Experimental and theoretical studies of the photophysics of 7-amino-3-phenyl-2H-benzo[b] [1,4]oxazin-2-one in homogeneous solvents and β-cyclodextrin aqueous solutions

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Estudios teóricos y experimentales de la fotofísica de la 7-amino-3-phenyl-2H-benzo[b][1,4] oxazin-2-ona en disolventes homogéneos y soluciones acuosas de β-ciclodextrina

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RESUMEN

El comportamiento fotofísico de la 7-amino-3-fenil-2H-benzo[b][1,4]oxazin-2-ona en solventes orgánicos y en soluciones acuosas de β-ciclodextrinas, se estudió usando métodos de fluorescencia estacionaria y de química computacional.

En medio homogéneo, el espectro de fluorescencia muestra un notable efecto solvatocrómico que produce grandes corrimientos de Stokes. El análisis de los corrimientos de Stokes usando relaciones lineales de energía libre de solvatación y la ecuación de Lippert-Mataga, indican que se produce un aumento del momento dipolar en el estado singulete excitado y la participación de un estado de transferencia parcial de carga en el proceso de desactivación. La incorporación de 7-amino-3-fenil-2H-benzo[b] [1,4]oxazin-2-ona en la cavidad de β-ciclodextrina fue monitoreada observando el incremento en la intensidad de fluorescencia en función de la concentración de ciclodextrina. Los datos de fluorescencia analizados usando gráficos de Job y la ecuación de Benesi-Hildebrand son compatibles con la formación de un complejo 1:1. La constante de asociación obtenida a partir de gráficos de Benesi-Hildebrand fue igual a 597 M^{-1} a 298 K. Además, los valores de los parámetros termodinámicos determinados de la dependencia de la constante de asociación con la temperatura, muestran que el proceso de inclusión es controlado por la entalpía. Estudios de acoplamiento molecular sugieren que la estabilidad del complejo resulta de interacciones de van der Waals favorables en el interior de la cavidad y de una interacción de enlace de hidrógeno entre el sustituyente amino de la oxazinona y un grupo hidroxilo localizado en el borde más angosto de la cavidad de la ciclodextrina. Las mismas conclusiones se obtuvieron al utilizar la metodología de Mecánica Molecular-Area Superficial de Poisson Boltzmann para determinar las contribuciones energéticas a la energía libre total para el proceso de inclusión.

Palabras clave: Ariloxazinonas; efecto solvente; sondas fluorescentes; β-ciclodextrina; complejo de inclusión; constantes de asociación.

SUMMARY

The photophysical behavior of 7-amino-3-phenyl-2H-benzo[b][1,4]oxazin-2-one was studied in organic solvents and in aqueous solutions of β-cyclodextrin using steady-state fluorescence and computational chemistry methods. In homogeneous media, fluorescence spectra show a noteworthy solvatochromic effect leading to large Stokes shifts. Linear solvation energy relationship and Lippert-Mataga equation analysis of the Stokes shifts indicate an increase of the dipolar moment in the singlet excited state and the participation of a partial charge-transfer state in the deactivation process. Incorporation of 7-amino-3-phenyl-2H-benzo[b][1,4]oxazin-2-one into the β-cyclodextrin inner cavity was monitored by observing the increase of fluorescence as a function of the cyclodextrin concentration. Analysis of fluorescence data in terms of Job plots and the Benesi-Hildebrand equation are indicate the formation of a 1:1 complex. The binding constant obtained from Benesi-Hildebrand plots was 597 M^{-1} at 298 K. Also, the values of thermodynamics parameters determined from the dependence of the binding constant on the temperature show that inclusion is an enthalpy-driven process. Docking studies suggest that the complex stability is due to favorable van der Waals interactions within the cavity and a hydrogen bond interaction between the amino substituent and hydroxyl groups located in the narrow rim of the cavity. The same conclusion was achieved employing the Molecular Mechanics Poisson-Boltzmann Surface Area methodology to determine the energy contributions to the total free energy for the inclusion process.

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Key words: Aryloxazinones; solvent effect, fluorescent probes, β-cyclodextrin, inclusion complex, binding constants.

RESUM

El comportament fotofísic de la 7-amino-3-fenil-2H-benzo [b] [1,4] oxazin-2-ona en solvents orgànics i en solucions aquoses de les β-ciclodextrines, es va estudiar utilitzant mètodes de fluorescència estacionària i de química computacional. En un mitjà homogeni, l'espectre de fluorescència mostra un notable efecte solvatocròmic que produeix grans corriments de Stokes. L'anàlisi dels corriments de Stokes utilitzant relacions lineals d'energia lliure de solvatació i l'equació de Lippert-Mataga, indiquen que es produeix un augment del moment dipolar en l'estat singulete excitat i la participació d'un estat de transferència parcial de càrrega en el procés de desactivació. La incorporació de 7-amino-3-fenil-2H-benzo [b] [1,4] oxazin-2-ona a la cavitat de β-ciclodextrina va ser supervisada observant l'increment en la intensitat de fluorescència en funció de la concentració de ciclodextrina. Les dades de fluorescència analitzades mitjançant les gràficas de Job i l'equació de Benesi-Hildebrand són compatibles amb la formació d›un complex 1:1. La constant d›associació obtinguda a partir de gràficas de Benesi-Hildebrand va ser igual a 597 M^{-1} a 298 K. A més, els valors dels paràmetres termodinàmics determinats de la dependència de la constant d'associació amb la temperatura, mostren que el procés de inclusió és controlat per l'entalpia. Estudis d'acoblament molecular suggereixen que l'estabilitat del complex resulta d'interaccions de van der Waals favorables a l'interior de la cavitat i d'una interacció d'enllaç d'hidrogen entre el substituent amino de la oxazinona i un grup hidroxil localitzat en la part extrema més estreta de la cavitat de la ciclodextrina. Les mateixes conclusions es van obtenir en utilitzar la metodologia de Mecànica Molecular-Àrea Superficial de Poisson Boltzmann per determinar les contribucions energètiques a l'energia lliure total pel procés d'inclusió.

Paraules clau: Ariloxazinones; efecte solvent; sondes fluorescents; β-ciclodextrina; complex de inclusió; constants d'asociació.

INTRODUCTION

Cyclodextrins (CD) are cyclic oligosaccharides, the most common consisting of six (α-CD), seven (β-CD) or eight (γ-CD) glucopyranose units linked by α-(1,4) bonds. These toroidally-shaped capsules produced from starch have a special structure characterized by a hydrophilic exterior and an internal hydrophobic cavity. The size of the internal cavity (5-8 Å) for the most common CDs, depends on the number of glucopyranose units. These distinctive structural characteristics confer the CDs their unique ability to form inclusion complexes in aqueous medium with a wide variety of hydrophobic guest molecules [1-3]. Covalent bonding is not involved in the complex formation, only physical forces accounts for the complexes feasibility [4,5]. The stability of inclusion complexes in aqueous solutions is mainly due to van der Waals forces and hydrophobic interactions that help the hydrophobic moiety of the guest molecule to be included within the hydrophobic CD cavity [6,7]. Thus, the organic guest can enter entirely into the cavity or in part depending on the polarity, molecular size and geometrical factors. The complexation phenomenon often involves remarkable variations in stability, degradation paths, reactivity and bioavailability (in the case of drugs or pro-drugs) of the guest molecule. Also, the photophysical and photochemical properties of the included molecule can change noticeably because of the non-polar microenvironment provided by the CD cavity. Formation of supramolecular complexes of fluorescent molecules with CDs leads to a dramatic increase of the fluorescence quantum yield of a probe [8,9]. Also, the rates of photochemical reactions decrease notably when CD inclusion complexes are formed due to the geometric constraints imposed on the guest molecule [10,11]. Moreover, the binding of guest molecules within the host cyclodextrin is not fixed or permanent but is rather a dynamic equilibrium, therefore measurements of the inclusion constant allow determining the thermodynamic parameters associated to the complex formation.

Over the last years, our group has studied the photophysical and photochemical behavior of a family of hydrophobic dyes, the aryloxazinones [12,13]. These compounds behave in very similar way than coumarine derivatives. Their fluorescence is characterized by intense and broad emission bands, large Stokes shifts indicating a significant sensitivity to the polarity of the environment, high fluorescence quantum yields and short fluorescence lifetimes [14, 15]. Also we found that these compounds are essentially photostable in the absence of additives. Their photophysical properties and large photostability in air-equilibrated solutions suggest that aryloxazinone derivatives could be valuable molecules to be used for several applications such as laser dyes, fluoro-ionophores, fluorescence markers and probe molecules for the examination of electron transfer processes and solvation effects as well as for singlet oxygen photosensitization [12,13, 16-19]. However, the use of organic solvents (the simplest approach) is incompatible with biologically- or environmentally-relevant applications, and is also frequently discouraged by economic and environmental considerations. The search for fluorescent molecules with high photostability and brightness in water is a topic of high interest since water is environmentally benign and biologically relevant. Popular strategies for solubilizing hydrophobic fluorescence molecules rely on the use of stabilizing, solubilizing, disaggregating and enhancing additives. In this context we have studied the formation of supramolecular systems between benzo- and naphthoxazinones and β-cyclodextrin. As a first step in this path we are interested in assessing the sensitivity of their properties to the inclusion within a hydrophobic cavity in aqueous meda. We report in this paper an experimental and theoretical study of the inclusion complex formed between 7-amino-3-phenyl-2H-benzo[b][1,4]oxazin-2-one (7-ABZ), Figure 1, and β-cyclodextrin. The aims of this study were (1) to determine the stoichiometry and binding constants, K_1, of the complex, (2) to determine the thermodynamic parameters associated to the inclusion process, and (3) to determine, through computational studies, the optimum geometry of the complexes in order to estimate the van der Waals and electrostatic contributions to the binding energies of the host–guests complexes.

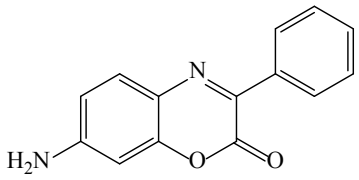


***Figure 1**. Structure of 7-amino-3-phenyl-2H-benzo[b][1;4]oxazin-2-one.*

EXPERIMENTAL

Reagents

All experiments were performed with analytical or spectroscopic grade chemicals. β-CD (obtained from Merck, 99.9% HPLC) was used as received. The fluorescent probe 7-amino-3-phenyl-2H-benzo[b][1,4]oxazin-2-one was synthesized as described earlier [14] and its structure was confirmed by means of ^{1}H NMR. The purity of the dye in these samples has been established by thin layer chromatography. Distilled water was purified trough a MilliQ system. Citrate buffer solutions (pH 3 and pH 5, 0.1 M) were prepared according to the current procedures. All other solvents used were of the commercially available spectroscopic grade.

Apparatus

Absorption spectra were recorded in a Unicam UV 4 spectrophotometer using 1 cm quartz cells. Data were processed with Vision software. Steady-state fluorescence measurements were performed with a PC1 photon counting spectrofluorimeter from ISS, equipped with a thermostatic rectangular cell holder. The experiments were carried out in the range 25–50 ρC, using a bath/circulation thermostat Haake K. The fluorescence spectra of each set of solutions were measured using identical experimental conditions. According to the maximum in the absorption spectrum, the excitation wavelength was set at 400 nm.

Solution preparation

The dye stock solutions were prepared by dissolving an appropriate amount of benzoxazinone in methanol. The standard solutions of β-CD in the corresponding buffer were prepared with concentrations in the range 1- 10 mM. In order to study the influence of the β-CD on the fluorescence dye intensity at different pH's, in all experiments the concentration of the fluorophore was kept constant.

Inclusion complex preparation

Typically a set of 8 – 10 solutions was prepared containing β-CD in the range 0 – 10 mM and a constant concentration of 7-ABZ. To prepare the final solution, 100 μL of a stock solution of 7-ABZ 30 mM in methanol were added into 15 ml PTFE test tube and the solvent was removed with a nitrogen stream. 10 mL of a solution of β-CD in milliQ water or citrate buffer at the appropriate concentration were then added to the dry 7-ABZ. The solutions were homogenized in an ultrasonic bath at room temperature by 2 min and finally shaken at controlled temperature in a Hangzhou model MSC-100 thermoshaker for 24 hrs. (650 rpm).

Determination of the Complex stoichiometry

The method of continuous variations was employed to determine the complex stoichiometry [20]. Briefly, the fluorescence intensity of the guest at the concentration *M*, *F(M)*, was measured after equilibration of solutions prepared with different molar ratios 7-ABZ:β-CD. From Jobs plots of *F(M)* against the molar fraction *X* of the guest in the solution, the stoichiometry *(n*) was obtained from the *X* value corresponding to the maximum value of *F(M)* according to equation (1):

$$X = (n+1)^{-1} \quad (1)$$

Association constants determinations

The association constant for 7-ABZ …β-CD complex was determined using the Benessi-Hildebrand method [21]. In the case of a complex with 1:1 stoichiometry, equation (2), the equilibrium constant is described by the equation (3)

$$7\text{-}ABZ + \beta - CD \rightleftharpoons [7\text{-}ABZ \cdots \beta - CD] \quad (2)$$

$$K_1 = \frac{[7\text{-}ABZ \cdots \beta - CD]}{[7\text{-}ABZ][\beta - CD]} \quad (3)$$

As inclusion of 7-ABZ in the β-CD cavity enhances the fluorescence quantum yield of the guest in a fashion dependent on the β-CD concentration, values of K_1 can be obtained from the analysis of the fluorescence intensities in terms of the modified Benesi-Hildebrand equation (4) for 1:1 stoichiometry:

$$\frac{1}{F - F_0} = \frac{1}{(F_1 - F_0)} + \frac{1}{K_1(F_1 - F_0)}\frac{1}{[\beta CD]} \quad (4)$$

where *F* and F_0 are the fluorescence intensities of 7-ABZ in the presence and absence of β-CD, respectively, and F_1 is the expected fluorescence intensity when all guest molecules are included in a complex.

Molecular Modeling Procedures

Initial structure of 7-ABZ was built using Gaussian View O5X and then energy minimized using DFT method with the 6-31g** orbital base in the Gaussian 09 environment [22]. The restrained electrostatic potential (RESP) fitted charges, employed in the molecular modeling procedure, were assigned using the RED-III-4 program. The initial structure of β-CD was obtained from the Cambridge Structural Database (code BCDEXD10). AutoDock4 [23, 24], was used to accomplish the 200 docking runs by applying a Lamarckian Genetic Algorithm (LGA) to generate the population evaluated using the following parameters: a population of 150 individuals, a maximum of 2.5 x 10^6 energy evaluations and 27000 generations as an end criterion. The best docking score was selected for further studies.

The Amber 12 software package was used to carry out molecular dynamics studies. The complex obtained by molecular docking was solvated in a pre-equilibrated octahedral water box of 15 Å. The solvated complex was first minimized and then heated from 100 to 300 K in equilibration runs of 1 ns each. Finally, a production run of 10 ns was carried out. The energetic components were analyzed using the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) module as implemented in Amber 12.

RESULTS AND DISCUSSION

Photophysical behavior in homogeneous solvents

The absorption spectra of 7-ABZ are nearly independent on solvent polarity with the maximum of the lowest-energy absorption band, λ_{max}(Abs), centered around 400 nm and

a molar extinction coefficient around of 18000 M^{-1} cm^{-1}. Spectra calculations employing DFT formalism (B3LYP-6311g+ for structure optimization and ZINDO-S to calculate the Franck-Condon transitions) overestimate λ_{max}(Abs) by about of 10 nm, however analysis of molecular orbitals indicate a π-π* transition. These results are comparable to those reported for other benzoxazinone derivatives [25]. The polarity of the solvent has a large effect on the fluorescence spectrum of 7-ABZ. The red shift is quite large in polar solvents and the position of emission maximum, λ_{max}(Em), shifts from 451 nm in n-hexane to 524 nm in methanol. These shifts obtained in a set of 16 solvents, were analyzed in terms of the solvatochromic equation (5) introduced by Kamlet et al. [26, 27]:

$$\nu = \nu_0 + a\pi^* + b\alpha + c\beta + d\rho_H^2 \quad (5)$$

where υ is the position of the emission maxima in cm^{-1}, υ_0 is a constant for a given fluorescent molecule, π* accounts for dipolarities and polarizabilities of solvent, α is related to the hydrogen bond donor solvent ability, β indicates the solvent capacity as hydrogen bond acceptor, and ρH2 is the square of Hildebrand parameter, that accounts for the solvent cohesive energy density and models the cavity effects [28, 29]. The result of multilinear regression analysis by employing StatView 5.0 statistical software affords the equation (6):

$$\nu = 21941 - 1751\pi^* - 1005\alpha - 1280\beta \quad (6)$$

The equation (6) (number of data point N =16, correlation coefficient R=0.991 and Fisher index F = 230), shows that the values of λmax(Em) depend on the microscopic solvent parameters π*, α and β, indicating that the emission red shifts in solvents able to stabilize charges and dipoles as well as in solvents with high α and β values. This result is strong evidence that supports a substantial charge-separation process occurring in the first excited singlet state. The red shift can be interpreted in terms of an increased dipole moment of 7-ABZ upon chromophore excitation and subsequent solvent relaxation. Indeed a Lippert-Mataga plot [30,31] of the Stokes shift vs. the solvent polarity function, yields the difference of dipole moments between the excited- and ground states ($\mu* - \mu_0$), equal to 6.8 Debye. This value, was obtained using an Onsager cavity radius of 4.8 Å calculated from the minimized structure (B3LYP-6311g+) under Gaussian 09 environment. In addition, Mulliken charge analyses shows that the charge separation mainly involving atoms in the oxazinone ring with the dipole in the molecular plane oriented close to the carbonyl bond axis. These results are comparable to those obtained for previously studied compounds of the family [13].

Photophysical behavior in cyclodextrin solutions.

The complexation of a fluorescent molecule with CDs or CD derivatives in aqueous media usually causes an increase of the fluorescence intensity and a blue shift of the maximum of the fluorescence spectrum. This behavior is largely due to the hydrophobic nature of the CD cavity, conduct similar to the observed when the media is changed from polar solvents such as water or methanol to non-polar solvents such as hexane or benzene. 7-ABZ has a low fluorescence quantum yield in water that increases in a concentration dependent fashion when β-CD in added to the solution (Figure 2).

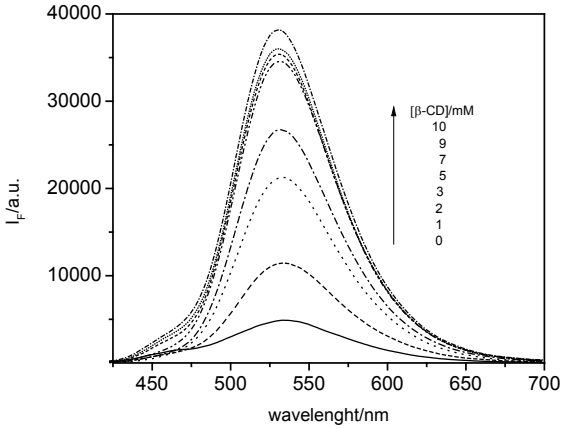
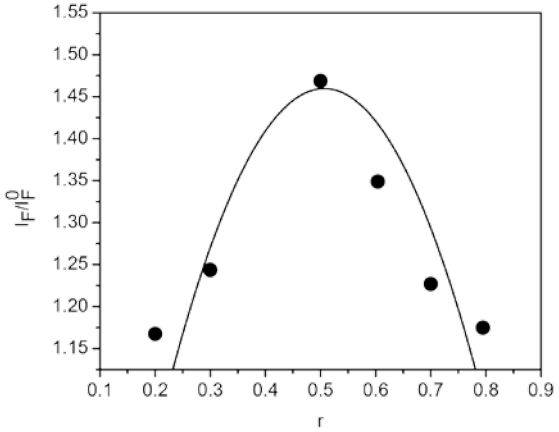


Figure 2. *Dependence of the fluorescence intensity of 7-ABZ on the β-CD concentration in aqueous solution (pH 6.0).*

Furthermore, the emission maximum of 7-ABZ shifts slightly towards the blue, from 538 nm in water at pH 6.8 to about 529 nm as the host concentration increases. The increase in the fluorescence intensity and the shift of the emission maximum to shorter wavelength indicate that the oxazinone derivative changes its environment upon interaction with the added β-CD. The inclusion of 7-ABZ in the β-CD cavity protects the fluorescent probe from the normal quenching in aqueous solution and modifies the rate of the competitive non-radiative processes that deactivate the excited singlet state, explaining the threefold increase of the fluorescence intensity when compared with the fluorescence observed in pure water.

The dependence of the fluorescence intensity enhancement on the β-CD concentration can be employed to determine the complex stoichiometry using the method of continuous variations. Figure 3 shows the Job plot obtained for 7-ABZ in pure water, where r = [7-ABZ]/([7-ABZ] + [β-CD]) was varied from 0.1 to 0.9 and plotted against the relative fluorescence intensity.



The Job's plot shows a maximum at r = 0.5, which indicates the formation of 1:1 inclusion complex between 7-ABZ and β-CD.

The enhancement of the fluorescence intensity measured as a function of host concentration can be also used to obtain the binding constant by means of the Benesi-Hildebrand approach [32-34]. To apply the method, the fluorescence intensity was monitored keeping the total concentration of

7-ABZ constant, whereas the concentration of β-CD was increased. The 1:1 modified Benesi-Hildebrand equation (4) was used to calculate the values of the equilibrium constant K_1 for the complex. Figure 4 shows the double reciprocal plot obtained for 7-ABZ in water at 298 K.

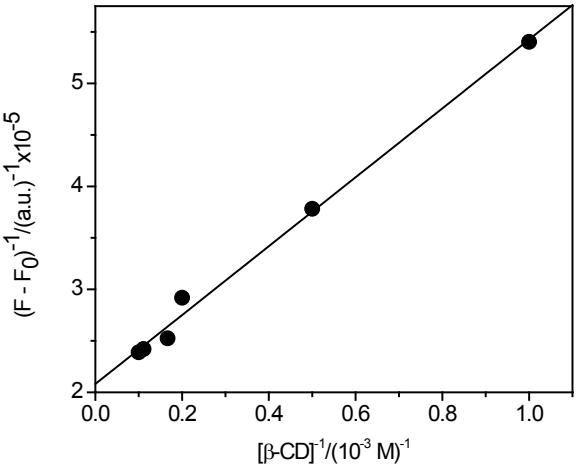


Figure 4. *Benesi-Hildebrand plot for the 1:1 inclusion complex formed between 7-ABZ and β–CD in aqueous water solution (pH 6.0*

The assumption of 1:1 association between 7-ABZ and β-CD gives a linear relationship with good correlation coefficients (r = 0.99) for all pH values and temperatures employed. A plot constructed assuming 1:2 association of 7-ABZ:β-CD shows a downward curvature as the CD concentration increases, which rules out the 1:2 stoichiometry. The values of K_1 were obtained from the ratio between the intercept and the slope of the Benesi-Hildebrand plot (Table 1).

Table 1. *Values of the binding constant and thermodynamic parameters for the inclusion of 7-ABZ in β-CD at different pH values. T = 298 K.*

	K_1/M^{-1}	ΔH^0/cal mol^{-1}	ΔS^0/cal mol^{-1}	ΔG^0/cal K^{-1} mol^{-1}
Water, pH = 6.8	597	-9500	-3780	-19.2
Citrate buffer, pH = 5	486	-7680	-3660	-13.5
Citrate buffer, pH = 3	156	-4730	-2990	-5.8

Data of Table 1 shows that binding constants are relatively high at all pHs and decrease as pH diminishes. As mentioned above, hydrophobic interactions are one of the major factors that promote the inclusion of hydrophobic guest into the cyclodextrin internal cavity and the inclusion process is more favorable for uncharged molecules than for charged ones. Consequently, lowering the pH values increases the protonation of the guest –mainly of the amino substituent in the benzene moiety– , improves its water solubility, and diminishes the relative importance of the hydrophobic effect leading to lower K_1 values. In addition, we measured the dependence of K_1 on the temperature in the range 25 to 45 ºC at all pH's. Values of K_1 diminish as the temperature increases, as expected for a process driven by van der Waals interactions, electrostatic interactions and hydrophobic effect. The enthalpy associated to the binding, ΔH0, was obtained from the slope of the classical plot of ln K_1 vs. 1/T that gives a straight line in the temperature range employed, with correlation coefficients values 0.88 and 0.99. Thermodynamic parameters ΔG0, ΔH0 and ΔS0 for the association of 7-ABZ with β-CD are included in Table 1. The negative value of enthalpy at all pH's, corresponding to an exothermic host-guest interaction, implies that the complex dissociates as the temperature increases and the enthalpy value could be a result of a few factors partially compensating each other: a) a diminution of hydrogen bonds in the system; b) release of water molecules off the β-CD internal cavity, and c) release of water molecules around the organic molecule. The entropy values are also negative indicating that the increase of entropy resulting from the hydrophobic effect is lower than the diminution caused for loss of translational and rotational degrees of freedom due to complex formation. As result of these negative changes in enthalpy and entropy, is concluded that the inclusion of 7-ABZ in β-CD is an enthalpy-controlled process. Moreover, we determined the dependence of the thermodynamic parameters for the inclusion process with the pH of the medium. All parameters, ΔG0, ΔH0 and ΔS0 increase as the pH diminishes and the energetics of the incorporation of the guest into the β-CD cavity is modified significantly. The inclusion process becomes less spontaneous at lower pH's because the increase of the fluorescent guest solubility decreases the exothermicity due to the lower release of well-organized water molecules surrounding the guest and the release of enthalpy-rich water molecules from the cyclodextrin cavity. Also, ΔS0 increases probably due to a greater compensation between the negative entropy contribution due to the loss of degrees of freedom and the entropy positive contribution originated by the hydrophobic effect.

Docking and Molecular Mechanics Studies.

Computational chemistry has been used to study a diversity of host–guest complexes involving CD [35-37]. Docking is a method of molecular modeling appropriate to predict the preferred the relative orientation of one molecule respect to a second when are bound to form a stable complex. To investigate the inclusion geometry of the complex formed between 7-ABZ and β-CD, molecular docking studies was carried out using the AutoDock4 program. The best docking score structure is shown in Figure 5.

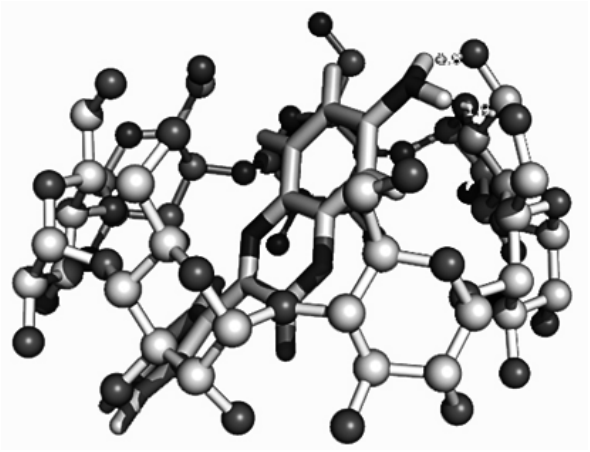


Figure 5. *The most stable structure obtained from the docking simulations of the inclusion of 7-ABZ in β-CD.*

The docking results shows that the favored relative orientation of the complex is one in which 7-ABZ inserts diagonally in the β-CD cavity with the phenyl substituent in

position 3 of the oxazinone ring oriented towards the wide edge of the truncated cone whereas the amino substituent is exposed to the inner surface near the narrow edge of β-CD. The hydrogen of the amino substituent is located at 1.9 Å of the oxygen of a hydroxyl group oriented towards inside the narrow edge of the cavity. This result suggests that two types of interactions contribute to the complex stability, van der Waals interactions in the inner of the cavity and a hydrogen bond between the amino and hydroxyl group. The presence of a hydrogen bond also allow to explain the increase in the fluorescence intensity because restrict the rotation of the amino group that is a well-known non-radiative deactivation path of the excited singlet of amino substituted dyes [38].

Table 2. *Contributions to the total free energy for the binding of 7-ABZ with β-CD calculated by the MM-PBSA method.*

Contribution	MM-PBSA/kcal mol^{-1}
ΔE_{vdW}	-24.03
ΔE_{Elec}	-13.36
ΔE_{PB}	-59.65
ΔE_{NPolar}	-15.78
ΔE_{Disper}	27.47
ΔG_{gas}	-37.39
ΔG_{solv}	-47.96
$\Delta G_{Binding}$	-85.34

The components to the total free energy are showed in kcal/mol: van der Waals interaction component (ΔE_{vdW}), electrostatic interaction component (ΔE_{Elec}), non-polar solvation component (ΔEN_{Polar}), polar solvation component (ΔE_{PB}), dispersion component (ΔE_{Disper}), interaction energy in the gas phase (ΔG_{gas}), solvation energy (ΔG_{solv}), binding energy (ΔG_{total}).

The binding energies (complexation energies) were evaluated with the MM-PBSA methodology [39, 40]. Using this method, inclusion free energies were estimated using the trajectories of each species from the same simulation run. The complexation energies were evaluated using structures coming from simulation runs on isolated 7-ABZ and β-CD monomers solvated with water molecules. Binding energies are thus computed as follows:

$$7-ANB_{isolated} + \beta - CD_{isolated} \rightleftharpoons \left[7-ABZ \cdots \beta - CD\right]_{complex} \quad (7)$$

According to equation (7), the interaction energy is calculated from free energies of three reactants:

$$\Delta G_{Binding} = \Delta G_{gas} + \Delta G_{solv} \quad (8)$$

ΔG_{gas} in equation (8) correspond to the sum of the van der Waals interaction component (ΔE_{vdW}) and the electrostatic interaction component (ΔE_{Elec}) whereas ΔG_{solv} was obtained from the non-polar solvation component (ΔEN_{Polar}), the polar solvation component (ΔE_{PB}) and the dispersion component (ΔE_{Disper}). The data of Table 2 shows that the van der Waals contribution (vdW) is more significant than the electrostatic interaction (el). It is expected that the Coulombic part of the electrostatic energy be large and favorable, due to interactions between amino group of the ligand and the hydroxyl groups of the host, but the release of high dielectric solvent molecules from these groups during binding diminishes

Table 3. *Contributions to the total entropy for the binding of 7-ABZ with β-CD calculated by the MM-PBSA method.*

Contribution	TDS/kcal mol^{-1} K^{-1}
$\Delta S_{Traslational}$	-12.44
$\Delta S_{Rotational}$	-9.80
$\Delta S_{Vibrational}$	5.18
DS_{Total}	-17.06

the contribution of electrostatic interaction component to the binding free energy. This suggestion is consistent with the loss in configurational entropy as consequence of the binding process. Table 3, includes the contributions to the total entropy to the binding process, obtained using the NMODE module of Amber 12 software. Data on Table 3 indicates that, the loss in configurational entropy is due to the decrease of the traslational and rotational degree of freedom. The increase in vibrational movements is not enough to compensate it. Considering the energy contributions to the free energy of the binding process, the MM-PBSA calculations indicates that the spontaneous inclusion of 7-ABZ in β-CD is driven mainly by entalphic factors.

In summary, photophysical behavior of 7-amino-3-phenyl-2H-benzo[b][1,4]oxazin-2-one in organic media is dependent on the solvent micro-properties. Emission shows an important Stokes shift in polar solvents, compatible with an increase of the dipolar moment in the excited singlet state and the participation of a partial charge-transfer state in the deactivation process. In aqueous β-cyclodextrin solutions, 7-amino-3-phenyl-2H-benzo[b][1,4]oxazin-2-one incorporates spontaneously to the cyclodextrin inner cavity by means of an enthalpy driven process. All thermodynamic parameters associated the inclusion process increases as the pH of the medium decreases and the binding of 7-ABZ to β-CD becomes less spontaneous.

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