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Study of dual encapsulation possibility of hydrophobic and hydrophilic drugs into a nanocarrier based on bio-polymer coated graphene oxide using density functional theory, molecular dynamics simulation and experimental methods

Sajad Moradi [a,b,c], Mojtaba Taran [b,c], Parviz Mohajeri [d], Komail Sadrjavadi [e], Farzaneh Sarrami [f], Amir Karton [f], Mohsen Shahlaei [g,*]

[a] Nano Drug Delivery Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[b] Department of Nano biotechnology, Razi University, Kermanshah, Iran
[c] Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran
[d] Department of Microbiology, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
[e] Pharmaceuticals Sciences Research Center, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran
[f] School of Molecular Sciences, The University of Western Australia, Perth, WA 6009, Australia
[g] Molecular Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

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ABSTRACT

Simultaneous loading of hydrophobic and hydrophilic drugs into a drug delivery system is a difficult task and challenges still remain due to opposite nature of drugs. So far, most of the drug delivery systems have been designed based on liposomes, dual emulsions or porous nano-silica cavities. The application of Graphene and its oxidized derivatives as nanocarriers (NCs) have grown rapidly in the past few years. The first part of this study, using molecular modeling methods, provides details on the possibility and the mechanism of simultaneous loading of two hydrophobic and hydrophilic drugs, Rifampicin and Isoniazid, into graphene oxide (GO). The results confirmed the possibility of simultaneous loading of drugs in GO. The binding energies, calculated at the B3LYP-D3/6-31G(d) level of theory, are: −46.5 and −14.0 kJ mol^{-1} for Isoniazid and Rifampicin, respectively. Drugs loading, as also evidenced in the second part of study experimentally. The drug-loaded NCs were coated with biopolymers of Chitosan and Gum Tragacanth. SEM results confirmed that GO-NCs have produced with a diameter < 100 nm. Also, the results showed that the final diameters of coated NCs fall in the range 120–130 nm. A loading of 42.7% and 22% was also achieved for Rifampicin and Isoniazid, respectively. The release of Isoniazid reaches 93% after 60 h, and for Rifampicin is 88% after 72 h. To determine the toxicity of the biopolymer coated NCs, MTT test was used. Also, the antimicrobial efficacy of coated NCs loaded with drugs was evaluated versus pure drugs against *Mycobacterium tuberculosis*. The results showed that the drug co-loaded NCs have the same efficacy as pure drugs in their MIC concentrations.

1. Introduction

Tuberculosis (TB), as a granulomatous chronic bacterial infection, is one of the most common debilitating and deadly disease which infects approximately 1.8 billion people across the world and is responsible for 1.5 million deaths per year [1,2]. Treatment of TB is still a major challenge and is further complicated by non-localized delivery of drugs, patient non-compliance, high dose, dosing frequency, the development of drug resistance and the adverse side effects. Hence, and to overcome these difficulties, some efforts would be welcomed in order to introduce new remedies and therapeutic strategies [3–5]. Rifampicin (RIF) and Isoniazid (ISN) are two of the four first-line agents in TB management [6–10]. It is known that high daily dosage of RIF and ISN solely cause some type of toxicity such as hepatotoxicity [11]. And also, it was reported that the combination of both drugs is may lead to a greater rate of hepatotoxicity in comparison to each drug on its own [12]. For these reasons, it was expected that loading of drugs on NCs was able to provide them at a longer time and with less doses. Stable therapeutic NCs can be used to release the Anti-TB drugs in a slow/sustained manner with controlling release kinetics, high drug loading efficiencies and enhanced intracellular delivery [13–17]. One of the main advantages of NCs is the possibility of the combinatorial drug delivery and

* Corresponding author at: Medicinal Chemistry Department, Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.
E-mail addresses: mohsenshahlaei@yahoo.com, m.shahlaei@kums.ac.ir. (M. Shahlaei).

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providing the simultaneous encapsulating of both hydrophilic and hydrophobic drugs in the same carrier. In this regard, the main purpose of this study is to address the above mentioned issues and co-delivery of RIF (hydrophobic) and ISN (hydrophilic) using a new drug delivery system to reduce cytotoxicities of free drugs.

It should be noted that the simultaneous encapsulation or the combination of different drugs possesses some advantages such as suppressed drug resistance [18], synergistic effects [17] improves the patient compliance, safety and therapeutic effect [19,20].Also it has the potential ability to fine tuning the relative amount of different drugs to the level of a single NC [21]. Several researches have been devoted to investigate the controlled delivery of Anti-TB drugs using degradable bio-polymers including poly (lactic *co* glycolic) acid (PLGA), poly (lactide cocaprolactone) (PCL), and PLGA *co* poly ethylene glycol (PLGA-PEG) copolymers [22–24]. Recently graphene oxide (GO) is introduced as an alternative candidate for design of drug/gene delivery systems [25–29]. This NC system show good biocompatibility, low toxicity, high efficiency loading, intelligent controlled release and also has many hydrophilic groups (including hydroxyl, epoxide and carboxylic groups) on its vast surface and hence can be well-dispersed in physiological environments and water [28]. Moreover, some literature demonstrated that functionalized GO-NCs with a bio-compatible polymer such as PEG, leading to higher stability of the delivery system, more efficient loading and lack of cellular toxicity [30–32]. In this study GO is coated by Chitosan (CS) and Gum Tragacanth (GT), in order to effectively enhance the desired properties for RIF/ISN delivery purposes. These two biopolymers with positive and negative charges are bio-compatible and safe [33,34].

In comparison with time consuming, laborious and expensive experimental studies, molecular dynamics (MD) simulations can be applied to understanding the detailed information on drug delivery systems and interactions exist between the drug and other molecules. Therefore, many efforts have been made to investigate the structures, interactions of drug delivery systems in atomistic details by using MD simulations [35–39].

In this work, the simultaneous loading of both RIF and ISN with different polarities in the GO-NCs was first explored by using MD simulation. Then, the GO-NC was synthesized and RIF and ISN were loaded together. In order to evaluate the performance of the new developed system, the extent of dual-drug loading and drugs release-pharmacokinetic was also determined and the cytotoxicity and antimicrobial potency of the developed functionalized GO-NCs were assessed.

2. Materials and methods

2.1. Chemicals

Graphite, H_2SO_4 98%, $KMnO_4$, H_2O_2 30%, Rifampicin, Isoniazid, Chitosan (medium molecular weight), and Gum Tragacanth were all purchased from Sigma Aldrich.

2.2. Computational methods

In order to get an insight about possible interactions between drugs and GO and also the mechanism of formation of co-loaded nanoparticles, molecular dynamics simulation (MDS) was used. MDS was carried out using Gromacs 5.1.1 package [40]. At first, the structure of the ISN, RIF and GO plates (based on a molecular formula of $C_{10}O_1(OH)_1(COOH)_{0.5}$) were constructed using ACD/Labs followed by converting into 3D structure [41]. Energy minimization and pre-optimization of all structures were done by the steepest descend method in Avogadro [42].

The geometries of all structures were optimized at the B3LYP-D3/6-31G(d) level of theory [43,44] using the Gaussian 09 program package [45]. Empirical D3 dispersion corrections [45] were included using the Becke–Johnson [46] damping potential as recommended [47] (denoted

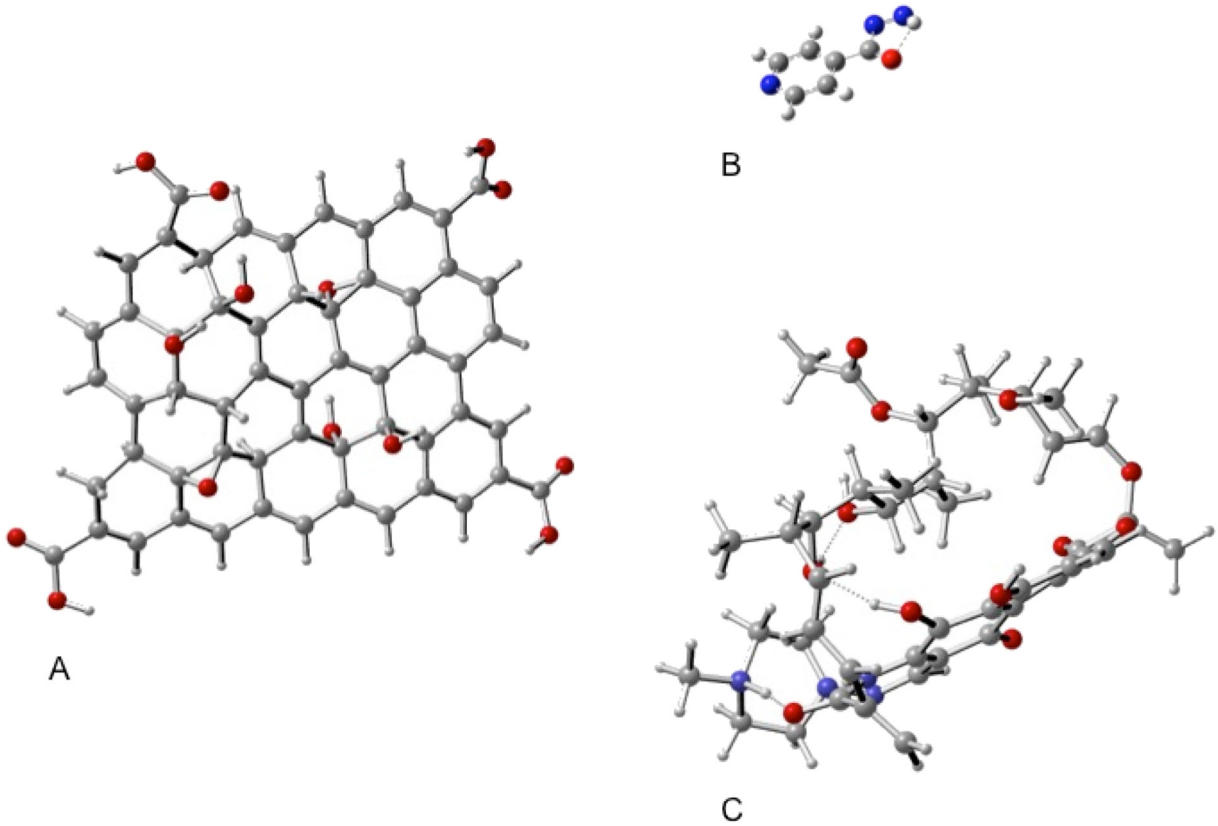

Fig. 1. B3LYP-D3/6-31G(d) optimized structures of A) GO, B) Isoniazid (ISN), and C) Rifampin (RIF).