameters of the ICs, which could be used to predict the most favorable βCD/IMI complex conformation. A detailed understanding of the interactions between the IMI and βCD at a molecular level is very relevant to support interpretation of experimental findings. The respective structures of five IC forms optimized at the PM3 level of theory are depicted in Figure 11.

Table 3. Relative Interaction Energy (ΔE) for the Inclusion Process of IMI and βCD Evaluated at the BLYP/6-31G(d,p)//PM3 Level of Theory According to the Equation $n\beta$CD + nIMI → $[\beta CD]_n$. . .$[IMI]_n$[a]

	ΔE	
inclusion complex	gas phase (kcal·mol^{-1})	aqueous phase (kcal·mol^{-1})
1:1 βCD/IMI (*form A*)	20.0	22.3
1:1 βCD/IMI (*form B*)	27.7	31.4
1:1 βCD/IMI (*form C*)	24.1	28.5
2:1 βCD/IMI	0.0	0.0
1:2 βCD/IMI	9.4	12.7

[a] Values in kcal·mol^{-1}.

Table 3 contains the relative interaction energy (ΔE) evaluated at the BLYP/6-31G(d,p)//PM3 level for the inclusion process. The most favorable of the 1:1 IC conformations was the βCD/IMI form A, being 7.7 and 4.1 kcal·mol^{-1} more stable in the gas phase than forms B and C, respectively. The reason for this slight stability, compared to the other conformations at the same ratio, can be attributed to a hydrogen bond formed between the chloride atom and the secondary hydroxyl group of the βCD (OH. . .Cl $\approx$ 1.7 Å) resulting in this form being energetically favored. It is opportunistic to mention that although form C is 3.6 kcal·mol^{-1} more stable than form B in the gas phase, the formation of this species was not detected by the 2D NOESY experiments.

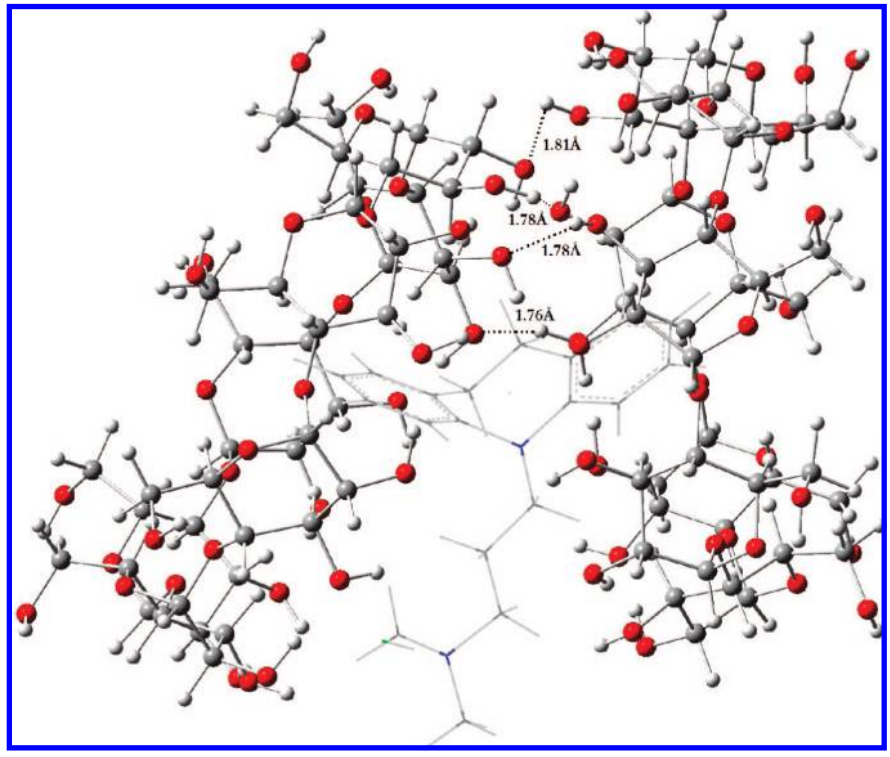

Figure 12. PM3 fully optimized geometry of the 2:1 inclusion complex of IMI and βCD. The hydrogen bond distances are quoted, and dotted lines were drawn to ease the visualization of the H-bonds.

From Table 3, we can observe that, among the five forms proposed, the global minima in both the gas phase and aqueous phase is the 2:1 βCD/IMI complex, with the IMI molecule placed between two βCDs molecules (Figure 11d). BLYP/6-31G(d,p)//PM3 calculations indicated that the formation of this orientation was the most energetically favored. For this configuration, the relative interaction energy (ΔE) in the gas phase was 9.4 kcal·mol^{-1} more stable than the 1:2 βCD/IMI complex. This can be explained by a meticulous structural analysis at the molecular level, which detected the occurrence of four hydrogen bonds between the secondary hydroxyl groups of the two βCDs molecules (Figure 12). These hydrogen bonds are the main forces responsible for the stabilization of the 2:1 βCD/IMI complex. A similar result concerning the major role of hydrogen bonds to stabilize the dimeric CDs structures was also observed in our previous study involving α-CD dimers.[34]

The solvent effect was also considered in the PCM method. The result supports the previous conclusion obtained in the gas phase concerning not only the energetic stability order of the species but also the higher stability of the supramolecular 2:1 βCD/IMI complex. In this case, the presence of the solvent medium increases the stabilization of the 2:1 complex form from 9.4 (gas phase) to 12.7 kcal·mol^{-1}, when compared to 1:2 βCD/IMI.

The theoretical relative energy results reported in Table 3, for the species in aqueous solution, can be regarded as a fair estimate of the enthalpy values for the inclusion process in solution. These theoretical data are quite useful for the experimentalist, since a direct relationship of the thermodynamic quantity with a specific spatial molecular arrangement for the IC is made, and so, an understanding of the intermolecular interactions in solution at a molecular level is possible. The theoretical data presented in Table 3 strongly indicate that the 2:1 βCD/IMI IC should be the preferred mode of interaction in water solution. Thus, this result is contrary to the E. Junquera and co-workers findings, in which the 1:1 stoichiometry was suggested as their preliminary conclusion.[32]

With this theoretical work, we can describe energetic parameters for the formation of the βCD/IMI complex at a molecular level which were in good agreement with the experimental findings. In addition, a systematic structural analysis indicated interactions of the aliphatic IMI hydrogens with the secondary hydroxyl groups of βCD, the distance encountered between them ranging 2.1−2.7 Å. This result corroborates the ^{1}H NMR spectral data, which demonstrated a high change in the chemical shift for these hydrogens when compared to aromatic hydrogens of IMI.

Finally, the possibility of aggregation was suggested by the experimental findings. Our research group has already begun a theoretical investigation of high molecular weight, supramolecular species, such as the 4:3 βCD/IMI complex.

5. Conclusion

In this article was demonstrated the supramolecular topology resulting from the noncovalent interaction between host (βCD) and guest (IMI) molecules. The FTIR-HATR experiments, continuous variation plot, and titration process indicated equilibria among 1:2, 1:1, and 2:1 βCD/IMI complexes.

Confirmation of the intermolecular interactions was obtained from 2D NOESY experiments, which provided the dipolar correlations among the IMI aromatic hydrogens within the βCD cavity. In addition, ^{1}H and T_1 experiments indicated that the IMI rings interact with βCD. However, a greater change in the chemical shift of the IMI aliphatic hydrogens was also observed, suggesting the possible inclusion of this part of the guest molecule in the βCD cavity. The DOSY spectra suggested the self-aggregation of the supramolecular structures, especially at

high βCD concentrations. This self-aggregation was also confirmed by DLS measurements.

The ITC curves demonstrated a high affinity between the host and guest molecules, with a large equilibrium constant. The stoichiometry ($N = 0.7$) obtained by ITC titration indicated that more than one complex is formed in solution, as was also suggested by FTIR-HATR spectroscopy. In addition, the inclusion process can be considered as being both enthalpy and entropic controlled.

The most stable calculated structure, in gas and condensed phases, was a 2:1 βCD/IMI complex with both IMI aromatic rings included in the cavities of two distinct βCD molecules. The formation of hydrogen bonds between the secondary hydroxyl groups of the two βCDs confers stability to this conformation.

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Supporting Information Available: Complete ref 37. This material is available free of charge via the Internet at http://pubs.acs.org.

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