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# The study of self-aggregation behavior of the bilirubin molecules in the presence and absence of carbon nanotubes: Molecular dynamics simulation approach

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ABSTRACT

In the present work, the bilirubin self-aggregation in the presence and absence of carbon nanotubes has been studied by molecular dynamics computer simulations. We focused on two distinct (5, 5) and (10, 10) single-walled carbon nanotubes. The effect of bilirubin's concentration and nanotubes' length and diameter on the aggregation and disaggregation of the bilirubin molecules was investigated. The radial distribution functions between bilirubin molecules and also bilirubin and carbon nanotube were calculated. The obtained results indicated that bilirubin molecules have a maximum interaction with each other at a distance of 0.4 nm and the presence of carbon nanotube decreased their interactions' intensity. Moreover, it was observed that these molecules interacted with carbon nanotube at a distance of 0.4 nm.



## 1. Introduction

Bilirubin (BR) is a yellow-brownish endobiotic product of breaking down of the heme's iron ring process in the α-carbon bridge position in the hemoglobin of red blood corpuscles [1]. Cleavage of heme's porphyrin ring occurs by hemoxygenase and releases carbon monoxide and a mixture of primarily the IXa isomer of BR, which is the substrate for cytosolic enzymes in producing BR IXa [2]. Hemoglobin can be broken down in other positions than aloha positions by different enzymes. In this case, the other BR isomers (IXB, IX) are formed. These two isomers are found in the mice bile [3]. It is noteworthy that isomer IXZ is not formed in mammalian circulation [4]. This yellowish pigment has conjugated and unconjugated conformations [1]. In fact, IXa isomer involving the 4Z–5Z structures is the unconjugated BR isomer. Because of the aromatic domains' presence and imposed conformational restrictions for intramolecular hydrogen bonding, the molecule is sparingly soluble in water while extremely bound to albumin in plasma. At low and physiological pH, the lion's share of BR species (80%) is uncharged, 17% approximately monoanion and 16% dianion. The determined $pK_a$ values are 8.12 and 8.44, respectively, for the mono- and dianion species [5]. Moreover, BR can play a role as a potent antioxidant and has an absorption spectral peak at 450 nm. This substance is light-sensitive. Therefore, the serum containers must be wrapped with aluminum foil and placed in the dark to avoid errors in the measurement of the serum's BR [1,4–6].

BR can turn into a dangerous molecule upon aggregation. The reason is that BR is non-polar and soluble in fat. Therefore, it can bind to proteins especially liver related proteins that can end up to liver malfunction and interrupting physiological processes. As a consequence, the BR concentration should be controlled in the metabolic pathways of the body [7,8]. In this respect, non-conjugated BR concentration in the blood should be around 342 μmol per liter, which is normally produced in healthy adults at about 250–300 mg/day concentration [9]. BR concentration in the blood can be raised due to different reasons such as cirrhosis and cancer of the liver, hepatitis, and biliary disease. Unfortunately, the excess amount of BR can penetrate into the brain and cause neurological or brain disorders, which may lead to mental or physical retardation or even worse, the death of the patient [10,11].

So it is necessary to dispose of excess BR from the blood. There are various ways to eliminate BR from the blood. For example, some drugs such as folic acid and Phenobarbital help in binding of BR to albumin in order to pave the way to solving it in water much easier. Another interesting solution is employing carbon nanotubes (CNTs) which are one of the masterpieces of nanotechnology. The properties of carbon nanotubes have caused researchers and companies to consider using them in several fields [10]. CNTs are made from carbon and hydrogen elements; moreover, their diameter is of about 1 nm which displays dimensional and chemical compatibility with biomolecules, such as DNA and proteins [11]. Because of these features, the carbon nanotubes have gained an enviable position in biomedical and pharmaceutical industry. CNTs have also shown considerable potentials, especially in the tissue engineering areas, drug delivery and thermal ablation [12–14]. They can play different roles as scaffolding materials supporting the bone cell and neuron growth [15–18], neurons and cardiomyocytes [19]. CNTs

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can absorb and convert electromagnetic radiation, near infrared (NIR) in particular; therefore, they can be used in photothermal therapy (PTT) or photoacoustic therapy against cancer cells [20–23]. Besides, CNTs have also been widely used as carriers for drug delivery to make this process better and simultaneously reduce drugs' undesirable systemic side effects. Indeed, CNTs have many fascinating features that make them striking drug delivery carriers. Therefore, due to the unique structure of CNTs, the excess amount of BR can be absorbed by carbon nanotubes and excreted from the blood.

Thus, not surprisingly, BR is a hot topic which has been the subject of many different studies and investigated by a wide range of experimental techniques such as, infrared spectroscopy (IR) [24], electron paramagnetic resonance (EPR), Fourier transform infrared (FT-IR) [25], ultraviolet–visible spectroscopy (UV/visible) [26], nuclear magnetic resonance (NMR) [27], circular dichroism spectroscopy (CD) [28], and CD in combination with the centrifugal liquid membrane (CLM) method [29], Raman spectroscopy [30] and spectrophotometric measurements [29]. In spite of the fact that these methods, in many respects, are helpful means with broad application for gathering scientific information, unfortunately, they have some limitations [31]. With the development in computer technology, molecular dynamics simulation methods have flourished and become a powerful tool for providing information at the atomic level. In addition to experimental studies, fortunately, BR has been the subject of many MD simulation studies. For example, Fedorova et al. performed quantum chemical calculation of BR's structural characteristics and its anion and also studied the interaction of BR and anionic forms in an aqueous solution by MD simulation. They revealed that the intramolecular hydrogen bonds in the BR molecules and ions are nonequivalent. Besides, they found that BR oxygen atoms are involved in hydrogen bonds between the BR molecule and water whereas, the BR nitrogen and hydrogen atoms are not contributed in the hydrogen bonds' formation [32]. Moosavi-Movahedi et al. investigated the interaction between HSA and BR by using molecular dynamics simulations and QM and QM/MM minimization methods. They observed a favorable electrostatic interaction between the lysine 195 of HSA and BR and also, a hydrogen bond between the oxygen atoms of BR's acetate groups and α-NH group of lysine 195 [33]. The investigation of BR's association behavior in the presence of CNT with the MD simulation method is desirable because it provides insight into the dynamics and structural properties of these substances which are onerous or even impossible to obtain by experimental means.

In spite of the numerous studies for over 60 years on the BR science, there are still many questions regarding its solubility and aggregation behavior, which are significantly important for its hepatobiliary excretion problem.

In view of the above, the aggregation behavior of BR has been studied in aqueous solutions of three distinct concentrations of 10, 20, 30 molecules and in the presence and absence of carbon nanotubes with different diameters of 5, 5 and 10, 10 nm by molecular dynamics simulations.

## 2. Simulation details

The nanotubes structures were designed in two dimensions of 5.5 and 10.10 nm by vegaZZ [34]. The ends of the nanotubes were saturated with hydrogen and optimized by OPLSaa force field [35,36]. Density functional theory method was used to optimize the BR's structure, and the atomic partial charges of BR were computed by the CHELPG (CHarges from Electrostatic Potentials using a Grid based method) method [37]. All MD simulations were carried out using Gromacs 4.5.5 package [38]. Nine simulation boxes with dimensions of $6 * 6 * 6\ nm^3$ of were defined. In three out of six boxes, CNT with dimensions of 5, 5 was located in the center of the boxes. Then, 10, 20 and 30 BR molecules were placed uniformly randomly within these boxes. Similarly, CNT with dimensions of 10, 10 was put inside in the three next empty boxes and 10, 20, 30 BR molecules were added, respectively. Finally, in the three remained boxes, respectively, 10, 20 and 30 BR molecules were inserted. All simulation boxes were filled with TIP3P water [39]. In order to neutralize the systems, an appropriate number of ions were added to each box. The components of simulation boxes for the given systems are briefly listed in Table 1.

To remove any undesirable contacts between atoms, the systems were energy minimized with 2500 steepest descent steps with LINC algorithm [40], while the cut-off values for van der Waals and short-range Coulomb forces were set to 1.4 and 1.9 Å, respectively. Then, the molecular dynamics simulations were performed on the systems for 1 ns in the canonical (NVT) ensemble [41]. During the course of simulations, the intermolecular (non-bonded) potentials were represented as the sum of Lennard Jones (LJ) forces and pairwise Coulomb interactions. The long-range electrostatic forces were computed using the particle mesh Ewald (PME) summation method [42,43]. The final simulations were run for 50 ns in the isobaric isothermal (NPT) ensemble [44] while a 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 [45]. Visual molecular dynamics (VMD) software was used for molecular visualization [46]. The average obtained structures from the simulations are given in Fig. 1.

## 3. Results and discussion

Here, the radial distribution function (RDF) which is a valuable tool to describe the structure of a system, especially of liquids, for BR and CNT and also the related figures of simulation boxes was used to show the aggregation behavior and position of BRs toward CNT more clearly.

### 3.1. Influence of concentration

Concentration is a key parameter in controlling the aggregation of self-assembling BR [47]. By investigating the concentration effects, aggregation mechanism of BR leading to BR crystal formation in brain

**Table 1**
An overview of studied systems.

| | Dimensions of box | Number of bilirubin | Span time of simulation(s) | CNT |
|---|---|---|---|---|
| System A | 6 * 6 * 6 | 10 | 50 | No CNT |
| System B | 6 * 6 * 6 | 20 | 50 | No CNT |
| System C | 6 * 6 * 6 | 30 | 50 | No CNT |
| System D | 6 * 6 * 6 | 10 | 50 | CNT (5, 5) |
| System E | 6 * 6 * 6 | 20 | 50 | CNT (5, 5) |
| System F | 6 * 6 * 6 | 30 | 50 | CNT (5, 5) |
| System G | 6 * 6 * 6 | 10 | 50 | CNT (10, 10) |
| System H | 6 * 6 * 6 | 20 | 50 | CNT (10, 10) |
| System I | 6 * 6 * 6 | 30 | 50 | CNT (10, 10) |