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Investigation of different methods for cisplatin loading using single-walled carbon nanotube

Amin Kazemi-Beydokhti[b], *Saeed Zeinali Heris*[a,*], *Mahmoud Reza Jaafari*[c]

[a] *Faculty of Chemical and Petroleum Engineering, University of Tabriz, Tabriz, Iran*
[b] *Department of Chemical Engineering, School of Petroleum and Petrochemical Engineering, Hakim Sabzevari University, Sabzevar, Iran*
[c] *Biotechnology Research Center, Nanotechnology Research Center, School of Pharmacy, Mashhad University of Medical Science, Mashhad, Iran*

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ABSTRACT

Cisplatin as an anticancer drug is frequently used in cancer treatment, but with a lot of side effects. In this study, we investigated three different protocols of cisplatin drug loading inside and outside of single-walled carbon nanotube as a nano-platform. The drug encapsulation, electrostatic interactions, and covalent binding of cisplatin were utilized in these complexes to evaluate the capability of drug–carrier interaction in each method. All nanotube samples were dialyzed after preparation in phosphate buffer saline at acidic and neutral conditions for better simulation of tumor environments and then, the rate of drug release was determined using drug concentrations from the inside and outside of dialysis bags in the samples. Cisplatin showed the maximum and minimum rate of release for covalent and encapsulated complexes, respectively. Also, the amount of cisplatin drug release was quite different for two compounds at the beginning and at the end of dialysis. These trends of drug release in each of these drug loading approaches in different pH environments can help us to manage the optimized circulation time of cisplatin in the human body and the maximum release of drug at the target position.

1. Introduction

Due to a large variety of physical and chemical properties of carbon nanostructures such as thermal, mechanical and electrical properties, it has widespread use as nanocarrier in various fields (Baughman et al., 2002; Bayazit and Kerkez, 2014; Dai, 2002; Li et al., 2013). Nanomedicine is one of this area, which has recently assigned a large part of this research (Kostarelos et al., 2009; Lacerda et al., 2006). Due to various shapes of these carriers like plane, cylinder and cone, different methods of preparation have been applied to use it in medicine (Ajima et al., 2005; Pastorin et al., 2006; Sun et al., 2008a). Particle size and shape of nanocarriers can change the amount of drug loaded and drug release of complexes (Bahrami et al., 2014; Caldorera-Moore et al., 2010; Mudshinge et al., 2011).

Carbon nanotubes (CNT) as one of these nanocarriers have been proposed as an efficient nanoparticle for transportation of different drugs to the target site, especially malignant tumors. The high mechanical strength, suitable electrical, optical, thermal and kinetic properties are the advantages while the toxicity is the main disadvantage of CNTs (Kostarelos et al., 2009). Thermal property of CNT can use as an effective parameter for thermal ablation of tumors (Chakravarty et al., 2008; Hashida et al., 2014; Kazemi-Beydokhti et al., 2015). The large number of drug delivery systems based on CNTs were recently synthesized to apply these properties and overcome the side effects of anticancer drugs (Bhirde et al., 2010; Dhar et al., 2008; Madani et al., 2011; Ren et al., 2012). Some techniques like PEGylation and functionalization were used to control the inherent toxicity of the CNT (Campagnolo et al., 2013; Rodrigues et al., 2013; Zhang et al., 2011).

* *Corresponding author*. Tel.: +98 411 33393158; fax: +98 511 8816840.
E-mail addresses: a.kazemi@hsu.ac.ir (A. Kazemi-Beydokhti), s.zeinali@tabrizu.ac.ir (S. Zeinali Heris), Jafarimr@mums.ac.ir (M.R. Jaafari).
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Cisplatin is one of these drugs, which has recently been used in the treatment of a large number of cancers. Size and activity of these molecules are the main advantages and inability to penetrate into tumor cells, rapid clearance, and poor drug distribution are the problems of cisplatin in the human body (Sui et al., 2014). Due to release of the drug at the target position, drug encapsulation can reduce the side effects of cisplatin on the kidneys, heart, lungs, etc. (Bessrour et al., 2012; Li et al., 2014). Prevention of drug structural change is the principle reason of cisplatin encapsulation, in order to retain the pharmacological activity of the drugs after arriving to the target sites (Guven et al., 2012; Li et al., 2012).

Covalent and non-covalent binding of drugs are another method for drug delivery of these systems. Although, drug covalent bonds keep drug tightly bound to carrier and increase the drug circulation time in the human body, but it would be more effective, if the drug delivery system can release the drug at the target position (Heister et al., 2012; Liu et al., 2007; Raffa et al., 2010). The inactive cisplatin prodrug using covalent binding of drug to —COOH group of functionalized CNT and PEGylation of phospholipids showed that it is possible to control the size of malignant tumor (Kazemi-Beydokhti et al., 2014). Feazell et al. (2007) applied this technique to bind cisplatin prodrug to the surface of single-walled carbon nanotube (SWCNT). They reduced the cytotoxicity of the complex to a hundredth of the free platinum (IV) complex. Different strategies and linkers are used to keep drugs efficiently on the surface of CNTs (Meng et al., 2012; Mo et al., 2015). Unlike the encapsulation method, due to the chemical structure change of covalently bound drug, the pharmacological activities may be subject to change. The best performance will be achieved when the maximum drug concentration reach to the target position in optimum time (Adeli et al., 2011; Bhirde et al., 2009; Liu et al., 2009).

Hence, three different formulations based on SWCNT and conjugated cisplatin were synthesized to evaluate three different loading of the drug in term of the drug release rate at different pH values, including the encapsulation, electrostatic attraction and covalent binding of cisplatin. Different characterization such as the TEM images, surface charge measurement, thermogravimetric analysis, and cumulative drug release were applied to investigate the capability of each system for better drug release rate. These findings can be useful for the synthesis of efficient complexes with high efficacy and low side effects.

2. Materials and method

Purified SWCNTs, prepared by a high-pressure CO conversion (Hipco) method, were purchased from the Nanointegris Company (USA), with 100–1000 nm in length and 0.8–1.2 nm in diameter (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-methoxy(polyethylene glycol)2000) (DSPE-PEG2000) and 1,2-dioctadecanoyl-Sn-glycero-3-Phospho-1-rac-glycerol (DSPG) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Cisplatin as an anti-tumor drug was obtained from the Tocris Co (USA). All other chemicals were purchased from Sigma–Aldrich and used without further treatment.

2.1. Synthesis of complexes

2.1.1. Drug encapsulation

The 5 mg cisplatin was dissolved in 5 ml of chloroform and dispersed in a bath sonicator (Bandelin, Germany) for 10 min. Cisplatin is sensitive to light and all the steps are done under dark conditions. The SWCNT (1 mg) was added carefully to the suspension and stirred for 48 h. During this process, several short-time bath sonications were applied to prepare better dispersion of CNTs. SWCNT have a high risk of inhalation and it is necessary to use a proper filter mask. Then, the suspension was filtered through a 0.1 μm PTFE membrane (Millipore, USA) and the excess cisplatin and other impurities were separated by several washings of precipitate with chloroform. The remained substance was placed in a round bottom flask and spun on a rotary evaporator at 70 °C.

In the next step, the prepared nano-platform was dispersed again in 10 ml chloroform and sonicated with the 5 mg DSPE-PEG2000 for 30 min. This phospholipid helped us to prepare better dispersion of the CNT in water and blocked the exit route of drug in the next step. After that, the compound was centrifuged at 18,000 × *g* for 2 h, yielding well-suspended complex in the supernatant. The 100 kDa Amicon centrifugal filters (Millipore, USA) were used to remove unbound phospholipids. The same filtration and drying were utilized to prepare final product and was denoted as FORM1.

2.1.2. Electrostatic binding

The pristine SWCNT were sonicated in an aqueous solution containing 1 mg SWCNTs: 2.5 mg DSPG: 2.5 mg DSPE-PEG2000: 10 ml of water for 2 h. The DSPE-PEG2000 can improve the dispersibility of nanocarriers and the DSPG phospholipid help us to make the electrostatic interaction between nano-platform and cisplatin molecules. The negative charge of DSPG is the key factor of cisplatin attraction. The mixture was then centrifuged at 24,000 × *g* for 2 h, yielding well-suspended complex in the supernatant. Unbound phospholipids were removed by repeated ultrafiltration through 100 kDa Amicon centrifugal filters (Millipore, USA).

In the next step, the prepared platform was sonicated again for better dispersion in an ultrasonic bath for 15 min. Afterwards, 0.5 ml cisplatin solution with concentration of 1 mg/ml was slowly added and dispersion was placed on a magnetic stirrer for about 24 h. Henceforth, this complex is referred to as FORM2.

2.1.3. Covalent binding

This complex was achieved by first carboxylating the surface of the nanotubes and providing functionality to covalently bind molecules to the surface of SWCNT. This complex with some modification was synthesized in our previous works (Kazemi-Beydokhti et al., 2014, 2015). First, oxidized nanotube was prepared by a reflux system through dispersion of SWCNTs in HNO_3–H_2SO_4 solution (3:1, v/v) at 70 °C for 4 h. Reflux apparatus was used to add enough energy for chemical surface modification of SWCNT. This combination of ultra-pure acid have high efficacy of oxidation on SWCNT. Like before, several short-time bath sonications were applied to disentangle SWCNTs from each other. Then, the mixture was diluted with double distilled water and filtered through 0.45 μm pore-sized membrane filter. All the impurities were separated by several washings of the precipitate. The 1 mg of the oxidized nano-platform was mixed again with 5 mg DSPE-PEG2000 and 10 ml of water for 2 h. The mixture was then centrifuged at 24,000 × *g* for 2 h and the supernatant was separated. Afterwards, the PEGylated oxidized SWCNTs were sonicated in an ultrasonic bath for 15 min and 0.5 ml of cisplatin solution (1 mg/ml) was slowly added. The mixture was placed on a magnetic stirrer 48 h. Due to free carboxylic groups on the surface of oxidized SWCNT, the covalent bonds will only occur in the oxidized complex. Like before, the same procedures were utilized for dialysis to separate the free cisplatin molecules. This formulation is referred to as FORM3. Fig. 1 shows the schematic synthesis of these complexes. The detail description of reaction mechanism has been explained in previous researches (Liming et al., 2003; Meng et al., 2009; Pasquini et al., 2012).