ANTIOXIDANT CAPACITY OF CARBOLINE INCLUSION COMPLEXES WITH mβCD Y βCD

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ABSTRACT

In the present study, the thermodynamic aspects and reactivity of a compound derived from Carboline (7-hydroxy-1-methyl-2,3,4,9-tetrahydro-1H-βcarboline-3-carboxylic acid) when it forms inclusion complexes with βCD and mβCD were described. For this purpose, computational modeling and tools such as Molecular Docking for obtaining the inclusion complex, Second Order Perturbative Analysis (E2PERT) and ONIOM2 (DFT/PM6) for thermodynamic analysis, interactions and reactivity were employed. As a result, it was obtained that the inclusion complexes are viable and stable in the modeled conditions (gas phase, 1 atm and 298K), but not spontaneous. For the process to be spontaneous and viable, the solvent effect and the desolvation of the hydrophobic cavity of the cyclodextrins were considered. On the other hand, the non-covalent interactions were described from the E2PERT analysis, where Hydrogen interactions are shown as donations and acceptance of Lewis and Non-Lewis orbitals. Finally, the global and local reactivity indices show that the ligand presents antioxidant activity of the SET and HAT type in the presence of the $OH\cdot$ radical, increasing this tendency when the compound forms inclusion complexes.

Keywords: Inclusion complexes; Carboline; Reactivity; βCD.

1. INTRODUCTION

Free radicals are a type of chemical species (atom or molecule) that are highly reactive and have a short half-life due to the presence of unpaired electrons, which can cause damage at the cellular level and even DNA [1]. Among the best-known pathologies produced by the action of free radicals (oxidative stress) are neurodegenerative disorders such as Alzheimer's [2,3], and Parkinson's [4,5,6], and somatic disorders such as cancer [7,8], hypertension [9], diabetes [10,11], and even aging.

Among the best-known radicals are reactive oxygen species (ROS) ($^1O_2, OH\cdot, RO_2\cdot$) [12], which are generated by various metabolic mechanisms in cells such as mitochondrial oxidative phosphorylation (In the case of ROS) [13]. One of the most reactive ROS is $OH\cdot$ which greatly promotes lipid peroxidation of cell membranes [14]. The generation of this radical is possible through the transfer of an electron, which can be contributed by cations, mainly transition metals such as Fe^{2+}, Cu^{+} or Mn^{+2}. The best-known reaction is the Fenton mechanism [15], which from the oxidation of Fe^{2+} (or Cu^{+}) promoted by H_2O_2 produces the OH· radical (eq. 1).

$$\begin{aligned} Fe^{2+} + H_2O_2 &\longrightarrow Fe^{3+} + OH^- + OH\cdot \\ Cu^{+} + H_2O_2 &\longrightarrow Cu^{2+} + OH^- + OH\cdot \end{aligned} \quad (1)$$

To combat free radicals, cells present endogenous protection mechanisms, mainly of the enzymatic type, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) [16,17,18]. But to complement the antioxidant activity, there are exogenous antioxidants, such as vitamins (vitamin A and E), carotenoids (lycopene and β-carotene), flavones and polyphenols, which can be supplied by the diet [19]. Therefore, the synthesis and discovery of new compounds is of particular interest to combat the aforementioned pathologies. One compound with potential antioxidant capacity is carboline derivatives, as demonstrated by Francik et al. [20] and Krüzselyi et al. [21].

Carbolines (specifically β-Carbolines) are a type of natural alkaloid derived from 9H-pyrido[3,4-b]indol, widely distributed in nature, present from Algae (*Dichothrix baueriana*) [22], to small Arthropods such as termites and scorpions [23,24], in which they fulfill varied tasks such as antioxidant activity (AOX) [25], free radical trapping (ROS) ($^1O_2$ and $OH\cdot$) [26] and antimicrobial (*Trypanosoma cruzi*, *Herpes simplex* and *Staphylococcus aureus*) [27,28,29].

For their part, cyclodextrins (CDs) are toroidal cyclic macromolecules derived from starch, which are made up of N units of α-1,4-D-glucopyranose ($N = 6, 7, 8$). Given the arrangement of these sugars, a hydrophobic cavity and a hydrophilic external face are generated by the arrangement of hydroxyl groups [30,31]. This amphipathic characteristic of CDs makes them potential candidates for the transport and modification of physicochemical properties of molecules such as their reactivity [32], forming host-guest inclusion complexes [33].

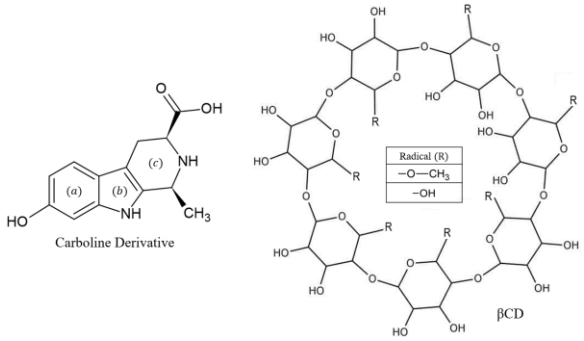

Figure. 1. Carboline derivative (7-hydroxy-1-methyl-2,3,4,9-tetrahydro-1H-βcarboline-3-carboxylic acid) [21] and mβCD (R: $O-CH_3$) / βCD (R: $-OH$)

The formation of inclusion complexes can be described by the thermodynamics of the process [34], which is mainly guided by Enthalpy (ΔH), Entropy (ΔS), Gibbs Free Energy (ΔG) [35,36,37], and the stability of the inclusion complex, which can be described by the interaction energy (ΔE_{int}) considering the *Basis Set Superposition Error* (BSSE), which is a phenomenon associated with the interaction and superposition of basis functions of interacting molecular systems [35,38,39].

$$\Delta E_{int} = E_{L/\beta CD} - \left(E_L + E_{\beta CD}\right) + BSSE \quad (2)$$
$$\Delta G^\circ = G^\circ_{L/\beta CD} - \left(G^\circ_L + G^\circ_{\beta CD}\right) \quad (3)$$
$$\Delta H^\circ = H^\circ_{L/\beta CD} - \left(H^\circ_L + H^\circ_{\beta CD}\right) \quad (4)$$
$$\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T \quad (5)$$

The thermodynamics of the inclusion process is directly related to the movement of the ligand in the cavity and the intermolecular interactions (non-covalent interactions, NCI), which can be described by NBO [40], and Second Order Perturbational (E2PERT) analysis [41,42], which is a numerical description of the stabilization phenomenon between donor orbitals (bonding, ϕ) and acceptor orbitals (anti-bonding, ϕ^*) of a molecule, so in an inclusion complex it can be described as a stability criterion given the interactions of electron densities between the ligand and the βCD [43]. Mathematically, the second-order perturbative analysis ($E_{ij}^{(2)}$) is of the form:

$$E^{(2)}_{\phi\phi^*} = -2\frac{\langle\phi|\boldsymbol{F}|\phi^*\rangle^2}{\varepsilon_{\phi^*} - \varepsilon_{\phi}} \quad (6)$$

$\boldsymbol{F}$ being the Fock operator, ϕ, ϕ^* being the natural bonding orbitals (NBO) bonding and anti-bonding, and $\varepsilon_{\phi^*}, \varepsilon_{\phi}$ being the energies of the bonding and anti-bonding orbitals [42].

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Similarly, it has been described that the thermodynamics of the inclusion complex formation process is influenced by the desolvation of the cyclodextrin cavity upon ligand (L) encapsulation [44,45]. Although the exact amount of water molecules in the cyclodextrin cavity is uncertain (approximately 11 water molecules) [46], it has been evidenced that the desolvation process generates a thermodynamically viable environment for the inclusion complex formation process, as both enthalpy and entropy favor spontaneity (more negative Gibbs function).

$$\mathrm{L} + 11\ \mathrm{H_2O} \cdot \beta\mathrm{CD} \longrightarrow \mathrm{L} \cdot \beta\mathrm{CD} + 11\ \mathrm{H_2O} \quad (\Delta H^{\circ} < 0, \Delta S^{\circ} > 0) \tag{7}$$

To describe the reactivity and antioxidant capacity (AOX) of compounds from the theoretical point of view, global and local reactivity indices can be used [47]. Among the global reactivity indices are chemical potential (μ) and electronegativity (χ) [48,49], chemical hardness (η) and Electrophilicity index (ω) [50]. The local reactivity indices, on the other hand, are the Fukui functions ($f_{(r)}$) [51], and the dual descriptor $\left(f_{(r)}^{(2)}\right)$ [52], which can be derived from the Fukui FMO approximations [51].

$$\mu = \left(\frac{\partial E}{\partial N}\right)_{v_{(r)}} \quad ; \quad \chi = -\left(\frac{\partial E}{\partial N}\right)_{v_{(r)}} \quad ; \quad \eta = \left(\frac{\partial \mu}{\partial N}\right)_{v_{(r)}} \quad ; \quad \omega = \frac{\mu^2}{2\eta} \tag{8}$$

$$f_{(r)}^{+} = \left(\frac{\partial \rho_{(r)}}{\partial N}\right)_{v_{(r)}}^{+} \approx \left|\psi_{(r)\,\mathrm{LUMO}}\right|^2 = \rho_{(r)\,\mathrm{LUMO}} \tag{9}$$

$$f_{(r)}^{-} = \left(\frac{\partial \rho_{(r)}}{\partial N}\right)_{v_{(r)}}^{-} \approx \left|\psi_{(r)\,\mathrm{HOMO}}\right|^2 = \rho_{(r)\,\mathrm{HOMO}} \tag{10}$$

$$f_{(r)}^{(2)} = \left(\frac{\partial f_{(r)}}{\partial N}\right)_{v_{(r)}} \approx f_{(r)}^{+} - f_{(r)}^{-} = \rho_{(r)\,\mathrm{LUMO}} - \rho_{(r)\,\mathrm{HOMO}} \tag{11}$$

Krüzselyi et al. [21] performed the elucidation of a new Carboline derivative (7-hydroxy-1-methyl-2,3,4,9-tetrahydro-1H-βcarboline-3-carboxylic acid) with antioxidant capacity, so, as a completeness, the present article aims to describe from the theoretical point of view the antioxidant mechanism presented by this compound, and its ability to form inclusion compounds with methyl-βCD (mβCD) and βCD. This description was carried out from the local reactivity indices such as Fukui functions ($f_{(r)}$) and Dual Descriptor $\left(f_{(r)}^{(2)}\right)$ and global reactivity indices, as well as the thermodynamic aspects of the process and the non-covalent interactions formed between the ligand and the host (βCD).

2. COMPUTATIONAL DETAIL

2.1 Optimization of Ligand and β-Cyclodextrin

The structure of β-Cyclodextrin (βCD) was obtained from the crystal structure of the β-amylase / β-cyclodextrin complex under the code 1BFN from the Protein Data Bank (PDB) database [53]. For the design of the ligand derived from Carboline and mβCD, GaussView 5.0.9 software was used for subsequent optimization in Gaussian' 09 revision E.01 [54], using the DFT (Density Functional Theory) level of theory with the long-range corrected functional WB97XD and the 6-31G+(d,p) basis set, which have presented satisfactory performances in modeling Non-Covalent Interactions [55].

2.2 Inclusion Complex (Molecular Docking)

Carboline inclusion complexes with βCD and mβCD were performed with PyRx software [56], in which a blind Docking methodology (in gas phase and under normal conditions of pressure and temperature, 1 atm and 298 K) was used. The conformational search site for the Carb/βCD complex was delimited a box of dimensions $x = -6{,}9350$ Å, $y = 27{,}4756$ Å, $z = 28{,}6702$ Å and a grid resolution of $0{,}375$ Å, while the Carb/βCD complex the box is of dimensions $x = -16{,}2228$ Å, $y = -0{,}3794$ Å, $z = 27{,}7888$ Å. The criterion for choosing the optimal conformer was the minimum interaction/coupling energy [57].

2.3 Thermodynamic Parameters

To obtain the interaction energy of the inclusion complex, a DFT level of theory was used with the Counterpoise correction tool which considers the *Basis Set Superposition Error* (BSSE). On the other hand, to obtain the thermodynamic parameters of the ligand, βCD and inclusion complexes in gas phase, their associated frequencies were calculated with a Semiempirical PM6 level of theory [43].

To model the host cavity desolvation process (βCD) and its influence on ligand encapsulation, the QST2 method was used, which utilizes the optimized structures of the reactants ($\mathrm{L} + 11\ \mathrm{H_2O} \cdot \beta\mathrm{CD}$) and products ($\mathrm{L} \cdot \beta\mathrm{CD} + 11\ \mathrm{H_2O}$), obtaining a transition state (ST), which, to confirm its nature, its imaginary frequency was considered.

2.4 Second Order Perturbative Analysis (E2PERT)

For the prediction of hydrogen (H bond) interactions and stability of the inclusion complex by the donation and acceptance of orbitals (Lewis and non-Lewis type orbitals), the Second Order Perturbative Analysis (E2PERT) was considered which was carried out in NBO 6.0 software [58], in conjunction with the ONIOM2 method with DFT (*High*) and Semiempirical PM6 (*Low*) levels of theory [59].

2.5 Local and Global Reactivity Indices

Local and global ligand reactivity indices were obtained via Fukui 4.1 software compatible with Gaussian '09 [60,61]. The calculation was performed through a singlepoint (SP) in Gaussian '09, and its output file was read in Fukui 4.1.

The ONIOM2 method was used for the reactivity indices of the inclusion complexes, for which two levels of theory (DFT/PM6) were considered. In the same way, the output file was read in Fukui 4.1 software.

To plot the local descriptors of the ligand (Dual Descriptor) the Fukui boundary molecular orbitals approximation was used, which were visualized in GaussView generating isosurfaces of density 0,002 u. a.

3. RESULTS AND DISCUSSION

3.1 Optimization and Inclusion Complexes

The systems (ligand and host) modeled and optimized with DFT WB97XD/6-31G+(d,p) can be visualized in Figure 2. On the other hand, it can be observed that, although the Carboline ligand was optimized prior to the formation of the inclusion complex, after molecular docking, rotations of the hydroxyl groups (alcohol and carboxyl) of the ligand can be evidenced, which is due to the semi-flexible docking methodology (flexible ligand and rigid cavity) [62,63].

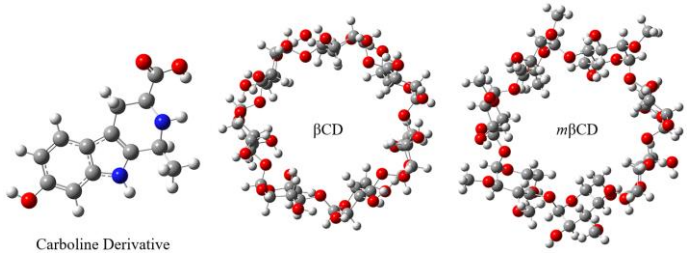


Figure. 2. Carboline-derived ligand, βCD and mβCD optimized.

The spatial conformation obtained from docking is identical in the C1 and C2 inclusion complexes, presenting the total immersion of the *a*, *b* and *c* rings of Carboline in the cyclodextrin cavity, whereby the hydroxyl group of *a* ring is disposed towards the primary hydroxyls, while the carboxyl of the *c* ring is disposed towards the secondary hydroxyls.

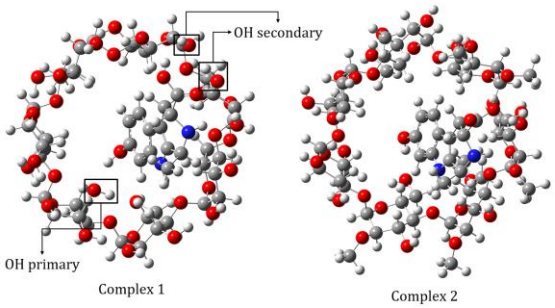


Figure. 3. Carboline/Cyclodextrin Inclusion Complexes generated by Molecular Docking

It should be emphasized that, although the molecular docking complexation is very useful for obtaining the possible conformer, there are also limitations and discordances, such as the complexation stoichiometry, that is, the amount of cyclodextrins that interact per unit of Carbolines, or the amount of Carbolines per unit of cyclodextrins. As the complexing process is mainly guided by the size of the ligand and the size of the cyclodextrin cavity (steric effects), mainly inclusion complexes with 1:1 or 1:2 stoichiometry is obtained [64], unless the ligand is of large size, in which case it is surrounded by more cyclodextrin units, as can be observed in hydrogels [65,66].

On the other hand, given the modeling conditions of molecular docking (gas phase), the solvent effect [67,68], which is of utmost importance, is not considered, since during the encapsulation process of the ligand the water molecules present in the cavity are displaced (desolvation), favoring the process from the thermodynamic point of view, since the reaction entropy increases, increasing the spontaneity (Gibbs free energy) [44,45]. Finally, the presence of water molecules in the environment would better describe the stability of the ligand in the cavity, given the formation of non-covalent interactions such as H bond or hydrophobic interactions [68].

3.2 Thermodynamic Parameters

The thermodynamic parameters of an inclusion complex are able to describe the stability and viability of the complex, in addition to the possible non-covalent interactions that stabilize the ligand within the cyclodextrin cavity, which, given the conditions, are van der Waals and H bond interactions. The thermodynamic parameters of the C1 and C2 complexes can be seen in Table 1.

Table 1. Thermodynamic parameters of the Carboline inclusion process with βCD and mβCD.

Complex	ΔE_{int} / (kcal/mol)	ΔG° / (kcal/mol)	ΔH° / (kcal/mol)	ΔS° / (kcal/mol)
C1 (Carb/ β CD)	-5,9	210,338	-285,067	-1,664
C2 (Carb/m β CD)	-7,2	383,963	-241,610	-2,101

From Table 1, it can be observed that the inclusion complexes are energetically stable given negative values of interaction energy ($\Delta E_{int} < 0$), since the more negative values of ΔE_{int}, the more stable and favorable is the complex formation [69] so, based on this, the complex C2 (Carb/mβCD) is more stable than C1 (Carb/βCD).

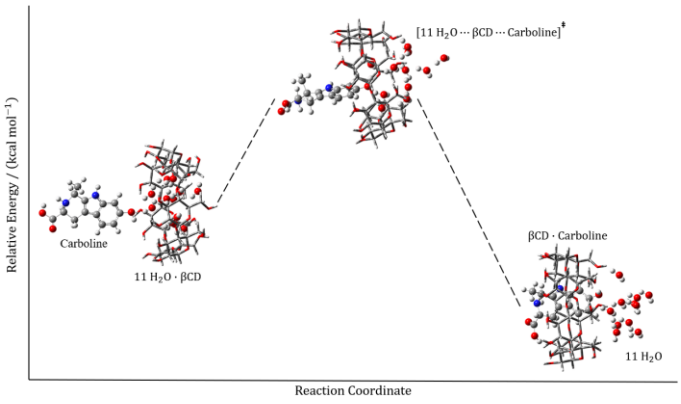
On the other hand, positive values of Gibbs free energy ($\Delta G^{\circ} > 0$) describes a non-spontaneous process under the modeling conditions (gas phase, 1 atm and 298 K), while negative enthalpy values ($\Delta H^{\circ} < 0$) describes an exothermic reaction, which is related to the non-covalent interactions generated between the ligand and the cyclodextrin cavity. Finally, negative and small entropy values ($\Delta S^{\circ} < 0$) describe steric hindrance and constraints on rotational and translational motion of the ligand in the cavity [35,36]. Therefore, the process of ligand complexation in βCD and mβCD is mainly guided by enthalpy.

Since water molecules play a role in the ligand encapsulation process, the thermodynamic parameters vary greatly compared to a gas-phase study. To describe the process of desolvation of the cyclodextrin cavity in the presence of ligand, table 2 shows the thermodynamic aspects of hydrated cyclodextrins ($11\ H_2O \cdot \beta CD$) during the process of inclusion complex formation.

Table 2. Thermodynamic parameters on inclusion complex formation considering the desolvation of the βCD cavity.

System	E/(kcal/mol)	H°/(kcal/mol)	G°/(kcal/mol)	ΔE/(kcal/mol)	ΔG°/(kcal/mol)
$carb + 11\ H_2O \cdot \beta CD$	95,787	95,783	94,383		
$[carb \cdots \beta CD \cdots 11\ H_2O]^{\ddagger}$	105,638	105,632	104,232		
$carb \cdot \beta CD + 11\ H_2O$	-10,298	-10,537	-11,637	-106,085	-106,020
$carb + 11\ H_2O \cdot m\beta CD$	94,977	94,963	93,461		
$[carb \cdots m\beta CD \cdots 11\ H_2O]^{\ddagger}$	102,439	102,433	101,433		
$carb \cdot \beta CD + 11\ H_2O$	-10,677	-10,682	-12,086	-105,654	-105,547

The thermodynamic parameters on the formation of the inclusion complex ($carb \cdot \beta CD$ and $carb \cdot m\beta CD$) and the transition state ($[carb \cdots \beta CD \cdots 11\ H_2O]^{\ddagger}$ and $[carb \cdots m\beta CD \cdots 11\ H_2O]^{\ddagger}$) can be observed graphically in Figure 4, where the energy barrier between reactants and transition state is relatively low. In turn, it can be observed that the relative energy of the reactants is considerably high compared to the products (inclusion complexes), which describes a highly stable system [70]. With the $carb \cdot \beta CD$ system being the most energetically stable, which agrees in part with the data in Table 1.



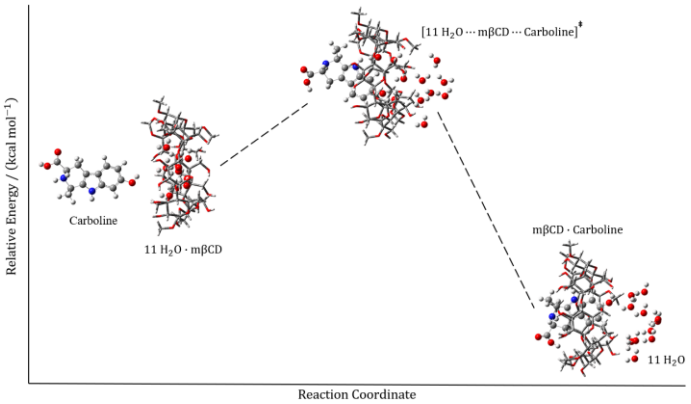


Figure. 4. Inclusion complex formation process between Carboline derivative and cyclodextrin. The desolvation of the cavity generates an energetically stable system given the entropic increase, which favors the spontaneity of the process.

3.3 Second Order Perturbative Analysis (E2PERT)

The second-order perturbative analysis (E2PERT) is a numerical description of the donation (Lewis-type bonding orbitals) and acceptance (non-Lewis-type

anti-bonding orbitals) of orbitals in a molecule or molecular system, so in an inclusion complex it can be considered as a stability criterion [43], and even a prediction parameter of the non-covalent interactions that are generated between the interacting species, specifically the Hydrogen (H bond) interactions [35]. In the following, the E2PERT analysis of the C1 and C2 complexes is presented.

Table 3. Second-order perturbative analysis (E2PERT) (**notation**: LP: non-bonding electron pairs, BD: bonding orbital, BD*: anti-bonding orbital).

Complex	Donor/Acceptor	Donor	Acceptor	$E^{(2)}$ / (kcal/mol)
C1 (Carb/βCD)	βCD → Carb	BD(1) O86 – H139	BD*(1) N159 – H163	2,27
		BD(1) C25 – H100	BD*(1) C158 – H175	8,11
		BD(1) C49 – H122	BD*(1) C148 – H169	1,54
		BD(1) O36 – H132	BD*(1) O164 – H165	2,43
	Carb → βCD	LP(1) O159	BD*(1) C3 – H80	1,16
		BD(1) O164 – H165	BD*(1) O36 – H132	2,41
		BD(2) C156 – C157	BD*(1) C14 – H90	1,73
		BD(1) N159 – H163	BD*(1) O86 – H139	7,91
		BD(2) C161 – O162	BD*(1) C5 – H82	3,17
C2 (Carb/mβCD)	mβCD → Carb	BD(1) O79 – H83	BD(1)* O189 – H190	2,71
		BD(1) O78 – C72	BD*(1) N180 – H184	1,26
		BD(1) C5 – H82	BD*(1) 157 – H172	2,78
		BD(1) O22 – H97	BD*(1) O166 – H167	1,81
		BD(1) C38 – H112	BD*(1) C159 – H173	1,24
		BD(1) O54 – H56	BD*(1) O185 – H186	1,15
	Carb → mβCD	BD(1) O189 – H190	BD(1)* O79 – H83	1,37
		BD(1) O185 – H186	BD*(1) O54 – H56	2,09
		BD(1) C157 – H172	BD*(1) C5 – H82	3,32
		BD(1) N180 – H184	BD*(1) O78 – C72	1,19
		BD(2) C160 – O161	BD*(1) C58 – H130	2,50

As a result of molecular docking, it can be observed that in complex C1 three hydrogen interactions are formed (H163 ⋯ O86, H162 ⋯ O52 and H165 ⋯ O36), while in complex C2 four are formed (H83 ⋯ O189, H184 ⋯ O78, H183 ⋯ O52 and H186 ⋯ O54). These interactions can be evidenced in Table 3, where the donation and acceptance of orbitals between ligand and cyclodextrin translate into hydrogen interactions, hence an aspect of stability and viability of the inclusion complex.

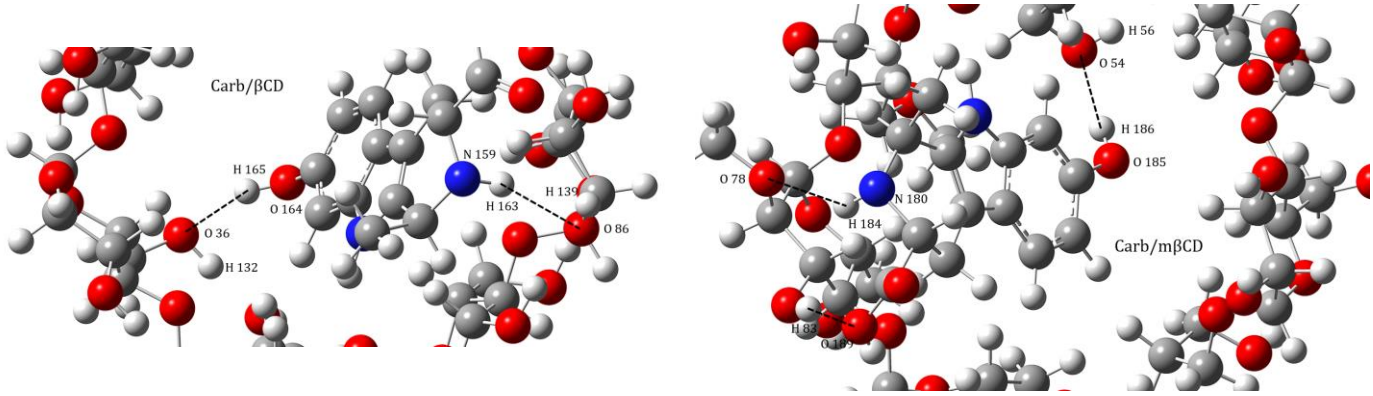


Figure. 5. Hydrogen interactions between carboline-derived ligand and βCD/mβCD cavity.

3.4 Local and Global Reactivity Indices. Antioxidant Capacity (AOX)

The antioxidant activity of a chemical compound refers to its ability to protect against short-lived and highly reactive radical species such as radical oxygen and/or nitrogen species, which can cause damage at the cellular and even DNA level [1]. Antioxidant mechanisms are varied depending on the structural nature of the compound and the radical. Mainly, antioxidant mechanisms can be classified into HAT (Hydrogen Atom Transfer), SET (Single Electron Transfer) or SPLET (Sequential Proton Loss Electron Transfer) [71,72].

The HAT and SET mechanisms can be described by the following reactions:

$$Carb - OH + R\cdot \longrightarrow Carb - O\cdot + RH \quad (12)$$

$$Carb - OH + R\cdot \longrightarrow Carb - OH\cdot^{+} + R^{-} \quad (13)$$

For a theoretical description of the antioxidant capacity (AOX) of a chemical compound, the global and local reactivity indices can be used, as shown in Table 4.

Table 4. Global and local reactivity indices of the ligand, inclusion complex and $OH\cdot$ radical with the WB97XD/6-31G+(d,p) functional.

Specie	**Atom**	$f^{-}_{(r)}$	$f^{+}_{(r)}$	$f^{0}_{(r)}$	$f^{(2)}_{(r)}$	μ / eV	η / eV	ω / eV
Carboline	1C	0,0938	0,0050	0,0494	-0,0887	-3,2145	8,2059	0,6296
	2C	0,0134	0,0956	0,0545	0,0822			
	3C	0,1344	0,0336	0,0840	-0,1008			
	4C	0,1083	0,0789	0,0936	-0,0294			
	5C	0,0528	0,2365	0,1447	0,1837			
	6C	0,0623	0,5096	0,2859	0,4474			
	28O	0,0003	0,1257	0,0627	-0,1260			
	29H	0,0000	0,0326	0,0163	0,0326			
	30O	0,0018	0,0128	0,0073	0,0110			
	31O	0,0783	0,0019	0,0401	-0,0764			
	32H	0,0013	0,0030	0,0005	-0,0017			
C1 (Carb/ β CD)	148C	0,0880	0,2781	0,1831	0,1901	-4,7725	8,0565	1,4136
	149C	0,0002	0,0617	0,0309	0,0616			
	150C	0,1094	0,0969	0,1032	-0,0124			
	151C	0,1550	0,1460	0,1505	-0,0091			
	152C	0,0575	0,0205	0,0390	-0,0370			
	153C	0,0276	0,0190	0,0233	-0,0086			
	166O	0,0001	0,0000	0,0001	-0,0001			
	167H	0,0000	0,0000	0,0000	0,0000			
	165O	0,0001	0,0002	0,0001	0,0001			
	168O	0,0348	0,0071	0,0210	-0,0277			
	169H	0,0016	0,0018	0,0017	0,0002			
C2 (Carb/m β CD)	169C	0,0858	0,2867	0,1863	0,2009	-4,3470	7,9931	1,1821
	170C	0,0002	0,0792	0,0397	0,0790			
	171C	0,1118	0,0764	0,0941	-0,0354			
	172C	0,1562	0,1421	0,1492	-0,0141			
	173C	0,0567	0,0260	0,0414	-0,0307			
	174C	0,0286	0,0183	0,0235	-0,0103			
	187O	0,0001	0,0000	0,0000	0,0000			
	188H	0,0000	0,0000	0,0000	0,0000			
	186O	0,0002	0,0001	0,0001	0,0000			
	189O	0,0380	0,0058	0,0219	-0,0322			
	190H	0,0012	0,0013	0,0013	0,0001			
$OH\cdot$						-1,9557	1,8987	1,0072

Of the global reactivity indices presented in Table 4, the one that shows the least variation with respect to the ligand in isolation and in inclusion complex is the chemical hardness (η), which can be understood as the resistance to deformation and/or electron density transfer. This parameter describes the reactivity based on the "Principle of Maximum Hardness" (PMH) [73], which states that more reactive systems have a lower chemical hardness than less reactive systems, which have a higher chemical hardness. From the PMH criterion, it can be observed that the Carboline-derived ligand increases its reactivity ($\approx$ 0,8%) when it is found forming inclusion complexes, being in the C2 complex where its chemical reactivity increases.

Electrophilicity (ω) is a measure of "electrophilic power", which is translated as the tendency to attract electron density and how it stabilizes the molecular system [74,75]. From Table 4 it can be seen that the ligand increases its Electrophilicity in inclusion complexes, thus increasing its electrophilic ability and stability by attracting electron density from the cavity [43].

To describe a possible antioxidant mechanism of the Carboline derivative one has to observe the Electronegativity ($\chi = -\mu$) and Electrophilicity, which are associated with the antioxidant mechanism SET [76]. Since Electrophilicity and Electronegativity measure the tendency to electron density attraction, it can be noted that the compound presents SET capacity, by its ω and χ values, and with an increase in this capacity when the inclusion complex is formed.

In the same way, global reactivity indices (mainly the chemical potential, μ) allow describing the tendency of electron density acceptor and donor, i.e., those species that behave as nucleophiles and electrophiles. In general, the electron density tends to move from the species presenting a higher value of chemical potential (less negative) towards the one presenting lower chemical potential [77]. By way of comparison, from Table 4 it can be seen that the $OH\cdot$ radical tends to yield electron density (nucleophile) toward the Carboline derivative (electrophile), in situ and in the inclusion complex, which is consistent with the SET capacity.

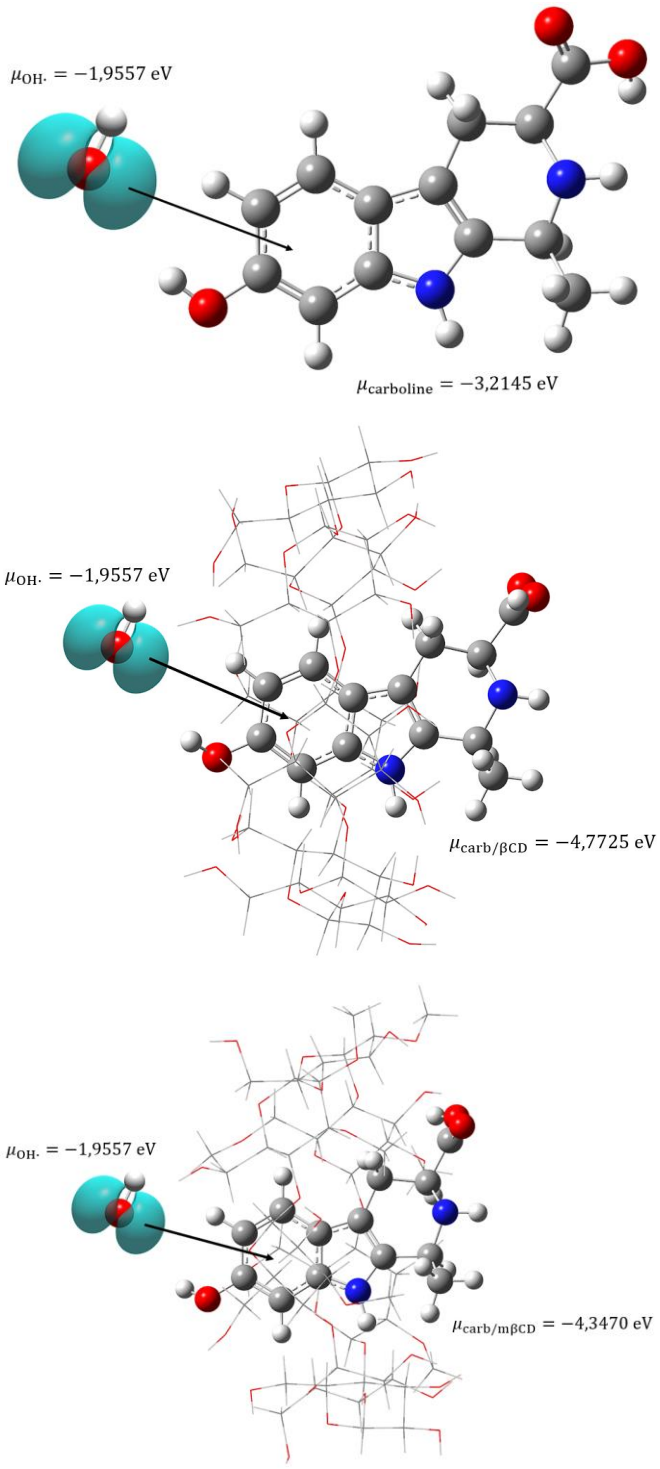


Figure. 6. SET mechanism produced by the Carboline derivative and $OH\cdot$ radical. The green lobe represents the nucleophilic tendency of the radical.

On the other hand, compounds that present hydroxyl groups in their structure are capable of generating HAT mechanisms [72,78], so the atoms of interest are Hydrogens and Oxygens, and their respective local reactivity indices. A fundamental characteristic of Hydrogens capable of performing HAT mechanism is their "electroacceptor" or "lability" capacity, which is given by positive values of $f_{(r)}^{+}$ and $f_{(r)}^{(2)}$ (sites susceptible to nucleophilic attacks). Based on this it can be noticed from Table 4, that the ligand presents HAT capacity in situ only with the labile hydrogen of the carboxyl group (29H), while in the cavity of the inclusion complex this capacity is lost, being exchanged by the lability of the hydrogen of the hydroxyl group (C1 – 169H, C2 – 190H). Figure 7 shows the local descriptors graphically, specifically the dual descriptor (DD).

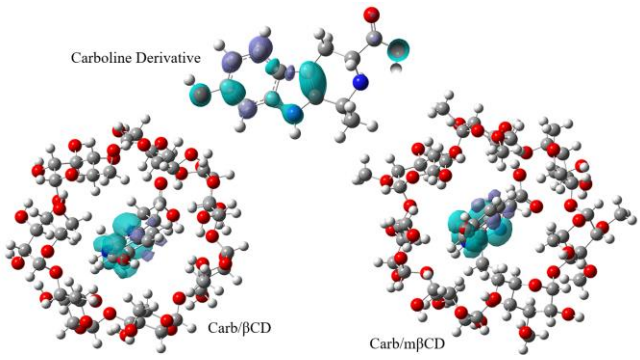


Figure. 7. Local reactivity indices (DD) of Carboline derivative in situ and in inclusion complex. The purple lobes represent electrophilic features, while the green lobes represent local nucleophilic tendencies.

Thermodynamically, the HAT mechanism can be characterized by the bond dissociation enthalpy (BDE) of the OH group [79,80,81]. This parameter can be calculated by the following equation.

$$BDE_{(OH)} = (H_{carb-O\cdot} + H_H) - H_{carb-OH} \quad (14)$$

Where $H_{carb-O\cdot}$ is the enthalpy of the Carboline derivative radical generated by homolytic abstraction of $H\cdot$, H_H is the enthalpy of the hydrogen atom, and $H_{carb-OH}$ the enthalpy of the Carboline derivative.

Table 5. Bond dissociation enthalpy (BDE) of the hydroxyl and carbonyl groups of the Carboline derivative and inclusion complex.

System	Active Site	BDE/(kcal/mol)
Carboline	$R-OH$	26,894
	$R-COOH$	19,273
Carb/ β CD	$R-OH$	30,802
	$R-COOH$	34,881
Carb/m β CD	$R-OH$	28,891
	$R-COOH$	27,911

According to Amić et al. [82] a lower BDE value is attributed to a higher tendency to donate a Hydrogen atom from an OH group, which is a primary descriptor of HAT antioxidant activity. From Table 5 it can be seen that the BDE values are consistent with the reactivity of the hydrogen atoms described by the local reactivity indices, where the hydrogen atom of the COOH group presents a higher lability. While the hydrogen of the Carboline derivative belonging to the OH group presents a higher tendency to homolytic cleavage. Given the high reactivity of the $OH\cdot$ radical towards the Carboline derivative, the abstraction of the labile hydrogen atom of the hydroxyl group with higher tendency to the HAT mechanism can be evidenced, as presented by Priya & Lakshmipathi [83].

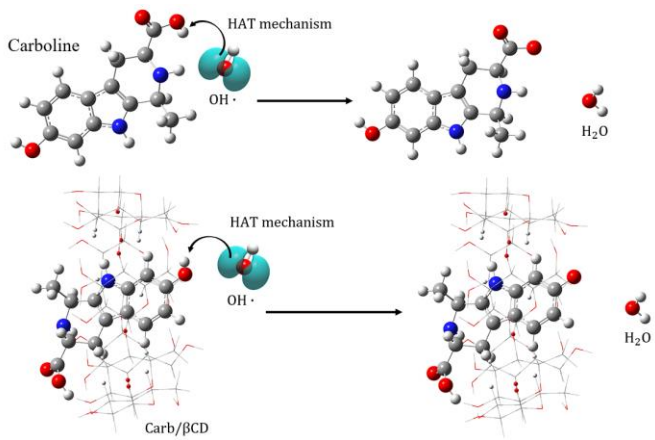


Figure. 8. HAT Antioxidant Mechanism of Carboline in situ and in inclusion complex against $OH\cdot$ radical. It can be evidenced that as a reaction product a water molecule and a reactive minor radical $(carb\cdot)$ are formed.

CONCLUSION

Based on the thermodynamic results, it can be concluded that the Carboline-derived compound is able to form inclusion complexes given its interaction energy values, but it is not a spontaneous process under the modeling conditions (gas phase, 1 atm and 298 K), and that it is mainly guided by the enthalpy of complexation (exothermic mechanism). This enthalpy value describes the non-covalent H bond interactions, predicted and modeled on the basis of the second order perturbative analysis (E2PERT). The spontaneity of the inclusion process is favored by cavity desolvation, forming energetically stable products.

For their part, the local and global reactivity indices describe the antioxidant capacity of Carboline, presenting a tendency towards SET and HAT mechanisms in situ, and with an increase in these capacities and activity when it forms inclusion complexes with βCD and mβCD. The SET capacity of Carboline could be established based on its overall reactivity values in the presence of the OH· radical, which, having a higher chemical potential, tends to yield electron density to sites susceptible to nucleophilic attacks and with resonance capacity, such as the benzene ring of the compound. For its part, the HAT capacity could be described from the BDE analysis, which shows the reactivity of the labile hydrogen atoms, susceptible to homolytic cleavages.

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Abbreviation: carb, carboline; βCD, beta cyclodextrin; mβCD, methyl beta cyclodextrin; ROS, reactive oxygen species, CD, cyclodextrin; BSSE, Basis Set Superposition Error; NCI, non-covalent interactions; E2PERT, Second Order Perturbational; NBO, Natural Bond Orbital; AOX, antioxidant capacity; HOMO, Highest Occupied Molecular Orbital; LUMO, Lowest Unoccupied Molecular Orbital; ST, transition state, ΔE_{int}, Interaction energy; PMH, Principle of Maximum Hardness, BDE, bond dissociation energy; HAT, hydrogen atom transfer; SET, single electron transfer; DD, dual descriptor.

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