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# Water behavior in the self-aggregation of the bilirubin molecules in presence and absence of carbon nanotubes: A Molecular Dynamics Simulation approach

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## ABSTRACT

This study illustrates the behavior of water molecules in bilirubin (BR)-carbon nanotube (CNT) systems by detailed analysis of several molecular dynamics simulations from the perspective of diffusion coefficients (D) and hydrogen bonding (HB). These analysis provide further understanding on the water network properties in the process of BR adsorption to treat or prevent hepatitis, biliary disease, liver cancer and some neurological and brain disorders. The results show that presence of BR and CNT interrupts the water network by confining the water molecules and decreasing the number of HBs that can be established. The best water and HB networking can be developed at high BR concentrations when CNT (10, 10) is available. Also, the most significant factor on increasing the water D is temperature. At higher temperatures, the BR molecules are adsorbed on CNTs and the enhanced CNT diameter helps BR molecules to advantage from higher adsorption surface to eliminate BR molecules from the water passage paths and diffuse, freely.

Keywords: Bilirubin, Carbon nanotube, Water, Hydrogen bonding, Diffusion coefficient

#### INTRODUCTION

During the twentieth century, knowledge concerning the physicochemistry of liquid water has increased enormously and several physical properties of it have been discovered. As an example, self-diffusion coefficients of liquid water at various temperatures were measured more than 50 years ago [1]. Another well-known feature is that water molecules participate in structures stabilized by a great variety of hydrogen bond networks [2,3].

Carbon nanotubes (CNTs) are one of the most active areas in new technologies. The extraordinary physical, chemical, and mechanical properties [4,5] of carbon nanotubes have made them attractive materials for numerous applications [6,7]. A key aspect of these applications is the interaction of the surrounding fluid such as water with the carbon nanotube.

The study of water confinement in CNTs is still associated with a high degree of theoretical importance to chemistry, biology, and materials sciences[8,9].

Actually, water-filled and water-permeable pores are present in biological cells, membranes, and surface of proteins [10] and in other relevant biological and geological [11] systems that may present a strict analogy with water confinement in carbon nanotubes [12].

Since CNTs have high surface area values per unit weight and a high capacity to adsorb various molecules, they are promising for efficient adsorption [13,14] of specific molecules. A potent molecules that can be adsorbed on CNTs is Bilirubin (BR). BR is a red-brown bile pigment that is a metabolite of heme produced from the senescent hemoglobin [15]. Its concentration can increase in several conditions such as hepatitis, biliary disease, and cirrhosis and liver cancers. The excess amount of BR can penetrate into brain and cause neurological and brain disorders, which may lead to mental or physical retardation or in severe cases, death of the patient [16,17].

This study focuses on the potential of carbon nanotubes (CNTs) as a new adsorbent to remove BR through molecular dynamics (MD) simulations. The first article, to the best of our knowledge, which was dedicated to MD simulation of water in CNTs was written by Gordillo and Mart [18]. This subject was further followed by many other researchers and motivated the present study in which we examine the structural properties of water surrounding BR adsorbed CNTs at various BR concentrations and temperatures by the means of MD simulations.

The choice of MD simulations relies on the fact thatsuch simulations can be as accurate as experiments, are much easier to perform at the nanoscale, have proven to be the most flexible tool for this kind of analysis and can provide many details on the subjects under study. MD simulation is a form of investigation where the motion and the interaction of a certain number of virtual atoms or molecules are studied. Molecular simulation is a very powerful toolbox in modern molecular modeling, and enables researchers to follow and understand structure and dynamics with extreme detail-literally on scales where motion of individual atoms can be tracked.

## MATERIALS AND METHODS

In order to start the simulations, the nanotube structures with (n, m) indices of (5, 5) and (10, 10) were designed by vegaZZ[19]. The end of the nanotubes were saturated with hydrogen and optimized by OPLSaa force field [20,21]. Energy minimized structure of BR and the atomic partial charges of BR were computed by CHELPG (CHarges from Electrostatic Potentials using a Grid based method) method [22].

All MD simulations were carried out using Gromacs 4.5.5 package [23]. A total number of nine simulation boxes with dimensions of  $6 \times 6 \times 6$  nm<sup>3</sup> were defined. In three out of the nine boxes, a CNT with dimensions of (5, 5) was located in the center of the boxes. Then, 10, 20 or 30 BR molecules were placed uniformly and randomly within these boxes. Similarly, CNT with dimensions of (*10, 10*) was put inside the next three empty boxes and 10, 20 or 30 BR molecules were added, respectively. Finally, in the remained CNT-free boxes, respectively, 10, 20 or 30 BR molecules were inserted. All simulation boxes were filled with TIP3P water molecules [24]. In order to neutralize the systems, an appropriate number of ions were added to each box. The components of each simulation box for the given systems are briefly listed in Table 1.

To remove the initial kinetic energy and any undesirable contacts between atoms in the simulation boxes, the systems were energy minimized with 2500 steepest descent steps using the LINCalgorithm [25], while the cut-off values for van der Waals and short-range Coulomb forces were set to 0.9 and 1.4 Å, respectively.He long-range electrostatic forces were computed using the particle mesh Ewald (PME) summation method [26,27]. Then, MD simulations of 1 ns in the canonical (NVT) [28] ensemble followed by 50 ns simulations in the isobaric-isothermal (NPT) [28] ensemble were invoked. The time step of 2 fs was used throughout the simulations. Temperature and pressure were kept constant at 300 K and 1.0 bar by coupling the systems to the external baths with Berendsen thermostat and barostat, respectively [29].

The intermolecular (non-bonded) potentials were represented as sum of Lennard Jones (LJ) forces and pairwise Coulomb interactions. Along the simulations, the carbon-water interactionswere consisted of a Lennard-Jones (LJ) term between the carbon and oxygen atoms where the LJ parameters of the potential,  $\varepsilon_{CO}$  and  $\sigma_{CO}$  are obtained from Bojan and Steele [30] and a quadruple potential between the carbon atoms and the partial charges on the water hydrogen and oxygen atoms according to Equation (1). In this equation,  $\alpha$  and $\beta$  run over all Cartesian coordinates (x, y, z), r is the distance between an O or H atomic charge sites and the quadrupole carbon site and  $\varepsilon$  is the coefficient of vacuum permeability.  $\delta_{\alpha,\beta}$  and  $\theta_{\alpha,\beta}$  are the delta function and quadruple moment tensor, respectively [31].

$$U(r) = \frac{1}{3} \frac{q}{4\pi\varepsilon} \sum_{\alpha,\beta} \theta_{\alpha,\beta} \frac{3r_{\alpha}r_{\beta} - r^2\delta_{\alpha,\beta}}{r^5}$$
(1)

Simulation system	Simulation box (nm <sup>3</sup> )	Number of BR	Simulation duration (ns)	Temperature (°C)	CNT	Number of
						Water
System A	6×6×6	10	50	25 and 125	-	3800
System B	6×6×6	20	50	25 and 125	-	3466
System C	6×6×6	30	50	25 and 125	-	3141
System D	6×6×6	10	50	25 and 125	CNT (5, 5)	3746
System E	6×6×6	20	50	25 and 125	CNT (5, 5)	3406
System F	6×6×6	30	50	25 and 125	CNT (5, 5)	3074
System G	6×6×6	10	50	25 and 125	CNT (10,	3746
					10)	
System H	6×6×6	20	50	25 and 125	CNT (10,	3406
					10)	
System I	6×6×6	30	50	25 and 125	CNT (10,	3074
					10)	

#### Table 1.An overview of the studied systems

## **RESULTS AND DISCUSSION**

This study first considers the stability and equilibrium status of the system and then analyzes the findings from two perspectives; hydrogen bonding (HB) and water diffusion coefficients (DC). The calculated water DC and HB are reported to concern the effects of temperature, BR concentration, presence or absence of CNT and CNT diameter.



Figure 1. RMSD evolution of water molecules with time in systems A to I, at 25 and 125  $^\circ\mathrm{C}$ 



Figure 2. Graphical representation of each simulated system, at 25 °C

### **Equilibrium and Conformational Stability**

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Conformational stability and equilibrium state of the system solvent, i.e. water molecules, were investigated by analyzing the time evolution of water root mean square deviation (RMSD) with respect to the initial fluid configuration. The RMSDplots of the simulated systems are shown in Figure 1. According to the plots as the simulation initiates, a sudden change is observed in the fluid structure. However, the water molecules adopt to the simulation conditions at the early stages and reach equilibrium with respect to the system components, so rapidly. The solvent equilibrium status is maintained to the end of the simulation period. So that the fluctuations in RMSD values are negligible for all systems and the RMSD lines sustain parallelism with the time axis. The snapshots of simulated systems after 50 ns of MD simulations are shown in Figures 2 and 3.



Figure 3.Graphical representation of each simulated system, at 125 °C.

#### Water Diffusion Coefficients

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The transport properties of water in the systems were concerned by considering diffusion coefficient of water molecules in the simulated systems. The diffusion coefficients were calculated from molecular dynamic simulations results by the means of the Einstein [32,33] equation (Equation 2).

$$D = \lim_{t \to \infty} \frac{1}{2k} \langle |r(t) - r(o)|^2 \rangle$$
<sup>(2)</sup>

In this equation, r(t) is the position of the center of mass of water molecules at time t and k is the Einstein's diffusion constant and therefore the DC is evaluated at mean square displacement (MSD) of water molecules' center of mass. The evaluated MSD values (see Figure 4) ascend steeply with time which demonstrates the high mobility and free traverse of water molecules inside the systems. The dramatic increase of values neglects does not permit reporting

an average MSD value. However, the MSD values are fitted in the linear equation (3) and the obtained MSD-time evolution trend is summarized as following: (3)

 $D (\text{nm.s}^{-2}.\text{N}^{-1}) = a (\text{nm.s}^{-1}.\text{N}^{-1})t(\text{ps}) + b (\text{nm.s}^{-2}.\text{N}^{-1})$ 25 °C:

D = 0.0320t + 4.1188; R<sup>2</sup>= 1.0000 A)

- D = 0.0310t 0.0967; R<sup>2</sup>= 1.0000 B)
- D = 0.0280t + 1.5624; R<sup>2</sup>= 1.0000 C)
- $D = 0.0246t 2.8701; R^2 = 1.0000$ D)
- $D = 0.0231t 3.2027; R^2 = 1.0000$ E)
- $D = 0.0244t 0.6345; R^2 = 0.9999$ F)
- $D = 0.0247t 0.7174; R^2 = 1.0000$ G)
- $D = 0.0230t + 5.8750; R^2 = 0.9999$ H)
- $D = 0.0226t + 2.4001; R^2 = 0.9999$ I)

125 °C:

- $D = 0.0667t 4.5224; R^2 = 1.0000$ A)
- D = 0.0605t + 11.373;  $R^2 = 1.0000$ B)
- D = 0.0596t + 0.3958;  $R^2 = 1.0000$ C)
- D = 0.0555t 4.8104;  $R^2 = 0.9999$ D)
- D = 0.0533t 1.102;  $R^2 = 1.0000$ E)
- $D = 0.0572t + 2.7362; R^2 = 0.9999$ F)
- D = 0.0567t 6.3608;  $R^2 = 1.0000$ G)
- $D = 0.0548t 5.205; R^2 = 1.0000$ H)
- D = 0.0544t 1.118;  $R^2 = 1.0000$ D



Figure 4. MSD evolution of water molecules with time in systems A to I, at 25 and 125  $^\circ\mathrm{C}$ 

Figure 4 and the slopes a of Equation 3 for systems A to I imply that as BR concentration increases, the rate of increase in water diffusion coefficients decrease. It means that the aggregates formed by increased number of BR molecules make a barrier in front of water free motion. For CNT (5, 5), the BR aggregates are more concentrated at the CNT instead of being dispersed at several positions. Thus, more free space is provided for water diffusion.

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The findings unravel that the most significant factor on increasing the water diffusion coefficient is temperature. Water molecules have 3 translational, 3 vibrational and 3 rotational, 9 total, degrees of freedom (DFs) which is far less than BR or CNT molecules. When the temperature increases, the added kinetics energy distributes in only 9 DFs for water molecules while the energy has to distribute over many DFs for CNT and BR. As a consequence, each water DF gains higher share of energy. It helps the water molecule to diffuse much faster than that of lower temperature, across the fluid.

Finally, the presence of CNT constraints water diffusion at any BR concentration since it occupies a great deal of space and alter the potential profile of the systems. At lower temperatures, D reduces upon increasing the CNT diameter. However at higher temperatures, the BR molecules become well organized on the CNTs and the enhanced CNT diameter helps BR molecules to advantage from higher adsorption surface to eliminate BR molecules from the water passage paths and increase the D values.

#### Hydrogen Bonding

Quantitative data on hydrogen bonding, which is a special type of attractive interaction that exists between an electronegative atom and a hydrogen atom bonded to another electronegative atom, was extracted from the simulation configurations. In general, each water molecule can form up to four hydrogen bonds, simultaneously.

In this paper, HB has been focused since HB networks of water molecules can aid interpretation and understanding of experimental findings. At the first step, the number of molecules with 0, 1, 2 and 3 HBs and total number of HBs present in the system were computed from 0 to 50 ns of simulation to determine the progress of HB at different time intervals and system configurations. The results for four randomly selected systems are displayed in Figure 5. In agreement with Figure 1 which illustrated equilibrium status for the solvent structure, Figure 5 shows that the number of molecules with 0, 1, 2 and 3 HBs and also the total number of HBs in the systems are constant. While negligible fluctuations are noticed in the number of HBs, the values can be averaged. The average number of HBs are reported in the bar-type plot of Figure 6.



Figure 5.Number evolution of molecules possessing HB in some simulated systems, with time



Figure 6. Average number of molecules possessing a number of HBs over 50 ns of simulations in systems A to I, at 25 and 125 °C

Though the average number of HB per molecule calculated in bulk water varies approximately from 2.3 to 3.8 according to the water model and the way used to define the HB [34,35], 3 HB values are so small that the presence of water molecules with 3 HBs can be ignored. This value is almost the same for all simulated systems and ranges from 5 to 8, approximately. This result is supported by former MD simulations of water inside the SWCNT [36] and other studies which have verified that the average HB of water molecules decreases in confined space and this value reaches approximately 1 to 1.5 bonds for small CNTs, where only a monomolecular layer of water is allowed. Therefore, presence of BR and CNT has irrupted the water network in a way that the confined water molecules advance no HB (see the orange bar in Figure 6) or only 1 or 2 HBs are observed for most molecules. Presence of 4 HBs for each water molecules has been avoided completely in every system of BR-CNT and BR.

When there is no CNT in the system, A to C, the number of molecules with higher orders of HB decreases. The reason can be that water molecules get trapped between BR molecules and the number of neighboring water molecules decrease. However at CNT presence, this trend break for 30 BR systems of F and I. Taking a look at Figures 2 and 3, declares that upon CNT presence and high concentrations of BR, BR adsorption on CNT is promoted. Consequently, water molecules are released to move freely and establish a stronger HB network.

Temperature elevation has increased the number of molecules with 0 to 3 HBs between water molecules and total number of HBs between system components, slightly. The main reason is associated with the increase kinetics energy of particles in the systems that provides the chance of water escape from BR aggregates and directing towards water networking.

At low and medium BR concentrations, increasing CNT diameter decreases BR adsorption on CNT and thus the potential of water molecules in developing more HBs. Meantime at high BR concentrations, BR adsorption on and orientation towards the CNT enhances for the (10, 10) CNT. As an outcome, water molecules succeed to spread the HB network, more efficiently. Increased BR adsorption on CNT (10, 10) arises from the electronic effects. It is also noteworthy that when BR concentrations increases to an optimum amount (here 30 BR molecules) aggregation of BR molecules increases but the presence of CNT motivates  $\pi$ - $\pi$  stacking, gradual withdrawal of BR aggregates towards the CNT and finally BR adsorption. This effect is more evident in CNT (10, 10) cases since the  $\pi$ - $\pi$  interaction between BR-CNT dominates that of the CNT wall rings.

## CONCLUSION

The present study is an attempt to declare water network properties in CNT-BR systems and during the procedure of BR aggregation in CNT free systems. This issue is investigated by considering water diffusion coefficients and HBs as important features of water networks through 50 ns MD simulations. The results show that although water molecules adopt to the simulation conditions and reach equilibrium with respect to the system components so rapidly, presence of BR and CNT interrupts the water network. So that, the confined water molecules advance no HB or only 1 or 2 HBs. The best water and HB networking can be developed at high BR concentrations when CNT

(10, 10) is available. Also, detailed analysis of diffusion coefficients showed that the aggregates formed by increased number of BR molecules make a barrier to water free motion and the most significant factor on increasing the water diffusion coefficient is temperature. At higher temperatures, the BR molecules are adsorbed on CNTs and the enhanced CNT diameter helps BR molecules to advantage from higher adsorption surface to eliminate BR molecules from the water passage paths and diffuse, freely.

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