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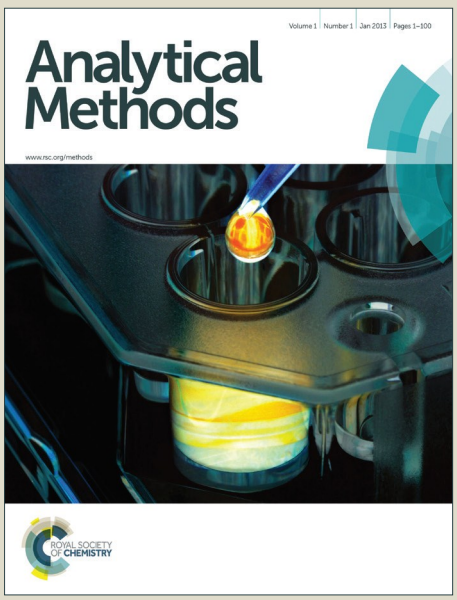

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Theoretical use of boron nitride nanotubes as a perfect container for anticancer molecule

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ABSTRACT

In recent years a great interest has emerged in the development of nanocarriers for drug transport. One of the major challenges is to obtain a drug delivery system able to control drug release profile, transport absorption and distribution, in a view of improving efficacy and safety. Herein, we present theoretical results based on density functional theory (DFT) to determine the best adsorption site of anticancer Ifosfamide molecule in boron nitride nanotube. For this functionalized system we determine the dependence of the adsorption energy on the displacement of molecules in the outer and inner boron nitride surfaces, together with their local morphological and charge modifications. Quantum simulations show that the most stable physisorption state is located inside the nanotube, with no net charge transfer between each subsystem, and no barrier

energy at the nanotube entrance. This demonstrates that chemotherapeutic encapsulation is the most favorable way for Ifosfamide to be vectorized.

Keywords: boron nitride nanotubes; therapeutic agents; DFT calculations

1. Introduction

Nano-scale drug delivery systems are usually dedicated to target specific cells in tissues or organs, and most of them are focused on cancer chemotherapy. Incorporating chemotherapeutics drug in the accessible cavities of the nanocarriers and attaching receptors to the nanocarriers is the most promising way to target precisely disease cells despite some drawbacks.[1-4] The molecular size and the biodegradability of the nanocarrier are two very important factors since the eventual nanocarrier clearance from the body, after delivering the drug, will depend on these two parameters essentially. Nanotubes are very important geometric class of nanomaterials that are used in biotechnology and medicine. Various types of drug/agent nanocarriers have been investigated, among which a small number of such systems have been already commercialized or are in clinical studies.[5] In particular, carbon nanotubes (CNTs) have attracted a wide attention as carriers for biologically relevant molecules, because of their unique physicochemical and biological properties.[4, 6, 7] Among all the nanocarriers studied, boron nitride nanotubes (BNNTs) are structural analogues of CNTs nanomaterials. Their electrical properties are not dependent on their chirality and diameter since they have a large band gap of about 5.5 eV.[8] Their electrical insulation is very high, despite a high thermal conductivity.[9] Moreover, BNNTs have a high hydrophobicity, a strong resistance to oxidation and to heat as well as a strong radiation absorption resilience. Besides, they have been widely investigated in medical and biomedical applications [10, 11] after covalent or noncovalent functionalization of their outer surface to increase

their water dispersion.[12-14] Density functional theory (DFT) studies have been conducted on the noncovalent functionalization of BNNTs with benzaldehyde and seven different heterocyclic aromatic molecules. They have shown that weak interactions give rise to new impurity states within the band gap of pristine BNNTs suggesting the possibility of carrier doping through the selective adsorption of aromatic rings.[15] Moreover studies dealing with the interactions between BNNTs and different molecules (azomethine (C_2H_5N), anticancer agent (Pt(IV)) complex linked to an amino-derivative chain or Pt(IV) complex alone) have demonstrated the possibility to obtain physisorbed or chemisorbed states. The most stable physisorption state was localized inside the nanotube and the molecular chemisorption was possible above two adjacent B and N atoms of a BNNT hexagon only. Note that the attachment of an azomethine plus a subsequent drug did not perturb the cycloaddition process.[16] Moreover, some preliminary applications in biomedicine have emerged in the latest years showing no significant adverse effects for in vivo study when BNNTs were injected in rabbits at a dose up to 10mg/kg. All these data suggested the optimal biocompatibility of BNNTs, and thus open the way to their exploitation in nanomedecine.[17, 18] Ifosfamide (Ifos) is a cytotoxic anticancer chemotherapy drug belonging to the category of mustard gas derivatives. It undergoes in the DNA formation and blocks the cell replication without any selectivity for cancer cells, which implies a lot of secondary effects. Driving the route of Ifos action using appropriate nanocarrier could definitively increase the selectivity of the anticancer agent.

In this paper we report some new theoretical results concerning the adsorption of Ifosfamide (Ifos) molecule on the outer and the inner BNNT nanocarrier surfaces. Thanks to full DFT calculations, we investigate the energy behavior of Ifos molecule interacting with a BNNT nanotube. We demonstrate that the molecule is able to encapsulate spontaneously and to find a

stable geometry leading to strong adsorption into the inner (10,10) BNNT sidewalls. To demonstrate this, we investigated the energy stability of the functionalized systems through the changes of their morphology and their electronic structure thanks to partial density of states (PDOS) and Bader charge analysis.

2. Theoretical details

We studied system consisting of an armchair single-walled (10, 10) BNNT with finite-length cylindrical BN cage (i.e. diameter radius 13.94 Å, length 21.32 Å), composed of 180 B and 180 N atoms. In order to avoid boundary effects, hydroxyl groups (–OH), consisting of 40 O and 40 H atoms, were introduced onto the BNNT extremities, as depicted in Fig. 1.[14] BNNT nanotube and Ifosfamide ($C_7H_{15}Cl_2N_2O_2P$) drug molecule were studied using the same unit cell (i.e. 50 x 50 x 60 $Å^3$), where the last number represents the length of the unit cell along the tube axis. Given the large unit cell, the Brillouin zone was sampled using a single k-point at the center Γ. A basis set of localized atomic orbitals (double-ζ plus polarization functions), and norm-conserving pseudopotentials were employed.

The adsorption energy (E_{ads}) of adsorbed molecules (Mol) on the inner surface of the BNNT (Template) was derived from the energy difference between the different states of the system, namely, E_{ads} = E(Mol + Template) - E(Template) - E(Mol). A negative E_{ads} value denoted a more favorable interaction between the drug and the BNNT.

The total energies were obtained from *ab initio* calculations in the framework of Kohn-Sham realization of the density functional theory DFT.[19, 20] We used the Perdew-Burke-Ernzerhof (PBE) generalized gradient approximation (GGA).[21] for the exchange correlation density functional as implemented in the SIESTA package.[22] All calculations were performed without spin polarization. We use the self-consistency mixing rate of 0.1, a maximum force tolerance of

0.01 eV/ Å and a mesh cut off of 100 Ry (the variations of these parameters showed a very low perturbation of the total energies by less than 0.1%). The self-consistent cycles were stopped when variations of the total energy per unit cell and band structure energy were both less than 10^{-4} eV.

3. Results and discussion

a) b)

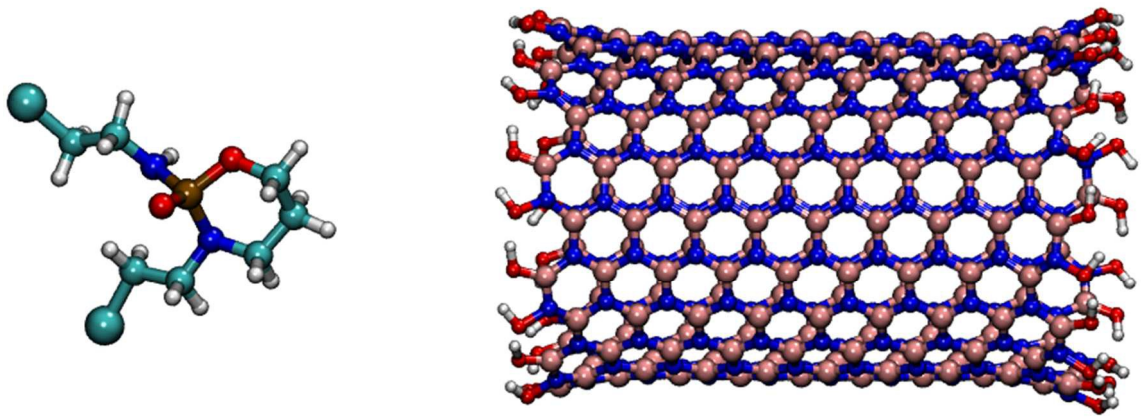


Fig. 1: Geometry of (a) Ifosfamide ($C_7H_{15}Cl_2N_2O_2P$), (b) single-walled (10,10) BNNTs with hydroxyl groups (–OH), (B, N, C, O, H, P, and Cl atoms are represented as pink, blue, small cyan, red, white, ochre, large cyan spheres, respectively).

To examine the interaction between the drug molecule and its carrier, we first placed and optimized the Ifos position on the outer surface of the BNNT. The adsorption energy obtained in this configuration was equal to -0.17 eV. While low, it confirms that the Ifos molecule could be stabilized in this position. However, this energy provided by physical interactions (no important charges transfer between the molecule and the BNNT) could not be enough to leave the molecule on the BNNT during its vectorization to the cancer cell.

The investigation of Ifos interaction with the inner surface of BNNT is realized considering the molecule centered along the principal axis of the BNNT. In all simulations, the molecule positions were optimized. The mean distance between the P atom of the molecule and the BNNT center of mass, denoted as d, was calculated for each calculation. Then, the position of the molecule was progressively changed along the tube axis in order to determine the interaction molecule-BNNT with d varying from -21 Å to 21 Å. For each distance, the whole structure was relaxed by allowing atom displacement to minimize the total energy. The adsorption energy of the interacting system is obtained by subtracting both the total energy of the isolated tube and the energy of the molecule in gas phase from the total energy of the combined structure after optimization. Fig. 2 shows the adsorption energy of the Ifos molecule as a function of the distance d. As observed in Fig. 2, the interaction presents an asymmetric adsorption energy pathway. We observe a first small energy well around d = -15 Å, when the molecule comes close to the nanotube entrance (position (0), Fig. 2). Then a small energy barrier appears (around 40 meV of height) which corresponds to the beginning of the molecule encapsulation. It is due to the oxygen and nitrogen atoms hampering the process of encapsulation. Then the molecule experiences a progressive insertion with important negative adsorption energies on the entire pathway (position (1) and thereafter, Fig.2). Between d = -12 Å and d = 6 Å, the adsorption energy curve presents a valley with a well around -0.6 eV for d= 0.0 Å (position (2), Fig. 2). It is the most favorable site (close to the geometric center of the nanotube). Note, that the energy slope is less rough after d= 6 Å and until the BNNT edge (i.e. d=12 Å) with an equivalent energetic barrier to the entrance one. We attribute the valley form of adsorption energy at the dissymmetric conformation of the molecule. This can be attributed also to the molecular

geometry, which is slightly tilted compared to the nanotube axis at the beginning and the end of the nanocarrier.

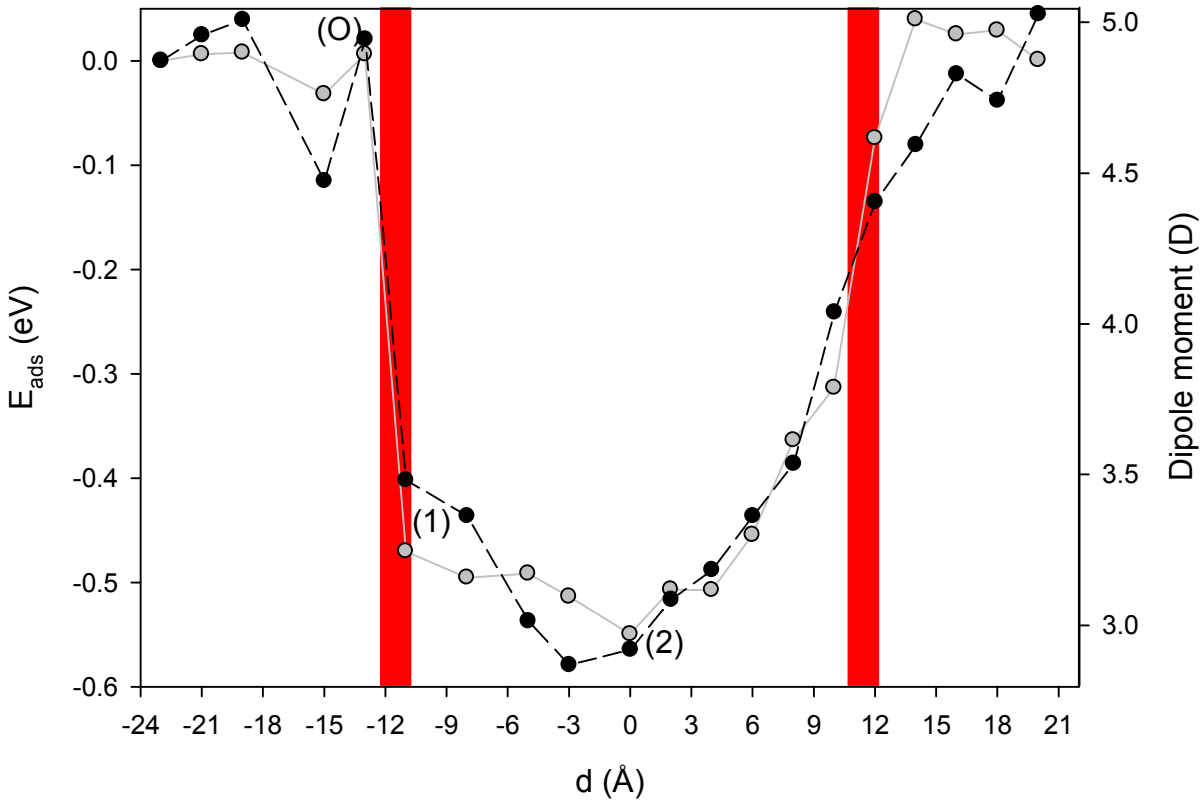


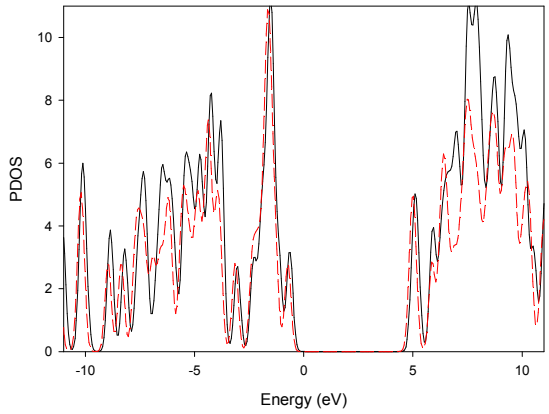
Fig. 2: Interaction energy of Ifos (grey curve) as a function of d, defined as the distance between the absolute position of P atom in the Ifos molecule and the center of mass of BNNT along the z axis. Red bars highlight the BNNT edges. Evolution of the total dipole moment (D) as a function of d (black dashed curve).

In order to have an insight of the interaction between Ifos molecule and the BNNT nanotube, we have sketched the Ifos PDOS molecule for the three most interesting positions (i.e. (O), (1), and (2) labeled on Fig. 2). To facilitate the observation of the evolution, PDOS of Ifos molecule

in gas phase was sketched in red on the different graphs (Fig. 3). In each table associated with the Fig. 3, we have reported the Bader charge evolution onto the different atoms of Ifos molecule and the total electric dipole of the system in Debye, depending on the positions of the molecule (Fig. 3).

Bader Total Charge on isolated Ifos: 84.00 e
Total Dipole on Isolated system: 2.51D -0.91D 4.07D

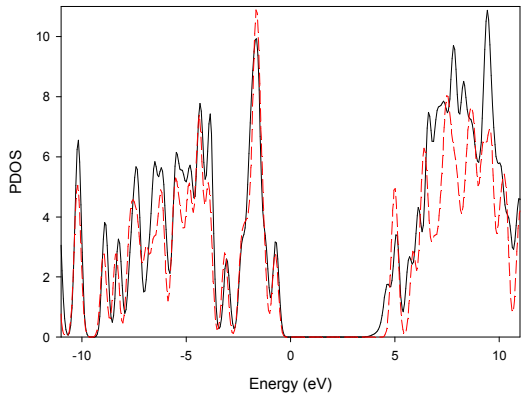
(O)



Bader charge variation (e) of atom					
P	N	C	O	H	Cl
0.01	**-0.30**	**0.04**	**0.05**	**0.03**	**0.00**
Bader Total Charge on Ifos (e) **: 84.05**					

Dipole (D)		
X	Y	Z
2.13	**-1.82**	**4.10**

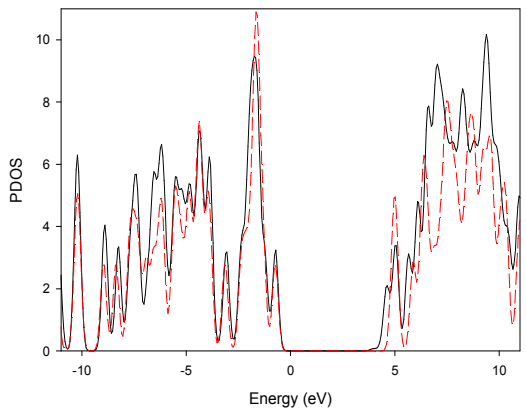
(1)



Bader charge variation (e) of atom					
P	N	C	O	H	Cl
-0.02	**-0.16**	**-0.32**	**0.06**	**0.16**	**-0.01**
Bader Total Charge on Ifos (e) **: 83.98**					

Dipole (D)		
X	Y	Z
1.97	**-1.23**	**2.59**

(2)



Bader charge variation (e) of atom					
P	N	C	O	H	Cl
-0.24	**-0.26**	**0.02**	**0.13**	**0.03**	**0.07**
Bader Total Charge on Ifos (e) **: 84.03**					

Dipole (D)		
X	Y	Z
2.05	**-0.97**	**1.83**

Fig. 3: Projected electronic density of states of the Ifos molecule in the three positions depicted in Fig. 2: (O) barrier, (1) well after the entrance of BNNT and (2) most stable site in the BNNT together with those of the molecule in gas phase (red curves). Fermi level was placed at 0 eV. The tables show the mean variations of the Bader charges of the Ifos atoms and the total electric dipole of the system.

The Bader charge evolutions during the Ifos transfer inside the BNNT show some variations for the O, N, C and P atoms with an intra molecular inversion of the variations in function of the position. On the contrary, we observe a weak charge variation on the Cl atoms. The intra charge variation induces a strong variation of the electric dipole of the system between the gas phase and the adsorption positions. The evolution of the dipole moment during the passage of Ifos inside BNNT is characterized by the same behavior than the energy path. It is almost constant (maximum of 0.5D of variation) at the BNNT approach and falls (about 40% of its value) when the molecule comes inside the potential well (Fig. 2, positions 1 and 2). Considering that the encapsulated drug molecules should be ultimately incorporated in human body, the role of the

solvent should be taken into account. Indeed, our DFT calculations did not take into account the dispersion repulsion terms and the presence of solvent. However, we have proven recently that the role of solvent in BNNT was to stabilize a carboplatin (another anticancer molecule) inside the nanocapsules without any event of molecule escape until the end of the simulations and concomitant energy value between DFT and all atom molecular dynamic simulation results.[23]

To visualize more precisely the physical interactions, we show in Fig. 4 the electron localization function (ELF) representation of the system at positions (O) to (2) of Fig. 2. This function produces informative patterns and describes chemical bonding in molecules and solids. It measures the probability of finding an electron near another electron with the same spin related to the Pauli Exclusion Principle.[24] The upper limit of the ELF representation corresponds to chemical bonding while the values lower than 0.5 correspond to electron-gas-like pair probability (i.e. no chemical bonding). As demonstrated in Fig. 4, no chemical interaction was involved in the strong confinement of the Ifos inside the BNNT.

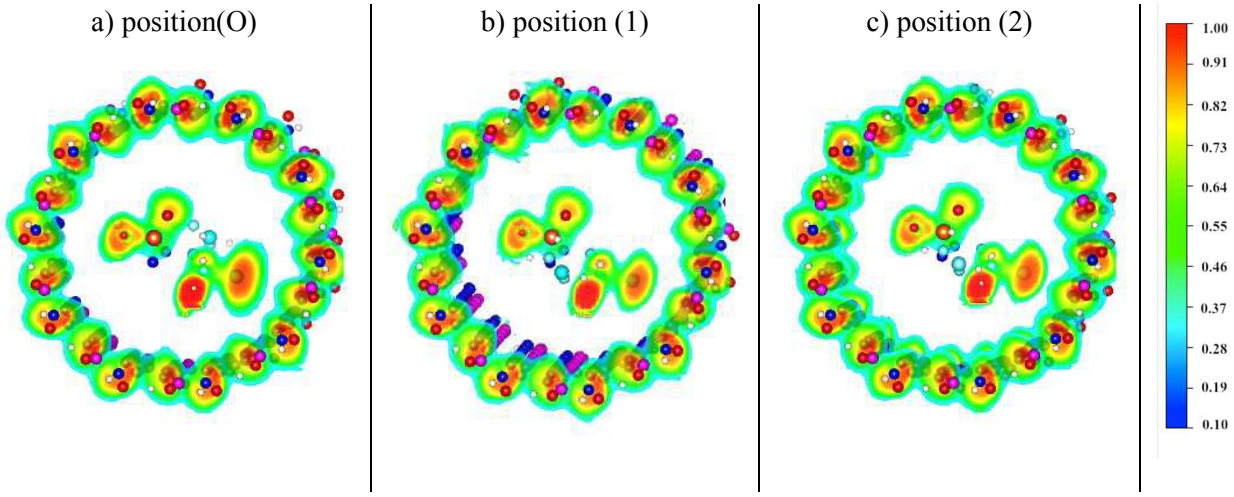


Fig. 4: ELF representation in position (O), (1) and (2) of Fig. 2. The values of each color are depicted in the diagram shown at right. The projection plane sketched is perpendicular to the nanotube principal axis and centered onto the P atom. Some atoms are not shown for clarity.

All these data do not show any chemical adsorption or bonding occurring between the molecule and the tube. Moreover, the total Bader charge variation of the Ifos molecule does not excess 0.05 e, which cannot be attributed to any significant chemical transfer between the molecule and the nanotube.

Note that the role of the chemical saturation of the dangling bonds at the both extremities of the nanotube could influence the energy valley of Ifos. Indeed, it is well known that to increase their dispersion in solvent and developing biomedical applications, two approaches are commonly used. The first one uses covalent attachment of a molecule and the other involves the physical adsorption of a molecular group onto the BNNT surfaces. In the first approach, the most common chemical modification was achieved through the –OH groups at the edges (or defects) of the BNNTs. Nevertheless, H groups can be held on the dangling bonds of nanotubes. To study the influence of this saturation, the energy valley was plotted in Fig. 5 when –H functions saturated the dangling bonds. Small differences are observed. First, the energy barrier at the entrance is translated due to the O atoms lack. Then, the inner position is characterized by a small shift in energy (around 50 meV) that leads to a less stable adsorption site in BNNT saturated with H groups.

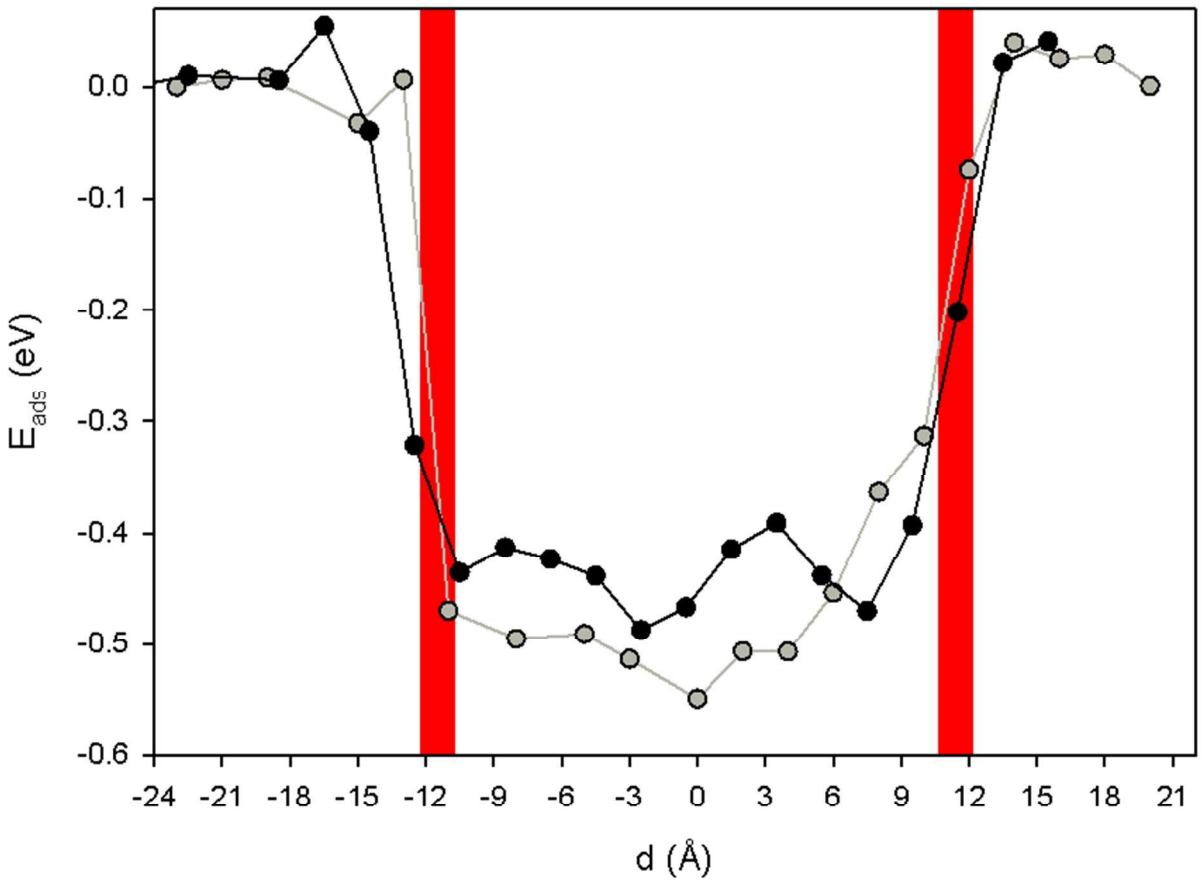


Fig. 5: Interaction energy of Ifos as a function of d for dangling bonds saturated with –H groups (black curve) or with –OH groups (grey curve). Red bars highlight the BNNT edges.

3.4. Discussion

In this study, we show that BNNT could contain inside their internal cavity Ifos molecules. More, we demonstrate with adsorption energy pathway estimated using quantum calculations that BNNT could catch anticancer Ifos molecules spontaneously. The small energy barrier observed at the entrance of the molecule could be crossed easily with thermal agitation at normal temperature. The analysis of the small electronic density of states and charges variations on the

atom constituting the anticancer agents leads to the conclusion that only physical interactions were responsible for the molecule and the nanopore surface interaction. This could be explained by the low reactivity of the BNNT support. Note that other nanocarriers (such as carbon nanotubes) can lead to easy chemical cycloaddition on their surface. The chemical bonds obtained between the molecule and its carriers increase the geometric stability of the anticancer agent but limit its release near its target unless a specific external excitation arises (infra-red pulse for example to break these bonds). In our case, the spontaneous encapsulation of the Ifos could prevent the molecule to be released anywhere since its thermodynamic state corresponds to the confined position. Indeed, as shown in Fig. 2 the entrance of the BNNT is quite easy to achieve (not very high barrier at the entrance, even in the presence of saturated –OH bonds) and the molecule falls irremediably into a large potential well (around -0.6 eV). To liberate the toxic molecules a third media should be reached (different from BNNT and vacuum) as for example a cancer cell if the BNNT outer surface would be functionalized by a specific target function. The release of the anticancer drug molecule near the cell should be due to thermal activation in order to let interacting the Ifos with the cancer cell. In our case an energy barrier of 0.6 eV should be passed to release the anticancer agent. We can note that the energy barrier obtained when the dangling bonds are saturated with –H groups is equivalent. Indeed, the small barrier observed in this case at the entry of the BNNT is compensated by a lower energy in the inner part of the BNNT which leads to the same difference in energy. This energy barrier could be compared to the work performed by Panczyck et al.[25] who used huge SWCNT as nanocarrier. In this study, the release of platinum anticancer agents was extremely rapid (below few minutes) near a cell. The estimation of the energy barrier for this release was about 25 kcal/mol (1.08 eV) to compare with other experiments developed by Li et al.[26] on SWCNT (or Tripisciano et al. on MWCNT[27]).

These latter did not reach such velocity to release drug molecules since 95% of the total encapsulated active molecules near the cancer cell were liberated in 6 hours. The stability of the drug molecule inside the carbon nanocarrier was thus stronger than in our case but the cancer cell presence was sufficient to release the confined molecules. Note that the large discrepancy observed between theoretical and experimental observations could be attributed to structural defects in the carbon structure. Our calculations estimate that the molecules should pass of an energy barrier of only 0.6 eV to exit the BNNT nanotube which tends to facilitate the release of the drug molecule. The residence time of the drug in the BNNT, assuming that the release is thermally active and follows an Arrhenius law, can be estimated to 18 minutes. While extremely low for human life, this could be sufficient at molecular level to transport the drug near its target and deliver the anticancer agent on site. It should be also underlined that contrarily to carbon nanotubes which are ended in equal proportion by zigzag, armchair or chiral structures, the BNNT ended structures are fully dependent on the synthesis methods. BNNTs grown directly from the vapor phase adopted an armchair configuration while those grown from laser-heating and carbon substituted methods are zigzag type. To study the role of the chirality, we have performed some calculations on positions (0), (1) and (2) for a zigzag BNNT saturated by -OH groups. The energies obtained in these positions were slightly different since we obtained 0.01eV, -0.44eV and -0.56 eV, for these three positions, respectively. These data show that the Ifos – BNNT interaction are independent of the chirality of the nanocargo.

4. Conclusion

In this study we have investigated the interactions between boron nitride nanotube and anticancer drug ifosfamide using full atoms DFT calculations. Our results show that the

incorporation of Ifos molecules into BNNT does not present any difficulties at room temperature. The BNNT nanocarrier represents thus a good candidate for loading this drug and hinders premature deactivation of Ifos before reaching the cancer cells. On the contrary of platinum agents, Ifos molecules present a large stable potential energy valley in its confined state, involving only physical interactions. Indeed, comparison of the density of states of the Ifos molecule in the gas phase with the density of states of Ifos incorporated inside BNNT shows slight discrepancies only. This particular molecular position protects the anticancer agent from any interaction with an external unwanted body and allows the experiments to use the external surface area of the nanovector for further chemical functionalization in order to target specifically the cancer cells.

Acknowledgments

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The table of contents:

The encapsulation of phosphorous anti-cancer drugs is spontaneous inside boron nitride nanotube.