

## CICLODEXTRINES INTERACTING WITH METOTREXATE VIA MOLECULAR MODELING<sup>1</sup>

### *CICLODEXTRINAS INTERAGINDO COM METOTREXATO VIA MODELAGEM MOLECULAR*

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#### ABSTRACT

The coupling of a drug delivery mechanism using nanotechnology is one of the most prevalent approaches in the development of more effective and rapid treatments. This work aimed to evaluate the adsorption capacity of pristine and functionalized cyclodextrins (CD) with the chemotherapeutic drug methotrexate (MTX) in order to decrease toxicity and increase the bioavailability and distribution of the drug mainly in the blood brain barrier. Some studies were carried out through ab initio computational modeling and simulation by the density functional theory (DFT). For all MTX configurations interacting with  $\beta$ -cyclodextrin ( $\beta$ CD), methyl- $\beta$ -cyclodextrin (M $\beta$ -CD) and hydroxypropyl- $\beta$ -cyclodextrin (HP $\beta$ -CD), the stability of the system was obtained with binding energies ranging from 0.28-0.32 eV, without chemical or structural changes that modify the characteristics of the drug. Through these studies, we can indicate that CDs can interact with MTX and form possible inclusion complexes.

**Keywords:** ab initio, chemotherapeutic, DFT, SIESTA.

#### RESUMO

*O acoplamento de um mecanismo de liberação de fármaco usando nanotecnologias é uma das abordagens mais prevalentes no desenvolvimento de tratamentos mais eficazes e rápidos. Este trabalho buscou avaliar a capacidade de adsorção de ciclodextrinas (CDs) puras e funcionalizadas frente ao fármaco quimioterápico metotrexato (MTX) a fim de diminuir a toxicidade e aumentar a biodisponibilidade e distribuição do fármaco principalmente na barreira hemato encefálica. Para tanto, foram realizados estudos através de modelagem e simulação computacional ab initio pela teoria do funcional da densidade (DFT). Para todas as configurações do MTX interagindo com  $\beta$ -ciclodextrina ( $\beta$ CD), metil- $\beta$ -ciclodextrina (M $\beta$ CD) e a hidroxipropil- $\beta$ -ciclodextrina (HP $\beta$ CD) a estabilidade do sistema foi obtida com energias de ligação que variam entre 0,28-0,32 eV, sem mudanças químicas ou estruturais que modifiquem as características do fármaco incluso. Através destes estudos podemos indicar que as CDs podem interagir com o MTX e formar possíveis complexos de inclusão.*

**Palavras-chaves:** ab initio, quimioterápicos, DFT, SIESTA.

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## INTRODUÇÃO

Methotrexate (MTX) is a small hydrophilic anticancer molecule widely used in Central Nervous Systems (CNS) neoplasms (AZADI et al., 2015). However, it has a low permeability in the blood brain barrier and presents high toxicity. These factors impair the treatment and quality of life of the patients, making it common for the treatment to be interrupted. Cyclodextrins (CDs) are cyclic oligosaccharides composed of glucose units bound by  $\alpha$ -1,4-type bonds with a hydrophilic outer surface and hydrophobic inner cavity, allowing the formation of inclusion complexes with lipophilic substances that increase their solubility in water (FERREIRA et al., 2013; SOUZA et al., 2013).

The most important CDs that present natural occurrence are  $\alpha$ -CD,  $\beta$ -CD,  $\gamma$ -CD, consisting of 6, 7 and 8 glucose units respectively, which adopt the chair conformation. The minimum requirement for forming the complex is the compatibility of sizes and geometries between the CD cavity and the guest compound (OLIVEIRA et al., 2009).

The molecular structure of  $\beta$ -CD becomes more advantageous due to its availability, inclusiveness, low cost and purity (VECSERNYÉS et al., 2014), however, it has a high toxicological restriction and low solubility. This factor can be bypassed when they are functionalized, such as, for example, M $\beta$ -CD and HP $\beta$ -CD that present lower toxicity and greater solubility compared to  $\beta$ -CD.

From this perspective, it is interesting to evaluate the interaction of the MTX chemotherapeutic with pristine and functionalized  $\beta$ -CD via computational simulation. The purpose of this study is to contribute to increase the solubility, bioavailability, decreased toxicity and improved penetration into the blood brain barrier.

Upon considering the contribution of  $\beta$ -CD systems to the study of drug delivery, the adsorption capacity of the interaction of the MTX molecule with  $\beta$ -CD, M $\beta$ -CD and HP $\beta$ -CD was analyzed. Both the structural modifications in the composition of the nanostructures and the different physical-chemical characteristics of these drugs were analyzed through the ab initio theoretical studies using the SIESTA code by DFT.

## MATERIAL AND METHODS

The electronic and structural properties of MTX interacting with  $\beta$ -CD, M $\beta$ -CD and HP $\beta$ -CD were simulated using ab initio calculations based on the density functional theory (DFT) (HOHENBERG; KOHN, 1964).

For all interactions, the cutoff of 200 Ry for the grid integration is used to represent the charge density. The atomic positions were fully relaxed until remaining forces acting on atoms dropped below 0.05 eV/Å (VENDRAME et al., 2013). The SIESTA code was used, which performs full self-consistent calculations by solving the Kohn-Sham equations using numerical atomic orbitals as basis sets and they were implemented using the SIESTA code (SOLER et al., 2002). The Kohn-Sham orbitals were expanded in a double-zeta plus a polarization function set (DZP) similar to that proposed by Sankey and Niklewski. The correlation energies were determined by the local density approximation (LDA), as proposed by Perdew and Zunger (PERDEW; ZUNGER, 1981). The interactions between the core and the valence electrons are described by improved Troullier- Martins pseudopotentials (TROULLIER, N.; MARTINS, J. L., 1991).

The binding energies ( $E_{bin}$ ) between MTX molecule with CDs are calculated with the basis set superposition error (BSSE) through the following equation (BOYS; BERNARDI, 1970):

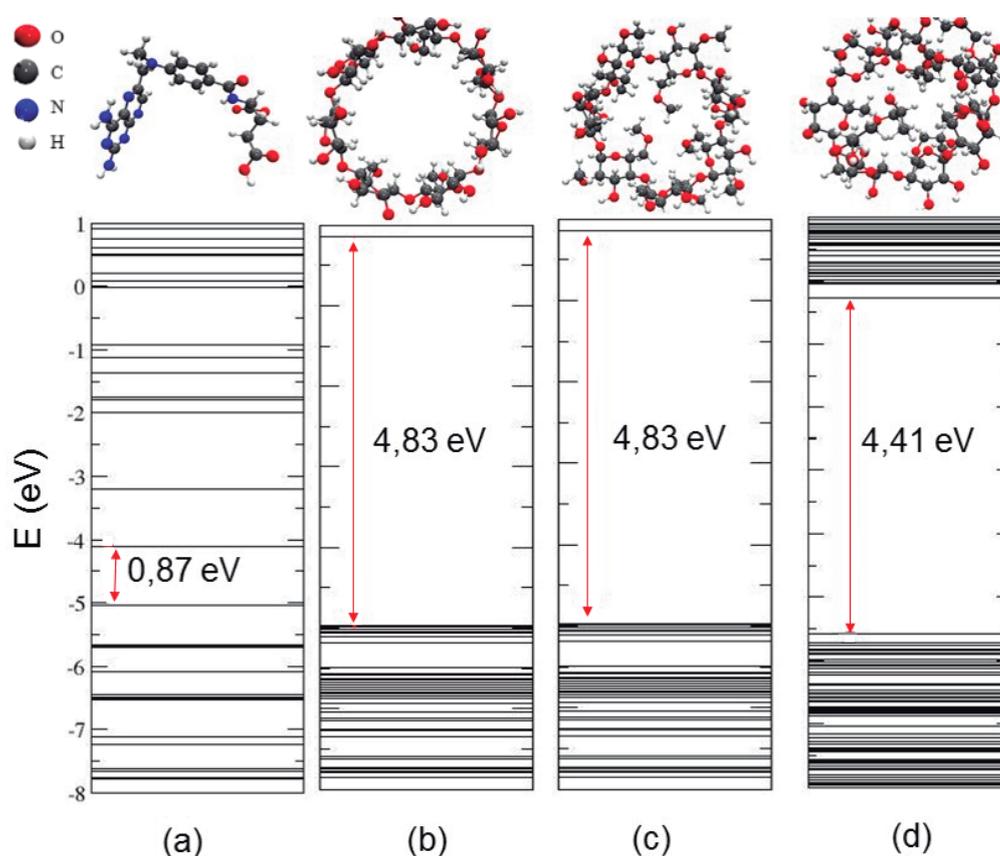
$$E_{bin} = - [E_T (CD+ MTX) - E_T (CD_{ghost} + MTX) - E_T (CD + MTX_{ghost})], \quad (1)$$

where  $E_{bin}$  is the total energy of the system,  $E_T (CD+ MTX)$  the total energy of the MTX molecule interacting with the CD,  $E_T (CD_{ghost} + MTX)$  is the total energy of the MTX molecule and  $E_T (CD + MTX_{ghost})$  the total energy of the CD.

## RESULTS AND DISCUSSION

First, the electronic and structural properties of the interaction of pristine and functionalized  $\beta$ -CD with MTX were evaluated. The equilibrium geometries and energy levels for MTX,  $\beta$ -CD, M $\beta$ -CD and HP $\beta$ -CD are shown in figure 1 (a), (b), (c) and (d), respectively. The molecules' structures, both the chemotherapeutic and the nanocarrier, were optimized with total relaxation of the atoms.

As shown in figures 1, a difference between HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) of 0.87 eV for (a), from 4.83 eV for (b) and (c) and 4.41 eV for (d).

**Figure 1** - Equilibrium geometries and energy levels for (a) MTX molecule, (b)  $\beta$ -CD, (c)  $M\beta$ -CD and (d)  $HP\beta$ -CD.

### $\beta$ -CD + MTX

Different configurations of  $\beta$ -CD interacting with MTX were analyzed. The most stable structures for these interactions are shown in figure 2. The MTX molecule was introduced into the larger and smaller inner cavity of  $\beta$ -CD, respectively ( $\beta$ -CD-MTX-I,  $\beta$ -CD-MTX-II).

In the  $\beta$ -CD-MTX-III interaction, we approximate the MTX molecule in parallel through the larger  $\beta$ -CD cavity. In the  $\beta$ -CD-MTX-IV interaction, the MTX molecule was approached on the  $\beta$ -CD walls.

In table 1, it is possible to observe the values of the binding energy, bond distance and charge transfers. The most stable configuration for the systems is when we approximate the MTX molecule of the  $\beta$ -CD molecule by 2.07 eV ( $\beta$ -CD-MTX-I). This high energy value is the final value for the whole complex, and we must consider all the established interactions between the MTX and the CD, as shown in table 1, where it also shows the two lower interaction distances of 1.64 Å ( $O\beta$ -CD-HMTX) and 2.03 Å ( $O\beta$ -CD-HMTX) for the more stable configuration  $\beta$ -CD-MTX-I.

Figure 2 - Structural configuration of  $\beta$ -CD interacting with MTX molecule.

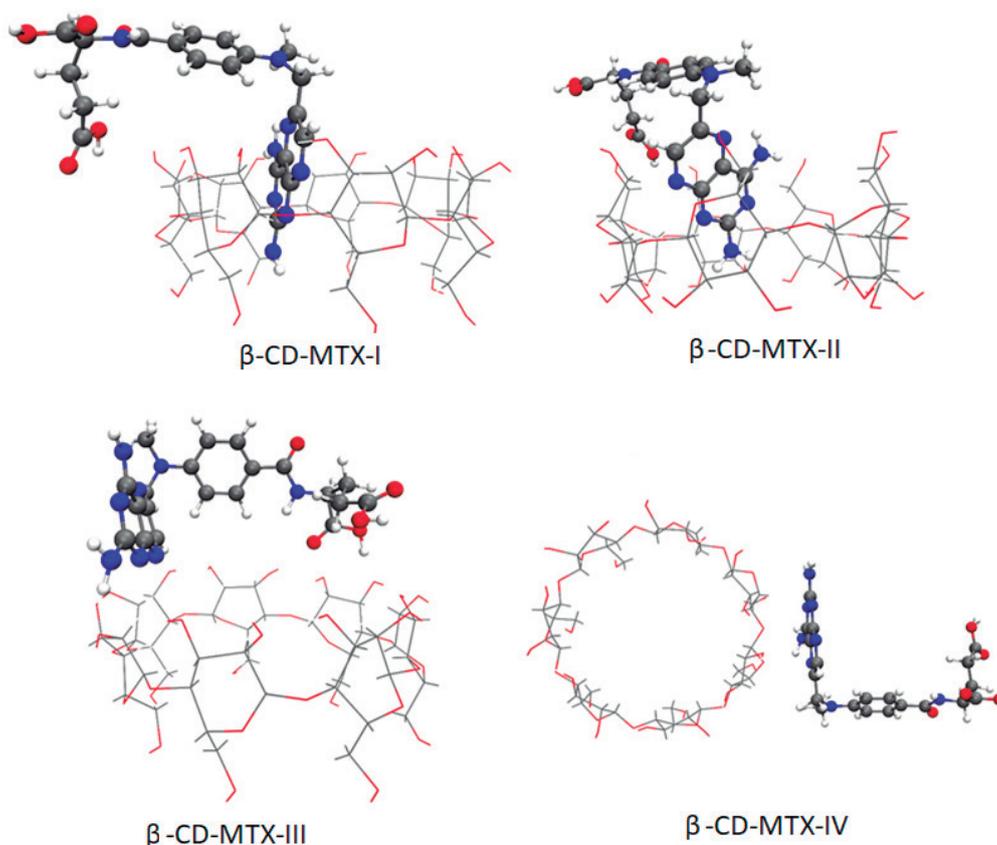
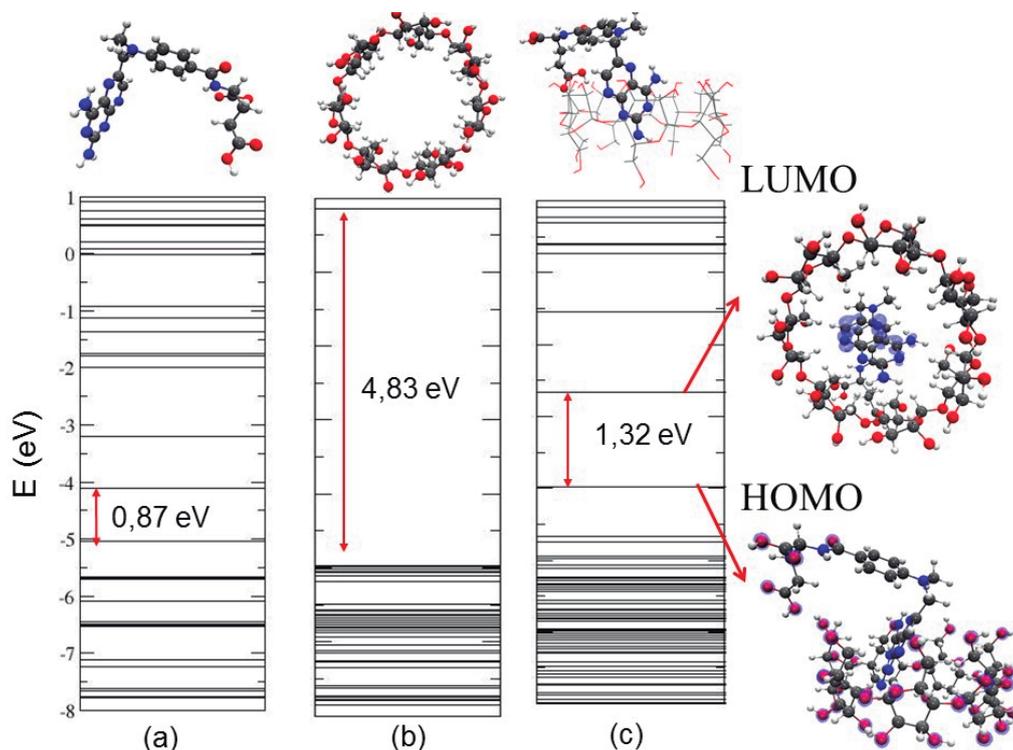


Table 1 - Bond distance, binding energy ( $E_{bin}$ ) and charge transfer ( $\Delta q$ ) for the MTX interacting with  $\beta$ -CD (positive values for charge transfer indicate that  $\beta$ -CD is an electron acceptor).

Configurations	Bond Distance (Å)	$E_{bin}$ (eV)	$\Delta q$ (e)
$\beta$ -CD-MTX -I	1.64 ( $O_{\beta-CD} - H_{MTX}$ )	2.07	0.06
	2.03 ( $O_{\beta-CD} - H_{MTX}$ )		
$\beta$ -CD-MTX -II	1.77 ( $O_{\beta-CD} - H_{MTX}$ )	1.57	0.03
	2.01 ( $O_{\beta-CD} - H_{MTX}$ )		
$\beta$ -CD-MTX -III	1.67 ( $O_{\beta-CD} - H_{MTX}$ )	1.67	0.04
	2.05 ( $H_{\beta-CD} - H_{MTX}$ )		
$\beta$ -CD-MTX -IV	1.72 ( $O_{\beta-CD} - N_{MTX}$ )	2.00	0.04
	1.67 ( $O_{\beta-CD} - N_{MTX}$ )		

After analyzing the charge transfer of the resulting configurations, we have  $\beta$ -CD as the electron acceptor. It is possible to observe a reduction of 3.51 eV in the HOMO-LUMO distance comparing the energy levels of  $\beta$ -CD, figure 3 (b), and after being adsorbed with MTX ( $\beta$ CD-MTX-I), figure 3 (c). When we analyze the charge distribution, (Figure 3 (c)), we have charge concentration on the oxygen atoms of the MTX molecule for HOMO. For LUMO, the charge is concentrated on the nitrogen of the MTX hexagons. In figure 3 (a), we present the optimized structure of the MTX molecule.

**Figure 3** - (a) Energy levels for the MTX molecule, (b) Energy levels for the  $\beta$ -CD-MTX-I, (c) Energy levels and electron density plots of the most stable configuration  $\beta$ -CD-MTX-I using orbital charge density isosurfaces  $0.0035 \text{ e}/(\text{\AA})^3$ .



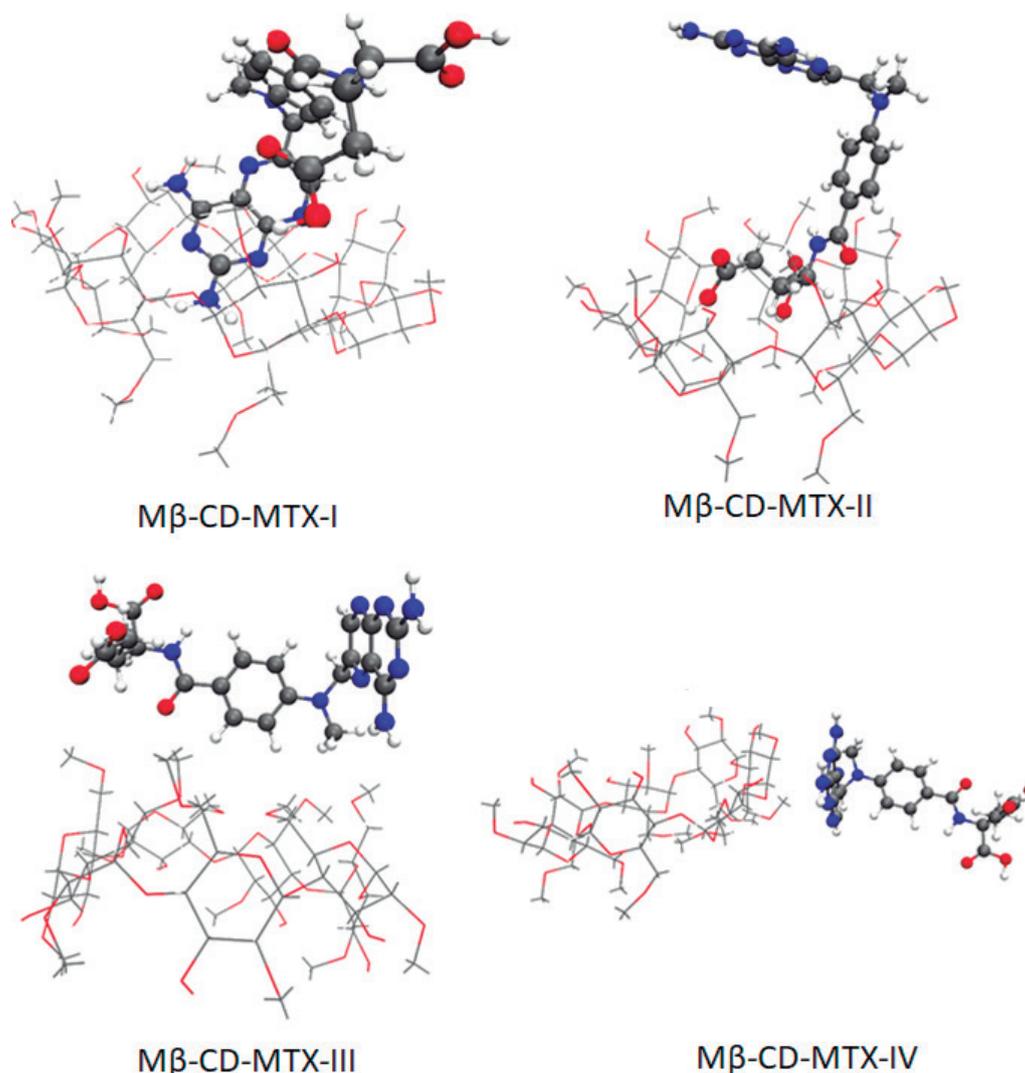
### M $\beta$ -CD + MTX

Different configurations of M $\beta$ -CD interacting with MTX were analyzed and the most stable structures for these interactions are shown in figure 4. Unlike  $\beta$ -CD, M $\beta$ -CD has radicals that hinder one of the entrances of the molecule, resulting in only one possibility of introduction of the MTX molecule. Therefore, we introduce the two ends of the MTX in the cavity of easier introduction of the M $\beta$ -CD, thus, we approximate the two hexagons formed by nitrogen and carbon atoms of the M $\beta$ -CD, respectively (M $\beta$ -CD-MTX-I, M $\beta$ -CD-MTX-II). In the M $\beta$ -CD-MTX-III interaction, we approximate the MTX molecule in parallel with the M $\beta$ -CD. In the M $\beta$ -CD-MTX-IV interaction, the MTX molecule was approached by the nitrogen rings on the outer wall of the M $\beta$ -CD.

The complexation by the end where we have the two hexagons formed by nitrogen and carbon atoms (M $\beta$ -CD-MTX-I) was the most stable when compared to the complexation by the opposite end, where we have the presence of oxygen atoms (M $\beta$ -CD-MTX-II). In table 2, it is possible to observe, for all the studied configurations, the values of the binding energies (calculated by eq. (1)), the relevant bond distances with the respective atoms involved and the charge transfers. The most stable configuration was the M $\beta$ -CD-MTX-I interaction with final binding energy of 2.82 eV. This is the final energy value for the whole complex. When we consider the closest distances between the MTX interacting atoms within the M $\beta$ -CD we have the distances of 1.77  $\text{\AA}$  (OM $\beta$ -CD-HMTX),

2.06 (OM $\beta$ -CD-HMTX). By analyzing the charge transfer of the resulting configurations, we have the M $\beta$ -CD as an electron donor.

**Figure 4** - Structural configurations of the M $\beta$ -CD interacting with the MTX molecule.

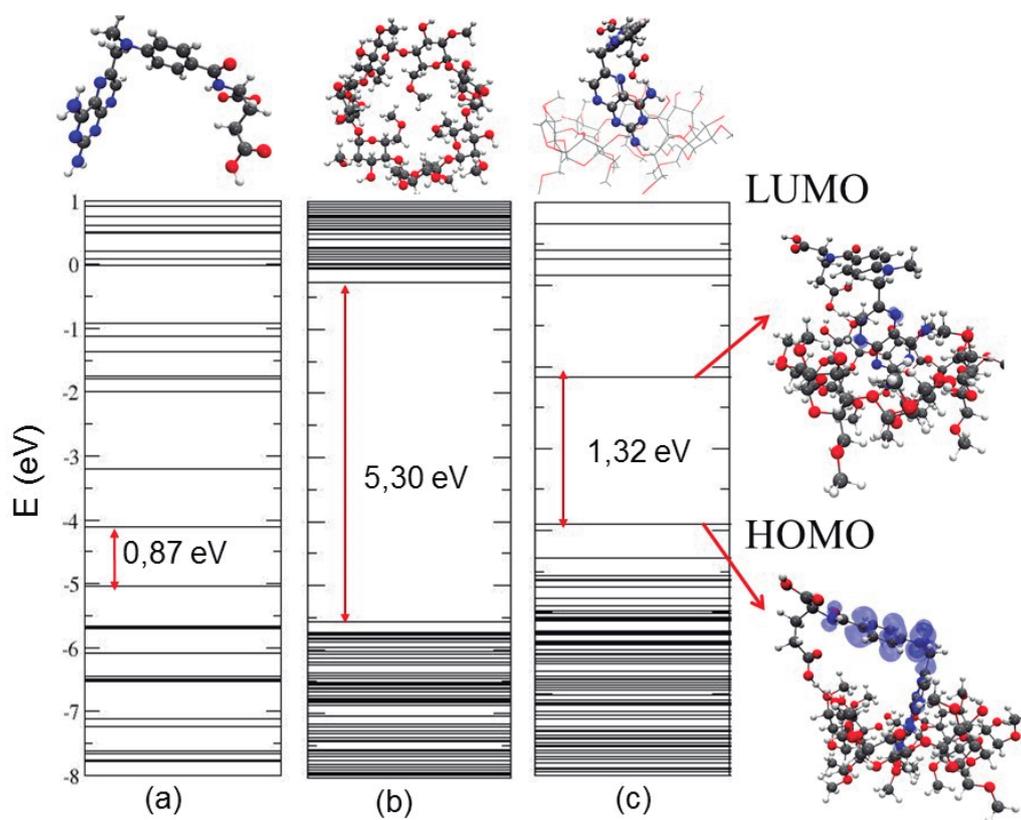


**Table 2** - Distance of interaction, binding energy ( $E_{bin}$ ) and charge transfer ( $\Delta q$ ) for the MTX interacting with M $\beta$ -CD (Negative values for charge transfer indicate that M $\beta$ -CD is an electron donor).

Configurations	Bond Distance (Å)	$E_{bin}$ (eV)	$\Delta q$ (e <sup>-</sup> )
M $\beta$ CD-MTX -I	1.77 (O <sub>M<math>\beta</math>-CD</sub> - H <sub>MTX</sub> )	2.82	-0.12
	2.06 (O <sub>M<math>\beta</math>-CD</sub> - H <sub>MTX</sub> )		
M $\beta$ CD-MTX-II	1.69 (H <sub>M<math>\beta</math>-CD</sub> - O <sub>MTX</sub> )	2.68	-0.09
	2.01 (O <sub>M<math>\beta</math>-CD</sub> - H <sub>MTX</sub> )		
M $\beta$ CD-MTX-III	1.95 (H <sub>M<math>\beta</math>-CD</sub> - H <sub>MTX</sub> )	1.84	-0.06
	2.12 (O <sub>M<math>\beta</math>-CD</sub> - H <sub>MTX</sub> )		
M $\beta$ CD-MTX-V	1.86 (H <sub>M<math>\beta</math>-CD</sub> - N <sub>MTX</sub> )	1.67	-0.08
	2.01 (O <sub>M<math>\beta</math>-CD</sub> - H <sub>MTX</sub> )		

We have a reduction of 3.98 eV in the HOMO-LUMO distance when we compare the energy levels of the M $\beta$ -CD, figure 5 (b), with MTX (M $\beta$ -CD-MTX-I) after having been adsorbed, figure 5 (c). When we analyzed the charge distribution (Figure 5 (c)), we observed in HOMO a contribution in MTX, more specifically in the hexagon formed by carbon atoms and in the nearby nitrogen atoms. For LUMO the charge concentrates on the nitrogen atoms of the MTX hexagons. In figure 5 (a), we present the optimized structure of the MTX molecule.

**Figure 5** - (a) Energy levels for the MTX molecule, (b) Energy levels for M $\beta$ -CD, (c) Energy levels and electron density plots of the most stable configuration M $\beta$ -CD-MTX-I using orbital charge density isosurfaces  $0.0035 \text{ e}^- / (\text{\AA})^3$ .



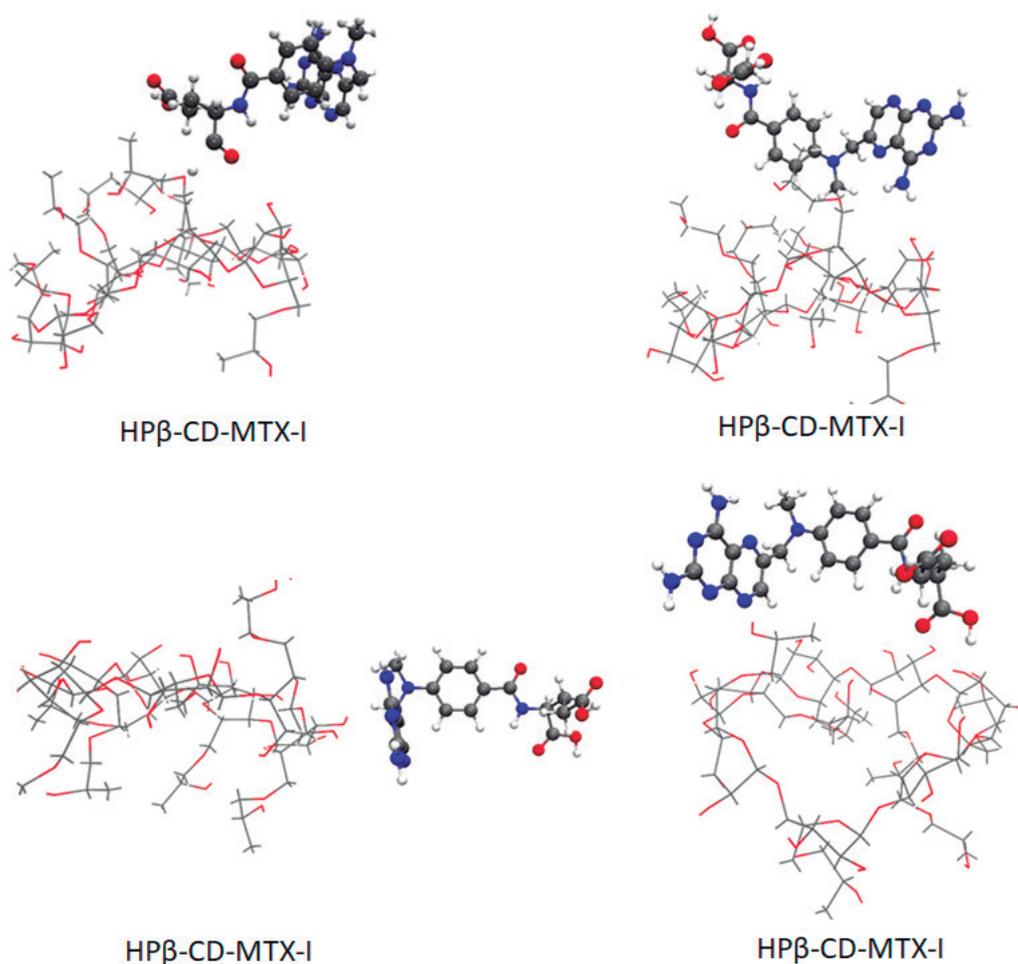
## HP $\beta$ -CD + MTX

Different configurations of HP $\beta$ -CD interacting with MTX were analyzed. The most stable structures for these interactions are shown in figure 6. We approached the MTX by the extremity where there are oxygen atoms (HP $\beta$ -CD-MTX-I), also by the opposite end where we have the two hexagons formed by atoms of nitrogen and carbon (HP $\beta$ -CD-MTX-II) in parallel with HP $\beta$ -CD (HP $\beta$ -CD-MTX-III) and at its ends (HP $\beta$ -CD-MTX-IV).

In table 3, it is possible to observe, for all the configurations studied, the values of the binding energies (calculated by eq. (1)), relevant bond distances with the respective atoms involved and charge transfers. The most stable configuration for the systems in which we approach the MTX molecule with

the HP $\beta$ -CD molecule was 2.68 eV (HP $\beta$ -CD-MTX-II). Considering the closest distances between the MTX interacting atoms within the HP $\beta$ -CD we have the distances of 1.69 (HHP $\beta$ -CD-OMTX) and 2.03 (OHP $\beta$ -CD-HMTX).

**Figure 6** - Structural configurations of HP $\beta$ -CD interacting with the MTX molecule.

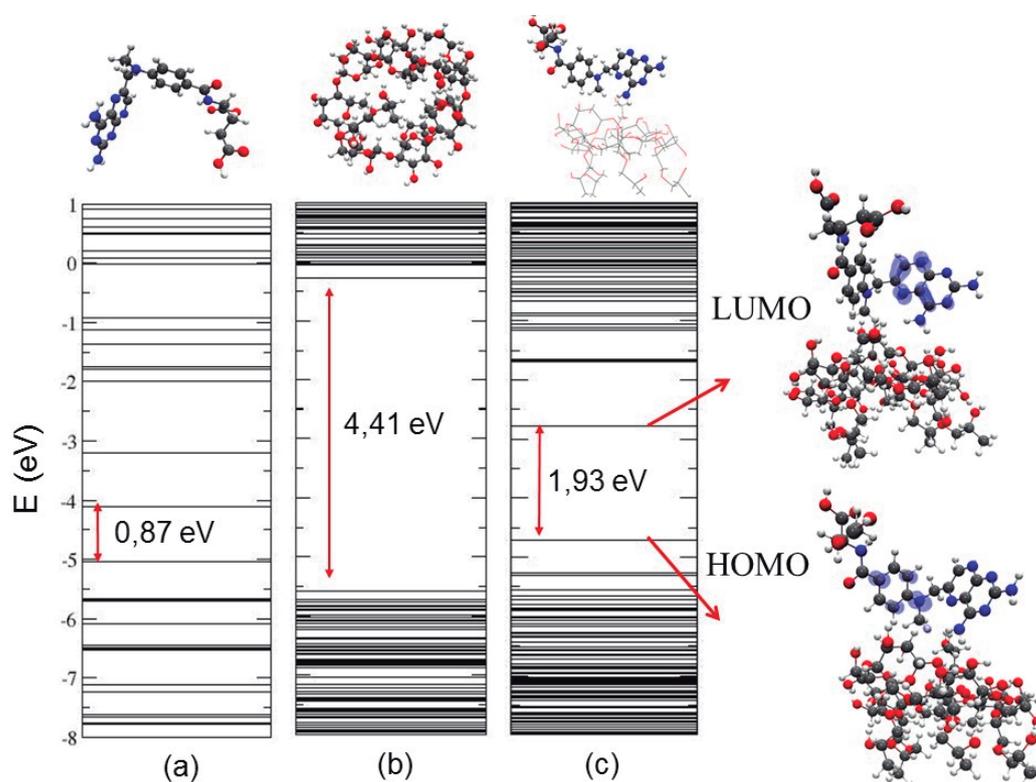


**Table 3** - Bond distance, binding energy ( $E_{bin}$ ) and charge transfer ( $\Delta q$ ) for the MTX interacting with HP $\beta$ -CD (Negative values for charge transfer indicate that HP $\beta$ -CD is an electron donor).

Configurations	Bond Distance (Å)	$E_{bin}$ (eV)	$\Delta q$ (e <sup>-</sup> )
HP $\beta$ -CD-MTX-I	2.03 (H <sub>HP<math>\beta</math>-CD</sub> - O <sub>MTX</sub> )	1.7	-0.06
	1.98 (O <sub>HP<math>\beta</math>-CD</sub> - O <sub>MTX</sub> )		
HP $\beta$ -CD-MTX-II	1.69 (H <sub>HP<math>\beta</math>-CD</sub> - O <sub>MTX</sub> )	2.68	-0.09
	2.03 (O <sub>HP<math>\beta</math>-CD</sub> - H <sub>MTX</sub> )		
HP $\beta$ -CD-MTX-III	1.65 (H <sub>HP<math>\beta</math>-CD</sub> - N <sub>MTX</sub> )	1.69	-0.04
	2.00 (O <sub>HP<math>\beta</math>-CD</sub> - H <sub>MTX</sub> )		
HP $\beta$ -CD-MTX-IV	2.03 (H <sub>HP<math>\beta</math>-CD</sub> - H <sub>MTX</sub> )	1.73	-0.04
	1.70 (O <sub>HP<math>\beta</math>-C</sub> - H <sub>MTX</sub> )		

We have a reduction of 2.49 eV in the HOMO-LUMO distance comparing the energy levels of HP $\beta$ -CD, figure 7 (b), and after being adsorbed with MTX (HP $\beta$ -CD-MTX-I), figure 7 (c). By analyzing the charge transfer of the resulting configurations, we have the HP $\beta$ -CD as an electron donor. When we analyze the charge distribution, (Figure 7 (c)), we have charge concentration in MTX in the hexagon formed by carbons and in the nitrogen closest to this site for HOMO. For LUMO the charge concentrates on the nitrogen of the hexagons of the MTX next to each other. In figure 7 (a), we present the optimized structure of the MTX molecule.

**Figure 7** - (a) Energy levels for the MTX molecule, (b) Energy levels for the HP $\beta$ -CD, (c) Energy levels and electron density plots of the most stable configuration HP $\beta$ -CD-MTX-I using orbital charge density isosurfaces  $0.0035 \text{ e}/(\text{\AA})^3$ .



## CONCLUSIONS

The study showed that there is interaction between the systems, without significant structural and electronic changes, which is of great interest for the use of nanostructures as carriers of these drugs. Although the energy values were high, the binding distances between the closest atoms in the interaction are not similar to a covalent bond distances. In addition, we should note the existence of more than one interaction occurring with CD and MTX.

All the interactions presented greater stability when the complexation was made by the greater cavity of the CD. When we compared the opposite ends of the MTX that were introduced in the CD, we realized that for both  $\beta$ CD and M $\beta$ -CD configurations, the most stable configuration occurred when MTX was introduced by the end of the hexagons formed by carbon and nitrogen

atoms. In relation to the charge transfer,  $\beta$ -CD presented a charge receptor behavior for all the configurations studied.

However, the configurations for M $\beta$ -CD and HP $\beta$ -CD presented a charge donor behavior. From our results, we had a physical adsorption on our system, which was expected, so we can use the CDs as a nanocarrier to transport the MTX without significant chemical or structural changes that modify the characteristics of the drug. Simulations with other chemotherapeutic drugs are of great interest for this study.

## ACKNOWLEDGMENTS

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