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COMPLEXATION OF [2-(2-BROMOPHENYLCARBAMOYL)PHENOXY]ACETIC ACID ETHYL ESTER WITH β-CYCLODEXTRIN

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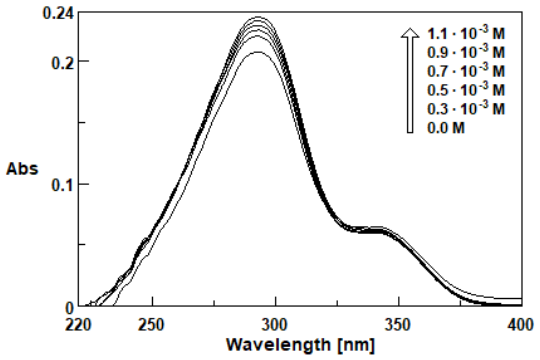
In order to study the behavior of ethyl esters with 2-hydroxy-benzamide structure in the presence of β-cyclodextrin (β-CYD), the [2-(2-bromophenylcarbamoyl)phenoxy]acetic acid ethyl ester was chosen as a representative compound. The evidence of obtaining the inclusion complex in the liquid phase, the 1:1 stoichiometry and the apparent formation constant (K=1211±111 L/mol) of ethyl ester/β-CYD inclusion complex was performed using absorbance measurements and the Benesi-Hildebrand equation. A β-CYD inclusion complex containing the ethyl ester as a guest was prepared using the kneading method and with the aliquot addition of ethanol. The product was characterized by ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR), which proves the formation of the inclusion complex where the benzamide part of the ethyl ester has been encapsulated in the hydrophobic cavity of β-CYD. The inclusion compound's geometry was established using molecular modeling, which also proved that the ethyl ester is included with the benzamide moiety inside the β-CYD cavity.



INTRODUCTION

Cyclodextrins are cyclic (α-1,4)-linked oligo-saccharides of α-D-glucopyranose which contain a hydrophobic central cavity and a hydrophilic outer surface.[1] The distinctive characteristic of cyclodextrins is their capability to form inclusion complexes with several organic molecules. Thus, cyclodextrins are capable to act as host molecules, being able to include a drug molecule (guest molecule) into their central cavity to form inclusion complexes. Through host-guest interactions between the central cavity of the cyclodextrin and a nonpolar molecule, the physical, chemical and biochemical properties of the guest molecule can be modified, so the application criteria of this guest molecule can also be improved.[2]

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Cyclodextrins empower the transport of slightly water-soluble drugs and of those which are chemical unstable within the body, changing the physico-chemical properties of some biologically active compounds in order to obtain therapeutically effective drugs. Development of a drug-cyclodextrin inclusion complex can, for instance, increase the water solubility, as well as the chemical and physical stability of the drug, and improve the drug distribution through biological membranes.[3,4]

Among the limitation in using the anticancer drugs, the poor biodisponibility caused by limited solubility in aqueous media represents a major problem. In the case of Albendazole, for instance, attempts have been made to resolve this problem by using co-solvents[5,6] or solid dispersions,[7] but, due to the irritation of the intestinal epithelium, the experiments have been discontinued. In another study, phase solubility diagrams were used to investigate the solubility enhancements in the case of Albendazole by using the β-cyclodextrin, hydroxypropyl-β-cyclodextrin and sulfobutylether-β-cyclodextrin. Complexes with sulfobutylether-β-cyclodextrin showed the highest potential to increase the apparent aqueous solubility and the rapid dissolution.[8] Complexation with cyclodextrins also proved to be the key for enhancing the biodisponibility of some new drugs, like difluorinated curcumin.[9]

Novel 2-hydroxybenzamide derivatives like esters, hydrazide and hydrazones were designed[10–14] and investigated for their antimicrobial activity[15,16] in our laboratory. Due to their low solubility in aqueous media, complexation with cyclodextrins seemed to be the best strategy for improving the aqueous solubility of such compounds. In this work, we studied the complexation of [2-(2-bromophenylcarbamoyl)phenoxy]acetic acid ethyl ester with β-cyclodextrin.

RESULTS AND DISCUSSION

UV-VIS spectroscopy

The absorption spectra are used to demonstrate the formation of the inclusion compounds.[17, 18] In Fig. 1, the absorption spectra of the [2-(2-bromophenylcarbamoyl)phenoxy]acetic acid ethyl ester and β-cyclodextrin are presented. It can be seen that β-CYD has almost no absorption throughout the studied domain, thus its absorbance can be neglected. Therefore, the absorbance of the inclusion compound (Fig. 2) is due to the ethyl ester alone.

By increasing the cyclodextrin concentrations while maintaining the ethyl ester concentration, it can be observed that the absorbance value increases. The increase of the absorbance value with the concentration of the β-CYD (Fig. 2) is due to the increase in the solubility of the guest molecule upon forming the complex, suggesting the formation of the inclusion complex between the ethyl ester and β-CYD.

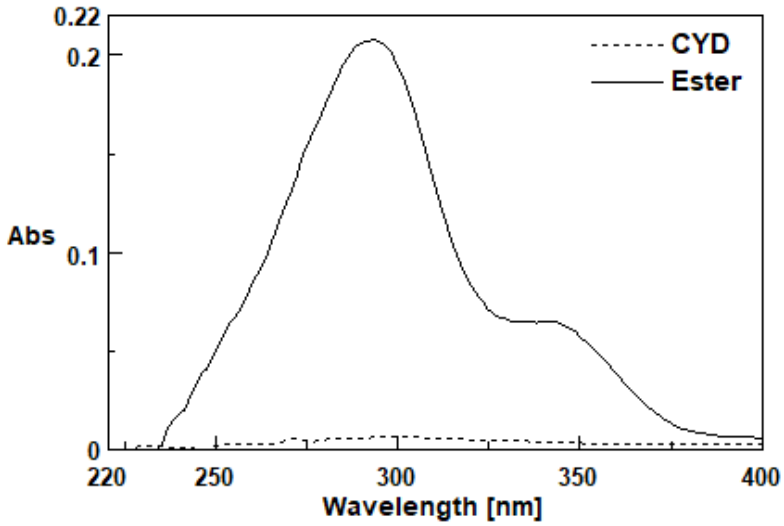


Fig. 1 – Absorption spectra of β-CYD and of the ethyl ester.

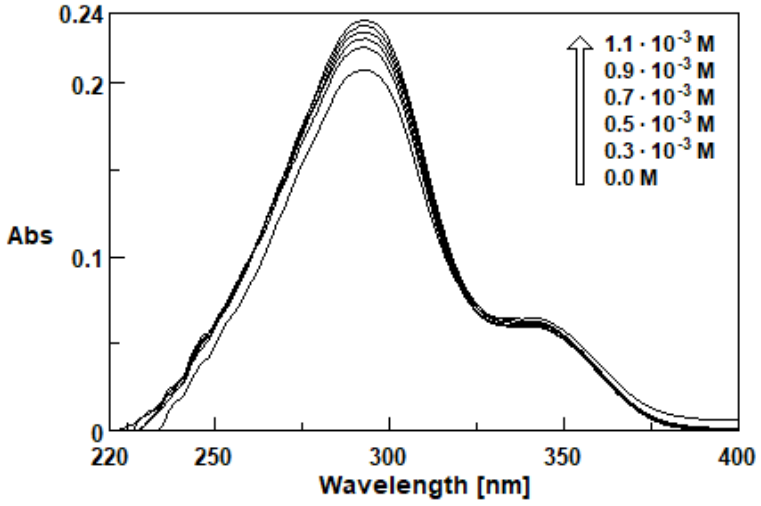


Fig. 2 – Absorption spectra of ethyl ester with various concentrations of β-CYD.

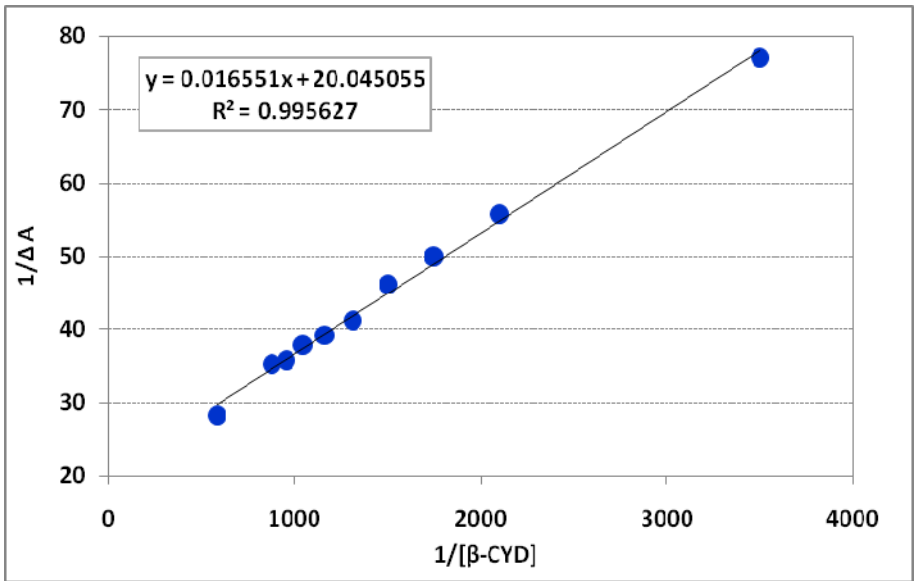


Fig. 3 – The Benesi–Hildebrand plot for the complexation of the ethyl ester and β-CYD at 293 nm.

In the meantime, the benzamide moiety of the ethyl ester is more likely to fit the hydrophobic cavity of β-CYD, so the stoichiometric ratio of the inclusion complex should theoretically be 1:1. This fact can be ascertained if a linear relationship is obtained from the reciprocal plot of 1/ΔA vs. 1/[β-CYD] based on the Benesi-Hildebrand equation (1).[19]

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon[G]_0 K[\beta - CYD]} + \frac{1}{\Delta\varepsilon[G]_0} \qquad (1)$$

where ΔA is the difference between the absorbance of the complex and the absorbance of the ethyl ester, Δε is the difference between the molar absorptivity of the complex and the molar absorptivity of the ethyl ester, $[G]_0$, K, [CYD], are the initial concentration of ethyl ester, apparent formation constant of the inclusion complex and the concentration of β-cyclodextrin, respectively.

In Fig. 3, the Benesi-Hildebrand plot for the complexation of the ethyl ester and β-CYD is presented. A good linear relationship was obtained for 1/ΔA vs. 1/[β-CYD], with $R^2 = 0.995627$. This linearity indicates that the stoichiometry ratio for the inclusion complex between ethyl ester and β-CYD is 1:1. Similar phenomena were observed by other researchers.[18, 20]

The parameters K and Δε can be evaluated from the Benesi-Hildebrand plot (Fig. 3), according to eqns. (2) and (3).[19]

$$K = \frac{(y - \text{intercept})}{(\text{slope})} \quad (2)$$

$$\Delta\varepsilon = \frac{1}{[G]_0 (y - \text{intercept})} \quad (3)$$

So, after the calculations, the values of K and Δε, for a 95% confidence interval, were established: K=1211±111 L/mol, Δε=873±65 $cm^{-1}\cdot L\cdot mol^{-1}$.

^{1}H-NMR spectroscopy

^{1}H-Nuclear Magnetic Resonance was used to characterize the β-CYD inclusion complex containing the [2-(2-bromophenylcarbamoyl)phenoxy]acetic acid ethyl ester as a guest, obtained using the kneading method. For an easier interpretation of NMR spectra, the numbering of β-CYD and of ethyl ester protons is presented in Fig. 4.

If the insertion of a guest molecule into the hydrophobic cavity of a cyclodextrin is accomplished, modifications of the chemical shifts of the guest and host molecules will appear in the NMR spectra. Generally, the most affected by these shifts are the protons H-3 and H-5, located in the inner cavity of the cyclodextrin.[20]

The ^{1}H-NMR spectra of both, free and complexed β-cyclodextrin, are depicted in Fig. 5. The chemical shifts of the host molecule (β-CYD) and the chemical shift change (Δδ), defined as the difference in chemical shift between the two states, free and complexed β-CYD,[20] are presented in Table 1.

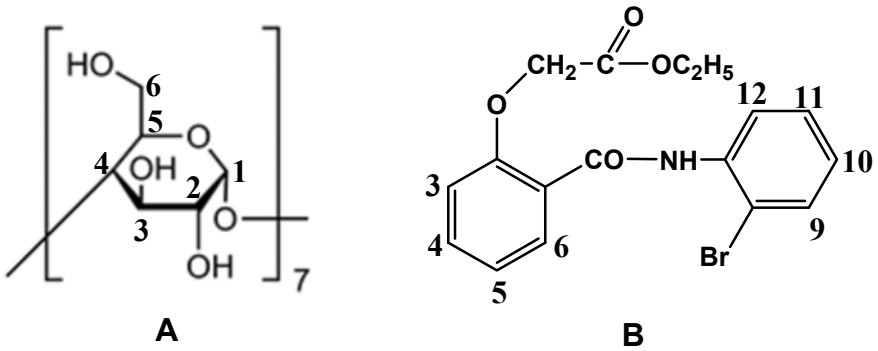


Fig. 4 – Protons numbering for β-CYD (A) and ethyl ester (B).

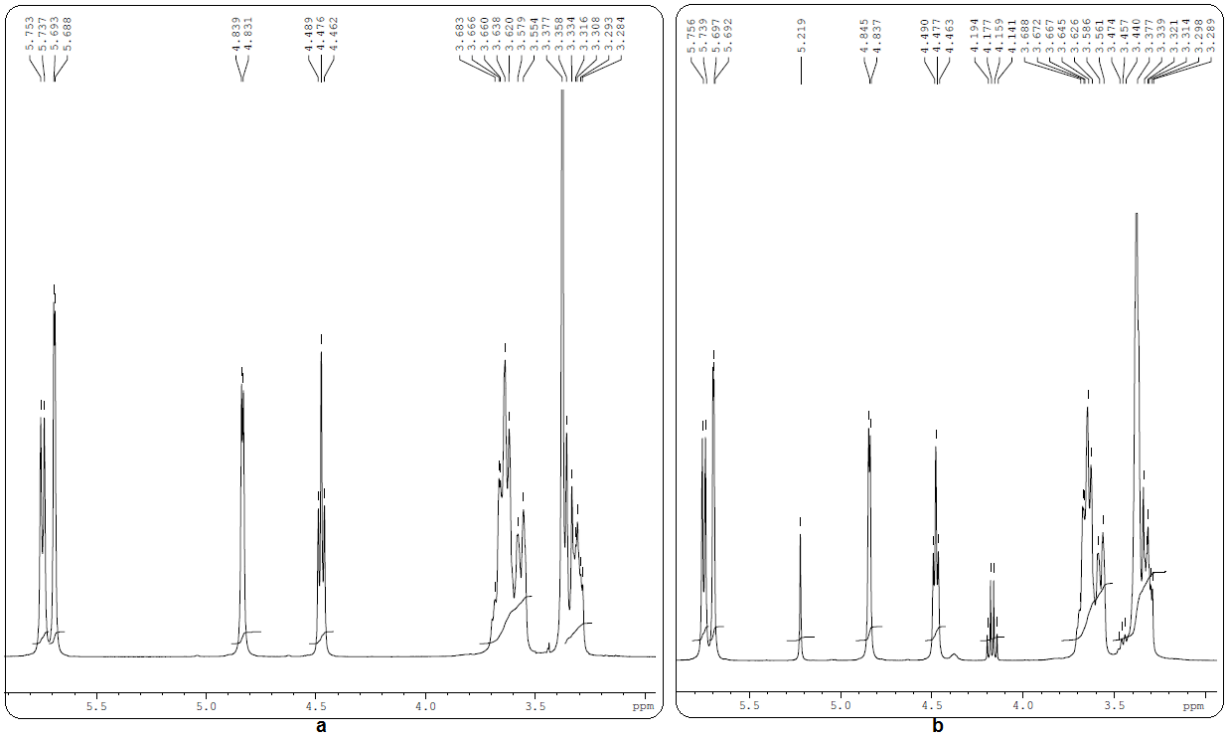


Fig. 5 – ^{1}H-NMR spectra of β-CYD, (**a**) free and (**b**) complexed.

Table 1

^{1}H chemical shifts (δ, ppm) corresponding to β-CYD protons in the free and complexed state with ethyl ester

^{1}H β-CYD	**H-1**	**H-2**	**H-3/H-6 (overlapped)**	**H-4**	**H-5**
Free	4.839	3.334	3.638	3.308	3.579
Complexed	4.845	3.339	3.645	3.314	3.586
Δδ	+ 0.006	+ 0.005	**+ 0.007**	+ 0.006	**+ 0.007**

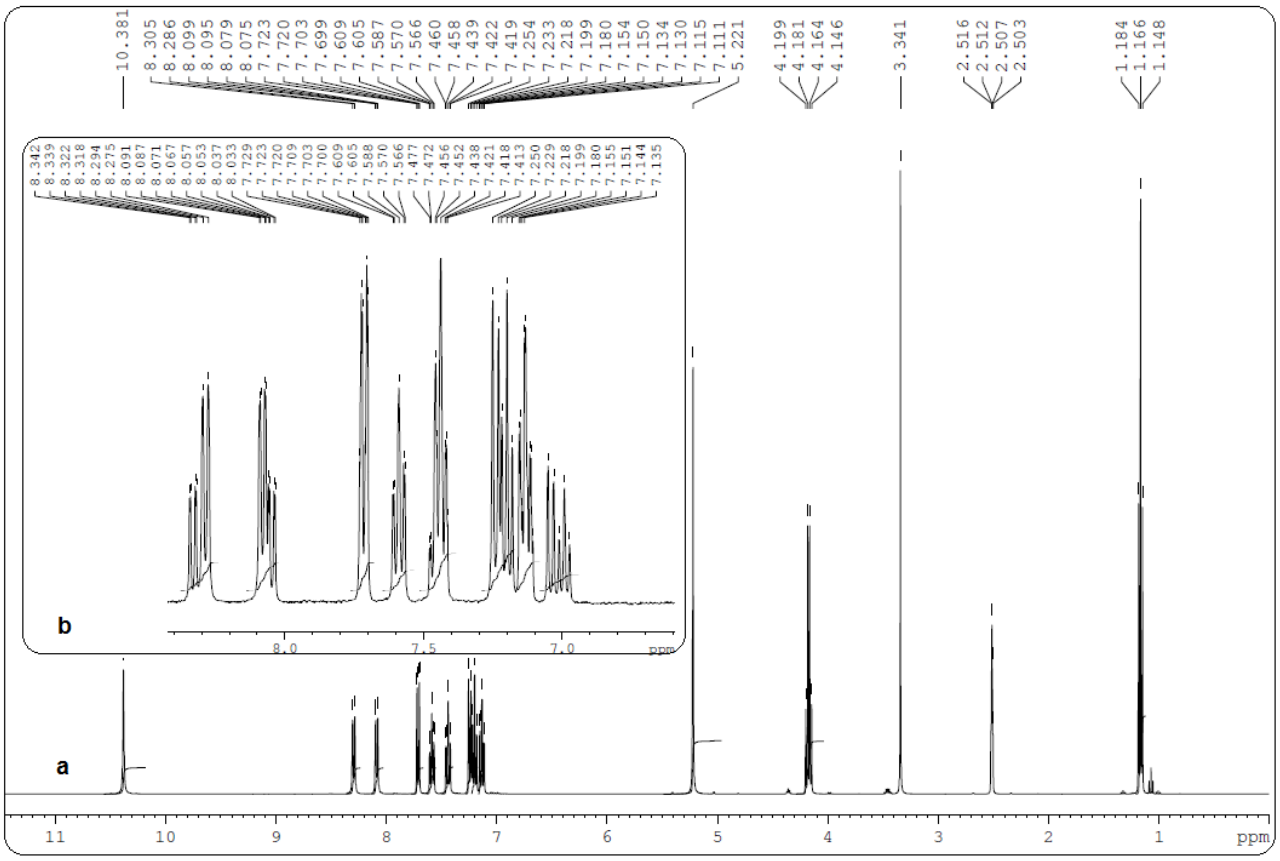


Fig. 6 – ^{1}H-NMR spectra of ethyl ester (**a**), free and (**b**) complexed.

Table 1 shows the chemical shifts observed for H-1, H-2, H-3, H-4, H-5 and H-6. As expected, the chemical shift change for inner located protons (H-3, H-5) is slightly higher compared to the protons located at the outer side of the β-CYD cavity, proving the formation of the inclusion complex between β-CYD and the ethyl ester.[20]

Fig. 6 shows the ^{1}H-NMR spectra of the free ethyl ester (a) and of the ester/β-CYD complex (b). The modified chemical shifts appear in the 7.0–8.3 ppm region of the aromatic rings.

The ^{1}H-NMR signals of the guest molecule (the ethyl ester) and the chemical shift change (Δδ) of the free and complexed ethyl ester are presented in Table 2.

The ^{1}H-NMR data presented in Table 2 showed minor modification of the chemical shifts which correspond to the aliphatic part of the ethyl ester, while the most affected by complexation seems to be the benzamide moiety ($\Delta\delta_{H\text{-}10}$= -0.111, $\Delta\delta_{H\text{-}12}$= -0.025), hence the hypothesis of encapsulation at this level.

Inclusion Compound Geometry

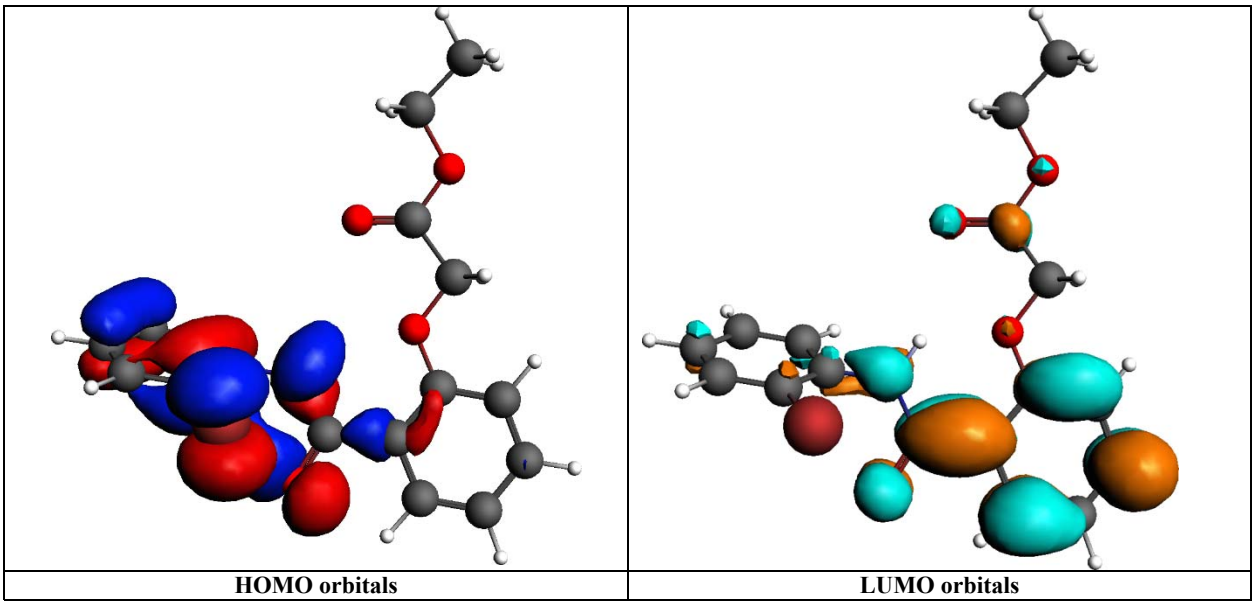
The geometry optimization of the ethyl ester was accomplished at BLYP/QZ4P level of theory. No imaginary frequencies were found, proving that a true minima structure has been obtained.

In order to obtain a detailed characterization of the investigated compound, a number of global reactivity parameters based on the energies of the frontier molecular orbitals have been computed. Also, the graphic representation of the HOMO and LUMO orbital is depicted in Fig. 7.

Table 2

^{1}H chemical shifts (δ, ppm) corresponding to ethyl ester protons in the free and complexed (with β-CYD) state

^{1}H ester	$CH_2\underline{C}H_3$	$\underline{C}H_2CH_3$	$OCH_2\underline{C}OO$	H-3	H-4	H-5	H-6	H-9	H-10	H-11	H-12
Free	1.166	4.173	5.221	7.244	7.588	7.199	8.300	7.711	7.133	7.440	8.087
Complexed	1.163	4.168	5.219	7.245	7.588	7.147	8.309	7.715	7.022	7.445	8.062
Δδ	-0.003	-0.005	-0.002	+0.001	0	-0.052	+0.009	+0.004	-0.111	+0.005	-0.025



HOMO orbitals | **LUMO orbitals**

Fig. 7 – The graphic representation of the HOMO and LUMO orbital of the ethyl ester.

Table 3

The global parameters of reactivity and the geometric descriptors computed for ethyl ester

Compound	Global parameters of reactivity			Geometric descriptors		
	μ (eV)	η (eV)	ω (eV)	Connolly accessible area (Å^2)	Connolly solvent-excluded volume (Å^3)	Ovality
ethyl ester	-3.81	1.77	4.10	602.8	267.5	1.566

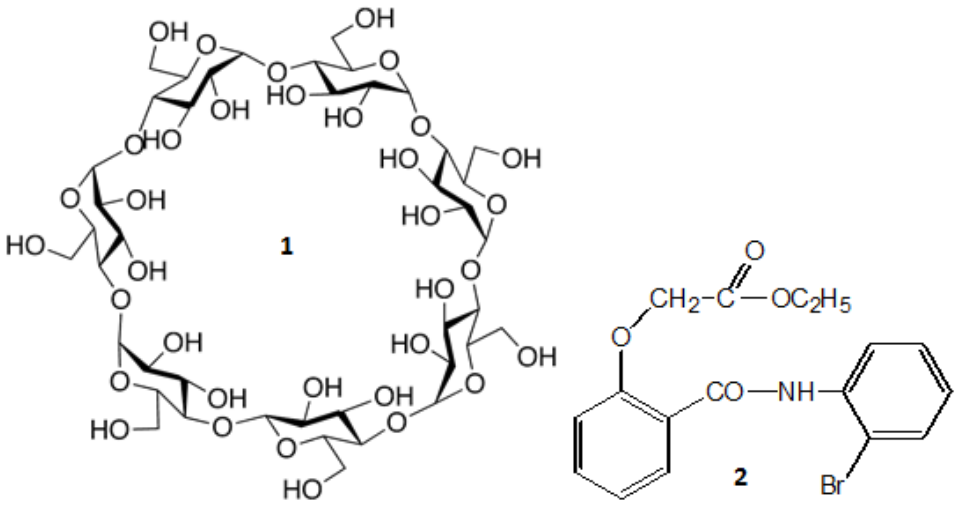


Fig. 8 – Structures of the two components of the ester/β-CYD binary compound.

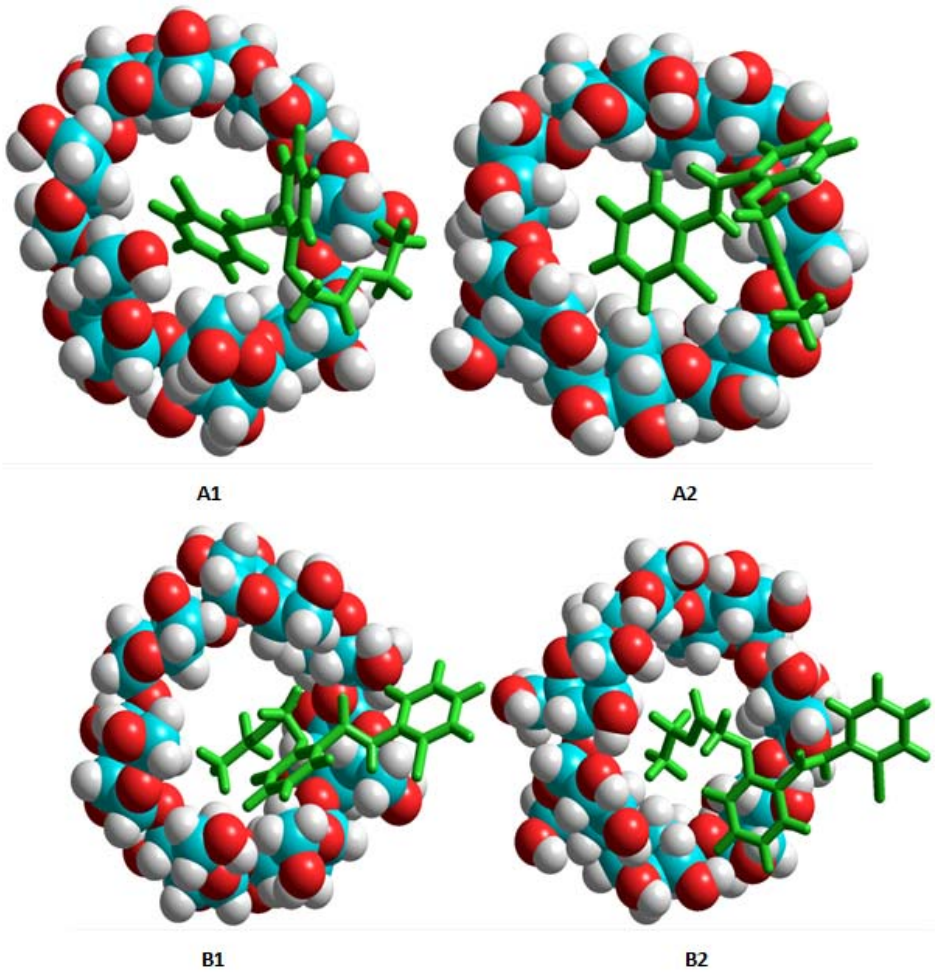


Fig. 9 – Molecular modeling of ethyl ester-β-CYD complex.

The results show that the frontier molecular orbitals are localized on the two aromatic rings: HOMO orbital appears at the phenyl cycle that bears the bromine atom, while the LUMO orbital is mainly located on the ester-bearing cycle. The energies of the frontier molecular orbitals were found to be -0.205 a.u. (HOMO) and -0.075 a.u. (LUMO), leading to a total HOMO-LUMO gap of 3.54 eV.

The molecular shape descriptors may bring valuable information regarding the interactions between the molecules. The Connolly accessible area outlines the surface that is accessible to the solvent, while the Connolly solvent-excluded volume represents the volume within the contact molecular surface. The ovality measures the deviation from the spherical shape, and also plays an important role in the ligand-receptor interactions.[21]

The computed values of the global parameters of reactivity[23] and geometric descriptors are presented in Table 3.

The central cavity of β-cyclodextrin (Fig. 8, 1), having a non-polar character, can include the non-polar molecule of the [2-(2-bromophenylcarbamoyl)phenoxy]acetic acid ethyl ester (Fig. 8, 2).

Depending on the binding alternatives between the guest compound and cyclodextrin, several patterns were chosen (Fig. 9). The first pattern (A1, A2) represents the complex formed by including the benzamide moiety of the ester within the cyclodextrin cavity (head variant), and the second (B1, B2) represents the complex formed by including the ester part (tail variant).

The complex binding enthalpy was calculated for each model (Table 4). The calculation relationship (4) is shown below and represents the difference between enthalpy of the complex and that of its component molecules, expressed in kcal/mol.

$$\Delta H=\Delta H(\text{complex}) - [\Delta H(\text{CYD}) + \Delta H(\text{ester})] \quad (4)$$

The complex binding enthalpy can provide information on the most likely binding alternative between the two components of the binary compound. The lower this enthalpy, the more stable the complex.

Table 4

Binding enthalpy of ethyl ester/β-CYD complex

Heat of formation (kcal/mol)						Binding enthalpy (kcal/mol)
CYD	**Ester**	**Model**	**Ester arrangement**	**CYD arrangement**	**Complex**	
-1457.89	-95.53	A1	head	primary hydroxyl groups, narrow rim	-1563.83	-10.41
		A2	head	secondary hydroxyl groups, wide rim	-1564.78	-11.35
		B1	tail	primary hydroxyl groups, narrow rim	-1558.20	-4.77
		B2	tail	secondary hydroxyl groups, wide rim	-1561.10	-7.68

The A2 model (benzamide moiety of the ester within the cyclodextrin cavity, secondary hydroxyl groups, wide rim) represents the form in which the complex is most likely to be found (ΔH = -11.35 kcal/mol).

EXPERIMENTAL

Materials and methods

The synthesis of [2-(2-bromophenylcarbamoyl)phenoxy] acetic acid ethyl ester was described in our previous work.[15] β-cyclodextrin was purchased from Sigma Aldrich. All the other reagents used in the study were obtained from Merck and were of analytical purity.

Synthesis of the inclusion complex of β-CYD and ethyl ester

The inclusion complex of β-CYD and ethyl ester was synthesized using the kneading method. Both components were mixed in the 1:1 molar ratio and then grinded in an agate mortar for 30 minutes; ethanol was added throughout the grinding process. The paste formed was kept in oven at 50 °C for 5 days. Then the solid has been removed from the mortar, placed in a vial and kept in a desiccator.

UV-VIS spectroscopy

A stock solution of the ethyl ester in the 1:1 vol. methanol:phosphate buffer (pH=7) was prepared at a concentration of $1.2 \cdot 10^{-3}$ mol/L. 0.1 mL of the stock solution is added to β-cyclodextrin solutions of varying concentrations ($3 \cdot 10^{-4}$-$2 \cdot 10^{-3}$ mol/L), so the final concentration of the ethyl ester in working samples is $5.71 \cdot 10^{-5}$ mol/L. The obtained solutions were kept overnight at room temperature in order to reach equilibrium. Then, the absorption spectra of the inclusion complex formed in solution at different concentrations of β-cyclodextrin were recorded using a Jasco V 530 UV-Vis spectrophotometer.

^{1}H-NMR spectroscopy

The ^{1}H-NMR spectra were recorded in $CDCl_3$ on an Avance DRX 400 spectrometer, Bruker, Germany, operating at 400 MHz. Chemical shifts (δ values in ppm) are expressed against tetramethylsilane (TMS) as internal standard.

Molecular modeling

The geometry optimization of the ethyl ester was accomplished at BLYP/QZ4P level of theory, by means of the ADF software. The graphic representation of the frontier HOMO and LUMO orbitals, as well as of the electron density, have been obtained by using the ADF software.[22,23]

The eqns. (5–7) have been employed for the calculation of the global parameters of reactivity,[24] namely the chemical potential (μ), hardness (η) and electrophilicity (ω):

$$\mu = (E_{HOMO} + E_{LUMO})/2 \quad (5)$$

$$\eta = (E_{LUMO} - E_{HOMO})/2 \quad (6)$$

$$\omega = \mu^2/2\eta \quad (7)$$

Descriptors like the Connolly accessible area, Connolly solvent excluded volume and ovality have been computed with the Chem3D Pro software.

The supramolecular architecture of the ethyl ester/ β-cyclodextrin binary compound was determined using the PM3 method of the HyperChem program. Each species (guest/host) was initially optimized with the PM3 method using the parameters mentioned below. Then the two molecules were oriented in 4 variants and geometrically reoptimized with the PM3 method.

The parameters used are:

- Convergence limit = 0.0000100 kcal/mol Iteration limit = 100
- Accelerate convergence = NO
- Optimization algorithm = Polak-Ribiere
- Criterion of RMS gradient = 0.0100 kcal/(Å·mol) Maximum cycles = 10000
- RHF Calculation

CONCLUSIONS

The increase of the absorbance value with the concentration of the β-CYD suggests the formation of the inclusion complex between ethyl ester and β-CYD. The 1:1 stoichiometry and the apparent formation constant (K=1211±111 L/mol) of ethyl ester/β-CYD inclusion complex were determined by using absorbance measurements and the Benesi-Hildebrand equation.

^{1}H-NMR spectra proved the formation of the inclusion complex, where the benzamide part of the ethyl ester has been encapsulated in the

hydrophobic cavity of β-CYD. Molecular modeling provided the inclusion compound geometry, in agreement with the experimental (^{1}H-NMR) data.

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