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Modifications of Carbon Nanotubes for Bio-Applications and Toxicity Evaluations

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Abstract

Multi Walled Carbon Nanotubes (MWNTs) were procured from Nanoshel, USA. Covalent functionalization was accomplished with three types of oxidants: (1) 70% Nitric acid under reflux (2) Sulfuric acid and Nitric acid (v/v = 3/1) under sonication (3) 20% Sulfuric acid and Nitric acid (v/v = 3/1) by heating. The non covalent functionalization was accomplished via di-imide activated amidation using N-(3-Dimethylaminopropyl)-N2 -ethylcarbodiimide hydrochloride (EDAC) and Bovine Serum Albumin (BSA).The covalent functionlized carbon nanotubes in combination with Non Covalent functionalized Carbon Nanotubes were readily dispersed in water, DMF and cell culture media to investigate their toxicity. Characterization of functionalized and dispersed Nanotubes were carried out by using FTIR, FESEM,and UV-vis spectroscopy.

Keywords : *Multi-walled CNT; Covalent Funtionalization; Non-Covalent Functionalization; Nanotoxicology*.

1. INTRODUCTION

Due to their exceptional electronic, optical, mechanical and chemical properties (Dresselhaus*et al.* 2001), Carbon Nanotubes have become the first choice for various applications, e.g. nanocomposites, nanoelectronics, field emission devices, probe tips, optical filters and biomedical applications like bio-sensors, bio-delivery, bio-imaging, tissue-engineering (Niraj*et al.* 2005). Functionalization and dispersion of these nanotubes is essential for their processing in various applications. They tend to form bundles and agglomerates in solution because of the van der Waals attraction forces between the nanotubes. Various covalent (Christopher *et al*. 2004) and non-covalent (Robert*et al*. 2003) modifications has been applied for functionalization and dispersion of the nanotubes, both in organic and highly polar solvents. The covalent approaches include controlled oxidation with strong acid (Jin *et al.* 2003), organic peroxides (Datsyuk*etal.* 2008), cyclo addition of nitrines (Christopher*et al.*2003), diazonium reaction (Christopher *et al.* 2003), and ball-milling (Yao *et al.* 2003). The covalent functionalization alters the electrical as well as chemical properties of nanotubes. The non-covalent approach includes dispersion of nanotubes in sodium dodecyl sulphate (SDS), sodium dodecyl benzene sulphate (SDBS) (Bystrzejewski*et al.* 2010), Tween surfactant (Valerie*et al.* 2003) ,dimethylformamode (DMF) (Nguyen*et al.* 2011) and Bovine Serum Albumin (Weijie*et al.* 2002). The non-covalent functionalization does not alter the internal electronic structure of the nanotubes. Based on the above background information, we did mild covalent functionalization of MWCNTs using 70% Nitric acid under reflux and 20% Nitric acid and

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sulfuric acid by heating. The non-covalent functionalization was done via di-imide activated amidation using N-(3-Dimethylaminopropyl)-N2-ethylcarbodiimide hydrochloride (EDAC) and Bovine Serum Albumin (BSA). The covalent ***functionlized carbon nanotubes*** in combination with Non Covalent functionalized Carbon Nanotubes were readily dispersed in water, DMF and cell culture media. This study was focused on covalent and non covalent functionalization and characterization of MWCNT using FTIR, FESEM,UV-vis spectroscopy and light microscopy as a pre-requisite for toxicological testing of these nanotubes.

2. EXPERIMENTAL

The Multiwalled Carbon Nanotubes (>95 wt%) were obtained from Nanoshel USA. In the first set of experiment, the pristine Multiwalled Carbon Nanotubes (P-MWCNT) 2.5mg/ml were refluxed under 70% HNO_3 at 120^0C in oil bath for 6 hours (R6-MWCNT), the refluxed MWCNT 10mg/40ml were then sonicated under acid mixture (3:1 ratio of H_2SO_4 and HNO_3) in bath sonicator (350 W, 40 KHz) for 3h (R6S3-MWCNT). In the second set of reaction, the P-MWCNT was sonicated under acid mixture (3:1 ratio of H_2SO_4 and HNO_3)in bath Sonicator (350 W, 40 KHz) for 3 hours and named as S3MWCNT. In the third set of experiment, the Multiwalled Carbon Naotubes 100mg/60ml were heated at 100^0C in 3:1 ratio of 20% H_2SO_4 and 20% HNO_3. The sample was drawn at 10, 20, 40, 60, 80 and 100 min., washed and named as H10-MWCNT, H20-MWCNT, H40-MWCNT, H60-MWCNT, H80-MWCNT and H100-MWCNT. In fourth set of experiment, 35 mg of MWCNT and 155mg of N-(3-Dimethylaminopropyl)-N2 - ethylcarbodiimide hydrochloride (EDAC) were added to 15 ml of 0.1M phosphate buffer saline (pH 7.4) and sonicated for 2 hours. 350 mg of Bovine Serum Albumin (BSA) were added and stirred at room temp for 24 hours. (Weijieet al. 2002). Dialysis against DI water in 12 k membrane tubing for 3 days. Finally centrifuged at 7800 rpm for 10 min to remove undispersed nanotubes and named as PB-MWCNT.

The H-MWCNT were actually H60-MWCNT which were heated for 40-60 min with stirring. All these treated MWCNTs were washed and dried (Yao *et al.* 2003). The appended functional group on MWCNTs was investigated by Fourier Transform Infrared (FTIR) spectroscopy using KBr pellet. The morphology of the MWCNTs were studied by using Field Emission Scanning (FESEM) Electron Microscopy on carbon tape with a thin layer of gold coating.

2.1 Estimation of MWCNT concentration

In order to estimate the concentration of MWCNT in supernatant after centrifugation, a standard curve were established using MWCNT (1mg/ml) dispersed in 1% BSA by stirring. The standard curve was obtained by scanning the sample in a quartz cuvettes using double beam UV-visible spectrophotometer (UV-1800 Shimadzu, Japan) between 250-800 nm range by subtraction method (Attal*et al.* 2006). The measured spectra shows that the intensity of the peak at 318 nm remains unaffected and independent of BSA concentration used in the reference cuvette. The intensity of the peak at 250 nm gradually decreases with increasing concentration of BSA in the reference.

3. RESULT AND DISCUSSION

FTIR spectra of reflux treated multi-walled carbon nanotubes for 6hours R6-MWCNT shows that the nanotubes are not appended well with the functional groups. It is almost similar to pristine multi-walled carbon

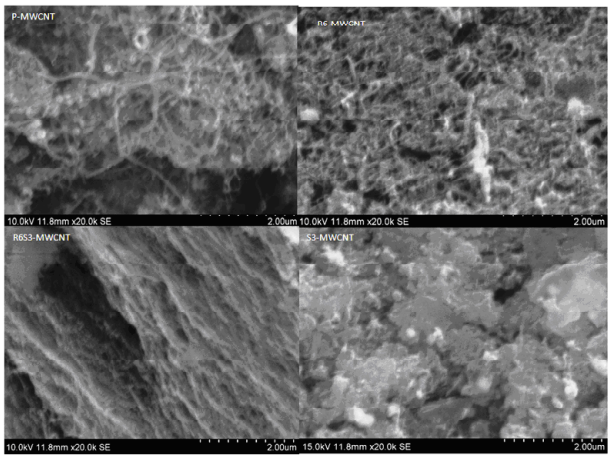

Fig. 1. : SEM images: P-MWCNTs, R6-MWCNTs, R6S3-MWCNTs, S3-MWCNTs

nanotubes P-MWCNT Fig 2. At the same time SEM image also confirms that the R6-MWCNT and P-MWCNT are physically similar except that some breakage in the R6-MWCNT Fig 1.

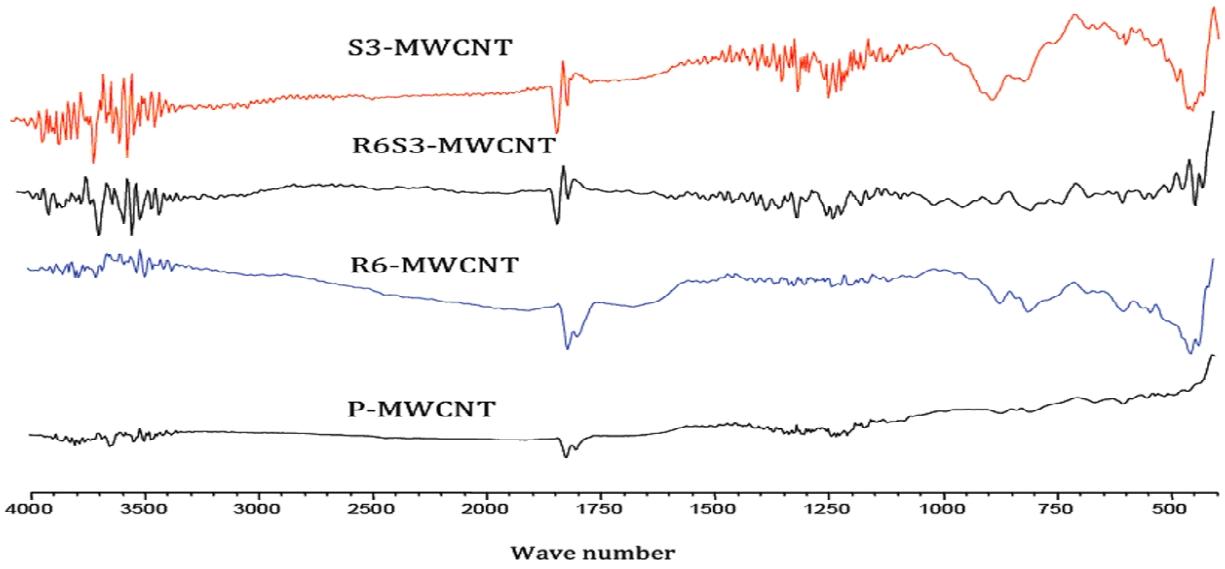

Fig. 2. FTIR spectra: P-MWCNTs, R6-MWCNTs, R6S3-MWCNTs, S3-MWCNTs

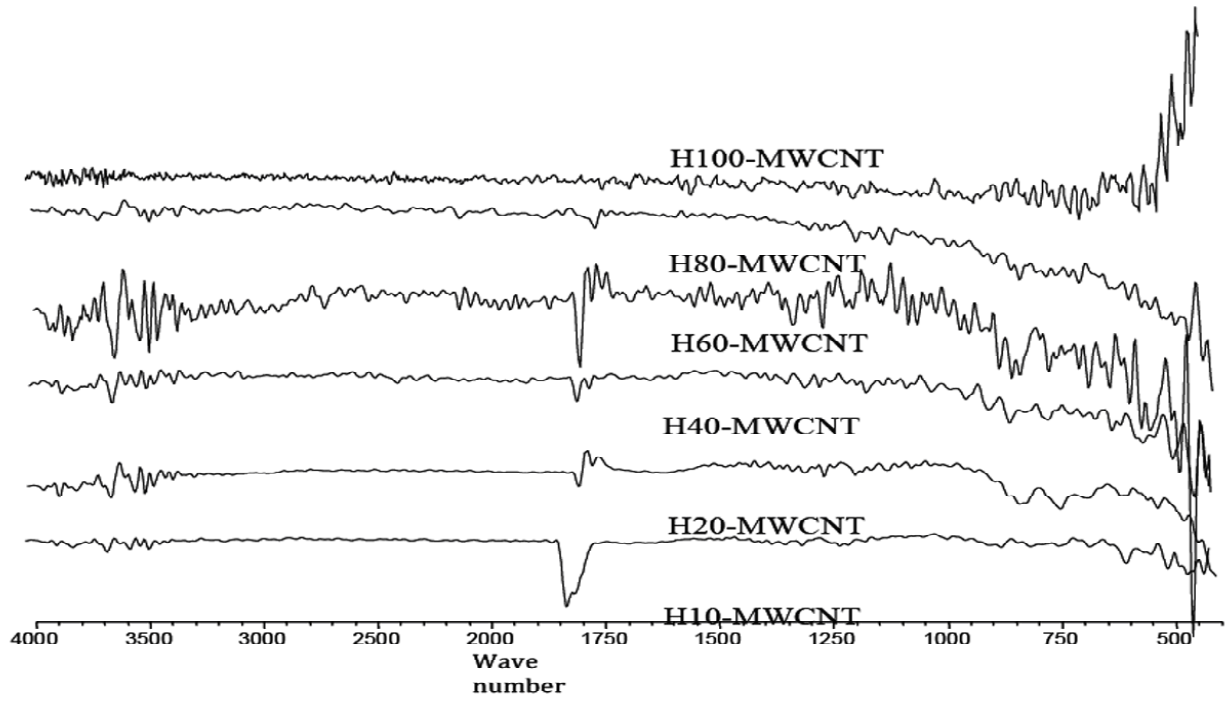

Fig. 3.: FTIR spectra:H10-MWCNTs, H20-MWCNTs, H40-MWCNTs, H60-MWCNTs, H80-MWCNTs, H100-MWCNTs

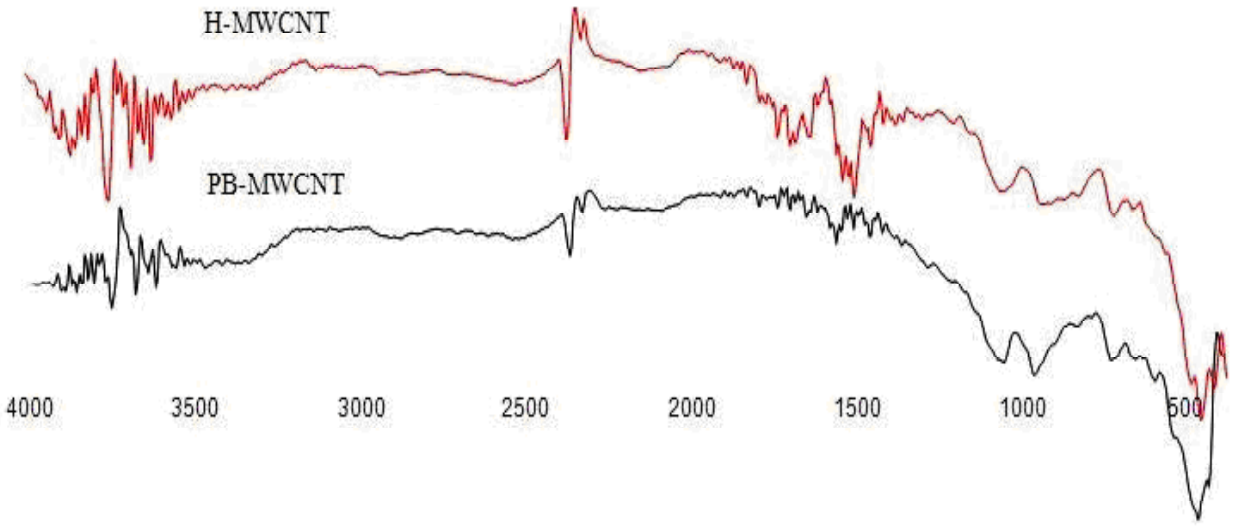

Fig. 4. : FTIR spectra of H-MWCNT and PB-MWCNT

When the 6h reflux treated MWCNT were sonicated for 3h R6S3-MWCNT, the FTIR shows the attachments of functional group Fig 2, but SEM image shows the change in the morphology of the nanotubes Fig. 1. The nanotubes started aligning together. When the muti-walled carbon nanotubes are sonicated for 3hours S3-MWCNT, the FTIR shows that the functional groups are appended Fig. 2, but SEM images revealed that there is a complete damage of the tubes Fig 1. Fig. 3 shows 60 min of heating H60-MWCNT result in well modification of the tubes,more number of functional groups appended to the nanotubes. Whereas heating for 80 min and 100 min caused the reduction of functional group attachments. That may be because of the reduction of surface area of the nanotubes due to breakage. The separation and dispersion of nanotubes primarily depend on the carbon nanotubes functionalization and dispersion methods adopted. Thin-long and curved structure and van der Waals forces are the factors result in the formation of nanotubes agglomerate. Fig 4 shows the di-imidation can well modify the MWCNTs which is readily soluble in water.

A BSA solution containing MWCNT in a concentration of 1mg/ml was used to setup the standard curve as shown in Fig 5. The final concentration of MWCNT in the supernatant was 580 µg/ml.

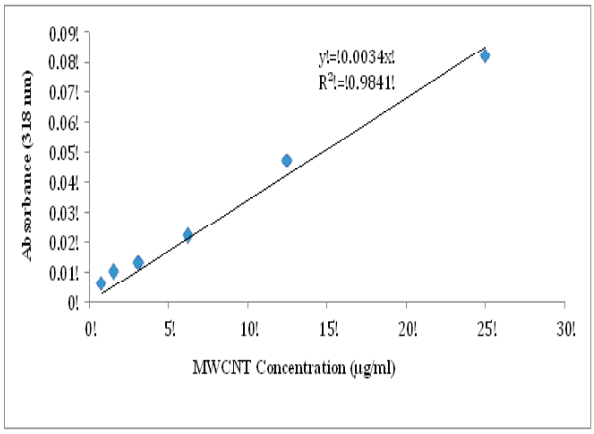

Fig. 5. : Absorbance standard curve established from a suspension of MWCNT dispersed in BSA 1%

4. CONCLUSION

The pristine carbon nanotubes entangled into aggregates of up to a few tens of mm. Entangled CNTs are difficult to disperse without damage but damage can be minimized by mild treatment. Reflux treatment of MWCNTs in 70% HNO_3 at 120^{0}C can be considered as the mild oxidative treatment with less damage. On the other hand, 60 min. heating of the MWCNTs at 100 ^{0}C in 3:1 ratio of 20% H_2SO_4 and 20% HNO_3 can also be the alternative option for the mild treatment of carbon nanotubes. Even one more alternative method we have tested for dispersion of MWCNTs i.e. bi-functionalization of nanotubes using covalent functionalization is by heating of the MWCNTs at 100 ^{0}C in 3:1 ratio of 20% H_2SO_4 and 20% HNO_3 in combination with non-covalent functionalization (di-imide activated amidation). We found that bi-functionalization is better than the above mentioned treatment methods. It leads to better modifications and well dispersion in water. Since these modified carbon nanotubes are readily dispersed in water, and the culture media, they can be used for their toxicity testing and other bio-applications.

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