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Functionalization of multi-wall carbon nanotubes with Metformin derivatives and study of their antibacterial activities against E-Coli and S. aureus

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Abstract

Bacteria can grow in different materials that are in close contact with humans, foods, etc., so, it is very important to control this matter in order to prevent risk of infections. Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. Multiwall carbon nanotubes (MWNTs) have interesting antibacterial activities and offering a promising new treatment preventing bacteria from becoming resistant. With this aim, we report for the first time three novel modified carboxylated multiwall carbon nanotubes consisting of MWNT–CO-Metformin, MWNT–CO-[Metformin][CuCl2] and MWNT–CO-[Metformin][AgNO3]. The functionalized carboxylated multiwall nanotubes were then characterized by FT-IR, Raman, TEM, EDX and Elemental analysis. Moreover, the antibacterial activity of all of compounds has been investigated against gram-negative Escherichia coli and gram-positive Staphylococcus aureus. These results show that nano compounds exhibited significant antibacterial activity and have a potential to be used as antibacterial agent.

Keywords: Carbon nanotubes, Functionalization, Metformin, Antibacterial activity

1.Introduction

Bacteria can grow in different materials that are in close contact with humans, foods, etc., so, it is very important to control this matter in order to prevent risk of infections. AMR is present in all parts of the world. New resistance mechanisms emerge and spread globally. AMR is resistance of a microorganism to an antimicrobial drug that was originally effective for treatment of infections caused by it [1]. In general, bacteria have the genetic ability to send and acquire resistance to drugs, which are used as pharmacological agents [2]. The microbial resistance represents a global concern and the outlook for the use of antimicrobial drugs in the future is still uncertain [3]. Recently, carbon nanotubes (CNTs) have attracted increasing attention in biomedical fields due to their unique structure and properties, including high aspect ratios, large surface areas, nanosized stability and rich surface chemical functionalities [4]. With these unique structures and properties, CNTs have been developed as promising nano platforms to

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immobilize biological or therapeutic molecules, such as proteins, antibodies, siRNA or drugs on their surface, and especially these functionalized CNTs are capable of crossing biological barriers independently of the cell type, which makes them suitable candidates for drug delivery systems [5,6]. The chemical modification of carbon nanotubes has received significant attention in recent decades [7,8,9,10,11]. Chemical functionalization is a common technique to increase dispersion stability and biocompatibility of CNTs [12]. CNTs have been proposed as multipurpose innovative transporters for drug delivery since they can be covalently or non-covalently attached to drug molecules and carry them throughout the body in a biocompatible way [13]. Modified carbon nanotubes have been widely studied for their antibacterial, antifungal and potential cytotoxic chemotherapeutic agents. Many factors may influence the antibacterial activity of carbon nano materials, including: electronic structure, size and surface chemical properties, as well as the interacting conditions between carbon nanomaterials and bacterial cells [14].

More recently, transition metal complexes have attracted attentions of inorganic, metallo-organic as well as bio-inorganic chemists because of their structural diversity, antibacterial activity and enormous number of biological applications [15]. The ability of metal to combine with ligands and then release ligand in specific process make them ideal candidate for use in biological system. It is well known that synthetic copper (II) complexes have been reported to act as potential anticancer and antibacterial agents [16]. The pharmacological activities of these metal complexes depend on the metal ion, organic ligands and the structure of the compounds [17]. Metformin hydrochloride (MF-HCl); (N,N-dimethyl-imido-dicarbonimidic diamide hydrochloride) or 1,1-dimethylbiguanide, it contains two imino (–C=NH) groups and the three amino groups (i.e. primary (–NH2), secondary (–NH) and tertiary ($-N(CH_3)_2$) as donating centers to immobilization metals [18].

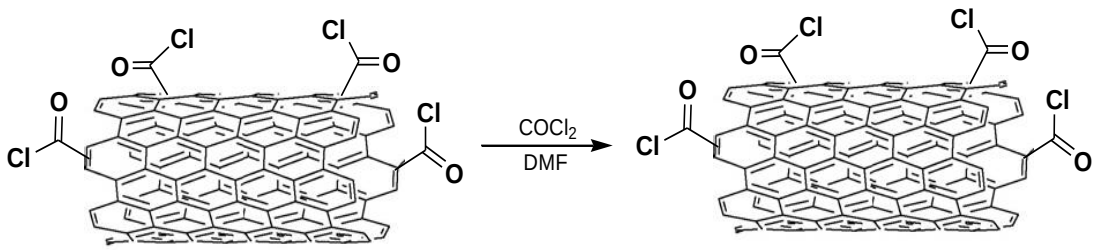
Metformin is commonly prescribed agent for the treatment of type II diabetes. It induces multiple beneficial effects such as weight loss, lipid reduction and lowering blood glucose levels [19]. It is an oral hypoglycemic agent, which enhances insulin sensitivity and is not effective in the absence of insulin [20]. It lowers blood glucose level in non-insulin dependent diabetes mellitus (NIDDM) patients by suppressing hepatic glucose output and enhancing peripheral glucose uptake. The mechanism of action involves binding of the polar biguanide hydrocarbon side-chain to membrane phospholipids, evoking a change in the electrostatic surface potential [21]. As an extension of this research field, we aimed to attach the new pharmacologic agents to the surface of carboxylated multiwall nanotubes, in order to take advantage of the MWNT's outstanding biological properties and to screen the final products for their antibacterial activity.

2. Experimental

All reagents and solvents were obtained from Merck Chemical Inc. (Darmstadt, Germany), and Carboxylic acid-functionalized MWCNTs (MWCNT–COOH with 95% purity, 20–30 nm in size) were purchased from Netrino Co. Ltd and used as received. The FT-IR spectra were recorded using KBr tablets on a Nexus 870 FT-IR spectrometer (Thermo Nicolet, Madison, WI). TEM measurement was carried out on the LEO 912 AB electron microscope.

2.1 Preparation of MWNT-COCl

60 mg of the MWNT-COOH was sonicated in 90 ml of DMF for 40 min to give a suspension. Oxalyl chloride (2.5 ml) was added drop-wise to the MWNT suspension at 0 ˚C under N_2. The mixture was stirred at 0 ˚C for 2 h and then at room temperature for another 2 h. Finally, the temperature was raised to 70 ˚C and the mixture was stirred overnight to remove excess oxalyl chloride (Scheme 1). After cooling to room temperature, the mixture was filtered through 0.2 μm pore size polytetrafluoroethylene membrane. The filtrate was washed with EtOH (3*100 ml), and then dried in an oven for 4 h at 60 °C [22,23].



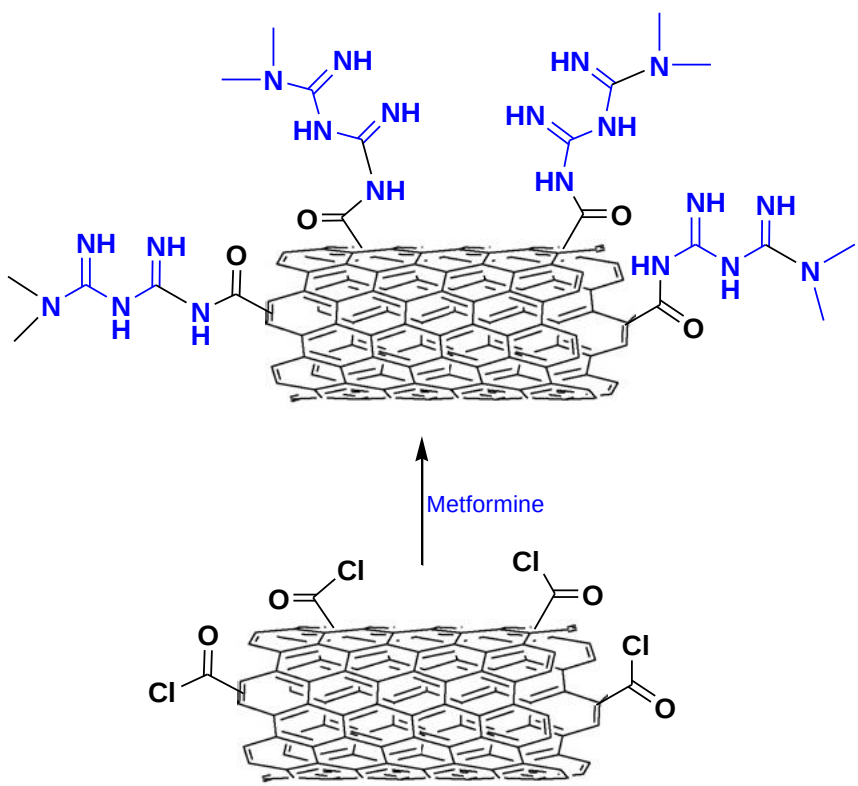
Scheme 1: Preparation of MWNT-COCl

2.2 Preparation of MWNT–CO-Metformin (MWNT-M)

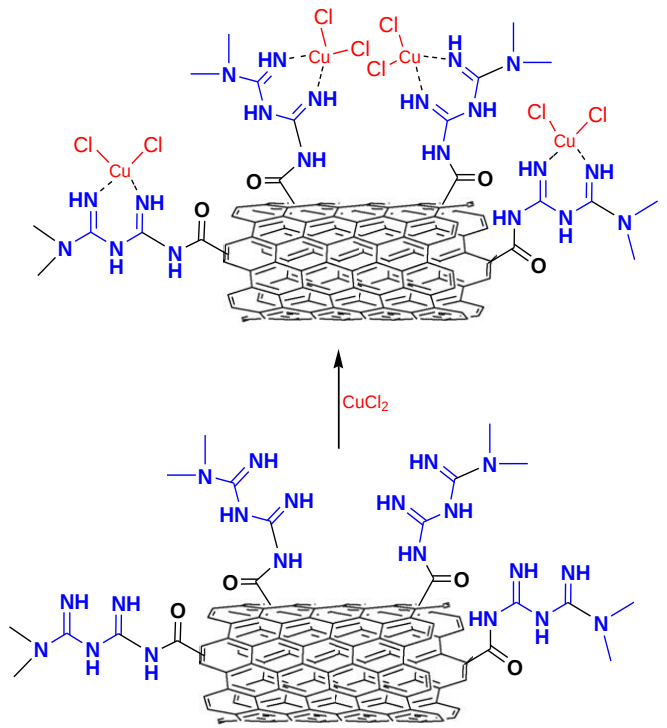
500 mg of Metformin compound was sonicated in 60 ml of DMF for 30 min and was added to suspension of 200 mg MWNT-COCl, and the mixture was refluxed at 80 ºC for 48 h (Scheme 2). Then the mixture was cooled to room temperature and filtered through 0.2 µm pore size polytetrafluoroethylene membrane. The filtrate was washed with MeOH (3*100 ml) and THF (100 ml). Subsequently, the black solid was vacuum dried at room temperature.

2.3 Preparation of MWNT–CO-[Metformin][$CuCl_2$] (MWNT-MC)

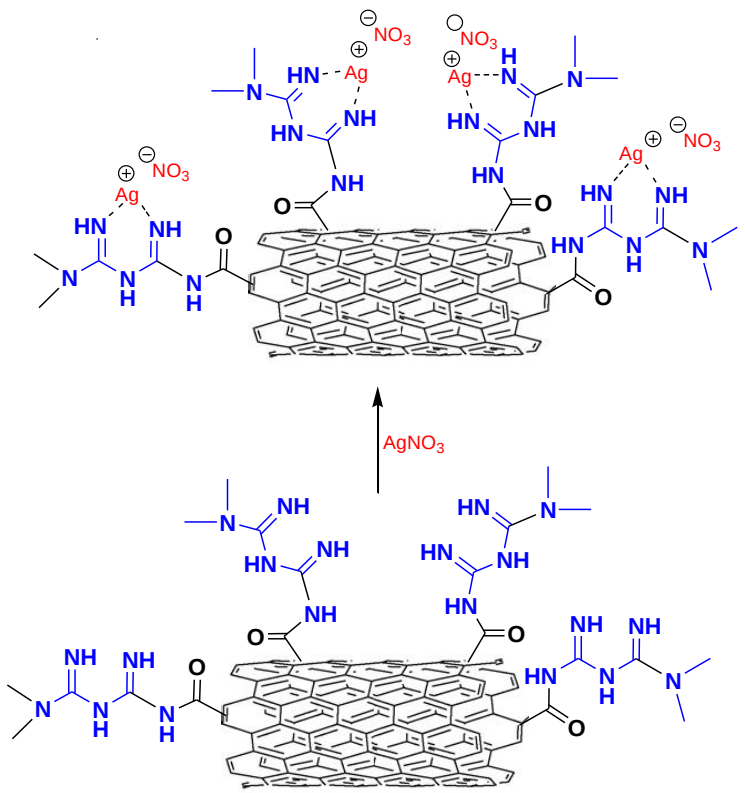
100 mg of MWNT-M was sonicated in 30 ml of DMF for 30 min. 20 ml of methanolic solution of $CuCl_2$. $2H_2O$ (100 mg) was added dropwise to the suspension (Scheme 3). The mixture was sonicated for one hour and stirred at room temperature for 24 h. Then the mixture was filtered through 0.2 µm pore size polytetrafluoroethylene membrane. The filtrate was washed with MeOH (3*100 ml) and THF (100 ml). Subsequently, the black solid was vacuum-dried at room temperature for 5 h.



Scheme 2: Preparation of MWNT-M



Scheme 3: Preparation of MWNT-MC.



Scheme 4: Preparation of MWNT-MA.

2.4 Preparation of MWNT–CO-[Metformin][$AgNO_3$] (MWNT-MA)

100 mg of MWNT-M was sonicated in 30 ml of DMF for 30 min. 20 ml of methanolic solution of $AgNO_3$. $2H_2O$ (100 mg) was added dropwise to the suspension (Scheme 4). The mixture was sonicated for one hour and stirred at room temperature for 24 h. Then the mixture was filtered through 0.2 μm pore size polytetrafluoroethylene membrane. The filtrate was washed with MeOH (3*100 ml) and THF (100 ml). Subsequently, the black solid was vacuum-dried at room temperature for 5 h.

2.5 Biological studies

All of the synthesized compounds were screened for their antibacterial activity against two strains of bacteria (Escherichia coli and Staphylococcus aureus). The antibacterial activities of the ligand and metal complexes were carried out using well diffusion method. Muller Hilton Agar was prepared and sterilized. 20 ml of media was poured into the petri-dishes and allowed to solidify. The wells were made carefully and these were completely filled with the test solutions. The test solutions of the studied compounds were prepared in DMF at a concentration of 1,000 μg/mL. The plates were incubated immediately at 37 °C for 24 hours and the diameter of the inhibiting area around each well was estimated which is described as the inhibiting effect against bacteria. The average of three diameters was calculated for each sample.

Table 1: Fourier transform infrared interpretation of the MWNT-COOH and products.

Type of functionalized MWNTs	Peak (cm^{-1})	Interpretation
MWNT-COOH	1568	**–C=C stretching vibration**
	1705	**–C=O stretching vibration (acid bond)**
	2450-3400	**–OH stretching vibration (acid bond)**
MWNT-M	1637	**–C=NH stretching vibration**
	1671	**–C=O stretching vibration (amide bond)**
	3436	**–NH stretching vibration**
	1320	**–N(Me_2) stretching vibration**
	3184	**–NH stretching vibration**
MWNT-MC	1557	**–C=C stretching vibration**
	1656	**–C=O stretching vibration (amide bond)**
	1093	**–N(Me_2) stretching vibration**
	3432	**–NH stretching vibration**
	1609	**–C=N-Cu stretching vibration**
	556	**Cu–N vibration**
MWNT-MA	1567	**–C=C stretching vibration**
	1605	**–C=N-Ag stretching vibration**
	1664	**–C=O stretching vibration (amide bond)**
	3418	**–NH stretching vibration**
	1227	**–N(Me_2) stretching vibration**
	3220	**–NH stretching vibration**
	529	**Ag–N vibration**

4. Results and discussion

FTIR spectroscopy was used to identify the chemical groups that were attached to MWNTs. Figure 1 shows the FTIR spectra of MWNT-COOH (1a), MWNT-M (1b), MWNT-MC (1c) and MWNT-MA. In spectrum (1a), the band at around 1568 cm^{-1} corresponds to the stretching mode of the C=C double bond that forms the framework of the carbon nanotube sidewall. The peaks at 1705 cm^{-1} and 2450 cm^{-1} to 3400 cm^{-1} apparently corresponds to the stretching modes of the carboxylic acid groups. The two bands at around 2850 cm^{-1} which are seen in all spectrums are attributed to the CH stretching of MWNT–COOH defects. In spectrum (1b), the two bands at 3436 cm^{-1} and 3184 cm^{-1} can be assigned to the N-H stretching modes and carbonyl peak in the spectrum (1b) shift to 1671cm^{-1} (as compared with 1705 cm^{-1} in spectrum (1a)) is a result of amide (C=O)NH linkage formation. Also the peak at 1637 cm^{-1} can be assigned to the C=N stretching. These could indicate that amidation reactions occurred between the amino groups of Metformin and the carboxyl groups on the surfaces of the MWNTs. The pick 1637 cm^{-1} in the spectrum

(1b) shifted to lower frequency 1609 cm^{-1} and 1605 cm^{-1} at the spectrums (1c) and (1d) and this indicate presence of C=N double bond coordinating of metal ion to N atom.

Furthermore, the nature of the metal-ligand bonding is confirmed by the newly formed bands at ~556 cm^{-1} and ~529 cm^{-1}, in the spectra of these compounds (MWNT-MC and MWNT-MA), which are tentatively assigned to Cu-N and Ag-N vibration. The detailed list of peaks and their assigned groups is provided in Table 1.

Raman spectra offer useful information concerning the slightly structural changes of MWNTs, especially the changes owing to significant Sidewall modification. As can be seen in Figure 2, the characteristic peaks of MWNT tangential modes, namely the D band at around 1330 cm^{-1} and the G band at around 1500 cm^{-1} slightly changed. We observed an increase in the ratio of intensities R = ID/IG in modified nanotubes. This indicates an increased disorder of the graphitic structure of the modified nanotubes, which shows that the nanotubes were covalently modified.

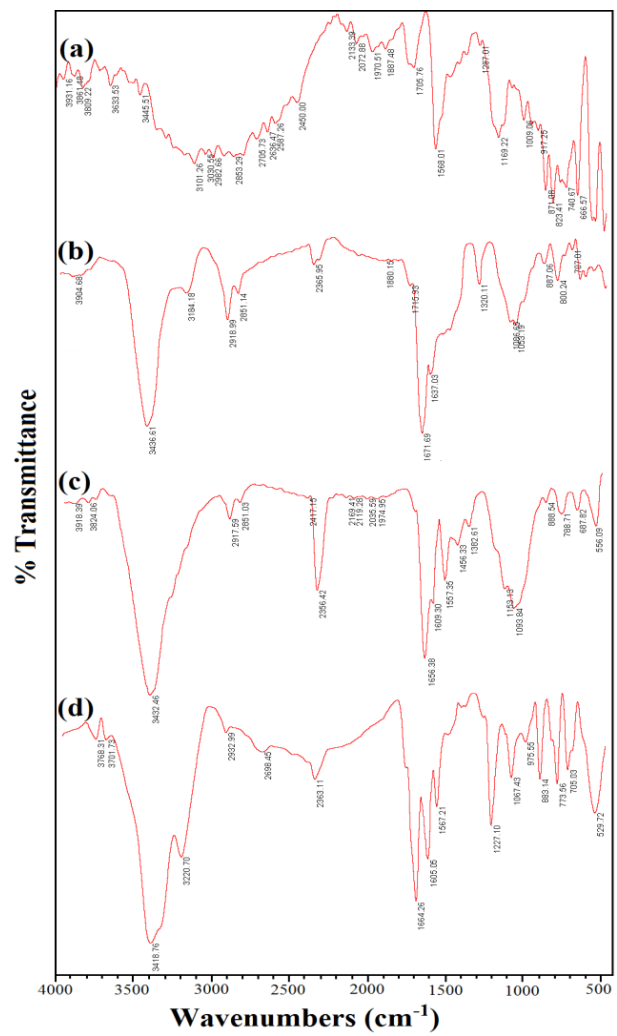


Figure 1. FT-IR spectra of functionalized carbon nanotubes: MWNT-COOH (a), MWNT-M (b), MWNT-MC (c) and MWNT-MA (d).

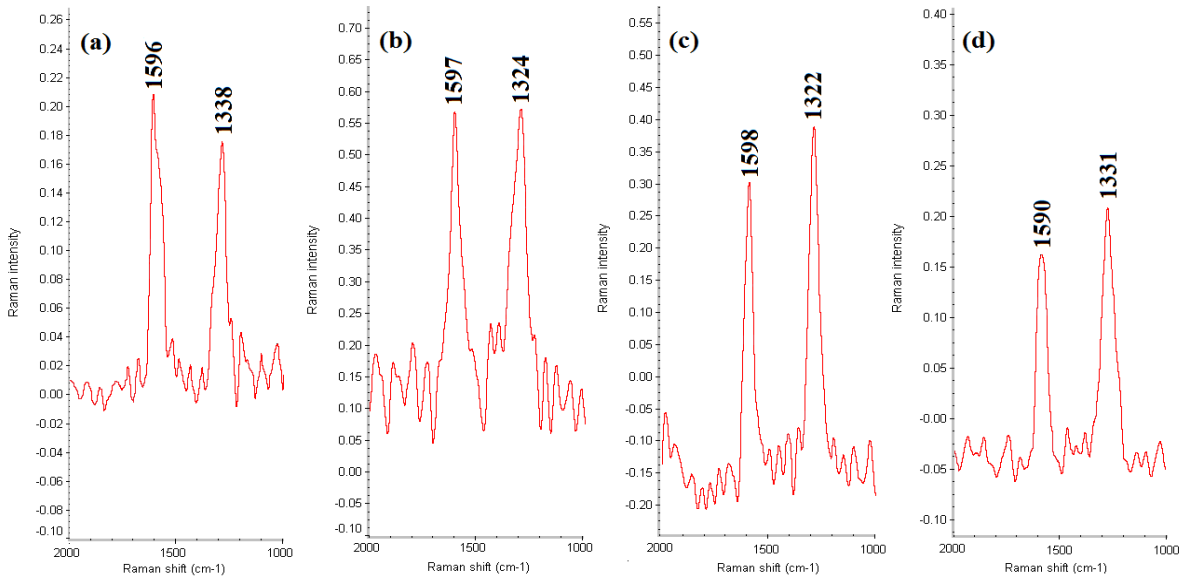


Figure 2. Raman spectra of functionalized carbon nanotubes: MWNT-COOH (a), MWNT-M (b), MWNT-MC (c) and MWNT-MA (d).

Table 2. Elemental analysis of modified MWNTs.

MWNT	%C	%H	%N
MWNT-COOH	93.47	0.34	0.00
MWNT-M	80.42	1.20	4.56
MWNT-MC	76.95	1.79	6.06
MWNT-MA	76.32	1.88	6.27

Elemental analyses of modified MWNTs are shown in Table 2. Apart from the carbon values, the changes of atomic percentages H (1.20%) and N (4.56%) in MWNT-M, H (1.79%) and N (6.06%) in MWNT-MC, H (1.88%) and N (6.06%) in MWNT-MA (as compared those in MWNT-COOH) indicated that MWNT-COOH is functionalized. On the other hand, the increase of percentage of N for MWNT-MC and MWNT-MA in comparing to MWNT-M confirms the formation of complex on the surfaces of the MWNTs.

Figure 3 presents TEM images of the MWNT–COOH (3a) and the MWNT-M (3b). The MWNT–COOH shows almost smooth surface (3a) while increased roughness of the functionalized CNT surfaces evident in TEM images of MWNT-M.

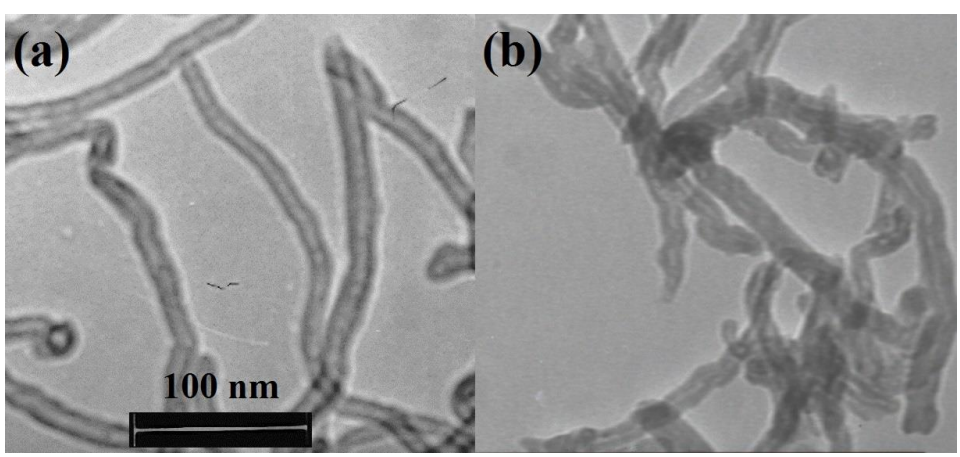


Figure 3. Transmission electron microscopy images: MWNT-COOH (a) and MWNT-M (b).

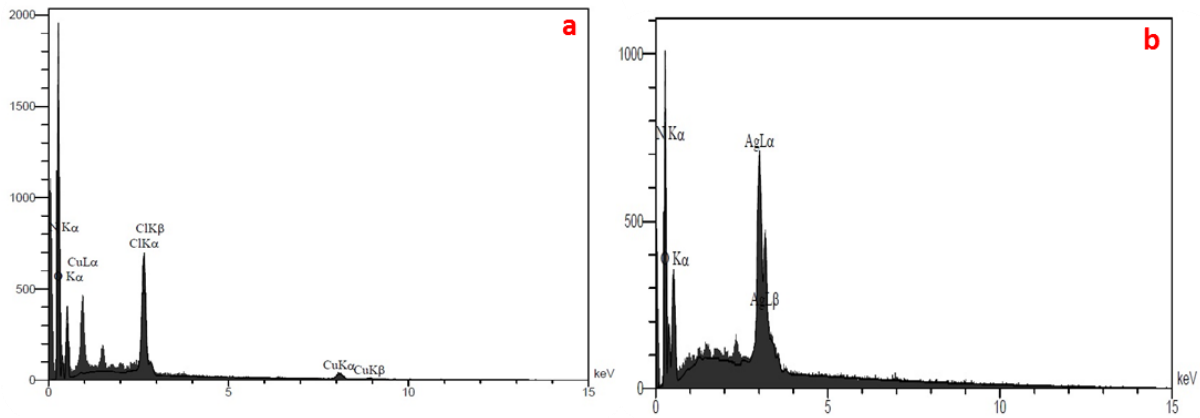


Figure 4. EDX analysis of MWNT-MC (a) and MWNT-MA (b).

Additionally, to confirm the presence of the cupric and silver ions, EDX spectra of the both nano composite were recorded. The spectra proved the presence of the Cu and Ag ions, and also carbon and nitrogen in the matrix (Figure 4).

The antibacterial activity of MWNT-COOH (A) as well as corresponding functionalized MWNTs MWNT-M (B), MWNT-MC (C) and MWNT-MA (D) and compounds Metformin (E), [Metformin][$AgNO_3$] (F) and [Metformin][$CuCl_2$] (G) were performed against one Gram positive (Staphylococcus aureus) and another Gram negative (Escherichia coli) bacteria, and the results are summarized in Table 3. The results indicate that the modified MWNTs show higher activity than MWNT-COOH against these two bacteria (Figure 5). These data also demonstrate that MWNT-COOH has no activity on the growth of S. aureus bacteria. Compounds C and D possessed a significant inhibitory effect against all the tested bacteria. The increase of the zone of inhibition for compound C when compared with corresponding ligands (compound E) is an indication that the modified MWNTs is able to decrease the population of Staphylococcus aureus bacteria. Moreover, Compound (C) present higher activity than other functionalized MWNTs against E. coli. Therefore, it should be noted that presence of metal ion in the structure of compounds C and D had a great influence on the suppression activity of these compounds on E-Coli. An improved activity was observed for the functionalized MWNTs of the present investigation. As these results are preliminary, further study on the antibacterial activity of these complexes is highly recommended.

In summary, we have introduced for the first time three novel modified carboxylated multiwall carbon nanotubes, as pharmacologic agents in order to observe the effect of different substituents attached to the surface of MWNTs on the studied bacteria. These modifications have been characterized by FT-IR, Raman, Elemental analysis and TEM. The antibacterial activity of the synthesized compounds has been evaluated against E-Coli and S. aureus. Functionalized MWNTs showed the ability to inhibit E-Coli and S. aureus. The most outstanding results were obtained from the activity of Compound MWNT-MC against E-Coli, which exhibited much higher antibacterial activity than other modified MWNTs. Therefore, compound MWNT-MC seems to be a very promising candidate for further tests which will be carried out in the near future.

Table 3: Antibacterial screening data of Compounds A-E against the tested bacteria (inhibition zone in mm)

Compound	E. coli	S. aureus
MWNT-COOH (A)	8	0
MWNT-M (B)	28	21
MWNT-MC (C)	38	32
MWNT-MA (D)	34	30
Metformin (E)	20	28
[Metformin][$AgNO_3$] (F)*	17	14
[Metformin][$CuCl_2$] (G)*	20	25

*Compounds (F) and (G) were synthesized according to references [19] and [20].

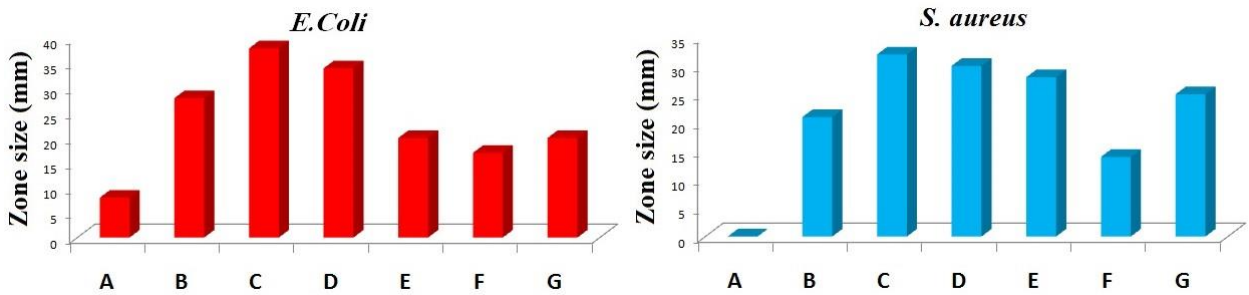


Figure 5. Average diameter of zones of inhibition for compound (A-G) against E-Coli and S. aureus bacteria.

5. Conclusion

In summary, we have theoretically investigated the incorporation of oxaliplatin molecule inside the semiconducting and metallic SWCNTs using the first-principles calculations based on vdW-DF methods. Various diameters of SWCNTs from 8 to 15 Å were considered. A full structural relaxation procedure was carried out for all the systems under study. The obtained results indicated that metallic SWCNTs exhibit slightly weaker interaction with the encapsulated oxaliplatin in comparison with the semiconducting one. However, the binding energies value and equilibrium distances obtained from first-principles calculations for the energetically favourable complexes are typical for the physisorption. Our results showed that semiconducting nanotube with diameter of about 11 Å is suitable for the encapsulation of oxaliplatin. In the case of metallic one the corresponding value is determined to be about 9 Å (Figure 3). A study of the electronic structure indicated that no significant hybridization between the respective orbitals of the host-guest entities takes place that explain the observed small interaction and weak charge transfer between the CNTs and the oxaliplatin. Our first-principles findings provide a molecular insight into understanding of the interaction between oxaliplatin and SWCNTs and hope to be helpful to the relevant experimental researchers who effort to realize suitable nanovectors for drug delivery.

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