
SUN-SNO-GUIDENANO Sustainable Nanotechnology Conference
9-11th March 2015, Venice

Ecotoxicity effects of multi walled carbon nanotubes

C. Piller¹, E. Gunter-Hoch¹, F. Ben-Ami¹, N. Vlachou², D. Perivoliotis², A.F. Trompeta², A. Skarmoutsou², Y. Benayahu^{1,*}, C.A. Charitidis²

¹ Department of Zoology, Tel Aviv University, Ramat Aviv, Tel Aviv, Israel

² Research Unit of Advanced, Composite, Nano Materials & Nanotechnology, School of Chemical Engineering, National Technical University of Athens, 9 Heroon Polytechniou St., Zografos, 15780, Athens, Greece

*yehudab@tauex.tau.ac.il

Abstract

Now-a-days the need for the manufacturing of engineered nanomaterials increases due to their performance, efficiency and decrease of total weigh in the applications they are used. However, not always large scale manufacturers or even small scale pilot lines established in regional laboratories follow regulations/limitations for the disposal of engineered nanomaterials waste. Consequently, there is a great possibility of polluting water that will eventually result in the effect on ecosystems micro and macro-organisms. In this study we will present the effect of carbon nanotubes (CNTs), one of the most widely studied engineered- nanomaterial on the water fleas, unicellular algae some macrofoulers. CNTs were synthesized via a thermal chemical deposition process, characterized and functionalized with different end groups to render them less toxic. CNTs length was measured ~5 μm and diameter ranged between 60-100 nm. Functionalization with different surfactants was conducted in order to render CNTs dispersal in polar and non-polar solvents and their effect on living organisms was assessed so as to estimate the potential effect in ecotoxicity and environment.

1 INTRODUCTION

Carbon nanotubes (CNTs) are one of the most appealing nanomaterials with unique physicochemical, mechanical, and electrical properties that find application in many different fields. The most widely used technique for CNTs synthesis is thermal chemical vapor deposition (T-CVD). As carbon precursors, solid, liquid or gas carbon sources such as CH_4 , C_2H_4 , C_2H_2 , C_6H_6 , $\text{C}_2\text{H}_5\text{OH}$ and camphor ($\text{C}_{10}\text{H}_{16}\text{O}$) can be used. As catalysts, typically, nanometer-size metal particles are used, in order to enable carbon source decomposition, leading in carbon nanotubes growth. The most commonly-used metals are Fe, Co, and Ni due to their high catalytic activity and relatively low reaction temperature. Camphor is considered an ideal carbon precursor to produce nanotubes due to its hexagonal and pentagonal carbon ring structure. Also, ferrocene as catalyst compound is a good precursor for the production of iron nanoparticles playing the role of catalyst in the formation of nanotubes. By this method both multi-wall CNTs (MWCNTs) and single-wall CNTs (SWCNTs) can be produced, depending on the temperature and other growth parameters (flow rate, pressure, catalyst concentration, etc.). The main advantage of CVD method is the large quantities of material that it is possible to produce, rendering this method the first candidate for industrial applications. However, impurities such as amorphous carbon, graphite nanoparticles and metal catalysts should be removed from the product. As a result, CNTs need purification that includes acidic treatment. The purification can also work as functionalization in some cases, providing to CNTs the desirable functional groups that improve their properties such as dispersibility. Dispersion of CNTs seems to be challenging, since the attractive forces between the aggregates, increase the possibility of nanotube entanglement and close packing. Nanotubes assemble into bundles or ropes, which contain hundreds of close-packed CNT tightly bound by van der Waals bonds. The dispersibility of CNTs in either water or organic solvents may be necessary for their chemical and physical examination [1-4].

However, CNTs may result in significant cell damage due to these unique properties that they present and especially due to their size that could be at the range of nanometers. Therefore, it is very important to understand the possibility of their effects in humans and other living organisms before CNTs are widely used in commercial products. Environments such as workplaces in which nanomaterial synthesis and storage may be considered unhealthy as CNTs have been reported to be associated with skin and lung diseases. Moreover, the disposal sites of such materials are also possible sources of exposure. Microorganisms that participate in the materials' decomposition are in the first line. It is important, therefore, to study the possibility of CNTs' having any antimicrobial effects as any disturbance of the ecosystem may cause worse impacts. Assessment of the risks and hazards of such materials is therefore urgent and needs to be comprehensively investigated despite the difficulties that may exist due to the many factors that ought to be taken into account and the complexity of such an investigation. The first factors in range that should be investigated is the length and the diameter of CNTs as it has been previously observed that the smaller the size the more interactions, such as cellular membrane disruption, exist [5].

2 EXPERIMENTAL PROCEDURE

2.1 *Synthesis of carbon nanotubes*

The growth of multi-wall carbon nanotubes (MWCNTs) was obtained by chemical vapor deposition (CVD) process. The CVD reactor consisted of a horizontal quartz tube (106 cm long, 3.6cm in diameter) housed in a cylindrical furnace 80 cm long, in which a constant nitrogen gas flow rate of 380 ml/min was maintained at atmospheric pressure. A pyrex flask containing the reagent mixture that composed by camphor (96% purity in weight, Aldrich) as carbon precursor and ferrocene (98% purity in weight, Aldrich) as catalyst compound in a 20/1 mass ratio was connected to the tube nearby the nitrogen inlet. A heater plate was located below the flask, to achieve the heating and sublimation of the reactants. Nitrogen gas flow was used to carry the gas mixture of precursors towards the center of the furnace, where pyrolysis of the gases took place at 850°C and carpets of CNTs were deposited on silicon wafers substrates. When the reaction was completed, the raw products were cooled down to room temperature in N₂ atmosphere.

2.2 *Carbon nanotubes purification and functionalization process*

After the synthesis, the raw products were milled and exposed at atmospheric air flow at 400°C for 1h, aiming at the removal of amorphous carbon. Afterwards, they were purified with constant boiling 5M HCl in a Soxhlet extractor in order to remove the remaining metal particles. Finally, the purified CNTs were washed with distilled water and dried in an oven. To activate the CNTs surface with –COOH groups, an acid solution mixture of 6M HNO₃:H₂SO₄ 1:3 was used. Then, the CNT/acid mixture (0.15 g CNTs/10 ml acid solution) was stirred for 48h at 80°C. The suspension was filtered and the black powder deposited on the filter is washed with distilled water, then with ethanol and acetone and dried in oven.

2.2.1 CNTs dispersions in different solvents

CNTs dispersion in different solvents was achieved using an ultrasonic bath filled with tap water, which operates at 37.5 kHz. The ultrasonic bath is programmed for 5min degassing and 1h ultrasonication. The samples were placed in test tubes which were immersed in the ultrasonic bath.

2.3 *Characterization techniques*

X-ray diffraction (XRD): measurements were performed at room temperature with Bruker D8 Advance Twin X-ray diffractometer equipped with a Cu K_α radiation source (wavelength = 1.5418 Å).

Thermogravimetric analysis: TGA experiments were conducted in oxidative atmosphere (atmospheric air flow: 120mL/min, heating rate: 5° C/min) at a Netzsch 409 EP instrument.

Scanning electron microscopy (SEM): morphology of CNTs was determined by SEM using Nova NanoSEM 230 (FEI company) microscope with W (tungsten) filament.

Transmission electron microscopy: TEM measurements were performed with a Tecnai G2 Spirit Twin 12 microscope (FEI) after the dispersion of CNTs in distilled water.

X-Ray photoelectron spectroscopy: XPS measurements were performed using a K-Alpha, Monochromated high-performance XPS spectrometer (Thermo Fisher Scientific).

Fourier transform infrared spectroscopy: FT-IR analysis was performed by using a ThermoScientific Nicolet 6700 Fourier Transform Infrared Spectrometer.

2.4 *Antibacterial activity*

After the synthesis and characterization of CNTs, their antibacterial activity was evaluated. *E. coli* was used as a microorganism model. The bacteria were propagated in Luria-Bertani (LB) medium at 37°C with shaking at 150 rpm until the OD₅₉₅ reached 0.5. OD₅₉₅ measurements were performed in an ultraviolet-visible

(Uv-Vis) spectrometer V-630iRM (JASCO) at 600 nm wavelength. Then, 2.5 % v/v of the bacterial culture was transferred into a 150 ml conical flask and mixed with non-functionalized MWCNTs to a final concentration of 0.0002 g / 100 ml, 0.0007 g / 100 ml, 0.0015 g / 100 ml and 0.0075 g / 100 ml. During incubation at temperature 37 °C with shaking at 120 rpm, OD₅₉₅ was measured every 30 min in order to observe the growing procedure. Then, 100 ml of the mixture from the lower concentration were plated on LB agar and the viable bacterial count enumerated after incubation of the plates for 16 h. Experiments were repeated five times.

3 RESULTS AND DISCUSSION

3.1 CNTs characterization

After the synthesis, purification and functionalization of CNTs, the structure, chemical composition and purity degree was investigated. In Figures 1a-d the XRD spectrum, SEM image, TEM analysis and TG graph of the produced CNTs are respectively presented. The main features of XRD patterns of CNTs are close to those of graphite (Figure 1a); a typical XRD pattern consisting of a few broad bands located near the (002), (100) and (110) reflections of graphite. The first peak at $2\theta \sim 26^\circ$ can be attributed to the (002) reflection of graphite while an asymmetric diffraction peak at $2\theta \sim 43^\circ$ is assigned to (100) reflection of graphite, which is typically observed for MWCNTs. The morphology of the produced carbonaceous materials was examined by SEM and TEM. In Figure 1b and c, the SEM and TEM images, respectively, of the produced carbonaceous materials at different magnifications are presented. The results revealed that the produced material consists mainly of hollow filamentous structures (MWCNTs) with diameter distribution between 60-100 nm. Thermogravimetric analysis (TGA) is used in order to evaluate the thermal stability, carbon content and the purity degree of the produced CNTs (Figure 1d). The initial weight loss of 1.5% observed at temperatures up to 450°C could be assigned to the burning of amorphous carbon material. The residual weight % at the end of the thermal oxidative curve was 5.90 and corresponds to the iron catalyst particles. The differential thermogravimetric analysis (DTA) curve showed only one narrow peak at 560°C indicating the high thermal stability in air atmosphere and the uniform graphitized structure of the CNTs produced. The overall purity of the material is around 92.6 wt. %.

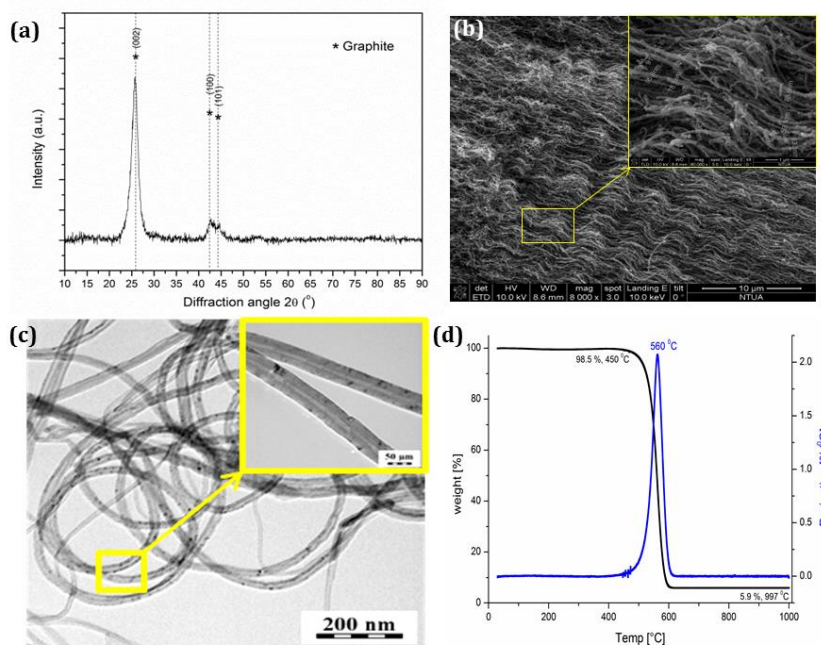


Figure 1 (a) XRD spectrum, (b) SEM image at 8000 and 60000 magnification, (c) TEM image and (d) TG analysis of the produced CNTs.

X-Ray Photoelectron Spectroscopy (XPS) and FT-IR analysis were used to study the effectiveness of the functionalization process. Table 1 presents that oxygen containing groups were successfully introduced (13.4% at.) in the CNTs' sidewalls. This result is also confirmed by the FT-IR spectra, where the characteristic peak of C=O bond at $\sim 1700\text{ cm}^{-1}$ is observed for the functionalized CNTs (Figure 2). The

presence of oxygen-containing groups facilitates the exfoliation of CNT bundles, and increases the solubility in polar media.

Table 1: XPS analysis of pristine and functionalized with oxygen containing groups

Chemical Bond	Pristine CNTs	Functionalized CNTs with -COOH
C-C (sp ³)	7.6	15.9
C=C (sp ²)	81.5	66.2
C-O	2.8	6.3
C=O	1.6	2.6
COOH		4.5

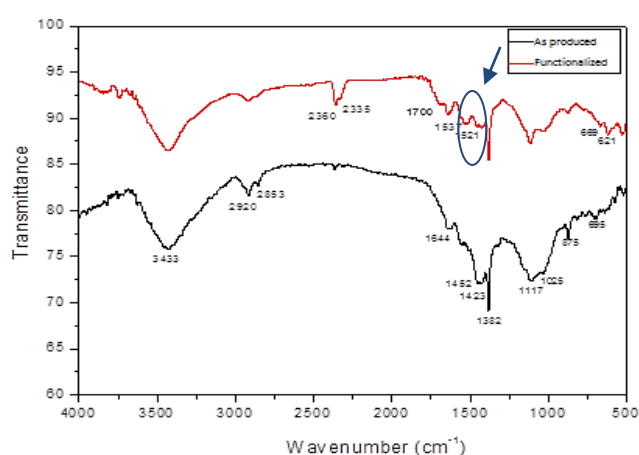


Figure 2 FT-IR spectra for pristine and functionalized CNTs.

3.2 Antibacterial testing results

Firstly, it was studied the effect of CNTs on 2.5 % v/v *E. coli* population¹ in Luria-Bertani (LB) medium and the growth curves were obtained, as shown in the Figure 3. It was observed that increasing the concentration of CNTs their dispersion in the medium was difficult, although the mixture was at continuous shaking. In addition, the higher concentration had as a result lower growth rate (μ_{max}), indicating the CNTs' obstruction to the bacterial growth. The smaller tested concentration that presented larger growth rate compared to the higher concentrated LB-CNTs medium, were plated on LB agar, and the viable bacterial count were enumerated after incubation of the plates at 37 °C for 24 h. The quantitative plating results after direct effect of CNTs on *E. coli* viability deposited onto LB – Agar plate are shown in Figure 4. It is observed that the lower concentration (0.0002g/100mL) presented prevention of the colonies' development. Generally, it was observed that the higher the concentration of CNTs, the lower interactions with the bacteria, probably due to the creation of agglomerates.

¹ We also studied the 0.5 %, 1 % and 2% but for the 2.5 % the obtained results of lag phase were desirable, i.e. smaller time values of lag phase. For the other tested *E. Coli* concentrations the lag phase was larger; hence, we continued our experiments with 2.5% *E. coli* solution from the initial LB medium.

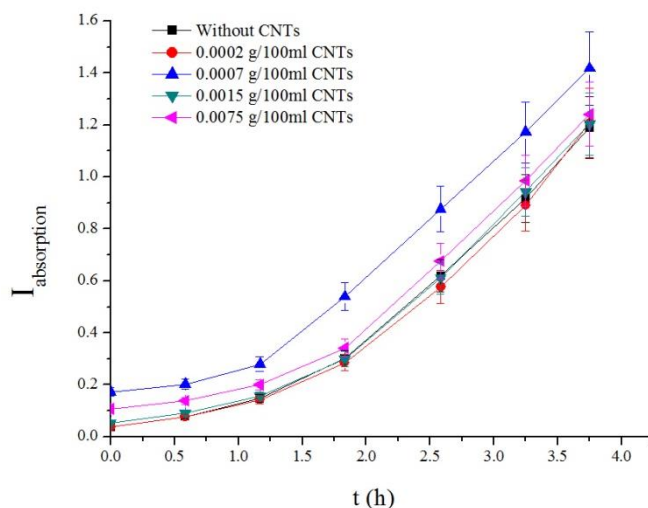


Figure 3 Growth curves after incubation of *E. Coli* (a) without CNTs (b) with 0.0002 g / 100ml MWCNTs (c) with 0.0007 g / 100ml MWCNTs (d) with 0.0015 g / 100ml MWCNTs and with 0.0075 g / 100ml MWCNTs.

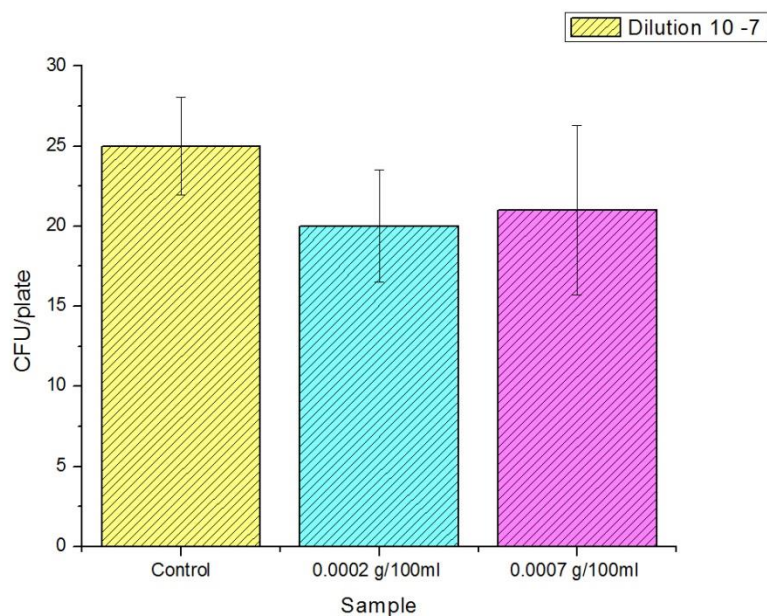


Figure 4 Results of quantitative plating after direct effect of CNTs on *E. coli* viability deposited onto LB – Agar plate. (The original bacterial concentration is 1.150×10^7 CFU ml⁻¹)

4 SUMMARY

The technical specifications of the produced CNTs are illustrated in Table 2. Main conclusions of the study are the following:

- ❑ **Multi Walled Carbon Nanotubes (MWCNTs)** were synthesized via **Chemical Vapor Deposition Method (CVD)** and **fully characterized** via Scanning Electron Microscopy (SEM), Transmittance Electron Microscopy (TEM), X-Ray Diffraction (XRD) and Thermogravimetric Analysis (TGA).
- ❑ MWCNTs with the **following characteristics** were produced:
 - 60 – 100 nm diameter
 - > 5 μ m length
 - 92 – 95 % purity
- ❑ The produced MWCNTs were **further purified** and **functionalized with oxygen containing groups** via chemical treatment with a mixture of strong acids in order to increase their **compatibility** with different solvents and matrices.
- ❑ FT-IR and X-Ray Photoelectron Spectroscopy results show that **oxygen containing groups (carboxyl groups) have successfully introduced** (13.4% at.) onto MWCNTs sidewalls.
- ❑ By increasing the concentration of CNTs it was quite difficult to fully disperse the CNTs, although the mixture (CNTs & *E. Coli*) was at continuous shaking (120 rpm).
- ❑ The sample with the higher CNTs concentration had lower growth rate (μ_{max}), indicating CNTs' low bacterial growth.
- ❑ The quantitative plating results indicated that the lower CNTs concentration appeared to prevent the colonies' development.
- ❑ The higher the concentration of CNTs, the lower interactions with the bacteria, probably due to the creation of agglomerates.

Table 2: Technical specifications of produced CNTs

Product	70 g MWCNTs
Type	Functionalized MWCNTs with -COOH
Form	Black powder
Purity (%)	93 – 95
External diameter (nm)	60 – 100
Length (μm)	~ 5
Dispersion	1mg MWCNTs/ml solvent after 1h ultrasonication
Toxicity (<i>E. coli</i> culture)	12.4 μ g/ml

5 REFERENCES

1. S. Porro, S. Musso, M. Vinante, L. Vanzetti, M. Anderle, F. Trotta, A. Tagliaferro, *Physica E* **37** (2007) 58–61
2. M. Kumar, Y. Ando, *Chem. Phys. Lett.* **374** (2003) 521
3. S. Musso, G. Fanchini, A. Tagliaferro, *Diamond Rel. Mater.* **14** (2005) 784
4. S. Porro, S. Musso, M. Giorcelli, A. Tagliaferro, S.H. Dalal, K.B.K. Teo, D.A. Jefferson, W.I. Milne, *J. Non-Cryst. Solids* **352** (2006) 1310
5. Y.-F. Young, H.-J. Lee, Y.-S. Shen, S.-H. Tseng, C.-Y. Lee, N.-H. Tai, H.-Y. Chang, *Mater. Chem. Phys.* **134** (2012) 279-286