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RESEARCH PAPER

A Molecular Dynamics Study of the Interaction Between Graphene as a Carrier and Gemcitabine as a Chemotherapy.

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A B S T R A C T:

Drug delivery is a technique or method of administering a pharmaceutical compound to earn a more satisfactory therapeutic impact for human diseases. Based on the good characteristics (e.g. high surface area, high capacity for loading a drug and high biological compatibility and degradability) of graphene nanoparticles, they are widely used in medicine, particularly drug delivery. Hence, in this research, a two-dimensional graphene sheet as a nanocarrier and Gemcitabine (GEM) as chemotherapy have been used to form a (graphene/GEM) system. Moreover, the interactions between the nanocarrier and GEM molecule were explored via Molecular Dynamics (MD) simulation. To investigate the interaction between GEM and graphene, dynamics of GEM molecule, radial distribution function (RDF), root mean square deviation (RMSD), radius of gyration (Rg), and solvent-accessible surface area (SASA) parameters are analyzed. Results indicate that GEM on the graphene has a more stable structure in comparison with the free GEM molecule. Therefore, the graphene/GEM system can be considered a useful therapeutic system for cancer treatment with minimum side effects.

KEY WORDS: Drug delivery system, MD simulation, Chemotherapy drug, Graphene nanoparticle, Gemcitabine.
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1. INTRODUCTION:

Several drawbacks of conventional drugs and their insufficient effectiveness for in vivo management have resulted in the development of nanomedicine and improvement of chemotherapy drugs. These defects hold non-specific targeting, abrupt chemical processes (metabolization) before acting on their targeted areas, low solubility of some medicines in a given solvent and their side effects.

Nonetheless, recent growth in nanotechnology and nanocarriers have provided a good opportunity in the biomedicine zone to improve therapeutic effect of drugs. These improvements include higher bio-distribution of medicines, specific selectivity and fewer side effects on normal cells using drug delivery methods (Hoseini-Ghahfarokhi et al., 2020). In the last twenty years, many nanoparticles of different sizes and designs have been investigated as carriers for the delivery of a useful chemotherapy drug to a targeted region. These nanocarriers hold metal nanocarriers, polymeric micelles, dendrimers, and inorganic nanocarriers (Mainardes and Silva, 2004, Lavan et al., 2003, Parveen et al., 2012).

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Several studies have been conducted on graphene and its products including Graphene Oxide (GO), as carriers of nano-drug delivery. The planar construction with high surface area, high capacity to load drugs, chemical and mechanical stability and good biological compatibility of Graphene Nanomaterials (GNs) rendered them as active carriers for different biomolecules such as DNA, proteins, antibodies, genes and anti-cancer drug molecules (Pan et al., 2012, Liu et al., 2013, Sun et al., 2008, Liu et al., 2011).

In 2008, Dai and collaborators showed that the GO nanocarrier affixed with Poly-Ethylene Glycol (PEG) presented admirable physiological stability and finite toxicity to the specific cells. Outcomes enrolled that the SN38 (7-ethyl-10-hydroxyl camptothecin) drug which is water-insoluble could be effectually loaded on the surface of the GO-PEG compound by a π-π stacking interaction. The GO-PEG-SN38 system with attractive water solubility indicated strongly increased cancer-cell damage ability in comparison with the drug alone. In another prosperous effort by the same team, it showed that the GO-PEG complex could be used for the carrying of another anticancer medicine Doxorubicin (DOX) (Sun et al., 2008).

Subsequently, Zhang et al. studied the delivery of two chemotherapy drugs by utilizing functionalized GO. They worked on two types of chemotherapy medicine, Camptothecin (CPT) and Doxorubicin (DOX), and loaded them onto Folic Acid (FA) affixed by GO-nanoparticle (FA-NGO) by π-π stacking and hydrophobic interactions. The system pointed to MCF-7 human breast cancer cells and was successfully able to target cells and finding out selective toxicity to those positive cancer cells (Zhang et al., 2010). In another investigation, graphene nanoparticle as the basis of chemotherapy drug delivery is used to transfer the chemotherapy cytokine and DOX drugs. This NP enables the subsequent delivery of the molecule of DOX and the cytokine to the nucleus and plasma membrane properly. The benefits of this system include enhanced therapeutic ability, decreased medicine resistance and other negative aspects (Jiang et al., 2015). Wang et al. proposed an exciting method to increase the therapeutic impact of GO nanoparticles by linking them with Gold Nano-Particles (AuNP) (Wang et al., 2011). They found out that the DOX molecule carried by AuNP-GO complex forbids HepG2 cells (cell lines from the liver tissue) development more tightly than DOX alone.

Hasanzade and Raissi, presented that the Paclitaxel (PTX) molecule on graphene surface is adsorbed more promptly than other agents. In addition, the attachment of PTX drug molecule on graphene surface is more powerful than the other studied systems (Hasanzade and Raissi, 2017). This effort showed that hydrophobic and π-π stacking interactions are the major active forces for the adhesion of the medicine on the graphene surface, whereas the adhesion of the PTX anticancer molecule on the GO-Chitosan (CS) surface is controlled by the forming of hydrogen bonds. It found out that the number of hydrogen bonds in the PTX-GO-CS complex is more than that of the PTX-GO system. This is because of the benefits of the chitosan functional group in bettering the adhesion of the therapeutic drug molecule onto the nanomaterial surface. These outcomes indicate that π-π stacking, hydrogen bond, and hydrophobic interactions have an important role in the adhesion of PTX chemotherapy drugs in graphene nanocarriers.

In other research, Quantum Dot (QD) nanoparticles with GO nanoparticles (GO-QD) was arranged in a manner to deliver a drug molecule for cancer treatment. To practice this nanocarrier complex in cancer therapy, the GO-QD nanocarrier conjugated with CS functionalized FA (FA-CS). They have revealed that the FA-CS-GO-QD system is an agreeable biocompatible carrier for targeted DOX chemotherapy drug delivery with a high therapeutic ability (De et al., 2018).

The main purpose of this study is to enhance the properties of the GEM chemotherapy drug by using a graphene nanoparticle sheet, make a model for the graphene-GEM molecule system, and studying the interactions between them. MD simulations are performed for studying the physical movements of atoms of the graphene/GEM system based on classical equations by interatomic potentials or molecular mechanics force fields. We expect that this method could enhance the effectiveness of the GEM drug on cancer cells and reduce the side effects of the drug.

2. MATERIALS AND METHODS

2.1. MATERIALS

In this study, a graphene nanoparticle as a carrier and GEM chemotherapy drug were used to form the graphene/GEM system as a drug delivery system. MD simulations were carried out to analyze their interactions using open-source code Large-scale Atomic/Molecular Massively Parallel Simulator (LAMMPS) software (Plimpton, 1995), which is developed to run a molecular system with only a few particles up to millions or billions (Sharma et al., 2019). MD simulations run via computing clusters at the Tarbiat Modares University, Tehran, Iran. The Virtual Molecular Dynamics (VMD) program was utilized for visualization of the systems and trajectories during the entire study of dynamics simulation of the systems and acquiring snapshots at different time frames.

2.2. METHODS

A Zigzag graphene sheet was built with length (2,2) nm along x and y axis, (0.1418 nm) in bond length and consisting of 240 carbon atoms using VMD package (figure 1a). a GEM as anticancer drug was built using ChemSketch software (figure 1b). Two MD simulations were carried out using LAMMPS package to study the dynamics and properties of free GEM and graphene/GEM systems in water-salt medium. To describe the bonded and non-bonded interactions between graphene, GEM, and water-salt atoms, molecules or ions in the simulated system, the CHARMM32 force field was specified to graphene nanoparticle from CHARMM Force Field Files - MacKerell Lab server (*https://www.charmm.org/archive/charmm/resources/charmm-force-fields/*). The force field for GEM anticancer drug was obtained from SwissParam server, which is available free of charge for academic users at (*https://www.swissparam.ch/*). In addition, the 6-12 Lennard-Jones was used as a potential energy model to compute all non-bond interactions during the simulations. Particle-Particle-Particle-Mesh (PPPM) approach (Ewald, 1921) was applied with the tolerance of 10^{-4} for long-range electrostatic interactions. Water molecules were described with

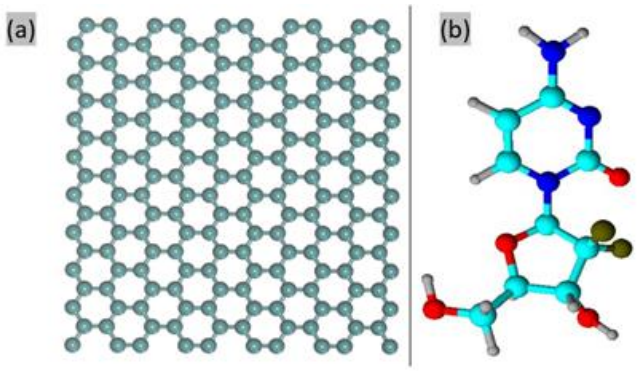

Figure 1: (a) A graphene sheet, and (b) a GEM drug molecule.

the Transferable Intermolecular Potential With 3-Points (TIP3P) potential energy model which is supported by the CHARMM force field (figure 2) (Haume, 2018). The primary simulation box is covered by unlimited copies of itself using periodic boundary conditions in all directions (González, 2011). The velocity Verlet algorithm (Haume, 2018) was utilized to calculate Newtonian equations of the motion in the simulation box. One femtosecond (1 fs) time step is used for both simulated complexes. Furthermore, the cut-off distances for non-bonded interactions were set to 8 and 10 Angstrom (A) to reduce the computational cost. The microcanonical ensemble (NVE) was specified which is a thermodynamic state described by a fixed number of atoms (N), fixed volume (V), and fixed energy (E). The Langevin method was used to hold the temperature of the system to stay at 300 K throughout the simulation.

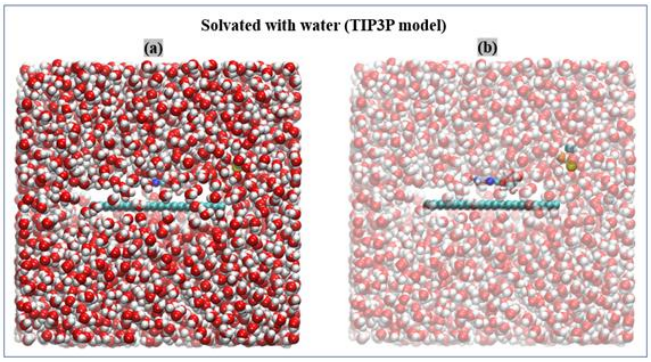

Figure 2: (a) Opaque style, and (b) Transparent style, of a typical water box with graphene/GEM system in the center, using TIP3P water model.

3. RESULTS AND DISCUSSION

3.1. RESULTS

The comparative simulations were carried out to investigate the molecular dynamics of free GEM and graphene/GEM complexes in a water-salt medium. Afterwards, various parameters were investigated by utilizing trajectory files of both

complexes acquired from simulation such as Root Mean Square Deviation (RMSD), Radius of gyration (Rg), Solvent Accessible Surface Area (SASA) and Radial Distribution Function (RDF), to estimate the conformational change, stability and degree of rigidity and compactness of the simulated complexes (Lamichhane and Ghimire, 2021).

3.1.1 RMSD analysis

To evaluate the degree of stability of the simulated systems in comparison with the initial conditions of the systems, RMSD values were calculated using VMD packages. Figure 3 demonstrates the RMSD values versus simulation time for both simulated complexes. It has been shown that RMSD for drug molecule with graphene nanoparticle is smaller in comparison with free GEM in a water-salt medium. Hence, this demonstrates that the graphene/GEM complex is mainly stable and their atoms has experienced a small deviation during the simulation. In addition, the average RMSD value of the graphene/GEM system is 6.5521 Å, while for free GEM system is 33.76966 Å. It is revealed that the average change in the whole structure of the graphene/GEM complex is less than free GEM during the simulation.

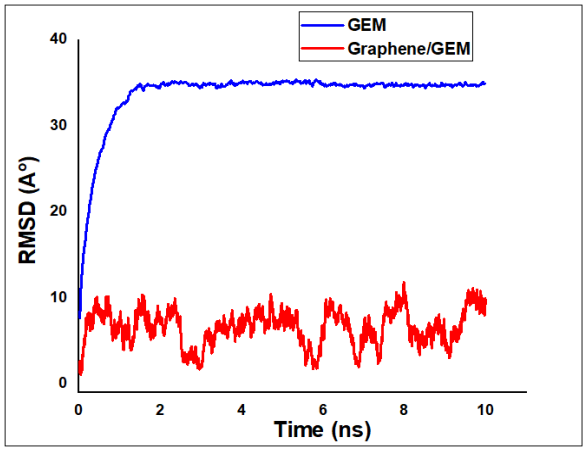

Figure 3: RMSD analysis of free GEM (Blue) and graphene/GEM (Red) complexes at 10 ns.

3.1.2. Rg analysis

To evaluate the degree of compactness of the drug molecule on the graphene sheet, The Rg values were computed for both different simulated complexes for 10ns simulation time. It is known as the root mean square distance of a group of atoms from their shared center of mass (Baig et al., 2014). Although the Rg values have slightly fluctuated for both systems, it is lower for the graphene/GEM system than the Rg values of the free GEM system (figure 4). Moreover, the average Rg value of the graphene/GEM system is 12.6357 Å, while for the free GEM system is 21.6454 Å. This decrease in the Rg values of the graphene/ GEM system is related to the interaction between atoms of the graphene sheet and GEM molecule.

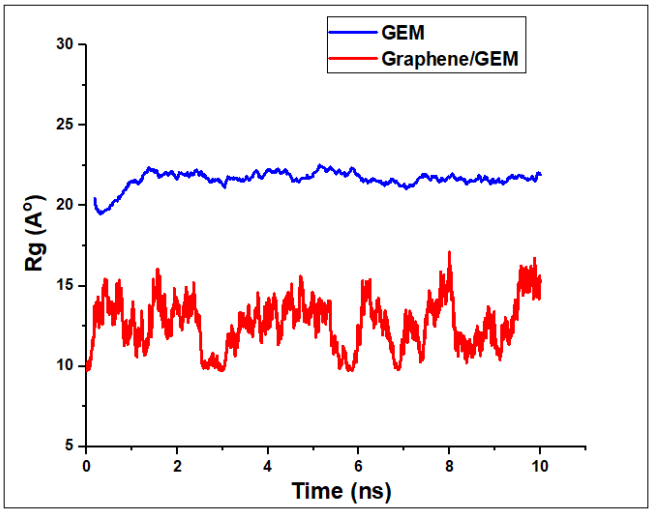

Figure 4: Rg analysis of free GEM (Blue) and graphene/GEM (Red) complexes at 10 ns.

3.1.3. SASA analysis

These above parameters have more affirmed by the SASA parameter which is known as the allowable surface area of a molecule to a solvent. The center of a solvent molecule is located in contact with an atom without permeating any other atoms of the molecule (Shrake and Rupley, 1973). Figure 5 depicts the computed SASA values for both simulated complexes. As noticed the figure, the SASA values for GEM in water-salt medium is converged to 14000 $Å^2$, while these values for graphene/GEM system have slightly fluctuated almost 2000 $Å^2$.

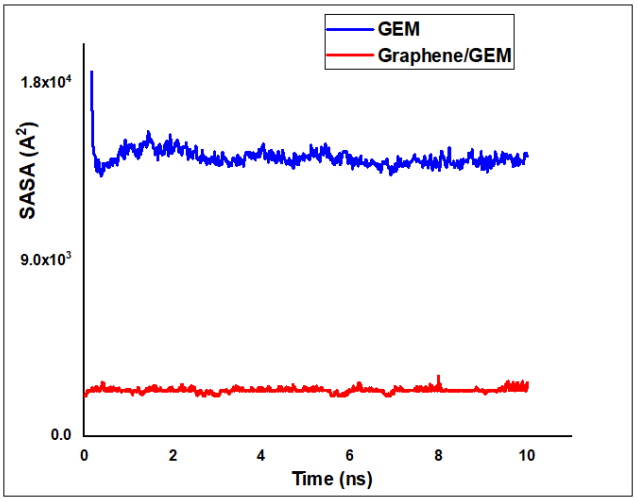

Figure 5: SASA analysis of free GEM (Blue) and graphene/GEM (Red) complexes at 10 ns.

In addition, the smaller SASA values of the graphene/GEM complex compared to the free GEM verify that the degree of compactness for the graphene/GEM system is higher than its degree for free GEM in the aqueous medium. This result is satisfied with the Rg result and shows the GEM placed on the graphene sheet to become more firmly compact.

3.1.4. RDF analysis

RDF is another crucial parameter which has been calculated in this study. It is a good quantity in MD simulation that can be utilized to investigate these attributes (Mansoori, 1993). Figure 6 depicts the computed RDFs for both simulated systems. Although, RDF values for the free GEM system are higher than the values for the graphene/GEM system as a whole. But the crucial discrepancy in the computed results is marked in the intensity of their peaks. The higher intensity of the peaks for the graphene/GEM system in comparison with the free GEM system ensures more interactions between the GEM molecule and graphene sheet. In addition, the difference in the number of peaks informed that hydrogen bonding has formed between GEM atoms and the hydrogen atoms of the graphene sheet. It indicates that more intermolecular hydrogen bonding between drug molecule and nanoparticle is helpful to raise its degree of rigidity. Therefore, the structure of the graphene/GEM system has a more rigid conformation and a higher degree of compactness compared to the free GEM system.

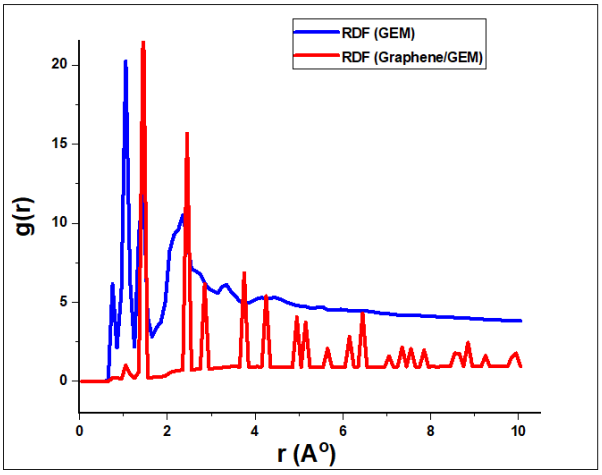

Figure 6: RDF analysis of free GEM (Blue) and graphene/GEM (Red) complexes at 10 ns.

3.2. DISCUSSION

The discoveries of this research are about the growth in the nanomedicine field, particularly drug delivery systems which are too valuable to reduce side effects of chemotherapy medicines. In this study, two comparative MD simulations were carried out on two molecular complexes. Afterwards, their results were analyzed and compared using some MD parameters. The parameters (RMSD, SASA, Rg, RDF) for graphene/GEM system illustrate higher and stronger electrostatic interactions between atoms of the drug molecule and the graphene sheet. In addition, they confirm that the energy level of GEM is directly related to its binding on the graphene surface. Therefore, the degree of compactness and stability for the graphene/GEM system is higher in comparison with the free GEM in an aqueous medium. Fortunately, the outcomes of this study are significantly consistent with the results of other recent studies.

4. CONCLUSIONS

In this study, the MD simulation method is utilized to carry out two simulations on the GEM molecule as an anticancer drug in two different environments (free GEM system and graphene/GEM system in an aqueous medium) at 300 K, based on the preparation methods. Several MD analyses explored using trajectories acquired

from the simulations, such as RMSD, Rg, SASA and RDF analysis and comparing the stability of both systems. Their outcomes are revealed the adsorbed drug molecule on the graphene surface as a nanoparticle via non-bonded interactions has conclusive results. For example, the RMSD values of the graphene/GEM system are lower in comparison with the values for the free GEM system. It is revealed that the average change in the whole structure of the graphene/GEM complex is less than free GEM during the simulation. Furthermore, the analyses revealed that the graphene/GEM complex exhibits a higher degree of molecular structure stability, compactness and rigidity. Therefore, the results indicate strong interactions via inter-molecular hydrogen bonding and π-π stacking between atoms of drug molecules and nanoparticle sheets, compared to the free GEM system during the simulation time.

In conclusion, the comparative simulation outcomes demonstrated the significance of adsorbed drug molecule on the nanoparticle surface compared to free drug molecule in an aqueous environment according to the degree of structural stability. Therefore, the graphene/GEM system can be considered a useful therapeutic system for cancer treatment with minimum side effects. Finally, our suggestion for the future is to perform more accurate MD simulations in the field of nanomedicine and use their results experimentally, to provide a great opportunity for further development of the drug delivery system.

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Conflict of Interest (1)

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