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# Design and development of multi-walled carbon nanotube-liposome drug delivery platforms

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ABSTRACT

The aim of this study is to design and develop delivery platforms made of liposomes and multi-walled carbon nanotubes (MWCNTs). We used different lipids with different main transition temperature (Tm) and differently functionalized MWCNTs with organic addends possessing either positive or negative charge. The phospholipids used for the formulations were 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (Tm = 41 °C) and L-α-phosphatidylcholine, hydrogenated Soy (HSPC) (Tm = 53 °C). By Differential Scanning Calorimetry (DSC), we studied the interaction between the DPPC and HSPC bilayers and MWCNTs. Liposome-MWCNTs delivery platforms prepared according to the protocol used in the literature. We used dynamic and electrophoretic light scattering in order to investigate the physicochemical characteristics of these mixed nanocarriers. The presence of MWCNTs causes alterations of the size of the conventional HSPC and DPPC liposomes. The ζ-potential values of mixed nanocarriers are near zero. This observation indicates the effective incorporation of MWCNTs into the lipid bilayer of liposomes. Fluorescence spectroscopy has been utilized to exact some qualitative information on the internal nanostructure and nanoenvironment of the lipid/carbon nanotube mixed structures. Finally, we conclude that we successfully prepare and completely characterize mixed nanocarriers composed of lipids and MWCNTs, with low toxicity as indicated by in vitro screening.


## 1. Introduction

In recent years, significant research effort has been devoted for the investigation of carbon nanotubes (CNTs) in biology and medicine toward their utility in bio-applications (Bianco et al., 2005; Pagona and Tagmatarchis, 2006; Kostarelos et al., 2009; Liu et al., 2009; Heister et al., 2013; He et al., 2013). Particularly, among the numerous outstanding characteristics of CNTs, their high aspect ratio, chemical stability, robustness, high drug carrier capacity and ability to penetrate cell membranes, render them ideal vehicles for delivering bioactive molecules, such as drugs, DNA and proteins (Peretz and Regev, 2012; Mendes et al., 2013). In general, biomolecules are conjugated to CNTs through well-established approaches either covalently, forming chemical bonds with the CNT scaffold, or non-covalently, by adsorption and/or wrapping onto the surface of CNTs or even by encapsulation, filling the empty inner cavity of CNTs (Battigelli et al., 2013). Specifically for medical applications, CNT-based materials need to disperse very well in water. However, pristine CNTs are insoluble in most common solvents as well as in aqueous and biological media due to their hydrophobic surface. In order to overcome this hurdle, functionalization of CNTs is absolutely required, which not only leads to solubility enhancement but also improves biocompatibility and minimize toxicity (Sayes et al., 2006; Lacerda et al., 2008; Bianco et al., 2011; Dumortier et al., 2006; Liu et al., 2013).

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Additionally, the size of CNTs plays an important role so that their length is necessary to be short in order to minimize toxicity issues (Kostarelos, 2008). Considering all the above, oxidation of CNTs is the method of choice for engineering the material, particularly when considering the availability of specific oxidation procedures that can simultaneously lead to shortening of CNTs and importantly introduces oxygen-based functionalities suitable to perform post-modification reactions toward the construction of advanced CNT-based biomaterials (Jain et al., 2011).

Current liposomology is a huge and still progressing area. Liposomes belong to colloidal dispersion systems and are characterized as lyotropic liquid crystals (Demetzos 2008, 2015). These particular liposomal lyotropic states are responsible for the mesophases taking place in phase transitions and are related to their thermal stress during phase transitions (Demetzos 2008, 2015; Pippa et al., 2015). Liposomes are clinically used delivery systems with many advantages such as biocompatibility and biodegradability (Allen and Cullis, 2013).

Pharmaceutical nanotechnology can provide challenges for producing innovative drugs based on bio-inspired nanostructures that can be employed as *advanced Drug Delivery nano Systems* (aDDnSs). Hence, mixed nanocarriers have attracted the scientific interest the last years with numerous applications in controlled-release of active pharmaceutical ingredients (Demetzos and Pippa, 2014). In particular, Karmchemski et al. designed CNTs liposome conjugates that could deliver a high dose of drug into cells using a low concentration of CNTs and thus CNTs related toxicity reduced significantly. Active targeting and delivery of drugs to desired cells and consequently preventing potential adverse systemic reactions was also achieved by CNT-liposome conjugates. Supramolecular nanotrains composed of CNTs and liposomes are used as smart biomimetic molecular–transport systems with lab-on-a-chip applications (i.e.) medical diagnosis, bionic computers and artificial biological networks (Miyako et al., 2012). Pereira and colleagues successfully developed hybrids composed of cationic liposomes and multi-walled carbon nanotubes (MWCNTs) for the simultaneous delivery of siRNA and anticancer drug to cancer cells. The functionalized MWCNTs incorporation did not affect the overall cationic surface charge of the final nanostructures formed and this is of great importance for the delivery of nucleic acids (Pereira et al., 2015). Lysine modified single-walled carbon nanotubes-liposomes conjugate loaded with the toxic anticancer drug doxorubicin was developed to ameliorate the anticancer effectiveness with a dual targeting mechanism (Zhu et al., 2015).

The aim of the current study is to design and develop innovative delivery platforms of therapeutic agents made of liposomes and MWCNTs. In this context, we employed different lipids, showing different main transition temperature (Tm) and different MWCNTs, properly modified to carry different surface functional groups. The phospholipids used for the formulations were 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (Tm = 41 °C) and L-α-phosphatidylcholine, hydrogenated Soy (HSPC) (Tm = 53 °C). Firstly, Differential Scanning Calorimetry (DSC) experiments were applied on lipid: MWCNTs bilayers, to evaluate the cooperativity of different materials, based on the system thermotropic behavior. Series of light scattering and imaging techniques were used to elucidate their physicochemical characteristics and morphology of the prepared hybrid systems respectively. The in vitro toxicity of liposome-MWCNTs delivery platforms was also evaluated.

## 2. Materials and methods

### 2.1. Materials

The phospholipids used for the chimeric formulations were 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine(DPPC) and L-α-phosphatidylcholine, hydrogenated Soy (HSPC). There were purchased from Avanti Polar Lipids Inc., (Albaster, AL, USA) and used without further purification. Multi-walled carbon nanotubes (MWCNTs) were obtained from Nanostructured and Amorphous Materials, Inc., (www.nanoamor.com; outer diameter 8–15 nm, length 500 nm; purity 95%). Chloroform and all other reagents used were of analytical grade and purchased from Sigma–Aldrich Chemical Co.

### 2.2. Preparation of MWCNTs

#### 2.2.1. Preparation of oxidized MWCNTs ***1a***

In a flask containing pristine 50 mg of MWCNTs, a freshly prepared piranha solution (50 mL, 4:1 v/v, 96% $H_2SO_4$/30% $H_2O_2$) was added. The reaction mixture was stirred upon heating at 70 °C for 7 h. After that period, the highly acidic mixture was carefully added to 1 L of deionized water and the suspension was filtered through a PTFE membrane filter (pore size 0.22 μm). The collected oxidized MWCNTs **1a** were washed onto the filter with deionized water, until the pH of the filtrate was neutral.

#### 2.2.2. Preparation of oxidized MWCNTs ***1b***

In a flask containing pristine 50 mg of MWCNTs, oleum (25 mL, 30% $SO_3$) was added and the mixture was stirred under a nitrogen atmosphere for 18 h. Then, a 1:1 mixture of $SO_3$/$HNO_3$ (25 mL) was slowly added into the above suspension with stirring in an ice bath to keep the temperature close to ambient conditions and then the resulting dispersion was heated at 65 °C for 2 h. After cooling to room temperature, the highly acidic mixture was carefully treated with deionized water (150 mL) and the suspension was filtered over a PTFE membrane filter (pore size 0.22 μm). The collected oxidized MWCNTs **1b** were washed onto the filter with deionized water to remove acidic residues, until the pH of filtrate was neutral.

#### 2.2.3. Preparation of anionic MWCNTs materials ***2a*** or ***2b***

Oxidized MWCNTs (15 mg of **1a** or **1b**) were dispersed in 10 mL deionized water through sonication for 15 min. Then, aqueous $NaHCO_3$ (24 mg in 5 mL $H_2O$) was added and the mixture was stirred for 18 h at room temperature. Finally, the suspension was filtered over a PTFE membrane filter (pore size 0.22 μm) and the collected material **2a** or **2b** were washed extensively onto the filter with deionized water until the pH of filtrate was neutral.

#### 2.2.4. Preparation of functionalized MWCNTs materials ***4a*** or ***4b***

A suspension of oxidized MWCNTs (15 mg of **1a** or **1b**) in 8 mL thionyl chloride was stirred at 75 °C for 24 h under $N_2$ atmosphere. Then, the reaction mixture was evaporated to dryness to remove the excess of MWCNT-based acyl chloride was obtained. Subsequently, *N-tert*-butoxycarbonyl-2,2′-(ethylenedioxy)bis-(ethylamine) **3** (200 mg) in dry THF (10 mL) was added and the reaction mixture was heated at reflux for 48 h. After cooling to room temperature, the solution was diluted with THF, filtered over a PTFE membrane filter (pore size 0.22 μm) and the collected solid materials washed extensively with MeOH and $CH_2Cl_2$ in order to provide the BOC -protected functionalized MWCNTs **4a** or **4b**.

#### 2.2.5. Preparation of functionalized MWCNTs materials ***5a*** or ***5b***

A solution of BOC-protected functionalized MWCNTs **4a** or **4b** (7 mg) in 7 mL $CH_2Cl_2$ was treated at room temperature with 10 mL TFA for 18 h. After that period, the highly acidic solution was evaporated to dryness, followed by addition of fresh dichloromethane with sonication and filtration through a PTFE membrane filter (pore size 0.22 μm) to afford MWCNT-based materials **5a** or **5b** after extensive washing with MeOH and $CH_2Cl_2$.