Synthesis of Multi-Walled Carbon Nanotubes Decorated with Zinc Oxide Nanoparticles for Removal of Pathogenic Bacterial

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Abstract- *A series of Zinc Oxide Nanoparticles (ZnONPs)-functionalized Multi-Walled Carbon Nanotubes (F-MWCNTs) Nano composites were developed as antibacterial. In this study, chemical oxidation of pristine MWCNTs were carried out with a mixture of strong acids (3H_2SO_4 98%:1 HNO_3 65%).The F-MWCNTs were used as templates to prepare hybrid material like ZnONPs decorated F-MWCNTs. Pristine MWCNTs, F-MWCNTs and (ZnONPs/F-MWCNTs) Nano composites powder were investigated using Fourier Transform Infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Anti-bacterial activity has been carried out using standard agar dilution (plate count) method against Escherichia coli (E. coli). This study demonstrated that (ZnONPs/F-MWCNTs) Nano composite has a powerful bactericidal effect against Escherichia coli (E. coli) at concentration 0.5 mg/ml after 3 hr, which led to speculation that the combination of ZnONPs and F-MWCNTs altered their toxicity and improved antibacterial property of Nano composite.*

Keywords- *Pathogens, carbon nanotubes (CNTs), ZnO, antibacterial activity*

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1. Introduction

The rapid emergence of some strains that resistant to traditional anti-microbial agents has complicated and extended the traditional treatment and caused to raise mortality risk globally. Moreover, some of the traditional anti-microbial agents are unable to cross certain cell wall thus, restricting treatment of intracellular pathogens. For this reason, the disease resulting from these resistant strains tend to persist in these cells. WHO prediction that waterborne and foodborne diseases (which conceder to be a global threat) taken together kill approximately $2.2*10^6$ of people every year. Nevertheless, the emergence of nanotechnology has come with the promising broad-spectrum nanoparticle (NP)-antimicrobial agents due to their vast functionalization and physiochemical characteristic. Indeed, nanoparticle-antimicrobial agents are able to unlock the restrictions experienced by traditional anti-microbial agents [1,2].

On the other hand, many waterborne diseases and Cholera exhibit multiple pathways of infection or features timescales, which can be modeled as indirect and direct transmission. A main globally health issue for Cholera and waterborne diseases involves understanding the methods of transmission in order to improve prevention strategies and control [3]. Usually in Iraq, deterioration of drinking water results from the traditional treatment modes, which are not always reliable and efficient. The pathogens associated to drinking water supply sources, it is important to remove them from water because it is related with the health. Traditional removal mode is by chlorine disinfection, which is considered less safe in many places, since it is carcinogenic on long term beside the big place and infrastructure in traditional units. Moreover, free-chlorine tends to react with some of Natural organic matter (NOM) (which conceder as substances found in natural water) to produce carcinogenic halogenated by-products like trihalomethanes [4].

The application of nanotechnology in field of the drinking water treatment provides a promising alternative to traditional water treatment modes. The application of nanotechnology in this field and pollution cleanup is promising, as showed by a number of bench scale and field-based (full scale and pilot) studies [5]. The adsorption technology of carbon nanotube (CNT) has the potential to

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support point of use (POU) based treatment method for removal of natural organic matter (NOM), cyanobacterial toxins and bacterial pathogens from water systems. Unlike many micro-porous adsorbents media, carbon nanotube possess high aspect ratio with fibrous shape, well-developed mesopores and large accessible external surface area, all contribute to the high removal inefficiency of these microorganisms and macromolecular biomolecules [6].

Many researchers are trying to improve the anti-microbial activity of CNTs by decorating its surface with other anti-microbial agents like metal and ceramic nanoparticles (NPs). The deposited nanoparticles on the modified surface CNTs such as Ag, TiO_2 and ZnO play an important role in inactivate or kill the pathogens [7-10].

In recent years, the strong antimicrobial ability of ZnONPs has attracted more attention because ZnO can release Zn^{2+} ions and produce the reactive oxygen species (ROS). Just as a coin has two sides, both the drug delivery and the antibacterial effects of ZnONPs become more and more attractive at the same time [9,11].

In the present work, ZnONPs decorated modified surface multi-walled carbon nanotubes (F-MWCNTs) was prepared. The functionalization of the pristine MWCNTs was carried out by chemical oxidation method. The functional groups have been exploited to capture ZnONPs. After decoration of F-MWCNTs with ZnONPs, the antimicrobial activity of the nanocomposites was evaluated against pathogenic bacteria like *E. coli*.

2. Experimental

I- Materials and Methods

Preparation of ZnONPs/F-MWCNTs hybrid was carried out as follow:

1- (0.2 gm) of pristine MECNTs (>95wt% carbon nanotube, USA, outside diameter: 8-15 nm, Length: 10-50µm) were dispersed in 100 ml of ($3H_2SO_4$ 98%:1 HNO_3 65%) using ultrasonic path at 40 ^oC for 2 hrs, the suspension was allowed to cool, after 20 hrs the acid mixture was diluted with deionized water and then filtered using a 0.22 µm filter paper. Finally, the filter paper was put in (100 ml) deionized water, which was then treated in an ultrasonic bath for 20 min to re-dissolve the sample.

2- (2 gm) of zinc acetate dehydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$) were dissolved in (100 mL) deionized water. Preliminary, the mixture was put in ultrasonic path at 40 ^oC for 15 min followed by continuously stirred at 65 ^oC for 1 hr to produce homogeneous and transparent solution. After that certain amount of monoethanolamine (MEA) was added drop wise to Zn-based solution to adjust pH at 9.5 with continuously stirred at 65 ^oC for 1 hr to produce white suspension.

3-Zn-based white solution was added gradually to F-MWCNTs solution with continuously stirred at 65 ^oC for 1 hr. Then, the mixture of ZnONPs/F-MWCNT solution was vacuum-filtered through a 0.22 µm filter paper and they were washed several times with deionized water. The ZnONPs/F-MWCNT nanocomposite was peeled off from the filter paper and dried at 100 ^oC under vacuum for 1 hr. Finally, the freestanding nanocomposite was annealed at 400 ^oC for 1 hr.

II- Characterization ZnONPs/F-MWCNTs nanocomposite

Fourier transforms infrared (FTIR) spectra of pristine MWNTs, modified surface MWNTs and ZnONPs/F-MWCNT nanocomposite were obtained using 8400S, Shimadzu model spectrophotometer to study the attached functional groups on the MWNTs surfaces. The surface morphology of pristine MWCNTs, modified surface MWNTs (F-MWNTs) and ZnONPs/F-MWCNT nanocomposite were studied with scanning electron microscopy (SEM, The VEGA EasyProbe), Besides, the SEM was introduced to observe the morphology of bacteria.

III. Antimicrobial assay

The antimicrobial activity of F-MWCNTs and ZnONPs/F-MWCNTs nanocomposite was evaluated against *Escherichia coli*. *E. coli* was diagnosed in center of nanotechnology and advanced materials /university of technology / Baghdad-Iraq. Estimation of antibacterial activity has been carried out using standard agar dilution (plate count) method against *E. coli*. The method involves firstly adding of a bacterial strain on a nutrient agar. Bacteria were grown at 37 °C.

The cells were suspended in 50 ml of normal saline to yield a bacterial suspension of ~10^7 CFU/ml (colony forming unit). Bacterial inactivation was evaluated using 0.5 mg/ml of each sample.

One-milliliter original bacterial inoculum was added into 9 mL (0.9%) sterile normal saline that containing the desired concentration of F-MWCNTs and ZnONPs/F-MWCNTs nanocomposite. The mixtures were cultivated at 37 ^0C and shacked at 150 rpm for 1, 3 and 24 hrs. After shacking periods the mixtures were diluted to obtain a suspension of countable colony forming unit (CFU/mL). Then100µl spread out on a solid Muller Hinton (M. Hinton) medium. Colonies were counted after 24 hrs incubation of the plates at 37 ^0C.

3. Results and Discussion

Figure (1 a, b, c) shows the FTIR spectrum of pristine MWCNTs, modified surface MWNTs and ZnONPs/F-MWCNTs nanocomposite. In Figure (1 a) we could not see any band compared with the modified surface MWNTs Figure (1 b). The modified surface MWCNTs shows new peaks in comparison with the FT-IR spectrum of the pristine MWCNTs, which lack the functional groups.

As shown in Figure (1 a), the presence of aliphatic hydroxyl bending 1520 (cm^{-1}) bands may result from oxidation during the proprietary purification of the raw material and/or ambient atmospheric moisture [12]. While, the generation of functional groups on the F-MWCNTs was confirmed using (FT-IR) spectra as shown in Figure (1 b). The peaks around 1520-1600 (cm^{-1}) are assigned to the O–H band in C-OH, and the peaks at 788 (cm^{-1}) are assigned to COOH [13]. Moreover, the stretching vibration of C-H at around 2900-3000 (cm^{-1}) which indicates the formation of carboxyl functional groups [14]. These FTIR results indicate that the functional groups have been successfully introduced onto the F-MWCNTs surface and make them easily dispersed in polar solvents such as water, ethanol, etc. [9, 10].

The other peaks refer to generated structural defects on to the F-MWNTs. The combination structural defects with the functional groups play an important role in the deposition Zn^{2+} onto the F-MWCNTs surface.

On the other hand, as shown in Figure (1 c) we could see there are forceful changes in the peaks compared with the modified surface MWNTs. Evanescence of peaks corresponding to structural defects and the functional groups ascribe to effectually deposition Zn^{2+} onto the F-MWCNTs surface. The upgrowth of ZnO on the modified surface MWCNTs was confirmed using (FT-IR) spectra. The peak at low range 475 (cm^{-1}) indicates the growth ZnONPs onto the surface of F-MWCNTs. our results are in agreement with other research [14, 15].

I. The Morphology of Samples

Figure (2 a, b) shows typical SEM images of the pristine MWCNTs and F-MWCNTs respectively. As shown in Figure (2 a) long nanotubes with relatively large agglomerates and closely packed MWCNTs are prevalent in pristine MWCNTs. Figure (2 b) shows functionalized MWNTs were gathered in state of stacking, which maintains its length after chemical oxidation.

The chemical oxidation introduces the functional groups such as (-COOH) on the surface of MWCNTs which it is necessary to attach ZnONPs to the surface of F-MWCNTs. Moreover, it is play important role in removing the impurities as amorphous carbon particles.

On the other hand, the deposition of ZnONPs on the modified surface MWCNTs is confirmed by SEM images as shown in Figure (2 c) . It can be seen that the ZnONPs with good dispersion and preferentially adhere onto the F- MWCNTs due to the electrostatic interaction between Zn^{2+} and (-COOH). Grain morphologies of ZnONPs can be observed from SEM images represented in figure (2 c), it was observed that the particles are spherical in shape with particles size less than 50 nm.

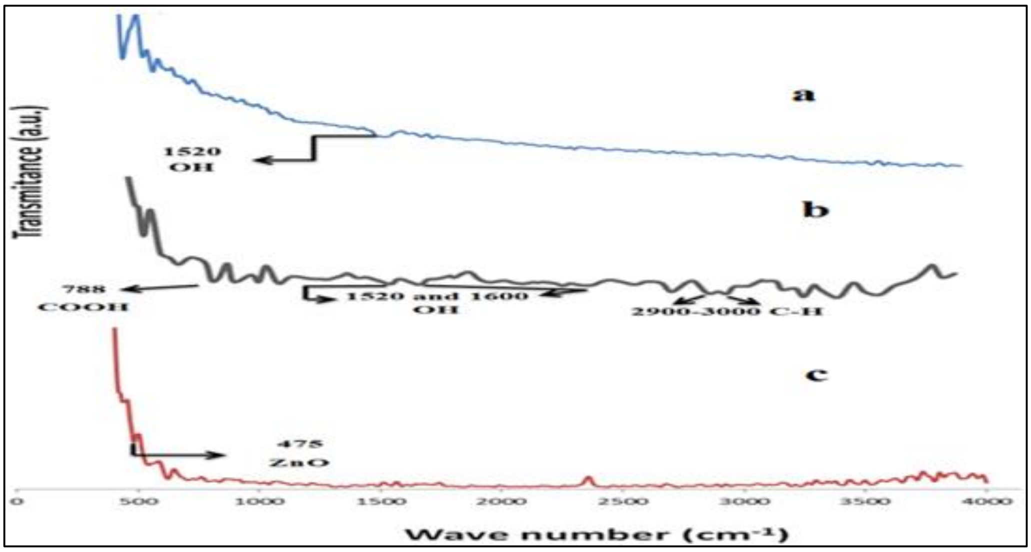


Figure 1: FTIR spectra of a) pristine MWCNTs, b) modified surface MWCNTs, and c) ZnONPs/F-MWCNTs nanocomposite.

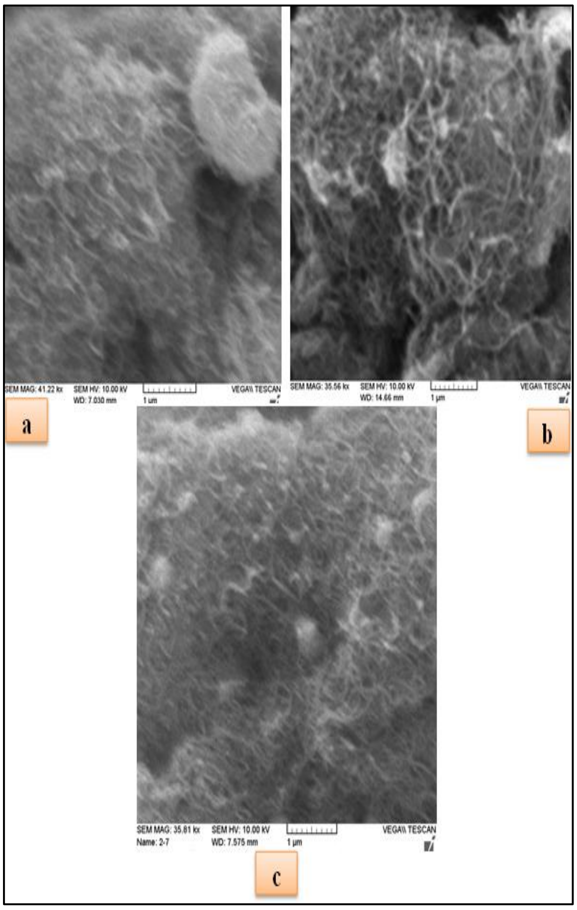


Figure 2: SEM images of a) pristine MWCNTs, b) modified surface MWCNTs, and c) ZnONPs/F-MWCNTs nanocomposite

II. Estimation of antibacterial activity

The antibacterial property with a fixed amount of F-MWCNTs and ZnONPs decorated MWCNTs were evaluated against *E. coli* bacteria at various exposure times (1, 3, 24 hrs). Figure (3 a-f) shows a typical image of *E. coli* incubated for 24 h at 37 ºC after treated with fixed concentration 0.5 mg/ml of F-MWCNTs and nanocomposite. As shown in figure (3 a-c), the number of colonies decreased gradually after treated with F-MWCNTs, while it is decreased severely after treated with nanocomposite at each exposure time (1, 3, 24 hrs) as shown in figure (3 d-f).

Besides, after *E. coli* had been cultivated with the fixed amount of F-MWCNTs and nanocomposite for 24 hrs, no bacterial colonies of *E. coli* presented on media as compared with control as shown in figure (3 c, f), which are corresponding to the antimicrobial activity of F-MWCNTs and nanocomposite. The antimicrobial property of ZnONPs decorated MWCNTs attributed to reactions between ZnO surface and water which led to produce reactive oxygen species (ROS) in the presence of ambient UV light. The generated hydrogen peroxide (H_2O_2) can destroy the cell membrane and kill the bacteria due to relatively weaker electrostatic interaction between ions and bacteria, while, the F-MWCNTs served as adsorbing materials for *E. coli* due to high surface area of MWNT. The results showed a significant reduction of CFU/ml after increasing time of shaking for both F-MWCNTs and nanocomposite. Moreover, Figure (4 a, b and c) shows the SEM images of *E. coli* cells as control, *E. coli* bacteria interacted with F-MWCNTs and nanocomposite, respectively. As shown in Figure (4 b) some of cells are setting on the top of F-MWCNTs representing excellent adsorption, while, Figure (4 c) revealed distinct morphological bacterial changes due to the interaction between *E. coli* with hybrid material.

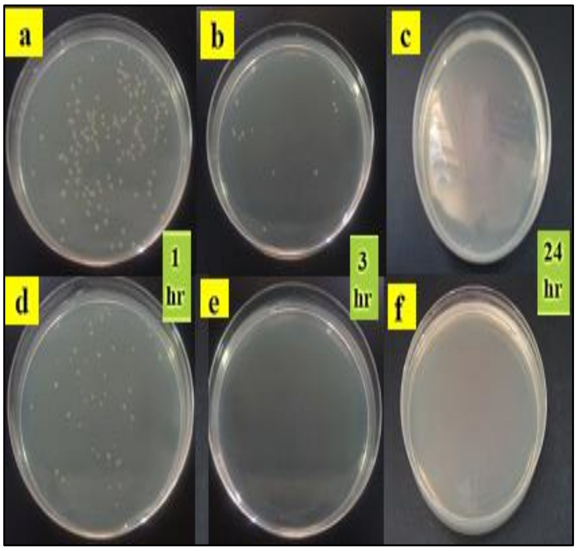
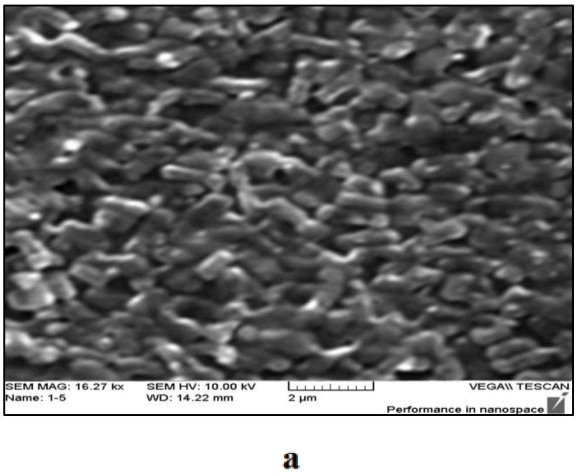


Figure 3: *E. coli* colonies incubated for 24 hrs at 37 ºC after different exposure times (1, 3, 24 hrs) using shaker incubator treated with 0.5 mg/ml of a-c) FMWCNTs, and d-f) ZnONPs decorated MWCNTs.



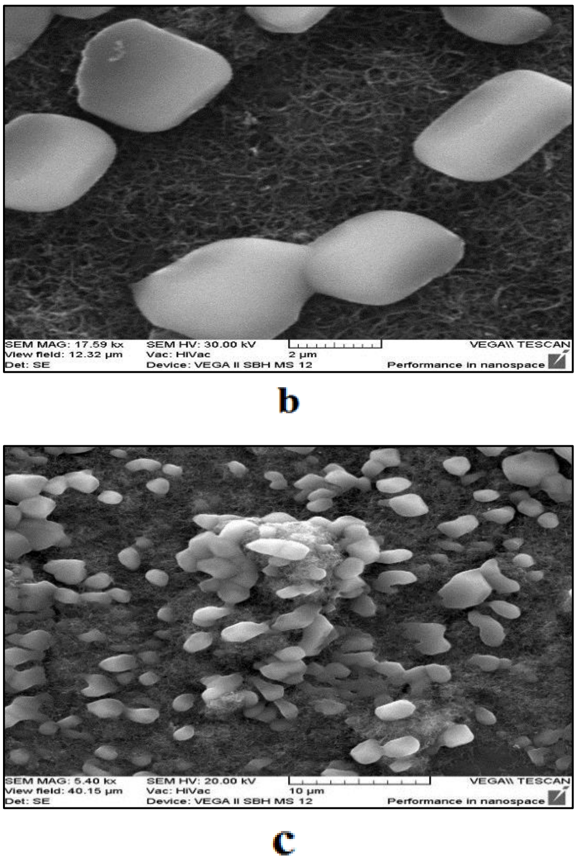


Figure 4: SEM images of a) *E. coli* cells as control, b) *E. coli* cells exposed to F-MWCNTs, and c) *E. coli* cells exposed to ZnONPs decorated MWCNTs with scal bar 10 m.

4. Conclusions

FTIR and SEM studies confirmed ZnONPs/F-MWCNTs nanocomposites were successfully synthesized. The SEM results showed that the ZnONPs were distributed uniformly on the surface of MWCNTs with negligible agglomeration. The FTIR results demonstrated that the functional groups have been successfully introduced onto the MWCNTs surface, which provided nucleation sites for deposition and dispersions of ZnONPs on the surface of MWCNTs. Moreover, the FTIR data confirms that dissolving zinc acetate dehydrate in deionized water and the reaction of solution with monoethanolamine (MEA) leads to the formation of ZnONPs and gets hybridized with MWCNTs during continuously stirred due to the electrostatic interaction between Zn^{2+} and functional groups. This study showed that F-MWCNTs and ZnONPs decorated MWCNTs have bactericidal effect against gram negative *E. coli* at 0.5 mg/ml concentration after 3 and 24 incubated periods. As well as, we demonstrated that the antibacterial activity of the hybrid can be attributed to the damage of cell membranes due to the opposite charges between the bacteria and the Zn^{2+} atoms in the ZnONPs/F-MWCNTs nanocomposites and these electrostatic forces between them may be the reason for their adhesion and bioactivity of hybrid material which leads to leakage of cell contents and ultimately cell death. The result of this research is of great interest for applications ZnONPs/F MWCNTs nanocomposites in water treatment as disinfection agents.

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