O8-3	**Ligand anchored surface engineered carbon nanotubes for targeting potential to cancer**	

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INTRODUCTION AND OBJECTIVES

According to the world cancer report issue by WHO in 2008, there were an estimated 12.4 million new cases of cancer worldwide and 7.6 million death. Nanomaterials possess fundamentally new properties and functions due to their small dimesnion. Carbon nanotubes (CNTs) were first fully described by Iijima S, 1991 in his TEM observations. CNTs are cylindrical molecules comprising hexagonal arrangement of sp^2-hybridized carbon atoms to make hollow inner cylinders formed by rolling single or concentric layer of graphene sheets into seamless cylinder. It could exist in two forms: Single walled CNTS (SWCNTs) and Multi walled CNTs (MWCNTs). Since their discovery great attention in drug deliver, because they could easily cross the plasma cell membrane without causing any toxic effect on the healthy cells due to their nanoneddle tubular shape structure. CNTs could bind to biomolecules with these possible mechanism, (1) Absoption of bioactives molecules with in the CNTs mesh,(2) Non-covalent or covalent linkages of bioctives to the external surfaces of CNTs by π- π stacking interaction, and (3) CNTs used as catheters. CNTs are uniques in physicochemical properties such as ultralight weight, high aspect ratio, easy surface modification, presence of π-π stacking interaction and endohedral filling could allow for higher drug payload. CNTs have been continuosly exploring as a drug delivery microcapsules for the biomedical application to cure the disease (Mehra, 2008 ; Jain, 2007 ;Mehra, 2010).

Doxorubicin HCl (DOX) is poorly water soluble drug anthracycline antibiotic anticancer agent. DOX is commonly administered intravenously in the form of commercially available injections Adriamycin and Rubex to maintain the therapeutics levels in blood. In addition, two PEGylated liposomal formulations of DOX i.e. Doxil and Caelyx, are also available in the market.

The main aim of present investigation is to explore the novel and innovative original research work of DOX loaded folic acid (FA)-polyethylene glycol (PEG)-MWCNTs formulation for optimized therapeutic response with reduced side effects associated to DOX and efficiently delivered to cancer cells.

MATERIALS AND METHODS

MWCNTs procured from Sigma Aldrich Pvt. Ltd. USA. DOX hydrochloride from Sun Pharmaceutical Vadodara, Gujarat INDIA. Folic acid (Himedia), PEG bi amine (Sigma). EDC, NHS, EDA (Himedia) and all other reagents were purchased from local supplier and used as received.

MWCNTs were further purified by oxidative treatment in oven at different time to remove metallic impurities presence in CNTs. The purified MWCNTs were further used for functionalization by following sequential steps *viz.* carboxylation by strong acid treatment H_2SO_4: HNO_3::3:1 (Piranha solution), acylation and amidation for amine modification. Finally, folic acid (a targeting ligand) was conjugated without spacer or spacer PEG-bis-amine on to the surfaces and ends of functionalized nanotubes. Doxorubicin, as an anthracycline antibiotic anticancer agent was physically loaded in *f*-MWCNTs and characterized by different physicochemical parameters such as electron microscopy (TEM, SEM), elemental, x-ray diffraction, FT-IR spectroscopy, zeta potential, entrapment efficiency, *in-vitro* drug release and toxicological profile (hemolytic, hematological and % cell viability assay). DOX was entrapped into the FA-PEG-MWCNTs at physiological pH (7.4) and determine the % entrapment efficiency. Finally, *in-vitro* release profile was studied by dialysis diffusion technique at different pH (7.4 & 5.3) and found a sustained manner at pH 4.0 upto 200 hr.

A-549 (lung epithelial cancer cell lines) was procured from National Center for Cell Sciences (NCCS), Pune, INDIA, cultured in RPMI-1640 medium and used for MTT assay, tumor induction by right flank method in Sprague Dawley strain for tissue biodistribution study.

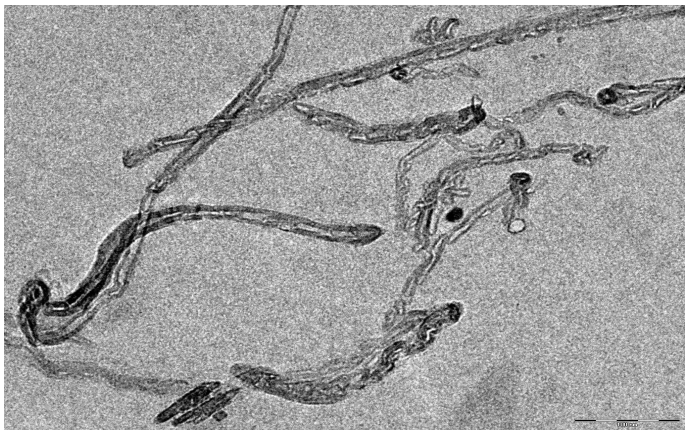

Figure 1. TEM of DOX/FA-PEG-MWCNTs

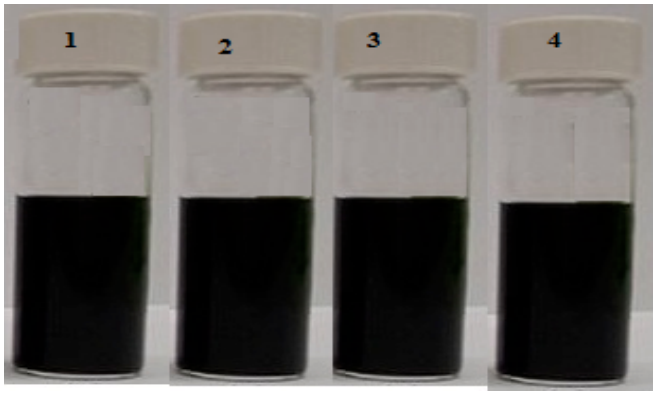

Figure 2. Formulation of FA-PEG-MWCNTs

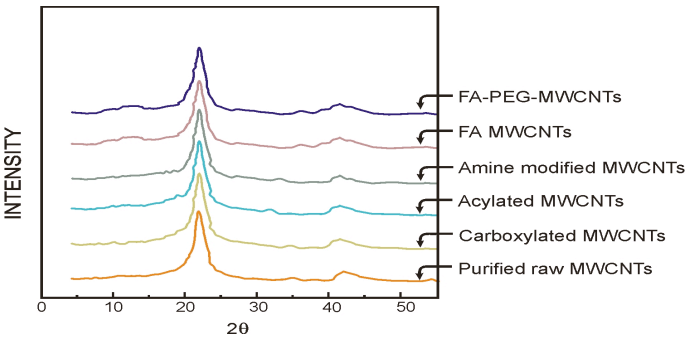

Figure 3 x-ray diffraction analysis of MWCNTs formulations.

RESULTS AND DISCUSSION

The different *f*-MWCNTs were characterized by FT-IR spectra, elemental, Raman, x-ray diffraction analysis; and electron microscopy (TEM, SEM). The data confirmed functionalization steps, suggest the attachment of folic acid without spacer or with PEG spacer to MWCNTs, which contained aromatic rings. X-ray diffraction analysis of different *f*-MWCNTs clearly indicated that even after the functionalization of MWCNTs, there was no change in tubular structure and interplaner spacing of MWCNTs. The TEM image was used to characterize CNTs in the nanometric range and surface morphology.

Entrapment efficiency (%) of doxorubicin hydrochloride in DOX/FA-PEG-MWCNTs formulation was performed at physiological buffer (pH 7.4) and found to be 92.0±1.4 due to π-π stacking interactions and microenvironment. PEG-bis amine chain make minor and major grooves for encapsulating of DOX molecules, make it biocompatible. *In- vitro* drug release study of final formulation was performed at different pH (7.4 & 5.3) from FA-PEG-MWCNTs formulation. The FA-PEG-MWCNTs could be a potential nano-carrier for cancer therapy due to their high entrapment capacity and depicts a controlled release at different pH value. Drug release from all formulations were dependent on the pH of the environment, being sustain at pH 5.3 lysosomal pH attributed to the greater hydrophilicity and lower retentivity. Slowest release in all cases was observed at pH 7.4.

DOX and DOX loaded FA-PEG-MWCNTs were used as a control and incubated with A-549 lung epithelium cancer cell and visualized by fluorescence microscopy. DOX itself produced fluorescence intensity. Data clearly suggest a dose dependent cytotoxicity response i.e. decrease in cell survival fraction by increasing the concentration of FA-PEG-MWCNTs and found to be more cytotoxic at the concentration tested.

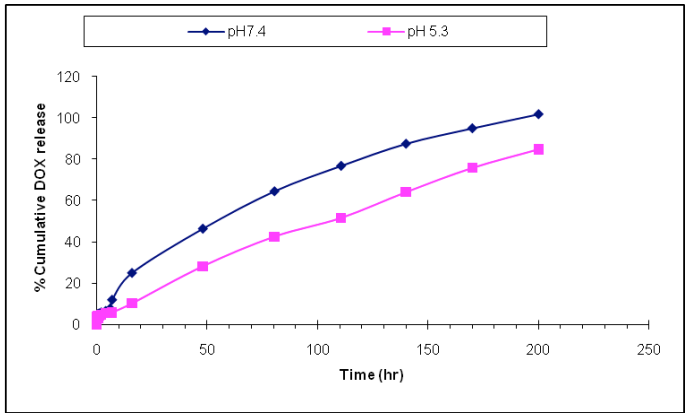

Figure 4 % cumulative DOX release from FA-PEG-MWCNTs formulation.

CONCLUSION

It can be concluded that DOX loaded FA-PEG-MWCNTs formulation shows better *in-vitro* release in a controlled fashion for optimized therapeutic response and also reduces the dose of doxorubicin so adverse effect could be reduced. CNTs could be the vector of choice because they not only provide an outer periphery for bioactive conjugation or loading but also provide an interior chamber for endohedral entrapment of bioactives. Such systems could provide the possibility of multidrug loading and delivery, which is a tedious task for other nanorange carriers

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REFERENCES

- Mehra et al. 2008. *Challenges in the use of carbon nanotubes for biomedical applications*. Crit Rev Ther Drug Carrier Sys.25:169-206.
- Zhang et al, 2009. *Targeted delivery and controlled release of doxorubicin to cancer cells using modified single wall carbon nanotubes*. Biomaterials. 30: 6041–6047.
- Mehra et al, 2011. *Carbon nanotubes: a new line to drug delivery*. Pharm Rev.09(49):75-80.
- Jain et al, *Carbon nanotubes and their toxicity*. Nanotoxicol.2007; 1(3):167-97.
- Jain et al, 2009. *Carbohydrate conjugated multi walled carbon nanotubes; Development and characterization.* Nanomed Nanotech Biol Med. 5:432-442.