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Loading of Anti Cancer Drug on Carbon Nanotubes

Seema Manchanda*, Madhuri Sharon, Maheshwar Sharon
Department of Chemistry Birla College of Arts, Science and Commerce, Kalyan, University of Mumbai Maharashtra, India

ABSTRACT

Carbon Nanotubes exhibit many unique intrinsic physical and chemical properties and have been intensively explored for biological and biomedical applications in the past few years. The presented work is a prelude in the direction of using Carbon Nano tubes as a vehicle for drug delivery to the desired sites. Anti cancer drug, Doxorubicin is loaded on functionalized Multiwalled Carbon Nano tubes at different pH conditions. Analysis done was FTIR, ATR, TEM, and UV Visible spectroscopy.

I. INTRODUCTION

Carbon Nano-tubes (CNT) are concentric shells of graphite formed by one sheet of conventional graphite rolled up into a cylindrical form. The lattice of carbon atoms of graphite sheets remains continuous around the circumference of the Nano tubes. Hence, Carbon nanotubes are fullerene-related structures closed at either end with caps containing pentagonal rings.
CNTs are of two types Single Walled Carbon Nano Tubes (SWCNT) and Multi Walled Carbon Nano Tubes (MWCNT) (Figure 1)

In **SWCNT** there are only tubules and no graphitic layers around them. Diameter of SWCNT is up to 2 nanometer where as length varies as per production procedure from 3 to 10 μm. The arrangement of carbon in a SWCNT can be of the arm-chair, zigzag, or chiral pattern. SWCNT are mostly produced in a bundle and then are separated by chemical or physical methods.

MWCNTs are stacks of graphene sheets rolled up into concentric cylindrical structures. Their diameter is in the range of 10 – 50 nm and length can be up to or more than 10 μm. The individual graphene sheets are separated by about 0.34 nm.

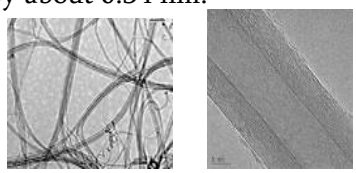


Figure 1. Single Walled (Left) and Multi Walled (right) Carbon Nanotube.

Whenever one thinks about loading any material to Carbon Nano tubes(CNTs), the thought that comes into the mind that (i) is it filling the lumen with the desired material or (ii) chemically attaching them onto the surface of the CNT.

Filling CNTs with metal like materials are easy as it can be done during the synthesis of CNT. However unloading them still remains a bigger task, as it is difficult to push out something from the 2-3 nm diameter lumen. Moreover, drugs are often temperature sensitive, hence their loading at higher temperature, at which CNT synthesis takes place is not possible.

So far as the attachment to the CNT surface is concerned, there are many possibilities (a) adsorbing

them on to the surface (b) attaching them to dangling bonds or (c) attaching through a linker or functionalized molecule.

Scientists have tried all such methods. Some of them are mentioned below.

Small drug molecules can be ***covalently conjugated*** to CNTs for *In vitro* delivery. Fluorescent dyes and drug cargoes were simultaneously linked to 1,3-dipolar cycloaddition functionalized CNTs via amide bonds for the delivery of an anti-cancer drug (Pastorin *et al* 2006) or an antifungal drug (Wiecowski *et al* 2005) into cells. Feazell and Lippard used non-covalently PEGylated SWNTs as a longboat delivery system to internalize a platinum (IV) complex, a pro-drug of the cytotoxic platinum (II), into cancer cells. The inert platinum (IV) pro-drug compounds developed by the Lippard's group are activated only after being reduced to the active platinum (II) form. SWNTs tethered with the platinum (IV) complexes through peptide linkages are taken into cancer cells by endocytosis and reside in cell endosomes, where reduced pH induces reductive release of the platinum (II) core complex, thus killing the cancer cells. The cytotoxicity of the platinum (IV) complex increases over 100-fold after attachment to SWNTs. They have also conjugated paclitaxel, a commonly used anti-cancer drug, to branched PEG-coated SWNTs via a cleavable ester bond (Liu et al 2008). The SWNT-PTX conjugate was tested both *In vitro* and *in vivo*.

Noncovalent conjugation; beside covalent conjugation, novel noncovalent supramolecular chemistry for loading aromatic drug molecules onto functionalized SWNTs by π - π stacking has been uncovered by (Liu et al 2007). Doxorubicin, a commonly used cancer chemotherapy drug, can be loaded on the surface of PEGylated SWNTs with remarkably high loading, up to 4 g of drug per 1 g of nanotube, owing to the ultrahigh surface area of SWNTs. The loading/binding is pH dependent and favorable for drug release in endosomes and lysosomes, as well as in tumor micro-environments with acidic pH. Similar drug loading behaviors have been reported for MWNTs (Ali Boucetta *et al* 2008), single-walled carbon nanohorns (Murakami *et al* 2006) and nano-graphene oxide (Sun et al 2008 and Liu *et al* 2008). The supramolecular approach of drug loading on CNTs opens new opportunities for drug delivery.

π - π stacking, for the delivery of aromatic drugs such as doxorubicin, which are directly loaded on the nanotube surface via π - π stacking, the functional groups on the SWNT coating molecules (e.g., PL PEG amine) can be conjugated with targeting molecules such as Arg – Gly - Asp (RGD) peptide for targeted delivery (Liu *et al* 2007).

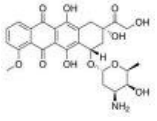
Encapsulation of drug molecules inside nanotubes; apart from drug conjugation and loading on the external surfaces of nanotubes, the lumen of CNTs may allow the encapsulation of drug molecules inside nanotubes for drug delivery. Fullerene balls (Kataura *et al* 2001), metal ions (Jeong et al 2003), small compounds such as metallocenes (Li *et al* 2005), and even DNA molecules (Kaneko et al 2007) have been encapsulated inside CNTs. Although a number of theoretical modeling studies predicted the insertion of biomolecules including chemotherapy drugs (Hilder *et al* 2007 and Hilder *et al* 2008) into CNTs, drug delivery by encapsulation of drugs inside CNTs has been rarely reported. Further experimental studies are still needed to examine the possibility of utilizing the encapsulation strategy in CNT-based drug delivery.

II. METHODS AND MATERIAL

Loading Doxorubicin on functionalized CNT

Drug Used:

Doxorubicin



- Structure:
- Molecular formula: $C_{27}H_{29}NO_{11}$.
- Mol. Wt. : 579.99
- M.P.: 204°C

- Appearance: Orange red crystalline Powder, hygroscopic
- Solubility: Soluble in water, slightly soluble in methanol.
- Storage: In air tight container

10 mg of f-MWCNT was suspended in 25ml of 0.2 mg/ml concentration of Doxorubicin in three different pH buffer solutions and kept overnight.
Calibration graph was plotted using the absorbance of 6 different dilutions of Doxorubicin (10, 20, 30, 40, 50 & 60 ppm) solutions. λ max was determined by scanning 0.05mg/ml solution of Doxorubicin from 400 to 580 nm using Spectrophotometer 169 of Systronics make.

To know whether Doxorubicin is loaded on f-MWCNT, FTIR, ATR and TEM was done.
Surface adsorption study was done by plotting a Calibration graph using 5 different concentrations of Doxorubicin (100,200,300,400,500 ppm) solutions.

5mg of f-MWCNT was added to 10ml of each concentration and absorbance of supernatant was taken after 24 hrs.
Graph of amount adsorbed *Vs* concentration of Doxorubicin was plotted and type of adsorption of drug on to the Carbon nano tubes was studied.

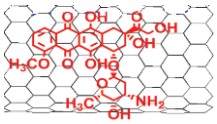


Figure 2 .Schematic diagram of Doxorubicin loaded on to the CNT surface

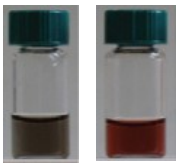


Figure 3. (Left) CNT in water and (Right) CNT + Doxorubicin in water

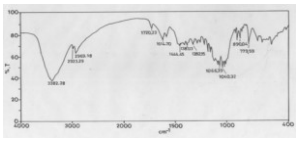
The loading capacity of Doxorubicin on CNT was determined by UV visible spectroscopy at 490 nm, which was calculated by the difference of Doxorubicin concentrations between the original Doxorubicin solution and the supernatant solution after loading.
CNT shows distinctly different loading capacity toward Doxorubicin at different pH values.

Table 1. Effect of pH on loading of Doxorubicin on F-MWCNT as recorded at 490 nm by measuring remaining amount of Doxorubicin in water (the amount of Doxorubicin was calculated using standard calibration graph)

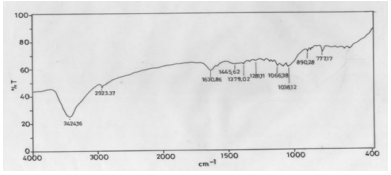
pH	Absorbance At 490 nm	mg/ml Doxorubicin remaining in waterin	Concn loaded (0.2-Concn) (mg/ml)	Amount loaded (mg)	%loading
4	0.152	0.045	0.155	3.87	77.5
7	0.121	0.036	0.164	4.10	82
9	0.140	0.0415	0.1585	3.96	79.25

To know Doxorubicin is loaded on f-MWCNT, FTIR, ATR and TEM was done

FTIRs



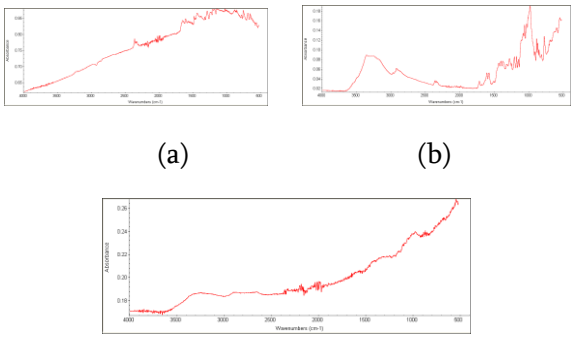
(a) (b)



(c)

Figure 4. FTIR of (a) Doxorubicin and (b) f-CNT (c) f-CNT loaded with Doxorubicin

ATRs



(a) (b)

(c)

Figure 5. ATRs of (a) f-CNT (b) Doxorubicin (c) Doxorubicin loaded on F-MWNT

TEM

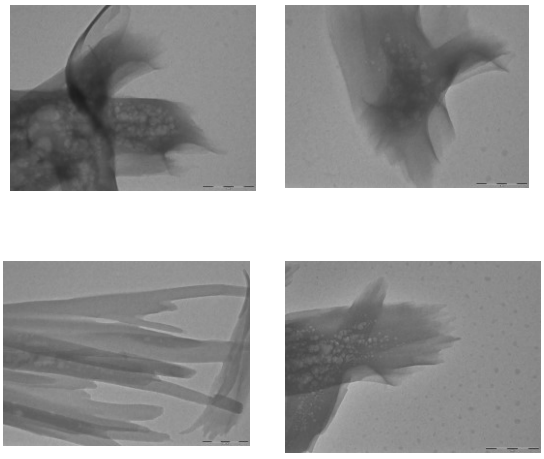


Figure 6. TEM of Doxorubicin

TEM of Doxorubicin shows that it cannot withstand high electron volt of transmission electron microscopy, as the images reveals that the drug degrades on the exposure of high electron volt. Therefore it was difficult to locate the drug on CNT by TEM.

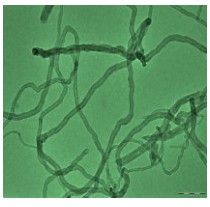


Figure 7. TEM of Doxorubicin on CNT

III. RESULTS AND DISCUSSION

FTIR of drug loaded on F-MWNT reveals the following facts:

- Peak at 1630.86 cm^{-1} in FTIR of MWNT loaded with Doxorubicin is shifted from 1633.82 cm^{-1} in FTIR of MWNT is due to the change in the environment of C=O group.
- Peak at 1066.39 cm^{-1} in FTIR of Doxorubicin is due to C-O stretching of ether group which is also seen in the in FTIR of MWNT loaded with drug (peak at 1066.38 cm^{-1}).
- Peak at 2923 cm^{-1} is due to C-H stretching.

ATR (Attenuated total reflection) of F-MWNT loaded with drug shows a broad peak at 3000-3500 cm^{-1} which is also seen in the ATR of Doxorubicin. This further proves the loading of drug onto the MWNT.
TEM of MWNT also shows the loading of drug, Doxorubicin onto the nanotube.

The FTIR spectra of CNT, Doxorubicin (Doxo), and CNT-Doxo nano hybrid show the following evidences. The peak at 1633.82 cm-1 corresponding to ν(C=O) in the spectrum of CNT and the C=O peak (1720.23 cm-1) for Doxo shift to a lower position at 1630.36 cm-1 after forming CNT- Doxo nano hybrid. This also indicates that Doxo be loaded onto CNT and the shift of characteristic peaks may be due to the hydrogen bonding between these two components. The -OH and -COOH groups on the CNT can form a strong hydrogen-bond with -OH and -NH2 groups in Doxo. Therefore, Doxo was non-covalently loaded on CNT simply by mixing them in aqueous solution with the aid of slight sonication.

The highest loading capacity is observed at the neutral condition, rather than acidic or basic conditions. The pH-dependent loading may be due to the different degree of hydrogen-bonding interaction between these two species under different pH conditions. –COOH of CNT and the

-OH of Doxo, -COOH of CNT and the $-NH_2$ of Doxo, -OH of CNT and the -OH of Doxo, and

-OH of CNT and the $-NH_2$ of Doxo (Table 2). Under acidic conditions, $-NH_2$ of Doxo forms

$-NH_3^+$ with H^+ and therefore cannot participate in hydrogen bonding. In this case, two kinds of hydrogen bonding can occur between -COOH of CNT and the -OH of Doxo, and -OH of CNT and the -OH of Doxo. Furthermore, the H^+ in solution would compete with the hydrogen- bond- forming groups and then weaken the above hydrogen-bonding interaction. Under basic conditions, -COOH of CNT exists as $-COO^-$ and cannot form a hydrogen bond with -OH or $-NH_2$ groups of Doxo. Two kinds of hydrogen bonding interaction can occur between -OH of CNT and the -OH of Doxo, and -OH of CNT and the $-NH_2$ of Doxo. Therefore, the strongest hydrogen-bonding interaction between CNT and Doxo is expected under neutral conditions, and the highest loading of Doxo on CNT is obtained.

Table 2. Groups That Can Form Hydrogen Bonds with CNT and Doxo at Different pH Conditions

pH conditions	CNT	Doxo
Acidic	-OH, -COOH	-OH,
Neutral	-OH, -COOH	-OH, $-NH_2$
Basic	-OH	-OH, $-NH_2$

ADSORPTION STUDY:

To know how many layers of Doxorubicin has been loaded on to CNT, graph of different concentrations of Doxorubicin against amount of doxorubicin adsorbed was plotted.

Table 3. Amount of Doxorubicin loaded on CNT at different concentrations.

Concn + 5 mg CNT	Absorbance	Corr. Concn (mg/ml)	Concn Adsorbed	Amount adsorbed (mg)
100	0.098	0.025	0.075	0.75
200	0.233	0.070	0.130	1.30
300	0.418	0.125	0.175	1.75
400	0.577	0.170	0.230	2.30
500	0.590	0.175	0.325	3.25

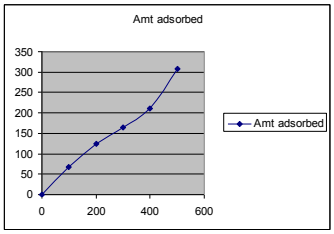


Figure 8. Graph of amount of Doxorubicin adsorbed Vs Different Concentration

The nature of graph shows adsorption of drug is multilayered adsorption which may be possible due to interactions within doxorubicin molecules also.

IV. CONCLUSION

With the advent of nanomaterials; interdisciplinary research has taken a big leap. The present work was a small effort to enter in this magnificent field of science having immense possibilities.

It was envisaged that the attachment of drug to the CNT surface could be by

(a) getting adsorbed to the surface

(b) or attached to the dangling bonds of CNT or

(c) may be by attaching through a linker or functionalized molecule.

FTIR of functionalized CNT showed the introduction of –COOH and –OH group on the surface of CNT; which was thought to be suitable for Doxorubicin attachment by non-covalent bonding.

pH was considered as important parameters for **Loading of Doxorubicin** and UV-VIS Spectroscopy, ATR, FTIR and TEM assessment was chosen as the method for assessing the loading.

When Doxorubicin was added to f-MWCNT suspended in water a change in color from black (of CNT) to orange red was noticed, this was due to the Doxorubicin. After attachment procedure, the Doxorubicin remaining in the solution was assessed by UV-VIS spectrum taken at 490 nm was taken, which revealed that loading occurred at all the 3 tested pH *viz.* 4, 7 and 9 maximum being at neutral pH of 7 (i.e. 82%) and minimum at pH 4 (i.e. 77.5%). The pH-dependent loading may be due to the different degree of hydrogen-bonding interaction between these two species under different pH conditions.

Both ATR and FTIR spectra indicated the loading of Doxorubicin onto CNT. Moreover, the shift of characteristic peaks that appeared in FTIR spectrum suggested it to be due to the hydrogen bonding between these two components. The -OH and -COOH groups on the CNT can form a strong hydrogen-bond with -OH and $–NH_2$ groups in Doxorubicin. Therefore, it can be concluded that Doxorubicin was non-covalently loaded on CNT simply by mixing them in aqueous solution with the aid of sonication.

TEM Analysis of Doxorubicin Loaded on CNT was not a suitable technique because during TE Micrography of Doxorubicin showed deterioration of drug molecule due to electron beams of TEM were bombarded on to them. While taking the TEM micrograph, the structure started to bubble and eventually bursted.

To find out whether adsorption of Doxorubicin on to MWCNT was single layered or multi-layered due to possible interactions within Doxorubicin molecules , Langmuir adsorption isotherm graph was plotted, which showed that adsorption of drug is Multi-layered.

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