

$C_{61}(\text{COOH})_2$ INTERACTING WITH THE ANTIVIRAL RIBAVIRIN¹

$C_{61}(\text{COOH})_2$ INTERAGINDO COM O ANTIVIRAL RIBAVIRINA

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ABSTRACT

Carbon nanostructures present unique properties that result from a variety of possible structural forms. Therefore, the application of such systems, in a functionalized or pristine form, as chemical or biological sensors have become a large field of study and application. Among the C_{60} derivatives, the $C_{61}(\text{COOH})_2$, has shown success crossing the external cell membrane. This makes the compound, in biological environment, ideal for drug delivery systems. In this work the interaction of $C_{61}(\text{COOH})_2$ with antiviral ribavirin was performed through *ab initio* simulations using the SIESTA code. The antiviral ribavirin is used for the treatment of chronic hepatitis C and, recently it has been used for the canine distemper disease. However, the delivery of the ribavirin in the central nervous system (CNS) still faces challenges due to its low penetration in the blood brain barrier (BBB). The results show that the ribavirin interact weakly with $C_{61}(\text{COOH})_2$ molecules. The interaction occurs through a physisorption regime, with values between 0.17 and 1.12 eV for the most stable configurations. This type of interaction is interesting so that the absorbent system remains connected to its carrier (in this case the $C_{61}(\text{COOH})_2$) with no alteration in the properties of pristine molecules.

Keywords: ab initio, DFT, fullerene, SIESTA, simulation.

RESUMO

Nanoestruturas de carbono apresentam propriedades únicas que resultam da variedade de possíveis formas estruturais. A aplicação de tais sistemas, funcionalizados ou puros, como sensores químicos e biológicos tornou-se um grande campo de estudo e aplicação. Dentre os derivados de C_{60} , o $C_{61}(\text{COOH})_2$, foi demonstrado com sucesso para atravessar a membrana celular externa. Isto torna o composto, em meios biológicos, ideal para entrega de fármacos. Neste trabalho estudamos a interação do $C_{61}(\text{COOH})_2$ com o antiviral ribavirina através de cálculos *ab initio* pelo código SIESTA. Este fármaco é utilizado para o tratamento da hepatite C crônica e estudos recentes tem destinado este fármaco para tratamento da cinomose canina. No entanto, a entrega da ribavirina no sistema nervoso central (SNC) ainda enfrenta dificuldades, devido à sua baixa penetração na barreira hematoencefálica (BHE). Os resultados mostram que a ribavirina interage fracamente com o $C_{61}(\text{COOH})_2$. A interação ocorre através de um regime de fisissorção, com valores entre 0,17 e 1,12 eV para a configuração mais estável. Este tipo de interação é extremamente interessante, para que o sistema absorvente permanece ligado ao seu transportador (neste caso, o $C_{61}(\text{COOH})_2$) sem que ocorra alteração nas propriedades das moléculas de origem.

Palavras-chave: ab initio, DFT, fulereno, SIESTA, simulação.

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INTRODUCTION

In the 80's, scientists discovered a molecule with soccer ball format, with 60 carbon atoms, which was given the name of buckyminsterfullerene (C_{60}) (KROTO et al., 1985). Since then, the fullerenes have become a very active field of research with a variety of possible derivatives.

Currently, the use of such nanosized functionalized or pristine systems, as chemical and biological sensors, have become an extensive area of study and application. Just the C_{60} interacting with drugs already encompasses a wide field and has added many studies, such as the use in the controlled drug delivery (GROBMYERA; KRISHNAB, 2012).

However, the high stability and low chemical reactivity of the fullerene nanostructures introduce some difficulties in working with these particles. These difficulties can be overcome through the functionalization process (BAKRY, 2007; SANTOS, 2010). The covalent functionalization of the carbon nanostructures can be made by combining chemical groups such as -OH and -COOH, through covalent bonds (FILHO; FAGAN, 2007; VELOSO et al., 2006).

These modified conformations can be used to facilitate the interaction of carbon nanostructures with organic and biological molecules (FU et al., 2002), with other chemical groups such as drugs or toxic molecules (DAI et al., 2012; KONG et al., 2000) and even to viruses and bacteria (FEDOROVA et al., 2012; TOLLAS et al., 2014). Among the biological activities there are those used in photodynamic therapy, neuroprotective activity, antimicrobial, antiviral and other biological targets (SHI et al., 2014).

Among the functionalized C_{60} , the $C_{61}(\text{COOH})_2$, will be the subject of study of this work. The $C_{61}(\text{COOH})_2$ succeeded in penetrating the outer cell membrane (FOLEY et al., 2002). This renders the compound in biological environment, ideal for drug delivery primarily in the brain. Theoretical studies of $C_{61}(\text{COOH})_2$ interacting with ascorbic acid showed good results with the $C_{61}(\text{COOH})_2$ as a promising candidate for the intracellular transport of vitamin C (ZANELLA et al., 2008).

Considering the contribution of these nanomaterials for the study and advancement of drug delivery, in this work, the structural and electronic properties of $C_{61}(\text{COOH})_2$, interacting with the antiviral ribavirin is studied by first-principles simulations. Ribavirin is an antiviral drug widely used to treat chronic hepatitis C. It has been extensively studied in animals that develop important viral infections similar to those of man (ZEUZEM, 2016). Currently, its application has focused on the treatment of canine distemper. In the experiment of Elia et al. (2008) ribavirin was effective in preventing the replication of canine distemper virus *in vitro* at low concentrations. The drug can cause mutations in the viral genome and interferes with RNA polymerase by competing with natural nucleosides, producing error in the virus chain termination (MANGIA, 2008). Ribavirin is quickly absorbed orally, widely distributed in the tissues, largely metabolized and excreted mainly in urine.

However, its penetration in the blood brain barrier is low (VIANA; TEIXEIRA, 2015). Because of this factor, its application in the brain is still challenge.

Therefore, this work aims to study the interaction of $C_{61}(\text{COOH})_2$ with the antiviral ribavirin, in an original way, through computer simulation using Density Functional Theory (DFT). The theoretical understanding of energetic and structural properties of these structures can assist in their use in future applications in the central nervous system providing better ways of interaction.

MATERIALS AND METHODS

The association of the ribavirin molecule with the $C_{61}(\text{COOH})_2$ was evaluated through *ab initio* calculations based on DFT (HOHENBERG; KOHN, 1964). Calculations were performed using the SIESTA (Spanish Initiative for Electronic Simulations with Thousands of Atoms) code which executes self-consistent calculations by solving the Kohn-Sham equations (SOLER et al., 2002). In all calculations the double-zeta basis set and the polarization function (DZP), with an energy shift of 0.05 eV for the numerical atomic orbitals are used (VENDRAME et al., 2013). To represent the charge density, the cutoff radius of 200 Ry was utilized for the integration grid in real space. The exchange and correlation potential was given by the local density approximation (LDA), according to the parameterization of Perdew and Zunger (PERDEW; ZUNGER, 1981; ZANELLA et al., 2008). The geometry optimizations were performed with the total relaxation of all atoms of the $C_{61}(\text{COOH})_2$, and the ribavirin molecule with the convergence criterion on all atomic coordinates of 0.05 eV/Å.

The binding energies (E_{bin}) were calculated using the basis set superposition error (BSSE) (PERDEW; ZUNGER, 1981). The BSSE was eliminated by adding ghost orbitals to the isolated adsorbate calculations. It is well known that BSSE is severe in calculating weak bonds. For example, van der Waals bonds cannot be reproduced correctly without a BSSE correction (TSUNEDA, 2014).

These ghost orbitals possess normal basis functions but do not otherwise affect the calculations, which ensures that the same degrees of freedom are available to the wave functions during any calculation (AHANGARIG et al., 2016). This correction is done by the counting method using “ghost” atoms, as the following equation:

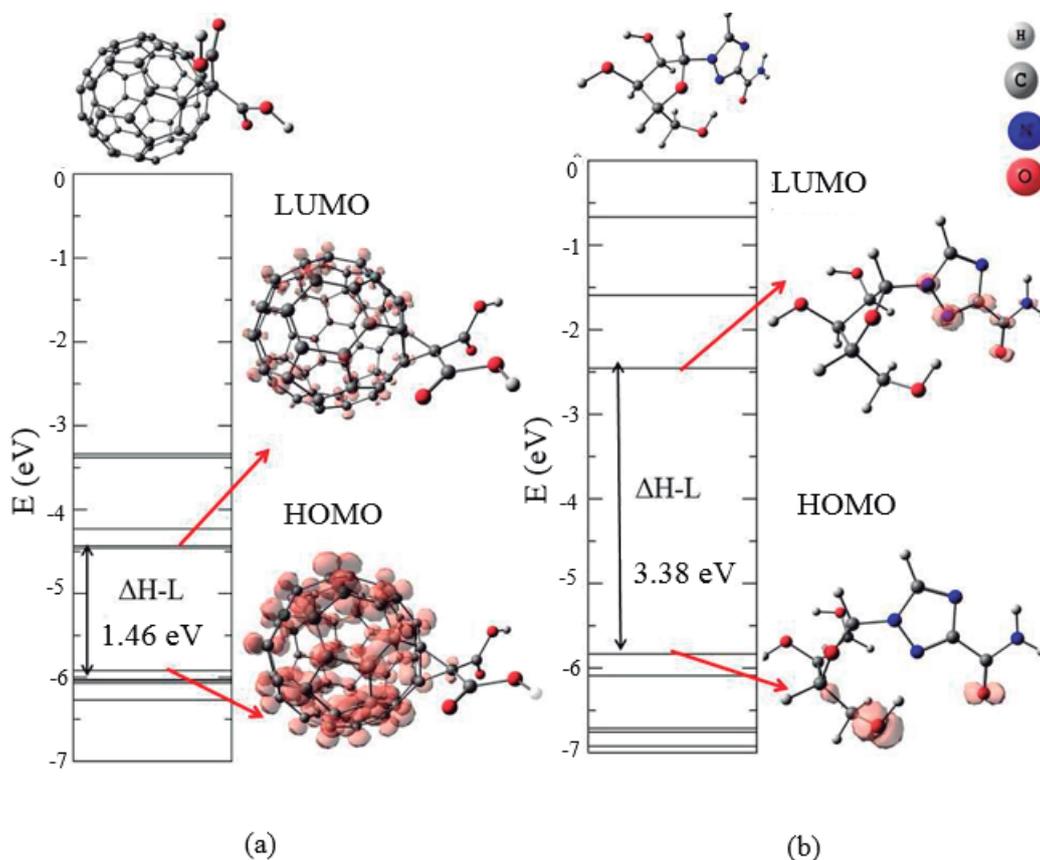
$$E_{\text{bin}} = - [E_{\text{T}}(\text{CN} + \text{Rib}) - E_{\text{T}}(\text{CN}_{\text{ghost}} + \text{Rib}) - E_{\text{T}}(\text{CN} + \text{Rib}_{\text{ghost}})] \quad (1)$$

where E_{bin} is the binding energy of the system, $E_{\text{T}}(\text{CN} + \text{Rib})$ is the total energy of the carbon nanostructure (CN) plus the ribavirin molecule, $E_{\text{T}}(\text{CN}_{\text{ghost}} + \text{Rib})$ is the total energy of the ribavirin molecule and $(\text{CN} + \text{Rib}_{\text{ghost}})$ is the total energy of the carbon nanostructure.

RESULTS AND DISCUSSION

The equilibrium geometry, charge plots and energy levels to $C_{61}(\text{COOH})_2$ and ribavirin are presented in figures 1 (a) and (b), respectively. It can be observed, as shown in figure 1 (a), a difference between HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) of 1.46 eV to $C_{61}(\text{COOH})_2$. The charge distribution in LUMO are homogeneous in the surface of C_{60} . The HOMO is located over the surface of the fullerene with contribution in bonds with the carboxylic groups. In figure 1 (b) it is observed a HOMO and LUMO difference of 3.38 eV for the ribavirin molecule. The charge distribution for LUMO is concentrated in the nitrogen atoms of the pentagonal ring and of the oxygen near this ring. For HOMO, the charge is directed to the oxygen atoms at the ends of the molecule.

Figure 1 - The electronic levels and local density of states for the (a) $C_{61}(\text{COOH})_2$ and (b) ribavirin. Isosurface value of $0,00782 \text{ e}/(\text{\AA})^3$.



Different configurations of $C_{61}(\text{COOH})_2$ interacting with ribavirin were analyzed. The more stable structures for these interactions are shown in figure 2.

The oxygen of one of the ribavirin pentagons is approximated to the hydrogen of the functionalization of the $C_{61}(\text{COOH})_2$ and the hydrogen of the other ribavirin pentagon with the oxygen of the $C_{61}(\text{COOH})_2$, Rib- $C_{61}(\text{COOH})_2$ -I. In Rib- $C_{61}(\text{COOH})_2$ -II the hydrogen is approximated to one

end of ribavirin with the oxygen of $C_{61}(COOH)_2$. In Rib- $C_{61}(COOH)_2$ -III it is approximated the oxygen and the hydrogen of the pentagonal ring to the hydrogen and oxygen of $C_{61}(COOH)_2$, respectively.

Finally, for hydrogen configuration it is approached the nitrogen with the pentagonal ring and hydrogen from one end of ribavirin with hydrogen and oxygen of $C_{61}(COOH)_2$.

Figure 2 - Structural configurations for the $C_{61}(COOH)_2$ interacting with ribavirin molecule: Rib- $C_{61}(COOH)_2$ -I, Rib- $C_{61}(COOH)_2$ -II, Rib- $C_{61}(COOH)_2$ -III, Rib- $C_{61}(COOH)_2$ -IV.

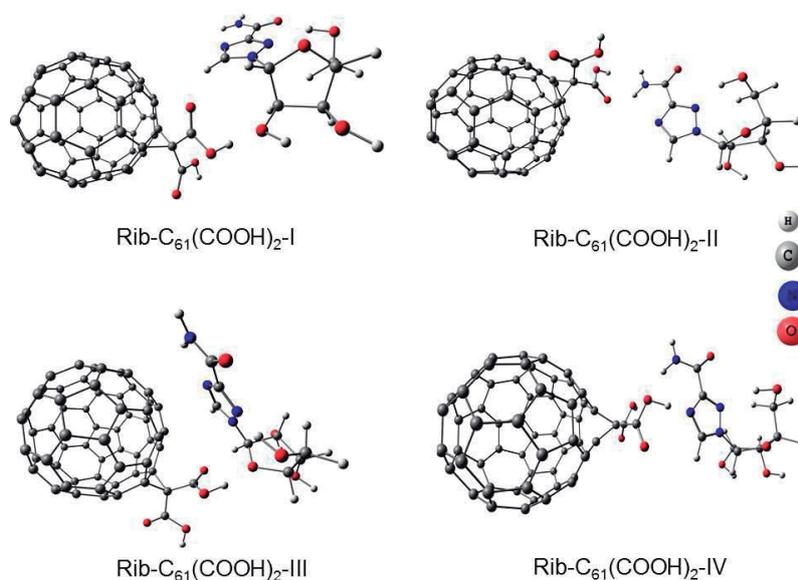


Table 1 shows, for all the configurations studied, the values of binding energies (calculated by Eq. (1)), relevant bond distances and charge transfers. The most stable configuration for the different systems studied was Rib- $C_{61}(COOH)_2$ -III. The bond distances were between 1.57 Å ($H_{CN} - O_{Rib}$) and 2.07 Å ($O_{CN} - H_{Rib}$). The binding energy obtained for the most stable system was 1.12 eV. This energy variation between the configurations can be related with the different atoms involved in the bonds and the number of the interactions between the systems.

Table 1 - Configurations, smallest distances, binding energies (E_{bin}) and charge transfers (Δq) for different configurations as shown in figure 2 (positive values indicate that the carbon nanostructure is an electron acceptor).

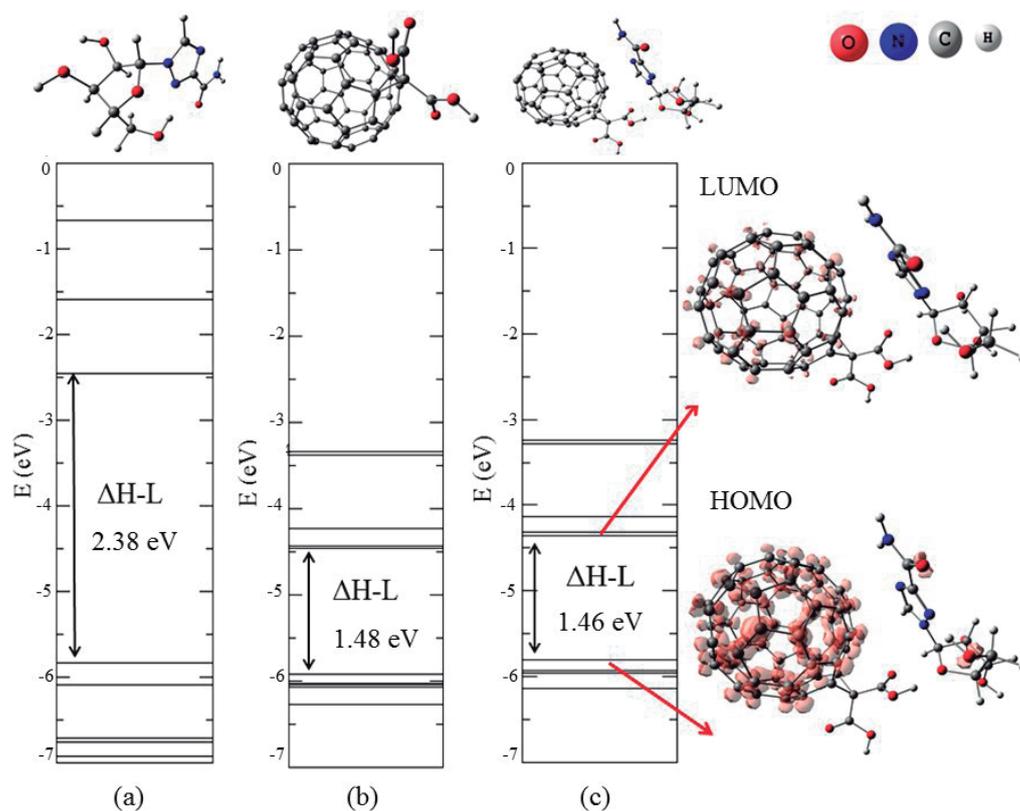
| Configurations | Distance (Å) | E_{bin} (eV) | Δq (e) |
|----------------------------|--|----------------|----------------|
| Rib- $C_{61}(COOH)_2$ -I | 1.99 ($O_{CN} - H_{Rib}$) 1.50 ($H_{CN} - O_{Rib}$) | 0.70 | 0.06 |
| Rib- $C_{61}(COOH)_2$ -II | 1.87 ($O_{CN} - H_{Rib}$) | 0.17 | 0.13 |
| Rib- $C_{61}(COOH)_2$ -III | 1.57 ($H_{CN} - O_{Rib}$) 2.07 ($O_{CN} - H_{Rib}$) | 1.12 | 0.04 |
| Rib- $C_{61}(COOH)_2$ -IV | 1.88 ($O_{CN} - H_{Rib}$) 1.37 ($H_{CN} - N_{Rib}$) | 0.63 | 0.19 |

Figure 3 (c) shows the energy levels, optimized structure and electronic charge density plot for the (Rib- $C_{61}(\text{COOH})_2$ -III). The electronic levels of $C_{61}(\text{COOH})_2$ and ribavirin, in pristine form, can be seen (Figure 3 (a) and (b) respectively).

It is observed that there was no significant changes in the energy levels of the most stable configuration studied in comparison with pristine $C_{61}(\text{COOH})_2$. The electronic charge density plots for LUMO in figure 3 (c) show a homogeneous charge contribution in the surface of $C_{61}(\text{COOH})_2$. For the HOMO, a small distribution near the oxygens in the ribavirin molecule is observed along with a homogeneous distribution in $C_{61}(\text{COOH})_2$, similar as $C_{61}(\text{COOH})_2$ pristine HOMO.

From the Mulliken population analysis, the charge transfers of the studied system show that the carbon nanostructure behaves as an electron acceptor with a charge transfer of $0.04 e^-$ of ribavirin to the $C_{61}(\text{COOH})_2$. This charge behavior is in accord with previous results in literature. (VENDRAME et al., 2013).

Figure 3 - The electronic levels of (a) $C_{61}(\text{COOH})_2$, (b) ribavirin molecule, and (c) Rib- $C_{61}(\text{COOH})_2$ -III configuration. Also in (c) the local density charge plot for HOMO and LUMO of the Rib- $C_{61}(\text{COOH})_2$ -III with isosurface value of the $0.0021 e^-/\text{\AA}^3$.



CONCLUSIONS

The interaction of $C_{61}(\text{COOH})_2$ with ribavirin was investigated using first-principles calculations. From the analysis of the Mulliken population, charge transfers obtained showed that $C_{61}(\text{COOH})_2$ behaves as an electron acceptor. There were no significant changes in the HOMO-LUMO difference

for interactions of ribavirin with $C_{61}(\text{COOH})_2$. The high values for the binding energies are results of the LDA approximation. The LDA approach to the exchange and correlation potential overestimates the binding energies, but describe very well the van der Waals interactions (GIRIFALCO; HODAK, 2002). Furthermore, systems interacting with the π type arrangement as carbon nanostructures in organic molecules are well-represented by this approach (TOURNUS et al., 2005). No covalent bonds are observed, confirming that the interactions between the systems occurs in a physical adsorption. From these results, it is concluded that the functionalized fullerene $C_{61}(\text{COOH})_2$ interacting with ribavirin can be a promising drug carrier system. Understanding the energy and structural properties of these structures can assist in the use of nanomaterials in future biomedical applications, providing the best forms of interaction, reducing the cost and time for possible future experiments. Considering these arguments, the functionalized C_{60} can be seen as a promising candidate for the transport of ribavirin molecule, and it has as the main objective a greater action in the brain.

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