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Antibacterial Activity of Treated- Multiwall Carbon Nan tubes and Their Characterization

Dr. M. R.Mohammed
Nanotechnology Advanced Material Research Center/ Baghdad
Dr. Adawiya J. Haider
Nanotechnology Advanced Material Research Center/ Baghdad
Email: adawiya_haider@yahoo.com ,
Duha S. Ahmed
Applied Physics Department University of Technology/ Baghdad

ABSTRACT

In this study, multiwall carbon annotates (MWNTs) were modified with concentrated sulfuric acid and nitric acid. The activity of f-MWCNTs toward the removal and inactivation of bacteria Gram negative Bacteria (E.coli) were examined. Fourier transformed infrared spectroscopy (FTIR) shows formation of oxygen containing groups such as C=O and COOH. Scanning electron microscopy (SEM) was used to characterize the treated -MWCNTs structure due to oxidation during acid treatment. Moreover, the treated MWCNTs exhibited antimicrobial activities towards E.coli using viable cell technique. Finally, these observations point to the potential use of MWNTs as building blocks for antimicrobial materials.

Keywords: Antibacterial, Carbon Annotates, Antimicrobial Activity, Fictionalization

الفعالية المضادة للكاربون النانوي المتعدد الجدران المعامل بالاحماض الكيمياوية و وخصائصها

الخلاصة

في هذه الدراسة تم تحسين مواصفات الكاربون النانوي المتعدد الجدران MWNTs عن طريق معاملته بالاحماض المركزة (حامض الكبريتك وحامض النتريك).وان فعالية الكاربون النانوي المتعدد الجدران MWNTs باتجاه اخماد او ازالة بكتريا ذات الصبغة السالبة *E*.coli تم فحصها. مطياف الاشعة تحت الحمراء (FTIR) اظهرتكون مجاميع فعالة مثل C=Oو COOH . استخدام المجهر الماسح الالكتروني (SEM) لدراسة خصائص تركيب MWNTs المعامل نتيجة الاكسدة خلال المعاملة بالاحماض.بالاضافة الى انه MWNTs المعامل اظهر فعالية بكترية مضادة باتجاه بكتريا *E*.coli باستخاد تقنية العد.واخير فان هذه الملاحظات تشير الى استخدام MWNTs كعوائق ضد المواد الجرثومية .

INTRODUCTION

Since the discovery of carbon nanotubes (CNTs), they have eventually revolutionized the future nanotechnologies area. CNTs as reported by Iijima (1991) and Bethune et al. (1993), are seamless cylinder-shaped macromolecules with a radius as small as a few nanometers, and up to several micrometers in length. The walls of these tubes are constructed of a hexagonal lattice of carbon atoms and capped by fullerene-like structures. The unique structure of CNTs can be divided mainly into multiwalled carbon nanotubes (MWCNTs) and single walled carbon nanotubes (SWCNTs). MWCNTs are composed of two or more concentric cylindrical shells of graphene sheets coaxially arranged around a central hollow area with spacing between the layers. In contrast, SWCNTs are made of a single cylinder graphite sheet held together by van der Waals bonds [1].

Since unfunctionalized CNTs are extremely hydrophobic and tend to aggregate quickly in aqueous solutions, one of the key strategies to enhance the colloidal stability of CNTs is to covalently attach either charged or hydrophilic functional groups on the surfaces of CNTs through chemical treatment. A popular approach is to expose the CNTs to strong oxidants or concentrated acids in order to create oxygen-containing functional groups, such as carboxyl and hydroxyl groups, on the sidewalls and tube ends of CNTs [2-3].

Antimicrobial materials are designed to kill, or at least prevent the growth of, microbial species (e.g. bacteria), and are beginning to make an impact on public health. With the rapid development of new biomedical devices and implants, antimicrobial surfaces are particularly important [4]. The antimicrobial activity of carbon nanotubes was first reported by Kang et al. [5], which considered aggregates of single walled carbon nanotubes (SWNT) in aqueous solution [5-8].

The objective of this study was to investigate the antimicrobial activity of different concentrations of functionalization MWCNTs with strong oxidizing agents improves dispersivity in aqueous solutions and mechanism of MWCNTs using samples inoculated with Escherichia coli (*E.coli*) and proved to destroy the bacteria successfully. The samples of functionalization MWCNTs were characterized by SEM and FTIR and the results of antimicrobial activities to bacterial cells (Escherichia coli) showed that the F-MWNTs of different concentrations have antimicrobial activity.

EXPERIMENTAL WORK

Functionalized of MWCNT-COOH

MWCNTs (0.1 g) were dispersed in mixture of concentrated sulfuric acid 95% H_2SO_4 and nitric acid 65%HNO_3 (3:1) under ultrasonication technique for 30 min to produce oxidized carbon nanotubes (MWCNT-COOH) . The samples were washed with deionized water (D.I) and dried at 70 °C for 24 h.

Characterization of treated MWCNTs and its Antimicrobial activities.

The surface morphology of raw and acid treated-MWNTs (F-MWNTs) is studied with scanning electron microscopy (SEM, The VEGA EasyProbe). Fourier transform infrared spectroscopy (FTIR, 8400S, Shimadzu) is used to study the attached functional groups on the MWNTs surfaces. In addition, the morphology of Gram negative Bacteria attachment on MWCNTs after chemical treatment can be studied by using SEM.

Antimicrobial activities of acid treated MWNTs

The Escherichia coli (*E*.coli) strain was kindly provided by lab of biotechnology-department of applied science, University of Technology - Baghdad, by using Muller –Hinton agar plates were seeded with tenth ml of suspensions of activated bacterial isolated separately. The bacterial suspension should be equal to McFarland solution, and using as control that equal to 10^7 CFU/ml. Some concentrations of MWNTs (1 ,3,7,9,12,15 mg/ml) were dispersed in 9mL of 0.9% NaCl, and then 1mL of the diluted cell suspensions was added, after that the mixed solution were kept in a shaker incubator at 37 ◦C (160 rpm) for(6-8h).Then one hundred microliters of the above mixed solution, with different active material concentrations, was spread on solid agar plates and incubated overnight at 37 ◦C, which needed to be sterilized at 120 ◦C for 15 min before inoculation, and the number of colonies was counted. Antimicrobial material free solid agar plates were used as control.

RESULTS AND DISCUSSION

Characterization studies of functionalized MWCNTs

FTIR spectroscope has been used to study the attached functional groups on treated (f-MWCNT) surfaces and chemical distribution of carbon nanotubes MWCNTs and functionalized (f-MWCNT) mixed with dried KBr powder and pressed to form the semitransparent pellets. The spectra were recorded in absorption, in range of 500 to 4000 cm^{-1}.

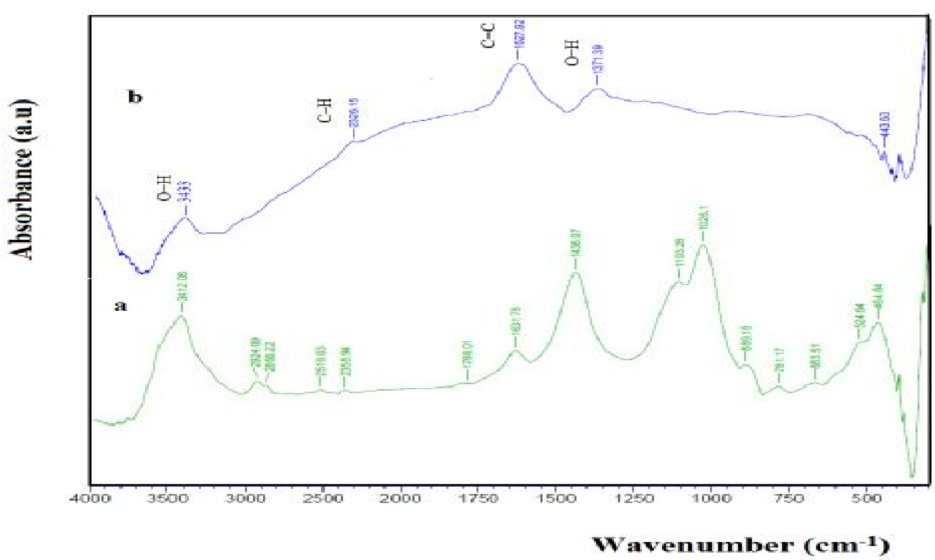

Figure (1) FTIR spectra of raw- MWCNTs (a) and acid Treated (f-MWCNT) (b).

Figure (1) shows the IR spectra of raw-MWCNTs and functionalized (f-MWCNTs) from 500 to 4000 cm^{-1}. Here one can observe typical bands that correspond to carboxyl groups -COOH and OH produced on the functionalized MWNT surface. a broad absorption band at 3433 cm^{-1} is correspond to the hydroxyl group. Band at 2326 cm^{-1} is due to symmetric stretching of CH stretching. The peak at 1627 cm^{-1} is assigned to conjugated C=C stretching of the CNTs. The peak at 1371 cm^{-1} is due to O–H bending deformation in –COOH [9].

These FTIR results indicate that the –COOH groups have been successfully introduced on the side wall of the MWCNTs and make them easily dispersed in polar solvents such as water, ethanol, etc [10].

Figure (2) shows SEM images of raw (a,b) and COOH-functionalized MWNTs (c,d) at different magnifications, respectively. In Figure (2a, b) long nanotubes with relatively well graphitized walls are observed. By comparison, Figure (2c, d) shows functionalized MWNTs were gathered in state of stacking, which maintains its length after chemical treatment. Figure (2c, d) also shows similar damage caused by acid treatment, with what appears to be short nanotubes produced by the breaking of some long nanotubes. This result indicates that the main parameter affecting the disentanglement of MWNTs is the chemical treatment as shown in Figure (2c,d). Moreover, formation of functional groups on the surface of nanotubes generates repulsion force which leads to debundeling of nanotubes and therefore causes surface increases [11].

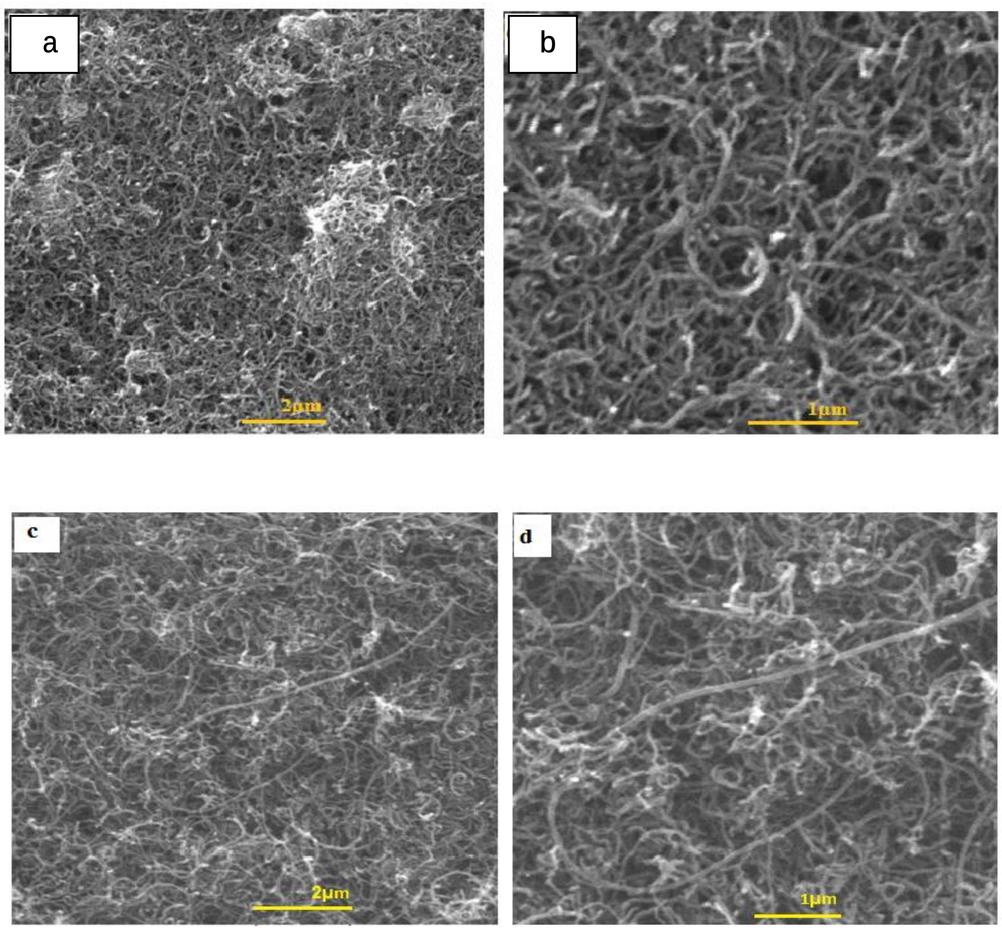

Figure (2) SEM images of MWCNTs: (a,b) raw-MWCNT and (c,d) functionalized-MWCNT at different magnifications.

Antibacterial tests.

With the large scale production of carbon nanotubes and their wide applications, their biosafety studies have attracted much attention. Recent years the cytotoxicity studies of CNTs extend from human cells to single celled organisms (bacterial cells) [6, 7]. To evaluate the biocompatiblity of the material, we construct the antimicrobial activity of traeted carbon nanotubes.

Figure (3) shows the typical images of E. coli incubated for 24 h at 37 ◦C after treated with various concentrations (1,3,7, 9,12,15) mg/ml of F-MWNTs was added to the solutions (shaker incubator for 8h) (b,c,d,e,f,g) and without any antimicrobial material (a) as control .

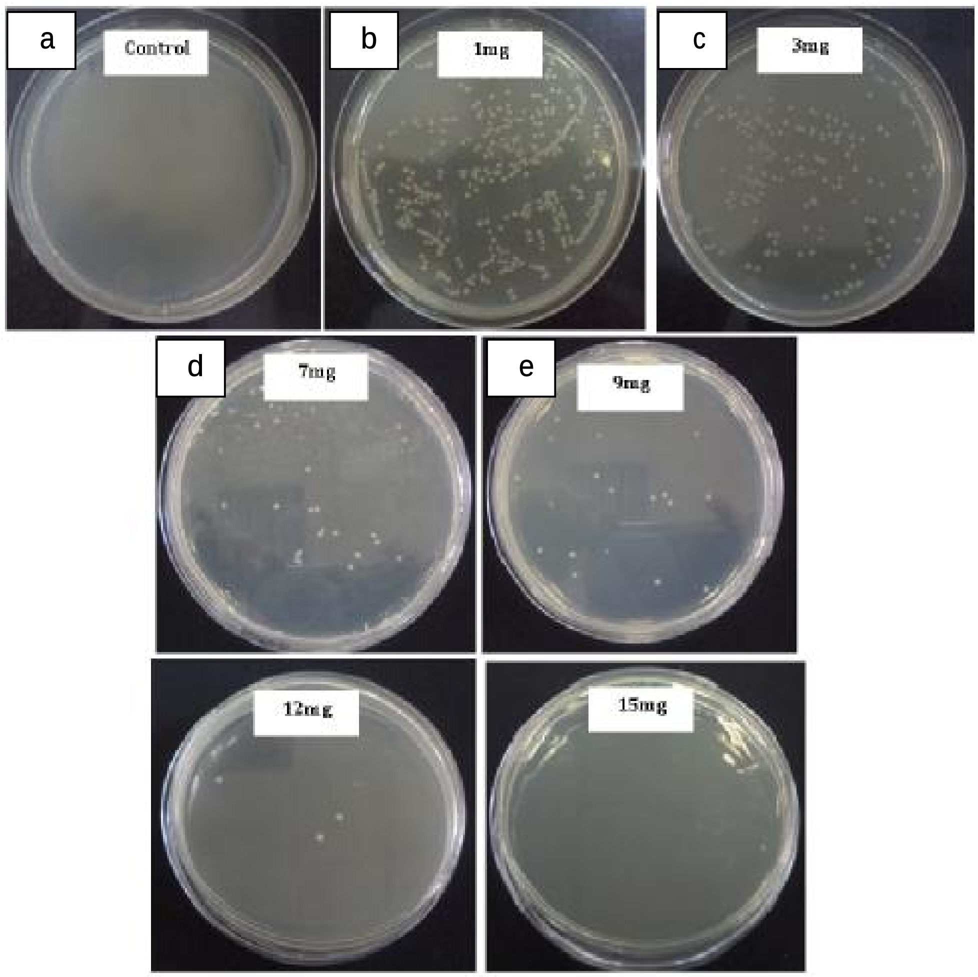

Figure (3) Digital photograph of *E. coli* colonies grown on nutrient agar plate as a function of F-MWNTs concentration. a) Control, b) 1mg/ml, c) 3mg/ml, d) 7mg/ml, e) 9 mg/ml, f) 12mg/ml , g)15mg/ml of F- MWNTs concentration.

Compared with control sample (a), there are only limited colonies as shown in Figure (3b, c,d,e) and there is no bacteria growth on N. agar media as shown in Figure (3f) which indicate that the MWNTs have obvious antimicrobial activity.

Moreover, Figure (4) represented the comparative graphic of F-MWCNTs against gram negative bacteria. Finally, from the results above the number of free bacteria decreased for all increasing carbon Wight, reaching reductions of 55-90% from the initial numbers of bacteria in the suspension and SEM images of treated MWCNTs by chemical acid and its contact with E.coli incubated for 60 min with normal is shown in Figure (5 a,b) which demonstrated the adhesion of bacteria on the surface of F-MWCNTs due the presence of functional groups during acid treatment as improved in FTIR analysis and resulting in direct bacteria-MWCNTs contact ant its inactivation.

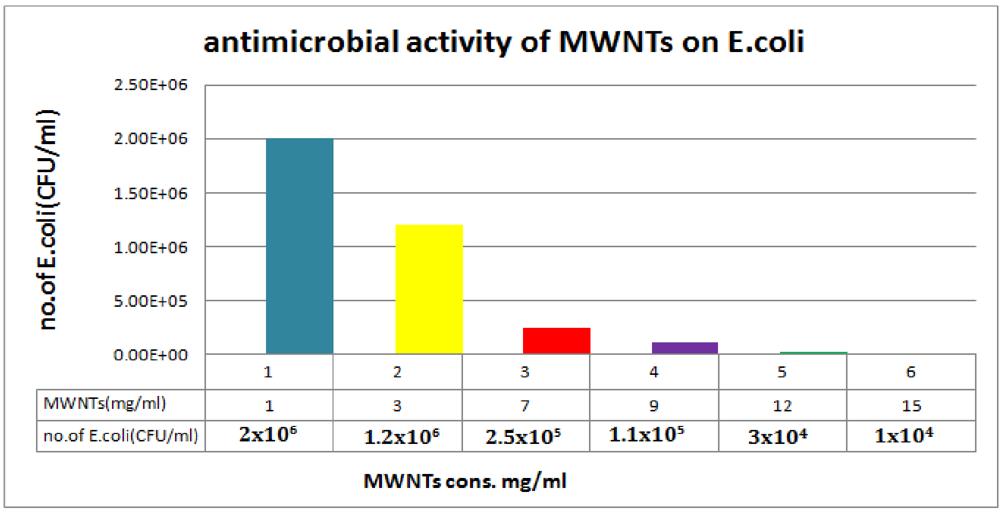

Figure (4) comparative graphic of F-MWCNTs against gram negative bacteria at different concentration of MWCNTs.

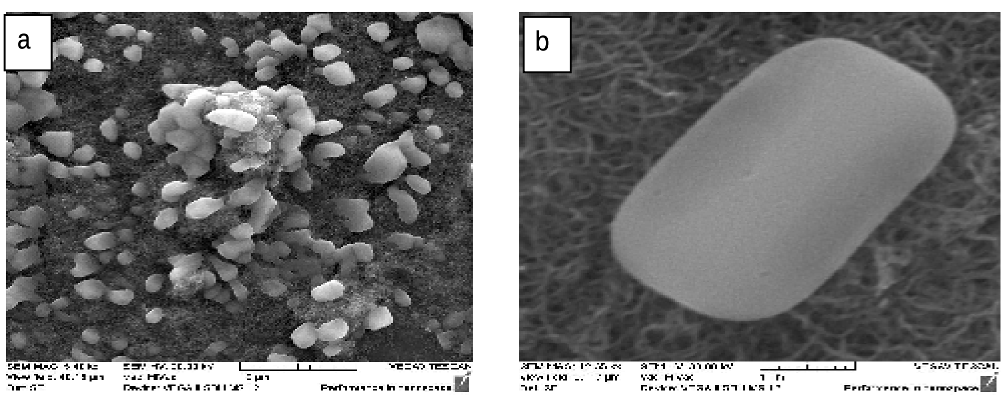

Figure (6) SEM images of MWCNTs and E.coli incubated for 60 min with normal saline prior to SEM imaging and after 8h of exposure time at different concentrations of treated-MWNTs a) 1mg,b)7mg.

CONCLUSIONS

Functionalized MWCNTs were obtained through treating MWCNTs by chemical method. The effect of acid treatment on development of functional groups on MWCNTs was investigated using SEM and FTIR that used to characterize the presences of functional groups on the outer-wall of MWCNTs. Also, the acid-treated MWCNTs exhibited antimicrobial activities to E.coli with increasing the concentration of MWNTs.

Moreover, the SEM images also showed the well interactions between the bacterial cells and acid- treated MWCNTs and the binding of- COOH molecules to MWCNTs increases its dispersing ability in aqueous solution.

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