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Biological Activity of Functionalized Carbon Nanotubes by Trimethoprim

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ABSTRACT

Multiwalled carbon nanotubes (MWCNTs) functionalization was carried out by using trimethoprim. A comparative study showed that functionalized MWCNTs are more active than trimethoprim. These results are confirmed with Fourier transform infrared spectroscopy and scanning electron microscope techniques. Investigation of prepared functionalized MWCNTs with the diameter range performed on three bacterial samples *P. aeroginosa*, *K. pneumoniae* and *P. mirabilis*. The results indicated that only *K. pneumoniae* was affected by functionalized carbon nanotubes as compared to trimethoprim.

KEYWORDS

Biological activity, Functionalized carbon nanotubes, Trimethoprim, Scanning electron microscope, Fourier-transform infrared spectroscopy.

INTRODUCTION

Carbon nanotubes display numerous special characteristic physical and chemical properties and have been investigated for natural and biomedical applications in the last few years [1]. Ultrasensitive recognition of natural species with carbon nanotubes can be acknowledged after surface passivation to repress non-particular authoritative of biomolecules on the hydrophobic nanotube surface. Surface-upgraded Raman spectroscopy of carbon nanotubes opens up a strategy for protein micro-array with discovery affectability down to 1 mol/L [2]. *in vivo* Biodistributions change with the functionalization and perhaps at the same time a size of nanotubes, with an inclination to aggregate in the reticulo endothelial framework (RES), including the liver and spleen, after intravenous organization [3]. In the event that all around functionalized, nanotubes might be discharged for the most part through the biliary pathway in excrement. Carbon nanotube-based medication has indicated *in vitro* and *in vivo* tests including conveyance of little meddling RNA (siRNA), paclitaxel and doxorubicin. Besides, single-walled carbon nanotubes with different fascinating characteristic optical properties have been utilized as novel photoluminescence, Raman and photoacoustic differentiate specialists for imaging of cells and creatures [4].

Carbon nanotubes (CNTs) are moved up consistent chambers of graphene sheets, showing unparalleled physical, mechanical, and compound properties which have pulled in huge enthusiasm

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for as far back as decade [5,6]. Contingent upon the number of graphene layers from which a solitary nanotube is created, carbon nanotubes (CNTs) are delegated single-walled carbon nanotubes (SWNTs) or multi-walled carbon nanotubes (MWNTs) [7]. For biomedical applications, surface science or functionalization is required to solubilize CNTs and to render biocompatibility and low danger. Surface functionalization of carbon nanotubes might be covalent or non-covalent. Compound responses shaping bonds with nanotube side walls are completed in the covalent functionalization case, while non-covalent functionalization abuses great cooperation between the hydrophobic space of an amphiphilic atom and the CNT surface, bearing fluid nanotubes wrapped by a surfactant. As medicine bearers, the dissolvability of CNTs in watery dissolvable is basic for gastrointestinal assimilation, blood transportation, emission, and biocompatibility and so forth; from now on, CNT composites required in restorative movement system must meet this central prerequisite. In this way, it is essential that such CNT scatterings be uniform and stable in a satisfactory degree, to procure exact obsession data. In such way, the solubilization of perfect CNTs in watery solvents is one of the key impediments in the way for them to be delivered as sensible prescription transporters owing to the fairly hydrophobic character of the graphene side dividers, joined with π-π associations between the individual tubes. These properties cause gathering of CNTs into packs. For the compelling scattering of CNTs, the medium should be fit for wetting the hydrophobic tube surfaces and changing the surfaces to decrease the tube's package advancement. In addition, functionalization has been shown fit for diminishing cytotoxicity, upgrading biocompatibility and offering an opportunity to extremity atoms of drugs, proteins or characteristics for the advancement of conveyance frameworks.

Carbon nanotubes can be more powerful and cost-proficient than conventional anti-toxin treatments. For instance, directed conveyance of amphotericin B to cells utilizing covalently functionalized carbon nanotubes is less expensive than utilizing customary liposomal amphotericin B. This settles on focused object the favoured decision for treating leishmanial diseases [8]. It has likewise been uncovered that the size and surface region of carbon nanomaterials are vital parameters influencing their antibacterial action; *i.e*., expanding the nanoparticles surface region by diminishing their size prompt enhancing their action for association with microscopic organisms [9,10]. For the most part, the antimicrobial movement of nanoparticles relies upon their arrangement, surface adjustment, inborn properties, and the sort of microorganism [11,12]. It has been suggested that carbon-based nanomaterials cause layer harm in microscopic organisms because of an oxidative pressure [13,14]. As indicated by ongoing examinations the physical connection of carbon-based nanomaterials with microorganisms, as opposed to oxidative pressure, is the essential antimicrobial action of these nanostructures [15,16]. A few *in vitro* considers have announced the effect of CNTs on multi-drug-safe bacterial contaminations, for example, CNT with width (≈ 30 nm) impact on Gram-negative and Gram-positive microbes by component of Electrostatic adsorption of bacterial layer because of positive charges of lysine bunches on CNT [17]. Single-walled carbon nanotubes with a distance across of 15-30 nm was found active as antibacterial on both Gram-negative and Gram-positive microorganisms [18]. Carbon-based nanomaterials, for example, carbon nanotubes, initiated carbons, fullerene and graphene are generally utilized as of now most encouraging utilitarian materials because of their high adsorption limits. Subsequently, graphene nano-sheets will challenge the current existing adsorbents; including different kinds of carbon-based nanomaterials [19]. Carbon nanotubes as adsorbent media have been turned out to be ready to expel an extensive variety of contaminants including microscopic organisms [20]. Multiwalled carbon nanotubes are probably the most alluring nanomaterials due to their surprising physico-chemical, mechanical and electrical properties and also their wide scope of potential applications. The expansion in business intrigue and ensuing large-scale manufacturing will prompt more prominent potential outcomes for cooperations of CNTs with people and the earth [21]. Understanding the toxicology and natural effects of CNTs is accordingly basic for the future utilization of these rising nanomaterials [22]. The principal was given by Kang *et al*. [9] which showed that the size (width) of carbon nanotubes is a key factor representing their antibacterial impacts and that the imaginable fundamental CNT-cytotoxicity instrument is cell layer harm by coordinate contact with CNTs [23]. In years 2004-2006, an intervention on trimethoprim use was conducted in Kronoberg County, Sweden, resulting in 85 % reduction in trimethoprim prescriptions. The investigation of dihydrofolate reductase (dfr)- genes distribution and integrons in *Escherichia coli* and *Klebsiella pneumoniae* and its effect of intervention on this distribution is conducted [24].

EXPERIMENTAL

Strong oxidizing agent mixture (NH_4OH/H_2O_2) was added to MWCNTs and sonicated to form carboxylated MWCNTs under reflux at 70 ºC for 4 h and then cooled at 4 ºC for 48 h (Fig. 1).

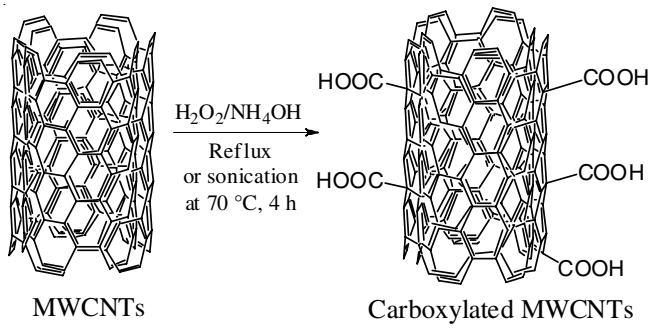

Fig. 1. Oxidation process for the surface of MWCNTs

Functionalization of oxidized MWCNTs was performed by mixing of 100 mg oxidized MWCNTs with trimethoprim in the presence of distilled thionyl chloride in dimethyl formamide (DMF) as a solvent. The mixture was refluxed for 4 h at 70 ºC. The refluxed mixture was cooled to room temperature and then washed three times with DMF. The mixture was filtered using polytetrafluoroethylene membrane (pore size 0.45 μm). The solid was washed with DMF and filtered again. This process removed any unreacted substance from the product.

The filtered sample was dried at 60 ºC for overnight under vacuum to obtain amine functionalized carbon nanotubes (Fig. 2).

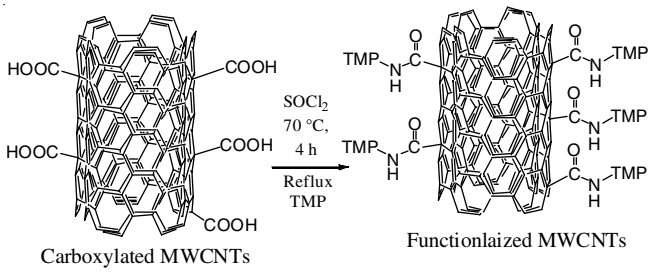

Fig. 2. Reaction of trimethoprim functionalization on the surface of MWCNTs

Three bacterial strains *viz*., *Pseudomonas aeroginosa*, *Klebsiella pneumonia* and *Proteus mirabilis* (Gram -ve) were tested in this study to investigate the effect of carbon nanotube particles (MWCNTs) combined with trimethoprim drug as an antibacterial agent. These bacteria were isolated from three patients with different diseases and didn't receive any antibiotic treatment before collecting specimens from Marjan Hospital, Hilla city, Iraq in January 2018, which were sent to the microbiology laboratory for routine culture, identification and sensitivity testing. Further identification tests performed for these bacterial isolates, including biochemical tests, culture and preserving of isolates were used [25,26].

To evaluate the antimicrobial activity of multiwalled carbon nanotube particles *in vitro* with three bacterial samples by the agar diffusion method CNTs particles powder was suspended in sterilized distilled water for achievement the interaction of MWCNTs particles with bacteria [21]. In this experiment, 100 ppm prepared from each of trimethoprim, oxidized carbon nanotube. Each tube remixed by using sonicatorin order to resuspend then directly apply the needed amount in the well of agar plate. All of them were tested in the same agar plate against these three bacterial samples. The inoculums size was adjusted so as to deliver final inoculums of approximately 10^8 colony forming unit (CFU)/mL from the grown bacterial culture of 24 h old for all strains to compare the turbidity of each sample to 0.5 McFarland standards, the broth of these microorganisms were cultured on a nutrient agar plates. After solidification of 25 mL in Mueller-Hinton (MH) agar medium in petri plates, hollows of four wells (5 mm diameter) were cut into the agar by cork borer, then all the collected pathogenic bacteria samples were tested on this agar, 0.1 mL of trimethoprim and CNTs solutions were applied in these four wells. All the petri dishes were incubated at 5-8 ºC for 2-3 h to permit good diffusion and then again incubated for 24 h at 37 ºC. The assessment of antibacterial was based on measuring the diameter of inhibition zone (mm) formed around the well.

RESULTS AND DISCUSSION

FTIR analysis: Fourier-transform infrared spectroscopy was used to monitor the surface functional groups present in functionalized MWCNTs. Potassium bromide pellets were used to characterize amine functionalized carbon nanotubes using (Tenser II) FT-IR spectrometer. The spectrum of carboxylated carbon nanotube shows the peaks at 3442.67 cm^{-1} (O-H) and 1634 cm^{-1} correspond to carbonyl group after the oxidation of MWCNTs (Fig. 3).

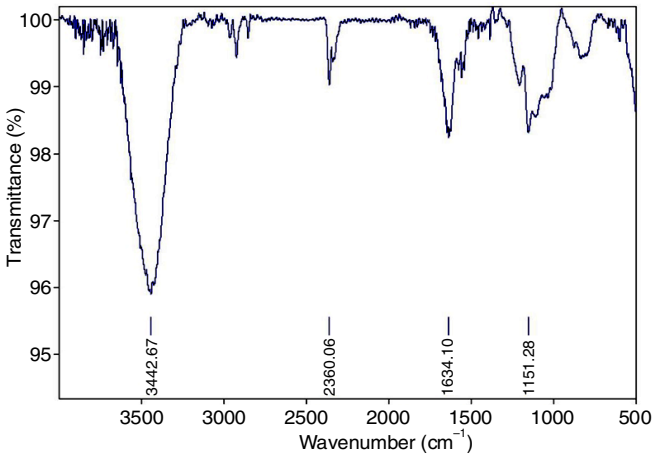

Fig. 3. FTIR spectrum of carboxylated MWCNTs

The spectrum of amine functionalized carbon nanotube approved the disappearance of double peaks of primary amine of pristine trimethoprim (N-H) at 3500-3300 cm^{-1}) and the appearance of carbonyl group at 1654 cm^{-1} (Fig. 4).

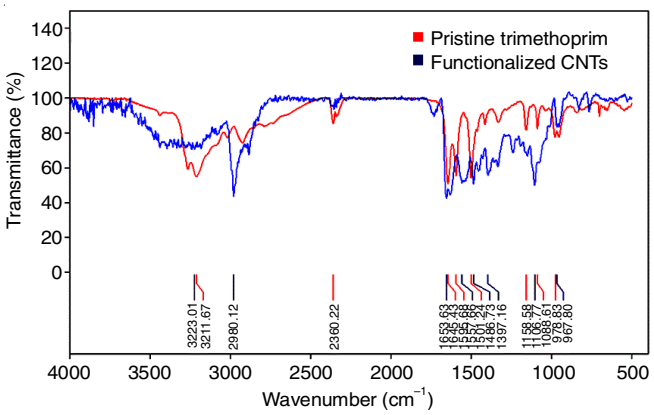

Fig. 4. FTIR spectrum of trimethoprim drug with functionalized MWCNTs

SEM: The average diameter of functionalized nanotubes with trimethoprim drug was found to be about 83.34 nm (Fig. 5).

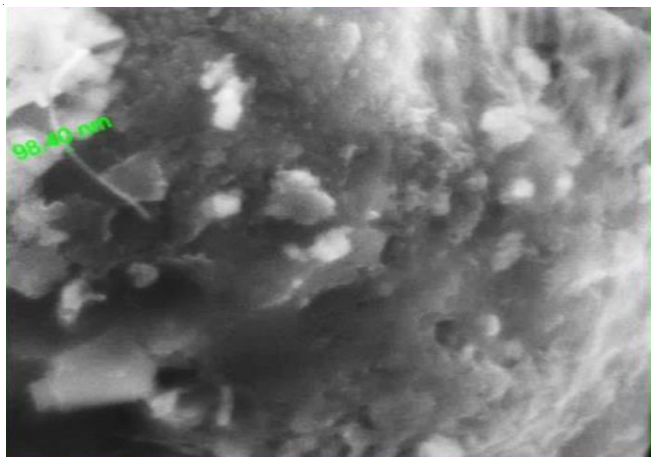

Fig. 5. SEM images of functionalized MWCNTs

Biological activity: The investigation of prepared MWCNTs with the diameter range from 20 to 30 nm performed by agar diffusion well method on three bacterial strains *viz*., *P. aeroginosa*, *K. pneumoniae* and *P. mirabilis*. The results indicated that only *K. pneumoniae* affected only by trimethoprim and carbon nanotube with trimethoprim, but not affected by carbon nanotube or oxidized carbon nanotube. While the other two bacterial strains *P. aeroginosa* and *P. mirabilis* didn't show any sensitivity to any kind of the investigated carbon nanotubes and even also to trimethoprim alone, which may be attributed to several factors related to bacteria as well as nanomaterials (MWCNTs) such virulence of pathogenic bacteria and preparation method of MWCNTs.

Conclusion

A comparison between amine functionalized carbon nanotubes and pristine trimethoprim, functionalized carbon nanotubes showed a high efficiency in inhibiting *K. pneumonia* bacteria more than pristine trimethoprim, while amine functionalized carbon nanotubes or pristine trimethoprim did not show the effect of *P. aeroginosa* and *P. mirabilis*. Further studies are needed to investigate the antibacterial mechanism and the physico-chemical, genetic factors involved in bacterial response which appear as sensitivity or resistance to the action of nanoparticles.

REFERENCES

1. S. Iijima, Helical Microtubules of Graphitic Carbon, *Nature*, **354**, 56 (1991); https://doi.org/10.1038/354056a0.
2. W. Zhou, C. Rutherglen and P. Burke, Wafer Scale Synthesis of Dense Aligned Arrays of Single-Walled Carbon Nanotubes, *Nano Res.*, **1**, 158 (2008); https://doi.org/10.1007/s12274-008-8012-9.
3. H. Ago, K. Petritsch, M.S.P. Shaffer, A.H. Windle and R.H. Friend, Composites of Carbon Nanotubes and Conjugated Polymers for Photovoltaic Devices, *Adv. Mater.*, **11**, 1281 (1999); https://doi.org/10.1002/(SICI)1521-4095(199910)11:15<1281::AID-ADMA1281>3.0.CO;2-6.
4. A.M. Jassm, F.H. Hussein, F.H. Abdulrazzak, A.F. Alkaim and B.A. Joda, Synthesis and Characterization of Carbon Nanotubes by Modified Flame Fragments Deposition Method, *Asian J. Chem*., **29**, 2804 (2017); https://doi.org/10.14233/ajchem.2017.20994.
5. A. Javey, J. Guo, Q. Wang, M. Lundstrom and H.J. Dai, Ballistic Carbon Nanotube Field-Effect Transistors, *Nature*, **424**, 654 (2003); https://doi.org/10.1038/nature01797.
6. Q. Cao and J.A. Rogers, Random Networks and Aligned Arrays of Single-Walled Carbon Nanotubes for Electronic Device Applications, *Nano Res.*, **1**, 259 (2008); https://doi.org/10.1007/s12274-008-8033-4.
7. S.S. Fan, M.G. Chapline, N.R. Franklin, T.W. Tombler, A.M. Cassell and H.J. Dai, Self-Oriented Regular Arrays of Carbon Nanotubes and Their Field Emission Properties, *Science*, **283**, 512 (1999); https://doi.org/10.1126/science.283.5401.512.
8. V.K. Prajapati, K. Awasthi, S. Gautam, T.P. Yadav, M. Rai, O.N. Srivastava and S. Sundar, Targeted Killing of *Leishmania donovani in vivo* and *in vitro* with Amphotericin B Attached to Functionalized Carbon Nanotubes, *J. Antimicrob. Chemother.*, **66**, 874 (2011); https://doi.org/10.1093/jac/dkr002.
9. S. Kang, M. Herzberg, D.F. Rodrigues and M. Elimelech, Antibacterial Effects of Carbon Nanotubes: Size Does Matter, *Langmuir*, **24**, 6409 (2008); https://doi.org/10.1021/la800951v.
10. C. Buzea, I. Pacheco and K. Robbie, Nanomaterials and Nanoparticles: Sources and Toxicity, *Biointerphases*, **2**, MR17 (2007); https://doi.org/10.1116/1.2815690.
11. M.J. Hajipour, K.M. Fromm, A. Akbar Ashkarran, D.J. de Aberasturi, I.R. Larramendi, T. Rojo, V. Serpooshan, W.J. Parak and M. Mahmoudi, Antibacterial Properties of Nanoparticles, *Trends Biotechnol.*, **30**, 499 (2012); https://doi.org/10.1016/j.tibtech.2012.06.004.
12. S. Gurunathan, J.W. Han, A.A. Dayem, V. Eppakayala and J.H. Kim, Oxidative Stress-Mediated Antibacterial Activity of Graphene Oxide and Reduced Graphene Oxide in *Pseudomonas aeruginosa*, *Int. J. Nanomed.*, **7**, 5901 (2012); https://doi.org/10.2147/IJN.S37397.
13. S.M. Dizaj, A. Mennati, S. Jafari, K. Khezri and K. Adibkia, Antimicrobial Activity of Carbon-Based Nanoparticles, *Adv. Pharm. Bull*., **5**, 19 (2015); https://doi.org/10.5681/apb.2015.003.
14. A. Manke, L. Wang and Y. Rojanasakul, Mechanisms of Nanoparticle-Induced Oxidative Stress and Toxicity, *BioMed Res. Int.*, **2013**, 1 (2013); https://doi.org/10.1155/2013/942916.
15. S. Kang, M. Pinault, L.D. Pfefferle and M. Elimelech, Single-Walled Carbon Nanotubes Exhibit Strong Antimicrobial Activity, *Langmuir*, **23**, 8670 (2007); https://doi.org/10.1021/la701067r.
16. A. Amiri, H.Z. Zardini, M. Shanbedi, M. Maghrebi, M. Baniadam and B. Tolueinia, Efficient Method for Functionalization of Carbon Nanotubes by Lysine and Improved Antimicrobial Activity and Water-Dispersion, *Mater. Lett*., **72**, 153 (2012); https://doi.org/10.1016/j.matlet.2011.12.114.
17. J.F. MacFaddin, Biochemical Tests for Identification of Medical Bacteria, Lippincott Williams and Wilkins: USA, edn 3 (2000).
18. J.G. Collee, A.G. Fraser, B.P. Marmiom and A. Simmon, Mackie and McCartney Practical Medical Microbiology, Churchill Livingstone Inc.: USA, edn 4 (1996), .
19. C. Perez, M. Pauli and P. Bazevque, An Antibiotic Assay by Agar Well Diffusion Method, *Acta Biol. Med. Exp.*, **15**, 113 (1990).
20. NCCLS (National Committee for Clinical Laboratory Standards), Methods for Dilution Antimicrobial Susceptibility Tests of Bacteria that Grow Aerobically; Approved Standard M100-S12, Wayne: PA (2002).
21. A.M. Allahverdiyev, E.S. Abamor, M. Bagirova and M. Rafailovich, Antimicrobial Effects of TiO_2 and Ag_2O Nanoparticles Against Drug-Resistant Bacteria and *Leishmania parasites*, *Future Microbiol.*, **6**, 933 (2011); https://doi.org/10.2217/fmb.11.78.
22. P. Cornejo-Juarez, D. Vilar-Compte, C. Perez-Jimenez, S.A. Ñamendys-Silva, S. Sandoval-Hernández and P. Volkow-Fernández, The Impact of Hospital-Acquired Infections with Multidrug-Resistant Bacteria in an Oncology Intensive Care Unit, *Int. J. Infect. Dis.*, **31**, 31 (2015); https://doi.org/10.1016/j.ijid.2014.12.022.
23. L.R. Arias and L. Yang, Inactivation of Bacterial Pathogens by Carbon Nanotubes in Suspensions, *Langmuir*, **25**, 3003 (2009); https://doi.org/10.1021/la802769m.
24. A. Brolund, M. Sundqvist, G. Kahlmeter and M. Grape, Molecular Characterisation of Trimethoprim Resistance in *Escherichia coli* and *Klebsiella pneumoniae* during a Two Year Intervention on Trimethoprim Use, *PLoS One*, **5**, e9233 (2010); https://doi.org/10.1371/journal.pone.0009233.
25. J.G. Yu, L.Y. Yu, H. Yang, Q. Liu, X.H. Chen, X.Y. Jiang, X.Q. Chen and F.P. Jiao, Graphene Nanosheets as Novel Adsorbents in Adsorption, Preconcentration and Removal of Gases, Organic Compounds and Metal Ions, *Sci. Total Environ.*, **502**, 70 (2015); https://doi.org/10.1016/j.scitotenv.2014.08.077.
26. T. Akasaka and F. Watari, Capture of Bacteria by Flexible Carbon Nanotubes, *Acta Biomater.*, **5**, 607 (2009); https://doi.org/10.1016/j.actbio.2008.08.014.