Functionalization, characterization, and antibacterial activity of single wall and multi wall carbon nanotubes

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Abstract
The raw-SWNTs and raw-MWNTs were chemically oxidized with a mixture of sulfuric acid and nitric acid under ultrasonic vibration. The functionalized-SWNTs (F-SWNTs) and functionalized-MWNTs (F-MWNTs) were characterized by using ultra violet-visible spectrophotometer, X-ray diffraction, and field emission scanning electron microscopy (FESEM). The antibacterial efficiency of SWNTs and MWNTs towards Escherichia coli (E. coli) as gram-negative bacteria was evaluated via viable count method and fluorescence microscopy. The results of viable count method showed that the SWNTs and MWNTs have higher inhibitory effect after being treated with H₂SO₄/HNO₃. The E. coli images under fluorescence microscopy exhibited that almost red color for dead cells, which confirms the efficient lethal ability of F-SWNTs and F-MWNTs.

Keywords: Carbon nanotubes, functionalization process, The antibacterial activity of CNTs, fluorescence microscope, pathogenic cells.

1. Introduction
Carbon nanotubes (CNTs) is one of the most useful substance as antibacterial agent due to its phenomenal properties such as high surface area, high microbial adsorption ability compared with other adsorbent materials, and high bactericidal ability comes from its tubular shape and presence of metals used as catalyzed in production procedure of CNTs [1,2]. In previous studies, CNTs have been applied by researchers on broad range of bacteria, both Gram negative and Gram positive [3,4]. But, one crucial parameter extensively influences on antibacterial activity of CNTs, which is the poor dispersion stability in many solvents [5]. The functionalization process of CNTs based on chemical treatment following by ultrasonic vibration can be utilized to increase dispersion stability of CNTs suspension in the solvent [6]. Using of aggressive acids leading to introduce oxygen groups with negative charge, which separate tubes aggregation from each other due to reducing the Van der Waals force.
between tubes [7]. In the present work, single wall nanotubes and multi wall nanotubes were functionalized with the mixture of H$_2$SO$_4$/NHO$_3$ and characterized by UV-vis spectroscopy, XRD, and FESEM. The antibacterial activity of CNTs against *E. coli* was assessed and discussed.

2. Experimental work

2.1. Chemicals

SWNTs (purity $\sim$99 wt%; diameter $\sim$1-6 nm; length $\sim$0.5-3 $\mu$m) and MWNTs (purity $>$95 wt%; diameter $\sim$7–16 nm; length $\sim$12-50 $\mu$m) were provided from (cheaptubes.com, Grafton, USA). Other chemicals includes anhydrous ethanol (C$_2$H$_5$OH, 99.9%), sulfuric acid (H$_2$SO$_4$, 95%), and nitric acid (HNO$_3$, 65%) were purchased from Sigma-Aldrich.

2.2. Functionalization of SWNTs and MWNTs

One gram of raw-SWNTs was oxidized with 100 ml in a 3:1 mixture of 65% H$_2$SO$_4$/HNO$_3$ to generate OH- and/or COOH functionalized-SWNTs (F-SWNTs). Then, the solution was placed in an ultrasonic device at 40 °C for 30 min, and diluted with deionized water, filtered through a 0.22 $\mu$m cellulose nitrate membrane, and dried at 120 °C for 14 h. The same step was repeated for raw-MWNTs to prepare functionalized-MWNTs (F-MWNTs).

2.3. Viable count method

The viable cell was assessed with the traditional count method [8]. The *E. coli* were seeded on Mueller-Hinton media at 37 °C for one day. The samples (raw-SWNTs, raw-MWNTs, F-SWNTs, and F-MWNTs) with concentration 1mg/ml were dispersion in 9 ml of saline and sterilized in an autoclave at 121 °C for 40 min [9,10]. Bacterial solution was adjusted to obtain cell samples with initial concentration about $10^7$ CFU/ml and fit with the McFarland turbidity solution. After that, 1ml of bacterial solution was added into the samples and kept in a shaker incubator at 37 °C (160 rpm) for 40 min. The solution of 1ml of samples-cell was serially diluted with saline (1:1000) [11,12]. The viable cell in each plate was assessed by spreading of 100 $\mu$l of the dilution onto Nutrient media plate for incubation at 37 °C. After 24 h, colonies on each plate were measured and viable cell number was evaluated as colony forming-units per milliliter (CFU/ml) [13,14].

2.4. Fluorescence imaging

The fluorescence microscope was utilized to image the dead and live cells after treating with CNTs. This done with acridine orange/ethidium bromide (AO/BO) dual staining[15,16]. At first, 40$\mu$l of cells-samples solution was added with 40 $\mu$l of the (AO/BO) fluorescent dye at
concentration 20 μg/ml for 10 min. Afterwards, a drop of the solution was spread on a glass slide, and fluorescent images were taken with the fluorescence microscopy [17].

3. Results and discussion

3.1. Optical properties

The optical properties of F-SWNTs and F-MWNTsdissolved in ethanol were measured as a function of wavelength in the range of 200 to 600 nm as depicted in figure 1. The samples show a feature absorption band located at highest intensity of spectra, which are assigned with π–π* transition of CNTs structure. The functionalization of CNTs causes red shift compared with raw-CNTs due to the electronic interaction of molecular orbitals of oxygen groups with CNTs, making a new molecular orbital and decreasing the band difference [18].

![UV-Vis spectra for F-SWNTs and F-MWNTs](image)

**Figure 1.** UV-Vis spectra for F-SWNTs and F-MWNTs

3.2. Structural properties

Figure 2 exhibits XRD patterns of F-SWNTs and F-MWNTs. The high dominate peaks located at 2θ = 25.68° for F-SWNTs and 2θ = 25.78° for F-MWNTs, can be ascribed to the (002) diffraction plane, which are match well with the graphite structure (JCPDS no. 01-0646). The weak intense peaks centered at 2θ = 43.96° for F-SWNTs and 2θ = 43.54° for F-MWNTs are characteristic peaks for the (100) diffraction plane. Since, the structure of SWNTs and MWNTs is protected even after undergoing the acid treatment. Besides, the XRD pattern of Raw-SWNTs shows broad diffraction peak at 2θ = 25.68° while narrow broadening peak at 2θ = 25.78° due to the small interlayer distance of 3.5 Å in SWNTs as compared to MWNTs [19].
3.3. Morphological properties

The morphological properties of F-SWNTs and F-MWNTs were characterized via FESEM analyses. Figures 3 (a) and (b) shows FESEM micrograph of raw MWNTs and F-MWNTs, respectively. While, Figures 3 (c) and (d) display FESEM micrograph of raw-SWNTs and F-SWNTs, respectively. It can be easily found that raw-MWNTs and raw-SWNTs images show aggregated nanotubes like bundles, due to Van der Waals force exists betweenthem. The F-MWNTs and F-SWNTs show less aggregates and tangled clusters with shorter tubes and more defective sites were found on the surface compared with than R-MWNTs and R-SWNTs.

Figure 2. XRD pattern for F-SWNTs and F-MWNTs.

Figure 3. FESEM images for a) R-MWNTs, b) F-MWNTs, c) R-SWNTs, and d) F-SWNTs
3.4. Antibacterial activity

The Antibacterial activity was done using the viable count method. At first, the antibacterial activity of the R-SWNTs and R-MWNTs alone was tested against *E. coli* as shown in Figures 4 (a) and (b). Both R-SWNTs and R-MWNTs were inefficient in killing the *E. coli* at 1 mg/mL after incubation. On the other hand, the number of *E. coli* colonies of decreases significantly after being treated with F-SWNTs and F-MWNTs as demonstrated in Figure 4 (a) and (b). This is causing by effecting of oxygen functional groups. The mechanism of CNTs bactericidal ability is mostly affected by length, residual catalyst, electronic structure, surface functional group, and surface chemistry [20,21,22]. Essentially, the length of CNTs is important during interactions with the bacteria membrane. Longer tubes have shown higher antibacterial activity than shorter ones. The short length of tubes are more likely to self-aggregate without including a large number of bacterial cells, while longer tube agglomerates are more bio-effective as they affect a larger number of cells in the aggregates as shown in Figure 5.

![Figure 4](image)

*Figure 4.* Survival *E. coli* cells incubated with a) R-MWNTs, b) R-SWNTs, c) F-MWNTs, and d) F-SWNTs.

![Figure 5](image)

*Figure 5.* *E. coli* viable number after incubated with R-MWNTs, R-SWNTs, F-MWNTs, and F-SWNTs.

Besides, in Figure (5) revealed that the R-SWNTs (before acid treatment) and F-SWNTs (after acid treatment) possessed higher antibacterial activity towards *E. colis* bacterial strains compared with F-MWNTs as well as R-MWNTs. These results were attributed to the physical interaction with the cell membrane, the large formation of cell-samples aggregates, and disruption of membrane function, hence resulting in cell death.
Figure 6 shows fluorescence microscope images of the *E. coli* cells after being treated with F-SWNTs and F-MWNTs. It can be seen that fluorescence microscopy image of the distinctive bactericidal action was displayed with almost all the dead cells with red color [23,24,25], which indicated F-SWNTs and F-MWNTs have a potential application as an antibacterial agent for inactivating pathogenic cells.

![Fluorescence images of E. coli incubated with a) F-MWNTs and d) F-SWNTs.](image)

**Figure 6.** Fluorescence images of *E. coli* incubated with a) F-MWNTs and d) F-SWNTs.

4. Conclusions

The raw-SWNTs and raw-MWNTs have been successfully functionalized by the mixture of sulfuric acid and nitric acid. The UV-Vis spectroscopy showed that the characteristic absorption band for SWNTs and MWNTs due to $\pi-\pi^*$ transition. The SWNTs and MWNTs hexagonal structure was confirmed by XRD pattern. The tube-like structure of SWNTs and MWNTs was successfully verified by FESEM images. Finally, the study demonstrated that the F-SWNTs and F-MWNTs have higher antibacterial performance in comparison with R-SWNTs and R-MWNTs.

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