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Comparison between Biological Activities of Commercial and Synthesized Carbon Nanotubes by Flame Fragments Deposition Technique

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Abstract:

Carbon nanotubes (CNTs) were synthesized via liquefied petroleum gas (LPG) as precursor using flame fragments deposition (FFD) technique. In vitro, biological activates of carbon nanotubes (CNTs) synthesized by FFD technique were investigated. The physiochemical characterizations of synthesized CNTs are similar to other synthesized CNTs and to the standard sample. Pharmaceutical application of synthesized CNTs was studied via conjugation and adsorption with different types of medicines as promote groups. The conjugation of CNTs was performed by adsorption the drugs such as sulfamethoxazole (SMX) and trimethoprim (TMP) on CNTs depending on physical properties of both bonded parts. The synthesized CNTs almost have the same performance in antibiotic activity compared with standard sample of CNTs (commercial CNTs).The products were effective antibiotic in the treatment as resistant bacteria, may higher concentration of CNTs would have antibacterial activity on multi-drug resistant bacteria such as Acinetobacter and also on resistant E.coli. The bioactivity synthesized and standard samples of CNTs were almost the same against different types of bacteria.

Key words: Biological Activity, Carbon Nanotubes, Flame Fragment Deposition, Sulfamethoxazole, Trimethoprim.

Introduction:

Nanotechnology is modern science relating to functional engineering systems of molecular, atomic, supramolecular scales below 100 nm. This science is not limited to a particular branch, in other words, it is not chemistry, biology, and technology or engineering due to nanotechnology has properties and aspects relating to all sciences of nature and life. Pharmaceutical nanotechnology has wide applications in disease diagnostics, therapeutics, and drug delivery (1).

Generally, there are many Nano-materials that have been discovered such as zinc nanoparticles, titanium dioxide nanoparticles, silver nanoparticles, carbon Nano-tubes, gold nanoparticles, and gold beacon. This tiny size molecules and particles could face scientific problems with large products (2).Recently, one unique form is discovered and known as carbon nanostructures (CNSs). This novel discovery will change different concepts in the applications of carbon.

Zhang *et al*. (3) reported that carbon nanotubes (CNTs) are cylinders of one or more layers of graphene (lattice). Single-walled carbon nanotubes (SWNTs) and multi-walled carbon nanotubes (MWNTs). Tanaka *et al*. (4) explained that CNTs have length about 1000 nm as diameter. Basically, CNTs structure consists of graphite cylindrical (graphene) rolled in a smooth cylinder with a diameter of the order of nanometer. Carbon nanotubes (CNTs) are considered as the allotropes of carbon with nanostructured cylinders (5). Such nanoparticles have exceptional properties, which are interesting for nanotechnology, electronics, optics and other materials science and technology fields.

The unique size of CNTs making them as drug transferor and biosensing platforms for treatment of diverse diseases (6). Bellucci*et al.* (7) studied characterization of CNTs by Atomic Force Microscopy (AFM) and regarded that evaluation of the nonmetric geometry and characteristic features of the nanostructures, homogeneity that means to find out the statistical distribution of the various nanomaterial's/structures present in a test sample, and dispersibility that determines the ability of the nanostructures to form stable suspension at certain

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concentration values as bundles or single elements. CNTs have the abilities to work with effective structures because of high capacities of drug loading and improved cell penetration qualities. They might be synthesized without or with end caps, which that without end caps the inside where the drug is captured with more accessible (8).Cao *et al.* (9) found that there is a qualitative relationship between the degree of nanotube alignments and the intensities of peaks of the XRD patterns. However, another study revealed that some problems have been raised with using carbon nanotubes in drug delivery systems for many reasons like the lack of solubility, clumping occurrences, and half-life (10). Bhirde*et al* (11) reported that minimum side effects carbon nanotubes in drug delivery due to effective uptake of these nanoparticles. Drug encapsulation may improve the physical and chemical effects of CNTs for example, enhancing water dispersibility, bettering bioavailability and reducing toxicity by carbon nanotubes(3). This work aimed on synthesis of CNTs via FFD techniques using liquefied petroleum gas (LPG) as a source of carbon (12-14) and studying their pharmaceutical applications.

Materials and Methods:

Material

All the chemicals used in this experiments were analytical grade and without any further purification. The precursor chemicals are liquefied petroleum gas (Hillah Gas Factory, Iraq), nitrogen and oxygen gases (Karl Kolb Gmbh, Germany), Acetone (BDH, England), Hydrogen peroxide (Barcelona, Spain), Muller Hinton agar (Himedia, India), Sulfamethoxazole and Trimethoprim (Shreeji Pharma International, India), and other solvents such as deionized water.

Synthesis of Carbon Nanotube from Liquefied Petroleum Gas

The carbon was obtained by burning a mixture of gases from Ethan, methane, isopropane, butane and isobutene by using flame fragmentation deposition (FFD) instrument. This process leads to produce carbon nanotubes/amorphous graphite carbon sixty. The FFD need to use two type of gases, LPG (as source of carbon) and nitrogen gas (to maintained the suitable flame form) first the gases will burn with the exits of oxygen in the instrument. FFD homemade reactor consists of 9 collection centers in which the crucible lays under each position.

Purification of Carbon Nanotubes

100mg of synthesized carbon nanotubes were taken and 70 ml of hydrogen peroxide were added and then the mixture was put in sonication apparatus for 1 hr. The suspension was put in refrigerator for 24 hrs at 8 °C, and then washed with DW twice. The sample was then dried by putting in the oven at 80 ˚C with stirring for 3.5 hr. 15ml of acetone were added and the suspension was sonicated for a period of 15 min. The sample was centrifuged for 15 min (6000) rpm finally burn in 275 ˚C for 2hr.

Preparation of Sulfamethoxazole and Trimethoprim solutions

500 ppm stock solutions of sulfamethoxazole, and trimethoprim were prepared in by dissolving 50mg in 100 mL of de-ionized water. The diluted solutions of drugs were prepared by taking required volume of stock solution and diluting by deionized water.

Adsorption Sulfamethoxazole and Trimethoprim on CNTs

20 mg of synthesized and standard samples of CNTs were weighted separately and added to 25mL of 500 ppm SMX solution separately. The trimethoprim was loaded on both forms of CNTs by the same way that SMX was loaded. The mixtures of TMP and SMS were shaken at RT for 2 hrs. Finally the mixture was centrifuged for 10 min at 6000 rpm, and then filtrates were diluted and scanned with UV-Vis spectroscopy analysis.

Biological Activity of Carbon Nanotube CNTs by Agar Well Diffusion Method

CNT particles were suspended in sterilized deionized water for achieving the interaction of the CNTs particles with the bacteria. In this work, different concentrations were prepared from mixture of trimethoprim, sulfamethoxazole, and carbon nanotubes. Suspensions of trimethoprim loaded on CNTs were prepared at concentrations (80, 120, 160, and 200 mg/L). As well as, CNTs suspension, TMP (50mg/L), and of SMX (50mg/L) solutions were prepared as control samples. Suspensions of sulfamethoxazole loaded on CNTs were prepared at concentrations (80, 120, 160, and 200 mg/L). Samples were shaken using sonicater for getting suspension. Then, well of agar directly is applied in the plate, all of them were tested against each bacterial samples. The inoculums size was adjusted so as to deliver final inoculums of approximately108 colony forming unit (CFU)/ml from the grown bacterial culture of a 24 hrs. old for all strains to compare the turbidity of each sample to the 0.5 McFarland standards, the broth of these microorganisms was culture on nutrient agar plates. Next, 25 mL of Mueller–Hinton (MH) agar medium were solidified in Petri plate's hollows of six wells (5 millimeter diameter). They were cut into the agar by cork borer then all the collected pathogenic bacteria samples were tested on this agar. 0.1 ml of

suspended TMP, and SMX loaded on CNTs were applied in these four walls. Petri dishes were incubated at 5-8 °C for 2-3 hrs and then incubated for 24 hrs at 37°C.

Results and Discussion:

Characterization of CNT

There are many techniques including scanning electron microscopy(SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) which have been implemented to reveal the structural details of CNTs.

SEM and AFM Measurements

The following techniques show methods used for determining carbon nanotubes basic parameters. Atomic Force Microscopy (AFM) images of the carbon nanotubes were obtained using AFM (Sartorius Arium 611). AFM images on the Fig.1 (A, B, and C), bundles of carbon nanotubes among carbon particles have been observed. AFM characterization refers to the highly morphology on the surface of CNTs and 3D of roughness to this surface as nano instrumentational. The morphological feature of the carbon nanotubes were determined by using Scanning Electron Microscopy (SUPRA- 55VP) using electron beam energy of 10 KV and 15 KV. SEM image shows existence as a needle structure (Fig1D) with highly agglomeration. This agglomeration is attributed to nanoparticles interfaces to the surface and high dimensional for the particle distance in the crystal size. The following figure represents the topography of the CNTs. The only measurable parameters are the size of carbon particles and diameter and length of bundles.

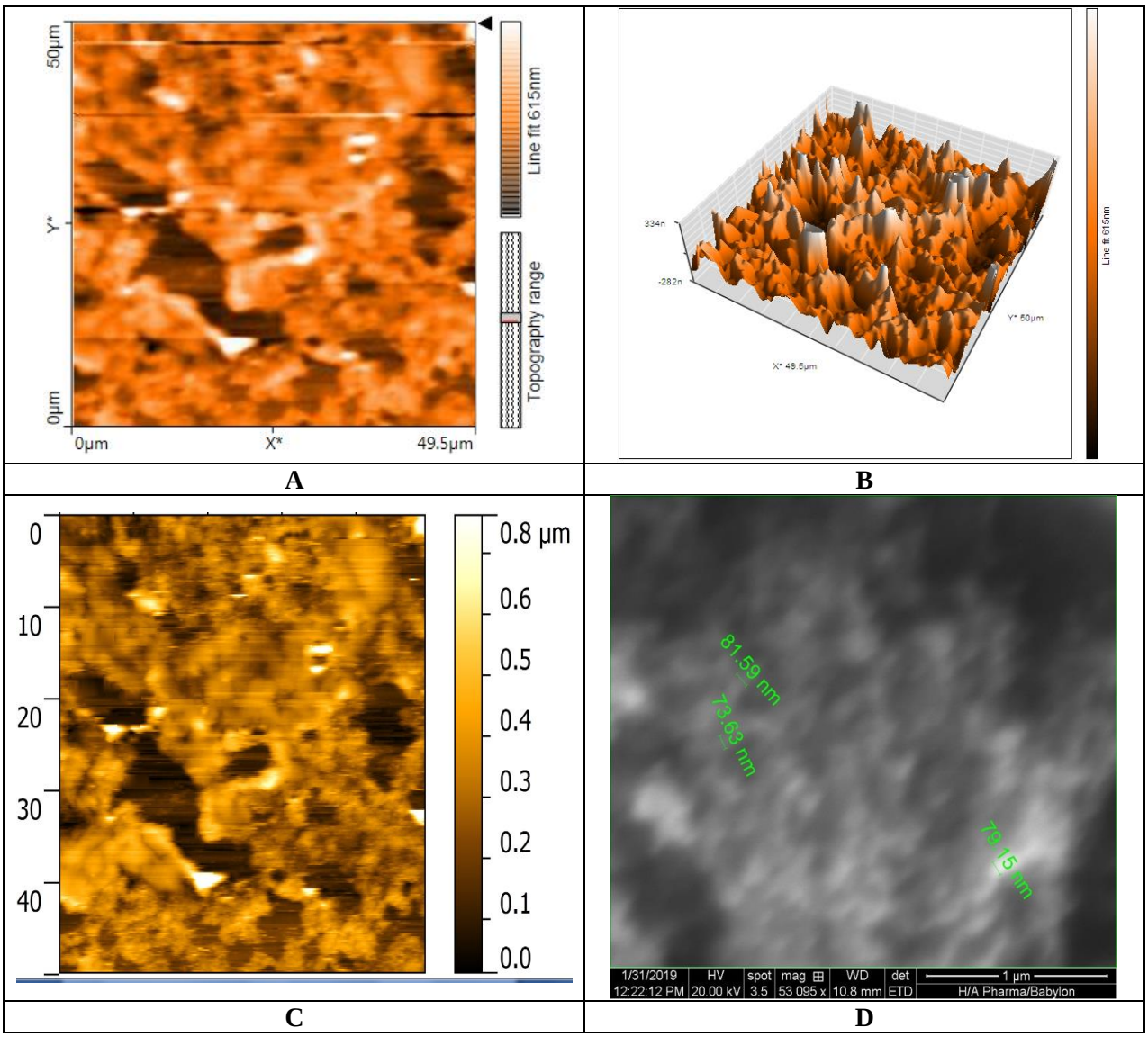
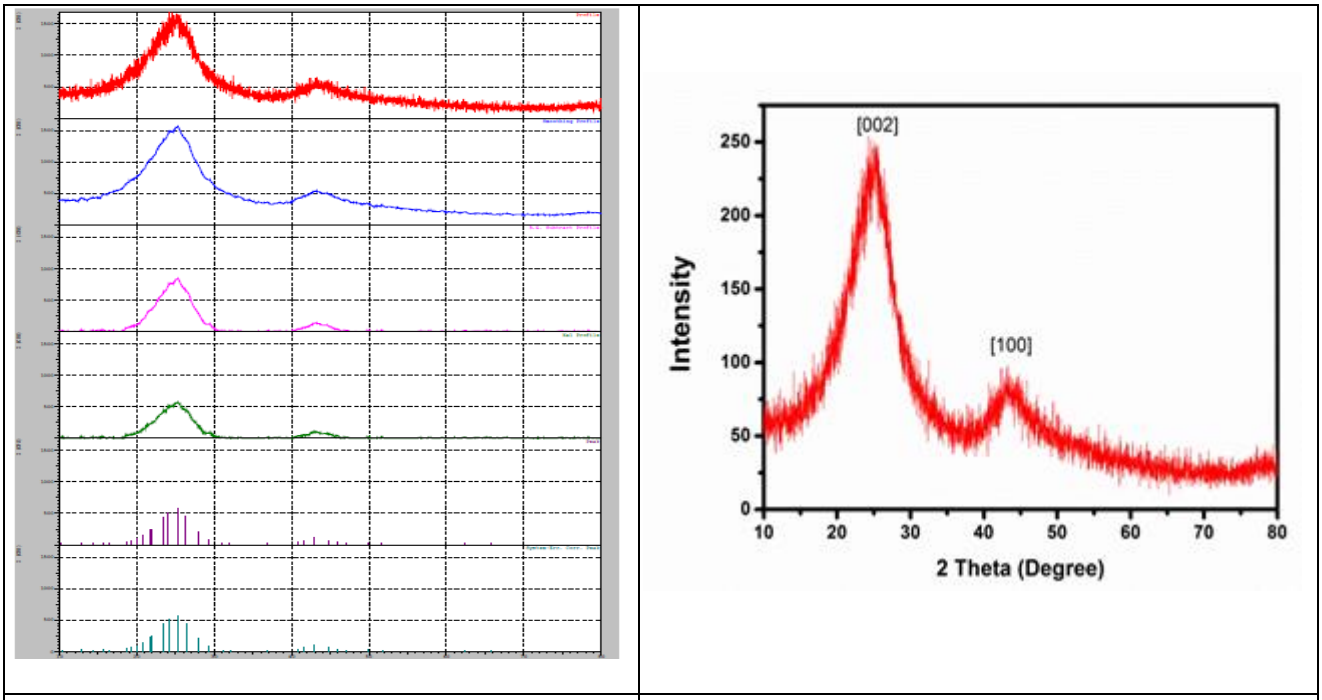


Figure 1. AFM and SEM images of synthesized CNTs

XRD measurements

The X-ray powder diffraction patterns of the carbon nanotubes were recorded using X-ray Diffractometer (Rigaku D/ Max Rapid) equipped with nickel filtered Cu-Kα1 radiation operating at 40 KV and 30 mA. XRD technique has been utilized to reveal most of the morphology and structural features of CNT aligned at different angles. The carbon atoms in CNTs act scatter light at different angles, but specific angles as 3D optical diffractions. Diffracted angles give information about the aligning graphene sheets of the CNT from the position and intensity of diffracted beams. XRD pattern of CNTs has shown (Fig.2 A and B) some distinct similarities to reference patterns, which are commonly found in the JCPDS (Joint Committee on Powder Diffraction Standards) library. Intensities of CNTs diffraction peak are based on CNTs' morphological orientation. From the Figure (2 A and B) X-ray beam strikes CNTs to produces (002) peaks with some parallel reflections. X-ray beams strike the empty central core of CNTs to produce some extra hexagonal peak arrays depending on the number of helix present. A peak of 002 occurs at integer $2\pi/C0$, when basal hexagonal carbon atomic networks and parallel nanotube stacking layers will generate reflections. Peak gives rise to the information on the spacing (C0) between the nanotube layers. (002) peak intensity can help to measure the CNT diameter with Debye-Scherer methods. Furthermore, Cao *et al.* (9) have been reported that peak (002) intensity decreases as the degree of aligned nanotubes increase.



A **B**

Figure 2. XRD pattern of synthesized CNTs

Adsorption of Sulfamethoxazole (SMX) and Trimethoprim (TMP) on Different Types of CNTs

The UV-Vis absorption peaks of sulfamethoxazole and trimethoprim solutions were recorded with UV-Vis spectroscopy (*Shimadzu*, 1800 UV/VIS Spectrophotometer) in the wavelength region of (200 – 400) nm at room temperature(Figure3). Results show that the absorbance of drug delivery coated decrease with increasing the linkage, this may be attributed to highly adsorption between the CNTs and drug (Fig.3). Trimethoprim alone has maximum absorbance at 0.093 at wavelength 274.20 nm(Table1). After loading trimethoprim on standard sample the maximum absorbance was decreased to 0.073 at wavelength 270.80 (Table 1).This means some of TMP drug was adsorbed on standard sample so the concentration of TMP was decreased and because the concentration was directly proportional to the absorbance, according to Lambert-beer law so the absorbance was decreased from (0.093) to (0.073).When synthesized CNTs were loaded with trimethoprim, the maximum absorbance became (0.074) at wavelength 270.8 nm. This means that some of the TMP was adsorbed on synthesized CNTs, so the concentration of TMP decreased and because the concentration was directly proportional to the absorbance so the absorbance decreased to 0.074 (Fig.3).

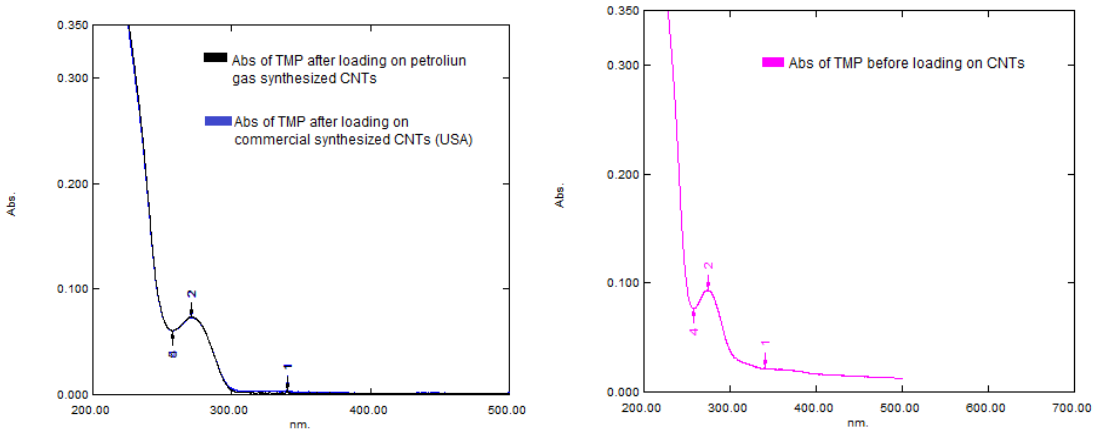


Figure 3. Absorbance of TMP solutions before and after loading on two types of CNTs

Table 1. Absorbance and Concentration of TMP solutions before and after loading on two types of CNTs.

Name of compound	λ_{max} nm	Abs.	Conc. (mg/L)
TMP alone	274.20	0.093	100
TMP loaded on commercial CNTs	270.80	0.073	82
TMP loaded on synthesized CNTs	270.80	0.074	87

By comparing of the standard CNTs sample results (Table 2) with the synthesized CNTs sample, no significant differences were observed (Figure4). However, the very small differences, may be due to advance in purification and synthesis process of the standard sample. SMX alone has maximum absorbance at (0.455) at wavelength (266.80) nm (Table 2). After loading SMX on standard sample of CNTs the maximum absorbance decreased to 0.281at wavelength 267.80 nm (Fig. 4). This means some of SMX drug was adsorbed on standard sample of CNTS so the concentration of SMX was decreased and because the concentration was directly proportional to the absorbance so the absorbance was decreased from (0.455) to (0.281) (Figure 4).When synthesized CNTs were loaded with SMX, the maximum absorbance became (0.340) at wavelength 267.60 nm (Table 2).

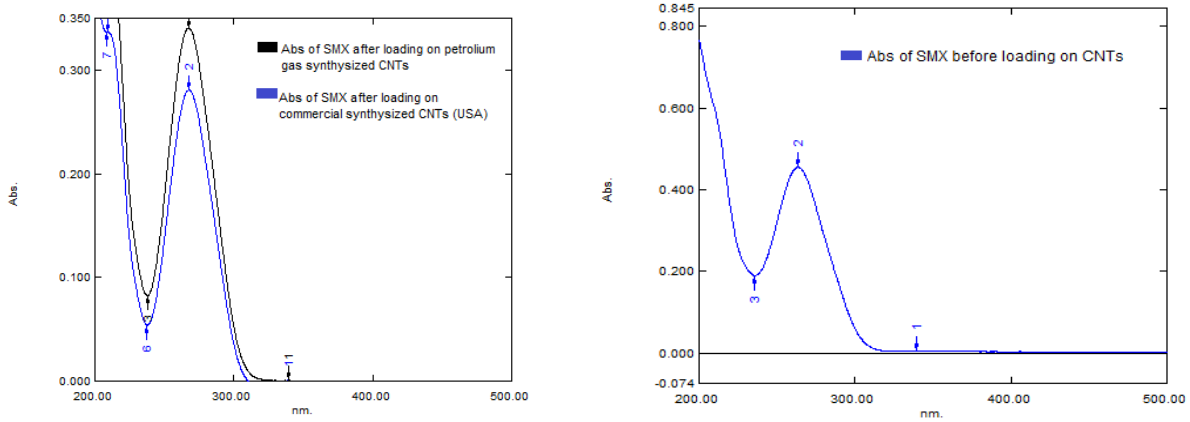


Figure 4. Absorbance of SMX solutions before and after loading on two types of CNTs.

Table 2. Absorbance and Concentration of SMX before and after loading on two types of CNTs

Name of compound	λ_{max}nm	Abs.	Conc.mg/L
SMX alone	266.80	0.455	100
SMX loaded on commercial CNTs	267.80	0.281	61.75
SMX loaded on synthesized CNTs	267.60	0.340	74.72

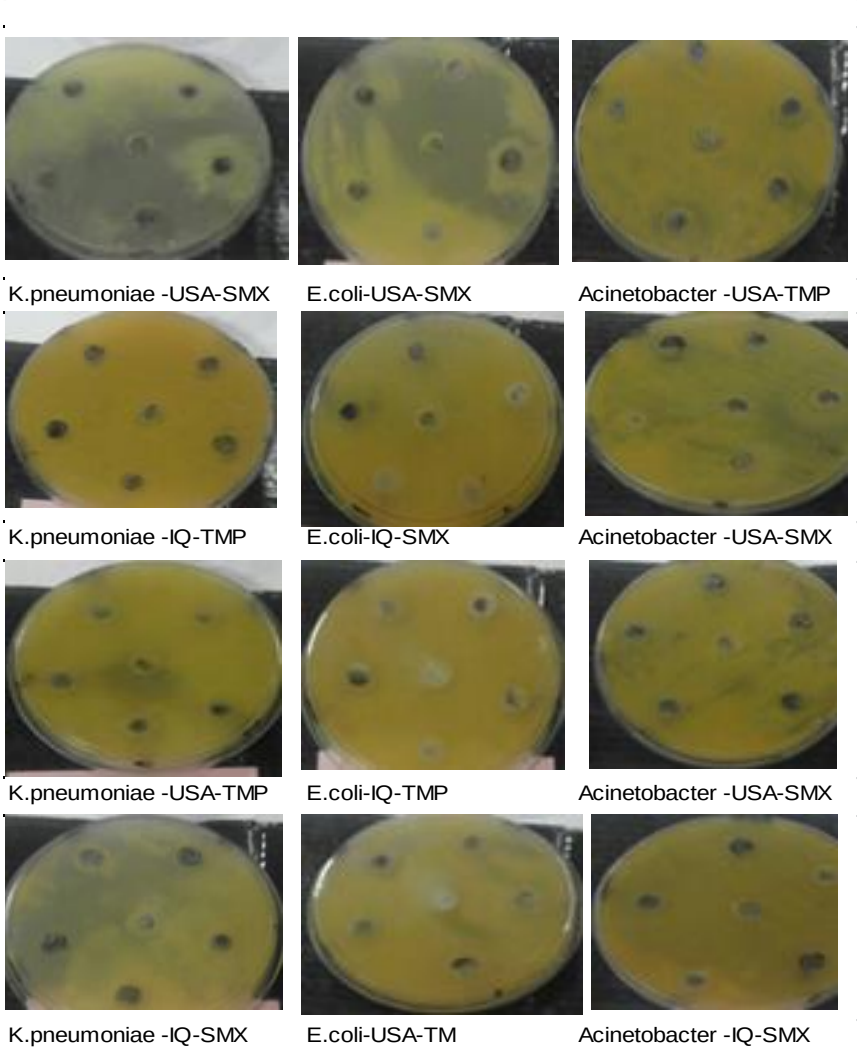
This means that some of the SMX was adsorbed on standard sample of CNTs so the concentration of SMX was decreased and because the concentration was directly proportional to the absorbance so the absorbance decreased. When makes comparison of the (USA-CNTS) results with standard sample of CNTs, it showed a little better

results, this may be due to advancement in purification and synthesis process.

Bioactivity of CNTs Loaded on Sulfamethoxazole and Trimethoprim

The assessment of antibacterial was based on measuring the diameter of the inhibition zone (mm) formed around the well. Most of the available antibiotics are modified versions of old one, that proved ineffective against MDR microorganisms, while there is still a strong need for novel antibiotics that can kill multi-drug-resistant (MDR)bacteria, new alternative techniques have been developed to detect and treat bacterial infections(15, 16).The use of nanotechnology in drug delivery has spread rapidly, with nanoparticles emerging as highly efficient delivery systems for targeted drug release(17).Present research revealed moderate to low sensitivity of bacteria by reagents, in general *K. pneumonia* more than another two bacterial samples, especially by (0,C and 4) of USA-SMX (Table-3) and only with (3 and 4) of USA-TMP (table-1), in addition, to that *K.pneumoniae* exhibited sensitivity to (2,3 and 0) of synthesized CNTs-SMX but at the same time *K.pneumoniae* didn't have any resistance to any reagent of IQ-TMP (Table 3).On the other hand, *E.coli* affected slightly by USA-TMPand only with C of USA-SMX (table 3), while *Acinetobacter* was not affecting by any reagent except C of USA-TMP. This moderate sensitivity of bacteria (*E.coli* and *Acinetobacter*) may refer to several factors related to bacteria and nanoparticles at the same time such as type of bacteria, virulence of pathogenic bacteria, different concentration CNTs of the preparation method of CNTs. Regarding most resistant bacteria in the current research, *Acinetobacter baumannii*, the importance of these bacteria relies in its ability to cause nosocomial infections and its increasing antibiotic resistance, the presence of resistance to antibiotic in *A.baumannii* complicates the implementation of efficient treatments, making the development of novel strategies to control the infections caused by such opportunistic microorganisms mandatory(18).

Table 3. The tested different type of bacteria in the present study



Sulfonamides are antimicrobial agents that have broad spectrum of activity, it is effective against the Gram-positive and certain Gram-negative bacteria, such as intestinal bacteria *Klebsiella, Escherichia coli, Shigella, Salmonella* and *Enterobacter* species(19).Sulfonamides differ in potency, but, not in the spectrum of the biological activity(20, 21).The combination of SMX/TMP is still considered as the most effective antimicrobial agent in the treatment of the infection caused by less common non-fermenters, except *Acinetobacter baumannii*. Against these microorganisms, sulfametrole/trimethoprim (SML/TMP) combination and sulfametrole (SML) are generally effective as SMX and SMX/TMP combination. The treatment of infections caused by bacteria can be more complicated due to the capability of the bacteria to createa resistance mechanism to the used antimicrobial drugs. Bacteria may be inherently resistant to antimicrobial drugs (inherent resistance) or may acquire resistance by *de novo* mutation or by acquired resistance (receiving the genetic material from resistant bacteria)(22).Bacterial resistance to sulfonamides and other antimicrobial drugs represents a serious problem. More frequent appearance of multi-resistant strains of *Staphylococcus Klebsiellapneumoniaee, Pseudomonas* and *Enterococcus*species in medical institutions causes treatment failure, which can lead to further complications, especially in children, old people and immune compromised patients. The use of sulfonamides and other antimicrobial medications in the various treatments leads to a continuous introduction into the environment, spreading over and maintaining drug bacterial (23).

The emergence of MDR pathogens is an increasingly significant global healthcare and economic drawbacks. Listed by the WHO as one of the top 3 threats to global public health more than 2 million of the American citizens suffer from antibiotic resistant infections at a direct cost of over $20 billion, with over 23,000 dying annually (24, 25).Analogous worldwide statistics are staggering, prompting intense multidisciplinary efforts by scientific and clinical communities to develop innovative products and tools to address the threat. The USA Center of Disease Control (CDC) has recently classified emergent resistant species as urgent, serious, or concerning (19). Resistance has developed virtually in every class of antibiotics in current uses [(21)]. The development of bacterial resistance to a given antibiotic is anticipated to evolve within an average of 50 years after initial use. Resistance to certain antibiotics (e.g. tetracycline, etc.), often develop in at least one bacterial species within a year of drug USA Food and Drug Administration (FDA) approval (25, 26).

Conclusion:

Further clinical studies are required to select the most appropriate and effective antibiotic in the treatment to resist bacteria, may be higher concentration of CNTs would have antibacterial activity on multi-drug resistant bacteria such as Acinetobacter and also on resistant *E.coli*. The application of the sulfonamides and other antimicrobial agents in the therapy leads to their continuous introduction into the environment, spreading over and maintaining drug resistance.

Conflicts of Interest: None.

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مقارنة بين الأنشطة البيولوجية للأنابيب النانوية الكربونية التجارية والمحضرة بتقنية ترسيب شظايا اللهب

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الخلاصة:
تم تحضير الأنابيب النانوية الكربونية (CNTs) في المختبر من غاز البترول المسال (الغاز المستخدم للطبخ في العراق) (LPG) كمصدر للكاربون باستخدام تقنية ترسيب شظايا اللهب (FFD). تم فحص النشاطات الحيوية للأنابيب النانوية الكربونية (CNTs) المحضرة بواسطة تقنية ترسيب شظايا اللهب ومقارنتها مع الانابيب الكاربونية التجارية. بشكل عام ، تتشابه الخصائص الفيزيوكيميائية للأنابيب النانوية الكربونية المحضرة مع الأنابيب النانوية الكربونية عن طريق الاقتران والامتزاز مع أنواع مختلفة من الأدوية كما تعزز المجموعات ، ولا سيما في الرحيل الدوائي. تم تنفيذ اقتران CNTs عن طريق الامتزاز للأدوية مثل sulfamethoxazole (SMX) و trimethoprim (TMP) على CNT اعتمادا على الخصائص الفيزيائية للأجزاء الرابطة. بينت النتائج بان فعالية CNTsالمحضرة لها نفس الأداء في نشاط المضادات الحيوية مقارنة مع العينة القياسية من CNTs (CNTs التجارية). كانت المنتجات المضادات الحيوية فعالة في العلاج مثل البكتيريا المقاومة ، قد يكون تركيز أعلى من CNTs النشاط المضاد للبكتيريا على البكتيريا المقاومة للأدوية المتعددة مثل كما Acinetobacter وأيضا على مقاومة E.coli. كان النشاط الحيوي المركب والعينات القياسية من الأنابيب النانوية الكربونية هي نفسها تقريبا ضد أنواع مختلفة من البكتيريا.

الكلمات المفتاحية: النشاط البيولوجي، الأنابيب النانوية الكربونية، ترسب شظايا اللهب، سلفاميثوكسازول، ترايميثوبريم.