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Supramolecular complex of fluoxetine with β-cyclodextrin: An experimental and theoretical study

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Abstract

In this work the complex formed between β-cyclodextrin (βCD) and fluoxetine (FLU) was investigated by experimental and computational methods. From Horizontal Attenuated Total Reflectance (HATR) was possible to verify a strong modification in the vibrational modes of βCD and FLU, indicating interactions between them. The Nuclear Magnetic Resonance (NMR) experiments confirm these interactions through the change in chemical shifts in ^{1}H spectra, reduction in longitudinal relaxation times values, and the Nuclear Ouverhauser Effect confirm the inclusion of aromatic rings of FLU into the βCD. The structures of the proposed inclusion compounds were optimized at PM3 semiempirical level of theory. In addition, single point calculations at the Density Functional Theory (DFT) level, using the Becke, Lee, Yang, and Parr functional and 6-31G(d,p) basis set, were used to determine the interaction energy for these structures. The DFT calculations identified the aromatic ring, which contains the CF_3 group as the most stable into the βCD by an amount of, 11.7 kcal mol^{-1}, in the gas phase. Polarized continuum model, at the DFT level mentioned, was used to investigate the solvent effect, and the results corroborated the gas phase analysis. A high equilibrium constant ($K \approx 6921 \pm 316$) and the stoichiometry, 1:1, were obtained by Isothermal Titration Calorimetry (ITC) experiments.

Keywords: Supramolecular complex; Isothermal Titration Calorimetry; Computational methods; β-Cyclodextrin; Fluoxetine; Inclusion compound

1. Introduction

Fluoxetine (FLU) hydrochloride (Fig. 1) commonly known as Prozac®, is a bicyclic antidepressant drug used medically in the treatment of unipolar mental depression, obsessive-compulsive disorder, bulimia and panic disorder (Hiemke and Härtter, 2000; Berzas et al., 2002; Heikkinen et al., 2002). In most countries, FLU was the first selective serotonin reuptake inhibitor (SSRI), which became available for clinical use employed in the treatment of depression (Preskorn et al., 1996). FLU is a potent SSRI, this uptake inhibition by FLU enhances serotonergic function, and as consequence the serotonin receptors are desensitized or down regulated after long time of FLU administration (Ali et al., 2005). Since the FLU approved in 1988, it has became the most widely prescribed antidepressant drug worldwide and the beginning of a new era of safe and effective treatment of patients with major depression.

On the other hand, a lot of side effects could be attributed to the use of FLU such as: rage and anxiety, which are possibly associated with the interaction between FLU and the pineal gland, and also restlessness and insomnia are observed as well (Uz et al., 2004). Other side effects are also reported like gastrointestinal (nausea and vomiting) and sexual adverse effects in most patients, both males and females (Claytona et al., 2006).

As strategy to reduce these drawbacks of many pharmaceuticals formulations, polymers, lipossomes and cyclodextrins

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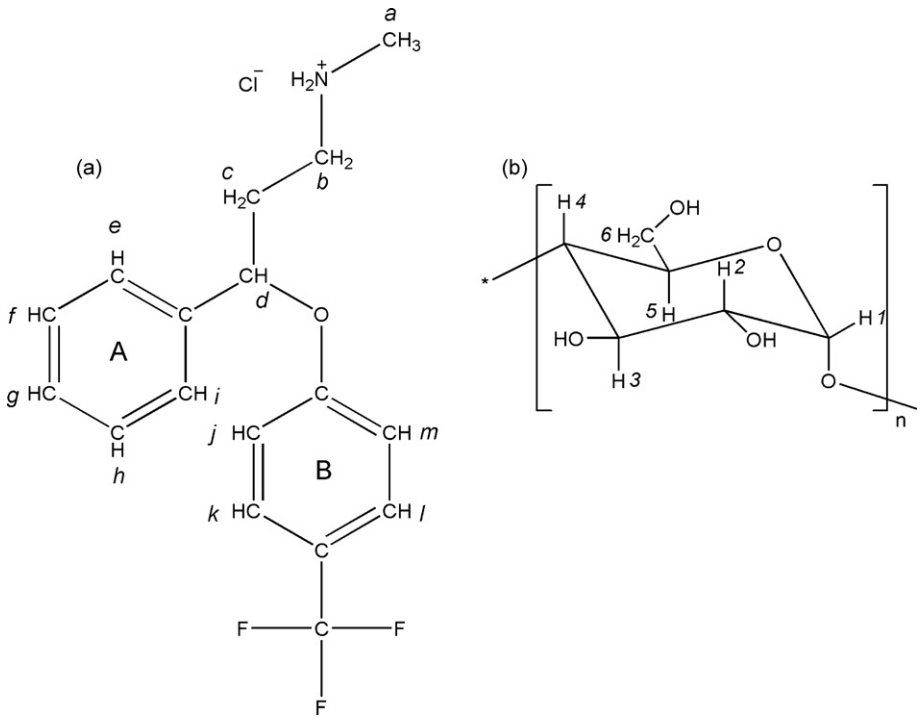

Fig. 1. (a) Structure of fluoxetine hydrochloride and (b) cyclodextrin monomer.

(CDs) have been used as controlled or sustained release systems (Hedges, 1998; Hirayama and Ukeama, 1999; Rajendrakumar et al., 2005; Shen et al., 2005; George and Abraham, 2006; Sajeesh and Sharma, 2006; Sudhakar et al., 2006; Loftsson and Duchene, 2007). These modified release systems can provide a reduction of drug concentration or reduction in the dose administration per day (Hedges, 1998; Hirayama and Ukeama, 1999; Irie and Ukeama, 1999; Loftsson and Duchene, 2007).

The CDs has attracted the attention not only in the pharmaceutical formulations but also in food and biological areas, since their ability to form inclusion compounds (ICs) with guests molecules either in solutions and solid-state (Hedges, 1998; Szejtli, 1998). In addition, CDs improve the therapeutic efficacy of poorly water-soluble drugs, enhance the physical and chemical stability, and also protect the guest molecules from degradation in the gastrointestinal tract (Hirayama and Ukeama, 1999; Irie and Ukeama, 1999; Stella et al., 1999; Zhang and Rees, 1999).

CDs are oligosaccharides most commonly formed by six, seven and eight glucopyranose units, denominated αCD, βCD and γCD, respectively. The structure of this class of compounds is often described as a truncated cone, which provides to CDs a hydrophilic exterior and a hydrophobic cavity (Szejtli, 1998). Thus a deep physical-chemistry characterization of these supramolecular complexes formed by CDs and a guest molecule, which are governed by noncovalent interactions such as: van der Waals forces, hydrogen bonds and hydrophobic interactions (Ariga and Kunitake, 2006; Denadai et al., 2006a,b; Daoud-Mahammed et al., 2007; Fung et al., 2007), is important to understand not only the IC association constant but also the geometry of the complex formed. In this sense, several methods have been used to investigate these kind of complexes, including: spectroscopy techniques such as modern Nuclear Magnetic Resonance (NMR), Fourier Transform Infrared (FTIR) and Horizontal Attenuated Total Reflectance (FTIR–HATR) (Lamcharfi et al., 1995; Schneider et al., 1998; Denadai et al., 2006b; Sun et al., 2006), Isothermal Titration Calorimetry (ITC) (Denadai et al., 2006b; Sun et al., 2006), thermal analysis (Mura et al., 2005) and also computational techniques (Liu and Guo, 2004; Nascimento et al., 2004, 2005; Castro et al., 2006).

In the present article the IC formed by FLU with β-cyclodextrin (βCD) was investigated in order to get more insights about the physico-chemical characteristics of the IC and also to propose geometry of the supramolecular system formed after the inclusion.

In the characterization of this supramolecular complex, modern NMR spectroscopy technique like as Nuclear Overhauser Effect Spectroscopy (NOESY), longitudinal relaxation times (T_1) and also protons (^{1}H) at various molar ratios of host:guest system were used. This system was also investigated by Horizontal Attenuated Total Reflectance (FTIR–HATR) in aqueous solution.

ITC was also used since this technique can provide the thermodynamics parameters of the inclusion process and indeed estimate the formation constant of the complex. To the best of our knowledge such study has not been reported in the literature.

Besides the experimental techniques, theoretical methods were also employed to optimize the proposed geometry and calculate the stabilization energy of each IC formed. Ever through quantum mechanical studies have already been reported for CDs (Liu and Guo, 2004), most of the theoretical calculations on CDs apply molecular mechanics methods, due mainly to the large size of these systems. In this context, some theoretical works attempt to find a suitable theoretical methodology to study these types of

molecules. At higher quantum mechanical level theory, Nascimento and coworkers used a sequential methodology, involving a semiempirical calculation for geometry and vibrational frequencies, following by a single point Density Functional Theory level (DFT) calculation in order to obtain the electronic plus nuclear repulsion energy (Nascimento et al., 2004, 2005; Blenke et al., 2007). Hybrid methods have also been recently used to calculate stabilization energies of ICs with modified CDs (Britto et al., 2004).

In this work, the main goal of the computational study is to elucidate the structures of the complexes and establish, on quantitative bases, the most stable form for the IC.

2. Materials and methods

2.1. Reagents and inclusion compounds preparation

FLU and βCD were obtained from Pharma Nostra, Minas Gerais (Brazil), and Xiamen Mchem, Xiamen (China), respectively. All ICs were prepared by the freeze-drying method in the molar ratio of: 0.5:1, 0.75:1, 1:1, 1.25:1, 1.5:1 and 2:1 βCD/FLU. In this method, the aqueous solution which contents the dissolved materials (βCD and FLU) was stirred for 4 h, after this the solution was frozen in liquid nitrogen and lyophilized (Savant ModulyoD—Freeze Dryer, Thermo Electron Corp., Waltham, MA, USA) for 48 h.

2.2. FTIR–HATR experiments

In order to investigate the vibrational changes upon host:guest interaction between βCD and FLU, ICs were prepared in different molar ratios by a titration process in Milli-Q® water. The first point of the titration was recorded to the βCD solution (1 mL at 12 mM) at the ZnSe cell. After this, successive injections of FLU solution (30 mM) were added. The FLU additions were injected at constant volume of 0.1 mL. During the titration the concentration of the βCD varied from 12.0 to 6.0 mM and FLU from 2.7 to 15.0 mM.

A Perkin Elmer spectrophotometer model, (Spectrum GX; Perkin Elmer, Boston, MA, USA) with a KBr beam splitter, equipped with HATR accessory with ZnSe cell (four reflections) was used. All spectra were recorded within a range of 4000–590 cm^{-1}, with a 4 cm^{-1} of resolution and 128 scans. The spectrum of pure water was done before each analysis as reference, and after it was subtracted from each spectrum obtained from the ICs. To reduce atmosphere effects, the sample detector was purged with N_2 before collection of the background, in each analysis.

All spectra were recorded in triplicate and all experiments were carried out at room temperature at 23.0 ± 1.0 °C. The ZnSe crystal was washed with distilled water and dried with soft paper after each measurement.

2.3. NMR experiments

The ICs prepared by 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 NMR spectroscopy technique.

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 *z*-gradient coil. ^{1}H NMR experiment was achieved using the WATERGATE (Piotto et al., 1992; Sklenár et al., 1993) technique for suppression the residual water signal. NOESY (Bax and Davis, 1969; Gil and Geraldes, 1987) (mixing time of 500 ms) and T_1 experiments were acquired using a standard experiment from the spectrometer library. The $\delta = 4.75$ water signal was used as reference.

2.4. Computational details

Exclusively, the ICs were evaluated by theoretical calculations, since the complete complexation reaction study demands a great computational effort, and the theoretical results provide information about energy and structure of βCD/FLU, proposed by experimental results.

The started structures were constructed from the X-ray data ((CSD): http://www.ccdc.cam.ac.uk/) available for βCD (Lindner and Saenger, 1982) and FLU (Robertson et al., 1988). The hydration water molecules were excluded from the βCD crystal structure, taking for granted that final product of the inclusion reaction does not contain water molecules inside the βCD cavity. To obtain the IC initial structure, the FLU molecule was included through the wider side of βCD cavity and the geometric center of the aromatic ring was placed exactly on the center of mass of βCD. These started geometries were named form A, when the monosubstituted aromatic ring is inside of the βCD cavity, and form B, when the aromatic ring which has the CF_3 group substituted is included into the βCD cavity.

The sequential methodology, using semiempirical and DFT approaches, was used here as described previously (Nascimento et al., 2004, 2005). The PM3 (Stewart, 1989) calculations, which has been successfully tested for CDs geometries (Britto et al., 2004), were carried out to find the global minimum structures of the two probable ICs (forms A and B), and used for frequency analysis, due to the computational coast of this kind of calculation for large systems, such as cyclodextrin complexes. The electronic plus nuclear repulsion energy was further calculated at the DFT level using the gradient generalized BLYP functional developed by Becke, Lee, Yang and Parr (Becke, 1988; Lee et al., 1988) with the Pople's standard split valence 6-31G(d,p) (Ditchfield et al., 1971), basis set. This sequential methodology (BLYP/6-31G(d,p)//PM3) was successfully used in theoretical works involving CDs (Nascimento et al., 2005) and their dimers (Nascimento et al., 2004).

The solvent effect was taken into account using the polarized continuum model (IEF-PCM) (Barone et al., 1997) in which the condensed phase is treated as a classical electronic medium represented by the solvent dielectric constant (for water $\varepsilon = 78.39$). The solute, in this case the IC, is placed in a virtual cavity of suitable shape to enclose the whole molecule. The PCM calculation was carried out at BLYP/6-31G(d,p) theory level.

All theoretical calculations were carried out at our laboratory using the Gaussian 2003 quantum mechanical package (Frisch et al., 2004).

2.5. ITC experiments

Calorimetric titrations were performed in duplicate with a VP-ITC Microcalorimeter (Microcal Company, Northampton, MA, USA) at 25 °C next the electrically and chemically calibration. Each titration experiment consisted of 51 successive injections of FLU aqueous solution (17.5 mM) into the reaction cell charged with 1.5 mL of βCD aqueous solution (1.0 mM), with time intervals of 300 s, which was sufficiently long for the signal to return to the baseline, to ensure the equilibrium for the system. The first injection of 1 μL was discarded to eliminate diffusion effects of material from syringe to cell calorimetric. The subsequent additions were injected at constant volume of 5 μL of FLU and the time of injection was 2 s.

The βCD concentration in the calorimeter cell varied from 1 to 0.86 mM and the concentration of the FLU from 0.0 to 2.9 mM. The ITC data were analyzed by the software supplied with the calorimeter (Microcal Origin 5.0 for ITC).

The peaks produced during titration were converted to heat output per injection by integration and correction for the cell volume and sample concentration. The dilution process of FLU was carried out in water to evaluate the heat of dilution to this molecule. In addition, the heat dilution curve was subtracted by the titration curve (FLU in βCD) to eliminate interactions between FLU and water.

Binding enthalpies, the stoichiometry and binding constant were obtained by nonlinear fitting (Wisman isotherm) incorporated in the software, which assumes a single set of identical binding sites.

3. Results and discussion

3.1. FTIR–HATR results

The spectra of the βCD aqueous solution in concentrations range from 12.0 to 6.0 mM subtracted from the pure water spectrum is depicted in Fig. 2. The analysis of the FTIR–HATR βCD spectra were carried out at 1600–800 cm^{-1} region, in order to study the characteristic vibrations ν(C–O–C) at 1031–1082 and 1300–1400 cm^{-1} attributed to δ(C–H) (Egyed, 1990; Denadai et al., 2006a). No significant changes in the vibration modes of βCD spectrum during the dilution process were observed. It is important to mention that these spectra are similar to the solid-state FTIR spectrum reported in the literature (Egyed, 1990).

The FTIR–HATR spectra of FLU is presented in Fig. 3, these spectra were carried out at 1600–800 cm^{-1} where the vibrational modes observed are related to: 1559–1505 cm^{-1} ν(C=C), 1328 and 1246 cm^{-1} strong bands of ν(C–F), 1180 and 1165 cm^{-1} ν(C–H) of aromatic ring, 1121 cm^{-1} ν(C–O–C) and 1069 cm^{-1} ν(C–N). The overall profile of FLU spectrum was also found similar throughout the dilution process.

According to the ICs FTIR–HATR spectra, presented in Fig. 4, a strong reduction in the ν(C–O–C) characteristic vibra-

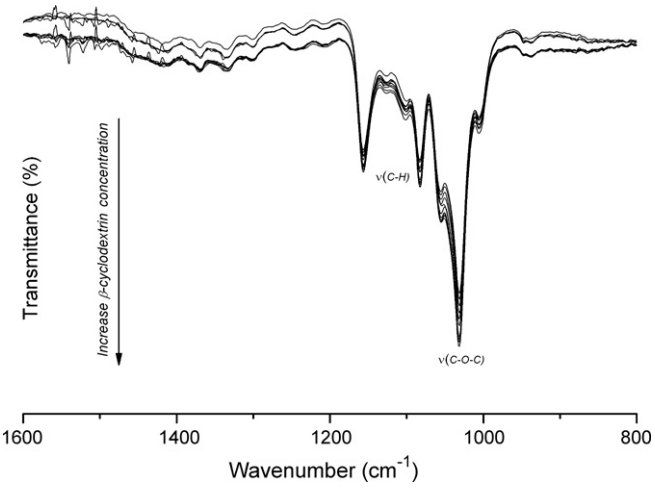

Fig. 2. FTIR–ATR spectra of βCD in water at concentration range from 12.0 to 6.0 mM at 1600–800 cm^{-1} region.

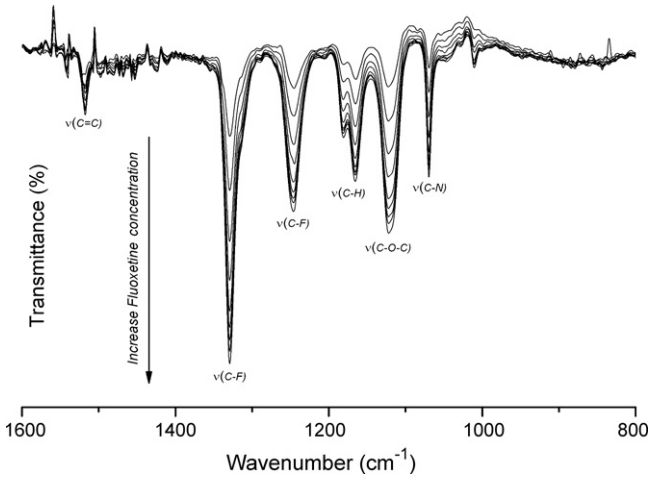

Fig. 3. FTIR–ATR spectra of FLU in water at concentration range from 2.7 to 15.0 mM at 1600–800 cm^{-1} region.

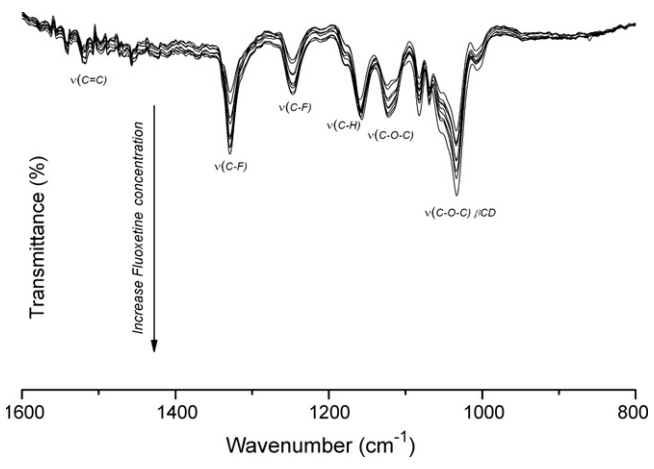

Fig. 4. FTIR–ATR spectra of IC βCD/FLU at concentration range from 12.0 to 6.0 and 2.7 to 15.0 mM, respectively, at 1600–800 cm^{-1} region.

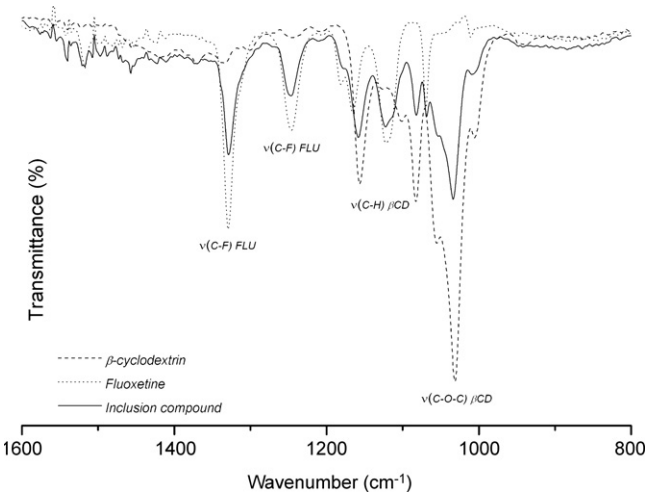

Fig. 5. Comparison of the FTIR–ATR spectra among of βCD 8.0, FLU 11.6 mM and the respective IC, at 1600–800 cm^{-1} region.

tion mode of βCD at 1031–1082 cm^{-1} upon the complexation with FLU was observed. This reduction in intensity of the ν(C–O–C) band in FTIR–HATR is described as a decreasing in the number of hydrogen bond when the guest compound replaces water molecules. In solid-state this is commonly reported as a sharpening not only of this vibration mode but also to the ν(O–H) vibration modes of βCD upon IC formation (Lamcharfi et al., 1995). However, the high intensity of the ν(O–H) from the water molecules in the FTIR–HATR spectra make so difficult to evaluate changes of these vibration modes from βCD, such as observed and discussed by FT-IR spectroscopy in solid-state.

In addition, when the IC spectra are compared with the pure FLU spectrum, reduction in the intensity of the ν(C–F) at 1328 and 1246 cm^{-1} and the ν(C–O–C) at 1121 cm^{-1} are also observed. These changes can be related with the interaction of the βCD with the aromatic ring B of FLU molecule. A short modification is also observed in the ν(C=C) vibration mode of the aromatic rings, compared to the pure FLU, which is an evidence of interaction between the aromatic rings (A or B) with the βCD.

In order to clarify the comparison between βCD, FLU and IC spectra, Fig. 5 presented the spectrum of each species at concentration of 8.0 mM for βCD, 11.6 mM for FLU and the respective IC.

3.2. NMR results

To confirm the interactions between the βCD with the FLU molecule, at different molar ratios, observed by FTIR–ATR in aqueous solution, NMR spectroscopy technique was also used.

The ^{1}H NMR experiments were performed to evaluate the changes in the chemical shifts (Δδ) among pure compounds (βCD and FLU) with the IC. The chemical shifts to the pure FLU and to the ICs in molar ratio of 1:1 and 2:1 are showed in Table 1. By comparing the ^{1}H NMR spectra of the IC, at molar ratio 0.5:1, 0.75:1, 1:1, 1.25:1, 1.5:1, and 2:1 of βCD/FLU, to the pure FLU spectrum (Fig. 6a), it can be clearly seen strong modifications in the chemical shifts at the aromatic region, which is in

Table 1
^{1}H NMR (at 400 MHz) chemical shifts of fluoxetine, inclusion compounds at 1:1 and 2:1 βCD/FLU in D_2O at 27.0 °C

Hydrogen	δFLU	δIC 1:1	Δδ[a]IC 1:1	δIC 2:1	Δδ[b]IC 2:1
a	2.68	2.72	−0.04	2.68	0.0
b	3.19	3.24	−0.05	3.23	−0.04
c	2.31	2.42	−0.11	2.41	−0.10
d	5.49	5.52	−0.03	5.47	0.02
Ring A	7.32	7.41	−0.09	7.37	−0.05
j, m	6.96	7.04	−0.08	7.00	−0.04
k, l	7.43	7.38	0.05	7.31	0.12

[a] Δδ = δFLU − δ1:1 βCD/FLU.
[b] Δδ = δFLU − δ2:1 βCD/FLU.

accordance with Ali et al. (2005) work, who evaluated different ICs formed by association of βCD and FLU (Ali et al., 2005). The hydrogen H_j and H_m (δ = 6.96) of aromatic ring B lost their equivalence in the presence of the βCD and when the concentration of βCD increases. It is interesting to note the change in the chemical shift of H_k and H_l from δ = 7.43 to δ = 7.31, which is a strong evidence of the inclusion of ring B in the βCD cavity. The coalescence of the protons of the aromatic ring A can also be observed, supporting the existence of some interaction between this molecular moiety of FLU and βCD. These modifications are caused by changes in the FLU electronic density upon the complexation with the βCD (Loftsson et al., 1993; Schneider et al., 1998; Denadai et al., 2006a), and suggest the inclusion of both rings. These different species, included through rings A or B, might exist in equilibrium (Ali et al., 2005), which most of the evidences, supporting the preference of form B.

Furthermore, a coalescence of the internal protons H_3 and H_5 (δ = 3.82–3.96) of βCD was also observed in all supramolecular complexes at different molar ratios Fig. 6b, and it is also important to emphasize the great change in the chemical shifts of the external protons H_2 and H_4 (δ = 3.51–3.65) when compared to the pure βCD spectrum. These changes are another evidence of the interaction between the species in solution, and all of these modifications in the βCD hydrogens could be attributed to the change in the electronic density caused by the interaction with the FLU molecule, these observations were also described in the literature for different system by (Denadai et al., 2006a).

The T_1 experiments were carried out to evaluate the molecular mobility of the FLU protons in the absence and in the presence of βCD at different concentrations. These values are presented in Table 2. For organic molecules the relaxation process occurs mainly by dipolar relaxation mechanism, thus the T_1 constant is inversely proportional to the molecular correlation time or directly proportional to the rotational diffusion coefficient (Denadai et al., 2006b).

Gradual reduction in the T_1 values when the concentration of βCD increases was observed (Table 2). This large reduction in the mobility of the FLU aromatic hydrogens is in accordance with the results obtained by ^{1}H NMR experiments and indicates the reduction of FLU mobility after the complexation with βCD, these observations were also reported by Denadai et al. (2006b) to hydrochlorothiazide associated to βCD (Denadai et al., 2006b). From Table 2 it is evident a greater reduction in T_1

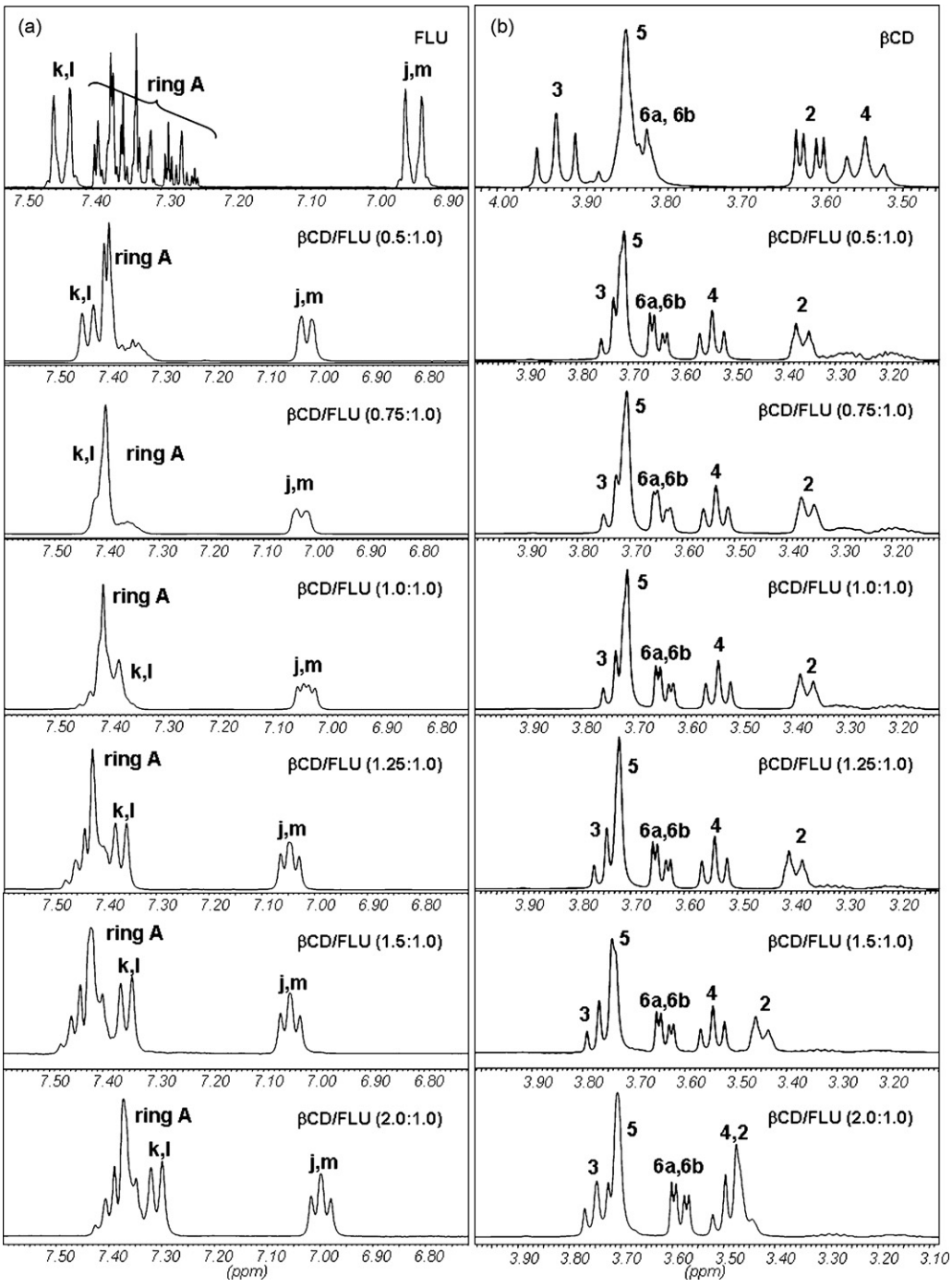

Fig. 6. Expansion of ^{1}H NMR spectra (at 400 MHz in D_2O) (a) FLU and the ICs at 0.5:1, 0.75:1, 1:1, 1.25:1, 1.5:1 and 2:1 of βCD/FLU molar ratios at $\delta = 6.80$–7.50 and (b) βCD and the ICs at 0.5:1, 0.75:1, 1:1, 1.25:1, 1.5:1 and 2:1 of βCD/FLU molar ratios at $\delta = 3.10$–3.90.

values to the hydrogens from ring B, suggesting their inclusion into the βCD cavity. Since the coalescence of the hydrogens from aromatic ring A is observed in the presence of βCD, the T_1 measurements to these protons were not carried out.

To confirm the inclusion of the FLU molecule through the aromatic region into the βCD cavity, the NOESY experiment was carried out to the ICs: 1:1 and 2:1 βCD/FLU. Nuclear Overhauser Effect (NOE) measurement is one of the most important tools to confirm the formation of the host:guest systems (Schneider et al., 1998), since this experiment is able to verify spatial correlation at short distance (less than 5 Å) that allows to get insights on the supramolecular complex geometry. The contour map expansion at 1:1 and 2:1 βCD/FLU molar ratios are presented in Fig. 7a and b, respectively.

Cross peak correlation between all FLU aromatic hydrogens (region at δ6.95 to δ7.44) to the internal (H_3, H_5 and H_6, region at δ3.59 to δ3.75) and with the external (H_2 and H_4 region at δ3.44 to δ3.76) protons of the βCD molecule was also observed when analyzed the contour map of all ICs in the molar ratio range from 0.5:1 to 2:1. These observations are in agreement with the

Table 2
1H NMR longitudinal relaxation times (T_1) values to fluoxetine, 1:1 and 2:1 βCD/FLU inclusion compounds

Hydrogen	T_1 (s) FLU	T_1 (s) IC 1:1	ΔT_1 (s)[a]	T_1 (s) IC 2:1	ΔT_1 (s)[b]
a	1.28 ± 0.01	1.14 ± 0.01	0.14	1.02 ± 0.01	0.26
b	0.62 ± 0.01	0.50 ± 0.01	0.12	0.40 ± 0.01	0.22
c	0.47 ± 0.01	0.38 ± 0.01	0.09	0.33 ± 0.01	0.14
d	0.79 ± 0.01	0.55 ± 0.01	0.24	0.51 ± 0.01	0.28
j, *m*	1.48 ± 0.01	–	–	0.87 ± 0.01	0.61
k, *l*	1.30 ± 0.01	0.81 ± 0.01	0.49	0.71 ± 0.01	0.59

–, no T_1 measurements.
[a] ΔT_1 (s) = T_1 (s) FLU − T_1 (s) IC 1:1.
[b] ΔT_1 (s) = T_1 (s) FLU − T_1 (s) IC 2:1.

results obtained by ^{1}H experiments, where strong modifications in these protons are observed caused by the inclusion of these aromatic rings in the cavity of the βCD.

The correlations observed between both aromatic rings of FLU with the internal and external protons of the βCD, to all ICs are indicative that a fast equilibrium might exist in solution. Three possibilities can be readily visualized for the species in equilibrium: (i) one βCD molecule interacting with the aromatic ring A, (ii) one βCD molecule interacting with the ring B (Ali et al., 2005) and (iii) two molecules of βCD interacting with both aromatic rings simultaneously. Due to the steric hindrance caused by the large volume of the βCD, the third possibility can be more improbable to happen. In addition, it is import to remember that higher supramolecular assemblies could be formed in aqueous solution, as observed in others works performed by our research group (Denadai et al., 2007), that can also explain all of these correlations observed in the NOESY contour map.

3.3. Theoretical results

In this work, theoretical calculations were carried out in order to determine the preferred arrangement for inclusion of FLU molecule (ring A or B) into the βCD cavity, in order to corroborate NOESY results that showed cross peak correlation with both aromatic rings of FLU. Our main goal is to propose the IC geometries and calculate the relative energies involving the two possible inclusion forms.

The ICs of two distinct modes of inclusion through ring A or B were fully optimized without any constraints and characterized as true minima on the potential energy surface by vibrational analysis. The structures obtained are showed in Fig. 8a and b. Besides the most stable in the IC form B the FLU geometry is quite similar to its crystallographic structure. The connection of the ring phenyl to the trifuoromethyl-substituted phenoxy ring is described by the torsion angle (C_{RB}–O–C–C_{RA}) which is 75.7° in the solid-state structure (Robertson et al., 1988). The included FLU calculated dihedral angle is 79.8° and 95.4° in the form B and in the form A, respectively. The energy difference calculated at the PM3 level predicts the form B as the most stable in gas phase by only 1.6 kcal mol^{-1}. This value is within the confidence limit of the method employed; therefore both isomers are equally probable at the semiempirical level. It is known that PM3 calculations are able to provide suitable equilibrium geometries but this method is not so satisfactory to determine the energy and electronic properties of these macromolecular structures (Britto et al., 2004; Nascimento et al., 2004, 2005). For that

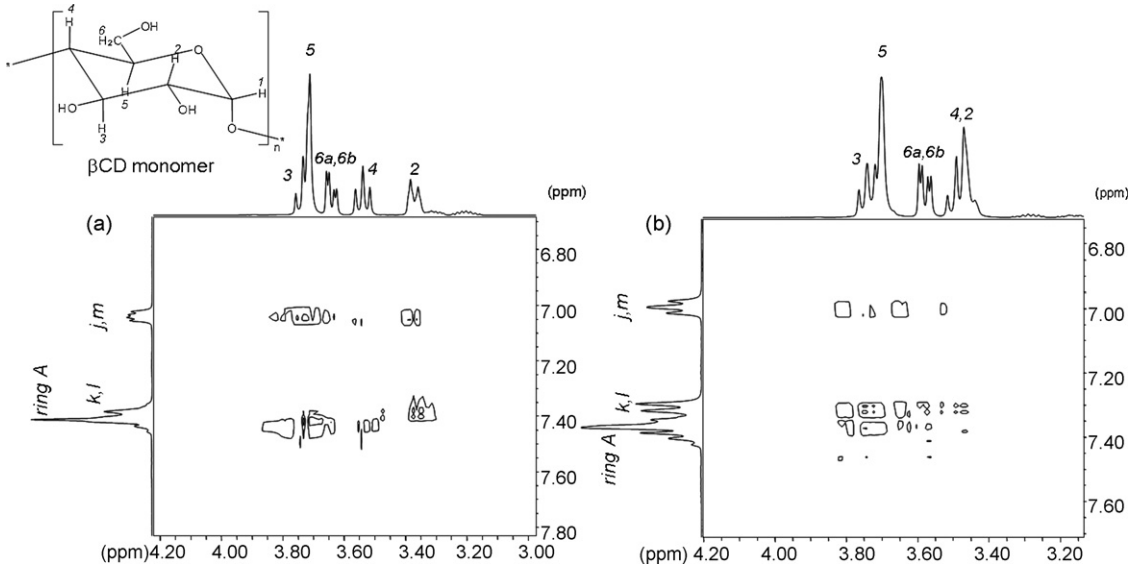

Fig. 7. Expansion of the NMR NOESY contour map (400 MHz, mixing time of 500 ms) in D_2O, at the aromatic region and the monomer of βCD (a) to the IC 1:1 βCD/FLU and (b) to the IC 2:1 βCD/FLU.

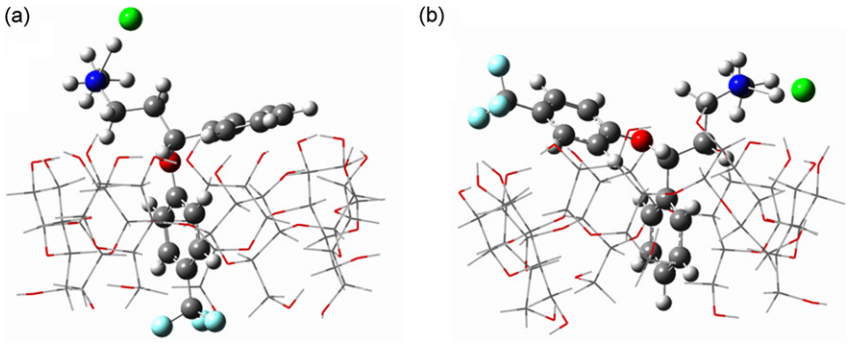

Fig. 8. (a) Structures obtained by theoretical calculations: (a) inclusion from aromatic ring B and (b) inclusion from aromatic ring A.

reason, single point calculations were also carried out at the DFT level, aiming to obtain more accurate electronic plus nuclear repulsion energy ($\Delta E_{\text{ele-nuc}}$) and define the most stable structure. In the BLYP calculations a greater energy separation for the two different forms of inclusion was found, 11.7 kcal mol^{-1}. This result defines the form B as the global minimum between the ICs theoretically studied, and corroborates the experimental proposal, indicating that form B is the most probable product.

The most stable structure, named form B, contains the aromatic ring B that with the CF_3 group, inside of the βCD cavity. Although there are no hydrogen bonds formally formed between the hydroxyl groups of βCD and the fluorine atoms from FLU molecule, probably the short distance (≈2.61 Å) between them should be responsible for the greater stabilization. For the IC which the aromatic ring A is included in the βCD cavity, a deep inclusion is prevented by the molecular moiety not included from the FLU molecule. This region of the FLU molecule produces a steric effect disfavoring the inclusion process.

The solvent effect was also taken into account by means of the PCM approach. The result supports the previous conclusion that the most stable IC is the form B. The presence of the solvent medium increases the stabilization of inclusion form B to 16.7 kcal mol^{-1} relative to form A, and extra stability of 5 kcal mol^{-1} due to the solvent effect.

3.4. ITC results

ITC was used to evaluated the thermodynamic parameters of the supramolecular host:guest interactions. Fig. 9a shows the titration of FLU in water and its titration in βCD solution.

It can be observed the FLU dilution is slightly exothermic. In the presence of βCD, the FLU titration curve assumes exothermic values demonstrating the host:guest interaction.

The pseudo sigmoidal profile obtained on the titration of FLU in βCD allowed the fitting of the experimental data with the Wiseman isotherm which the stoichiometry coefficient N (0.8), equilibrium constant K (≈6921 ± 316) and enthalpy

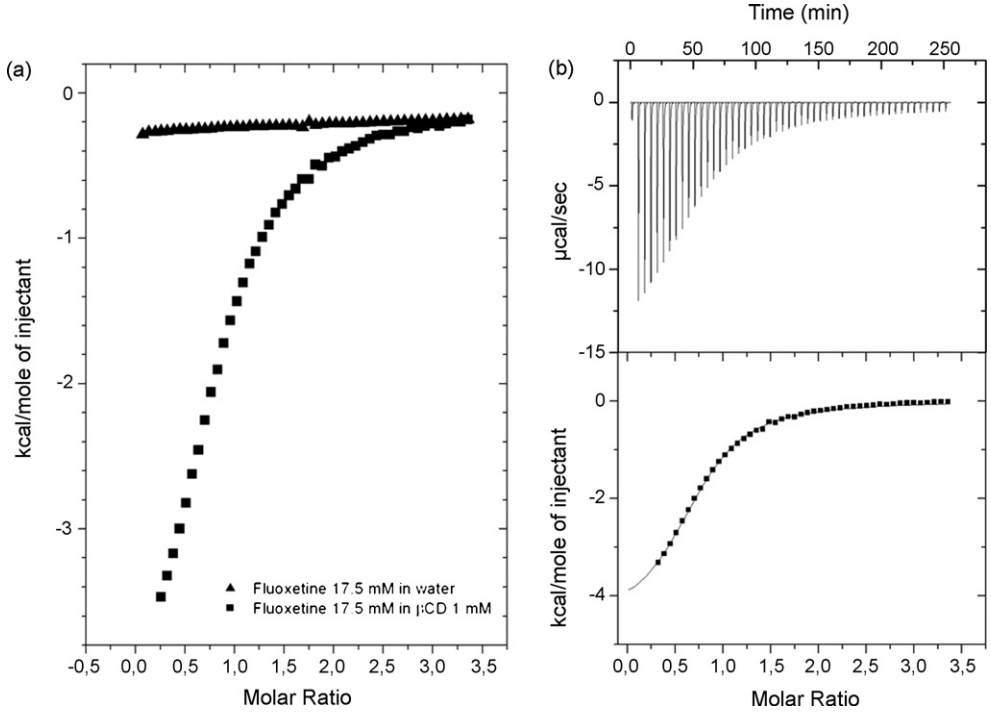

Fig. 9. (a) ITC experiments for FLU (▲) in water and (■) in βCD 1 mM. (b) Final figure: (_) raw data, (■) subtracted curve of titration, (- - -) nonlinear regression.

ΔH° (-4.1 kcal mol^{-1}) were calculated (Turnbull and Daranas, 2003). The calculations were made on the basis of "one binding site", since that the pseudo sigmoid fitting showed an equivalence point at 0.8 molar ratio approximately, which suggest a 1:1 βCD/FLU stoichiometry, not excluding other less favorable stoichiometries.

Through the thermodynamic equations (Klotz and Rosenberg, 2000), it was possible to evaluate, Gibbs energy ΔG° (-5.1 kcal mol^{-1}), and the entropic contribution $T\Delta S^{\circ}$ (1.0 kcal mol^{-1}).

The declivity of the ITC curve of the FLU in βCD solution (Fig. 9b), demonstrated the existence of a strong interaction between the species, if compared with other CD systems described in the literature (Rekharsky and Inoue, 1998; Turnbull and Daranas, 2003). This strong interaction was confirmed by the calculated equilibrium constant ($K \approx 6921 \pm 316$). This relatively abnormal strong interaction between the species could be related to the small size of FLU, which is well fit into the βCD cavity, this is the base of size/shape concept, important in the stabilization of ICs.

In addition, it is well reported related in the literature, the high affinity of the aromatic groups to accommodate in the cyclodextrin cavity, favoring the van der Waals interactions. In these senses, the enthalpy changes could be attributed to the binding of enthalpy-rich water molecules, released from the βCD cavity, with bulk water molecules (Szejtli, 1998), as well as the formation of cooperative van der Waals interactions between guest host and mainly, the electrostatic interaction between FLU and unpaired electrons of OH groups, explaining the higher enthalpic contribution.

The small entropic contribution was attributed to a balance of dessolvation 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 confers a greater molecular volume and a least rotational and translational degrees of freedom when compared to pure substances. This intense dessolvation was possible due to the small size of FLU and the strong ionic interaction with βCD.

4. Conclusions

In this paper the βCD/FLU complex was characterized by different techniques and the structure of this supramolecular system was proposed based on the experimental and theoretical results.

The FTIR–HATR spectrum suggests the formation of the IC in aqueous solution, since strong modifications in the vibrations modes of both βCD and FLU were verified when compared to the pure molecules. Since the ν(C–F) of FLU is modified upon the inclusion it is suggested that the inclusion may occur through the aromatic ring B.

This proposal is also supported by ^{1}H NMR, which showed significant change in chemical shift for aromatic hydrogens (H_k and H_l from $\delta = 7.43$ to $\delta = 7.31$) of ring B. The T_1 results also confirmed the interactions between βCD and FLU, by the large reduction of the T_1 values, mainly to the hydrogens H_k and H_l, of FLU in the presence of βCD. However, the NOESY contour map showed cross peak correlations for hydrogens from both aromatic rings (rings A and B) with internal and external hydrogens from βCD, suggesting equilibrium between ICs in solution.

In this sense, the theoretical calculations showed the inclusion through the aromatic ring B as more favorable in gas phase and also considering the solvent effect by an amount of 11.7 and 16.7 kcal mol^{-1}, respectively. In spite of the distance between hydroxyl groups of βCD and fluorine of FLU (≈2.61 Å), this interaction plays a primary role to stabilize the complex formed.

The ITC experiments demonstrate that this system has a high equilibrium constant ($K \approx 6921 \pm 316$) when compared to other host:guest systems with CD, and this process is exothermic ($\Delta H^{\circ} = -4.1$ kcal mol^{-1}) accompanied by an entropy increase ($T\Delta S^{\circ} = 1.0$ kcal mol^{-1}). It was also possible to estimate the stoichiometry as 1:1 for this system, although other stoichiometries are also possible to occur in solution.

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