Titanium Dioxide-Carbon Nanotubes Composites Immobilized Ag Nanoparticles: Enhanced Photocatalytic Bacterial Inactivation and Mechanistic Study

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Abstract: Ag nanoparticles modified TiO2-CNTs synthesized using polymeric template consisting of polyethylene glycol and polyvinyl alcohol (Tev) and loaded with different wt% of Ag (2%, 6%) were exploited to react hydrothermally (433 K for 48 h) with carboxylate functionalized SWCNT/MWCNT moieties. Several characterization techniques, including UV-visible diffuse reflectance spectroscopy, X-ray diffraction, HRTEM, and selected area electron diffraction (SAED), photoluminescence (PL), Raman, FTIR and N2 sorptiometry were used to obtain information about the morphology, crystalline phases, optical properties and surface texturing. The Antibacterial activity of synthesized photocatalysts towards Escherichia coli (Gram negative) and Staphylococcus aureus (Gram positive) was tested by performing bacterial DNA and agar well diffusion method under visible irradiation ($\lambda > 450$ nm, an average light intensity of 60 mWcm$^{-2}$). It has been shown that TevAg6-SWCNT verified the highest lethal action against bacterial growth compared to rest of samples due to delaying the recombination of electrons and holes, increasing the surface area value as well as decreasing the spherical nanoparticles of Ag into 3 nm diameter. A clue about the attack mechanism of nanocomposites while disinfecting bacteria indicates the existence of $\cdot$OH and O$_2^-$ radicals those played important role in the inactivation process. The role of Ag$^+$ ions, leakage of K$^+$ ions, TEM images; taken while irradiating bacterial strains in presence of TevAg6-SWCNT, as well as protein damage were thoroughly studied and correlated to understanding the visible light induced disinfection mechanism. Catalyst recyclability for 6 consecutive times retains more than 96% activity highlighting the catalyst stability.

Keywords: Ag/TiO2-CNTs nanocomposites, Bacterial disinfection; DNA damage; Surface active species, morphology change.

1. INTRODUCTION

Carbon nanotubes (CNTs) have attracted considerable attention because of their outstanding electrical, mechanical, magnetic properties, high surface areas and high chemical stability [1-2]. TiO2 is used widely as a photosensitizer for solving environmental problems. In particular, it has been used to remove different chemical pollutants from water as well as from air [3-5]. However, the low photocatalytic efficiency has restricted the application of TiO2, in practical water treatment [6-8] since it’s only active under ultraviolet irradiation. Composite materials containing CNTs and TiO2 have attracted considerable attention because of their distinctive properties including large surface area, high adsorption capacity as well as attenuating photoassisted reactions to emerge under visible light conditions [9-10]. Accordingly, anchoring TiO2 on nanocarbons obstructs the electron–hole recombination at the TiO2-CNT boundaries and thus yields superior photocatalytic performances compared to individual analogue [11-13]. On the other hand, the morphology and structure of CNTs allow them to act as specific templates for fabricating metal nanoparticle-CNTs composite [14-15]. It has been reported that Ag incorporated CNTs demonstrated high electrocatalytic activity towards hydrazine oxidation [16]. In contrast, the photocatalytic performance of Ag/TiO2 towards 4-nitrophenol reduction was also accomplished via the existence of Ag$^+/Ag^+$ species at the interface of TiO2 phases [17]. Ag$^2$-modified mesoporous TiO2 indicates high activity for herbicide degradation due to the formed Ag/TiO2 interface as well as decreasing Ag nanocrystallites size [18].

In present days, resistance to commercially available antimicrobial agents by pathogenic microorganisms has been increasing at an alarming rate and has become a serious problem [19]. Accordingly, there has been an upsurge for Ag containing compounds to work as antibacterial and antifungal based on their silver nanoparticles properties [19-22]. In daily life, human beings are often infected by microorganisms like bacteria, molds, viruses, etc. Research has been intensively carried out in antibacterial materials containing various natural and inorganic substrates over the last few years [23-26]. The mode of action of silver nanoparticles (Ag-NPs) on
bacteria is to produce reactive oxygen species such as \( \text{O}_2^\cdot \), \( \text{H}_2\text{O}_2 \), \( \text{OH}^- \) and singlet oxygen under photoaerated conditions [27]. In addition, Ag-NPs can bind to the DNA inside the bacterial cells preventing its reproduction [28] or interact with the bacterial ribosome inhibiting translation [29]. Accordingly, Ag-NPs have attracted considerable attention as antimicrobial agents [30]. Different techniques were used to synthesize Ag-NPs including chemical reduction of silver ions; with or without capping agents, thermal decomposition in organic solvents and photo reduction in reverse micelles [31-32]. However, the mentioned methods involve the use of toxic and hazardous chemicals that may pose environmental and biological risks [33]. In this study, the antibacterial activities of the Ag/TiO\(_2\)-SWCNT composites synthesized based on increasing visible light harvesting, decreasing the Ag aggregation and to take the benefit of high surface to volume ratio will be investigated together with their effect on the bacterial DNA using model cell types: *Escherichia coli* and *Staphylococcus aureas*. Structural variations, optical and morphological properties were examined for the fabricated CNT/TiO\(_2\) and Ag-CNT/TiO\(_2\) composites. X-ray diffraction (XRD), transmission electron microscopy (TEM), energy dispersive X-ray (EDX), N\(_2\) sorptiometry, FTIR, Photoluminescence, Raman and UV-vis diffuse reflectance spectroscopy were used to characterize the synthesized photocatalysts. The mechanism involved in the photocatalytic disinfection process was investigated via measuring \( K^+ \) leakage, protein separation and bacterial TEM images stucked on the composite surfaces.

2. EXPERIMENTAL

2.1. Catalyst Preparation

2.1.1. Mesoporous TiO\(_2\)-Swcnt and TiO\(_2\)-Mwcnt Catalysts

TiO\(_2\) nanoparticles were fabricated by self-assembly method according to the following procedure. Briefly, Polyethylene glycol (HO(CH\(_2\)_2O\(_n\))\(_{H-0.01\text{ M}}\) and polyvinyl-alcohol (CH\(_2\)_2-CH(OH)\(_v\)-0.01M) dissolved in a least amount of water (15 ml) and in a weight ratio of 7:3 were mixed thoroughly. Titanium isopropoxide (Ti(OCH(CH\(_3\))\(_3\))\(_{2\text{-29.3 ml}}\) was added into the above mixture via a drop wise manner with vigorous stirring for half an hour at room temperature. The resulting mixture was transformed into an autoclave lined with Teflon followed by hydrothermal treatment at 393 K for 48 h. After hydrothermal treatment, the product was recovered by centrifugation and then washed with deionized water. The solid was then dried overnight at 333 K and further calcined in air at 673 K for 6 h to remove the copolymer template. This sample was denoted as Tev, where the letter e expresses polyethylene glycol and v expresses polyvinyl-alcohol. Accordingly, the Tev catalyst was suspended individually in a mixture of ethanol-water and left under stirring for 2 h. This mixture was poured into SWCNT dissolved in ethanol to give a ratio comprised of 20% SWCNT relative to Tev, then sonicated at 313 K for 1.5 h. subsequently, it’s left under stirring overnight at room temperature and poured into a Teflon lined autoclave and heated under autogeneous pressure at 433 K for 48 h. Finally, it’s filtered, washed with absolute ethanol and distilled water, dried at 373 K overnight and then calcined at 673 K for 4 h. This sample was denoted as Tev-SWCNT.

2.1.2. Mesoporous TiO\(_2\)-Swcnt and TiO\(_2\)-Mwcnt Supported Ag Nanoparticles

Different weight % of Ag at a ratio of either 2% or 6% took place before titanium iso-propoxide admission to prepare samples denoted as TevAg\(_2\) and TevAg\(_6\). Accordingly, TevAg\(_2\) and TevAg\(_6\) catalysts were suspended individually in a mixture of ethanol-water and left under stirring for 2 h. These mixtures were poured into the suspension of MWCNT and SWCNT in ethanol to give ratios comprised of 20% MWCNT&SWCNT related to Tev then sonicated at 313 K for 1.5 h. Then, they left under stirring over night at room temperature. The mixtures were afterwards poured into autoclaves and heated under auto-geneouse pressure at 433 K for 48 h. Finally, the mixtures were treated as Ag free samples and denoted as TevAg\(_2\)-SWCNT, TevAg\(_2\)-MWCNT and TevAg\(_6\)-SWCNT.

2.2. Catalyst Characterization

X-ray diffraction analysis was performed on X-Ray Diffraction (XRD) spectrometer Model XRD 8030 from Jeol co., Japan. Maximum power is 3 kw and the unit is equipped with rotating stage and thin layer accessories. The nanostructured morphologies of the samples were examined using high resolution transmission electron microscopy (HRTEM) obtained by Tecnai G2 supper twin USA, with an accelerating voltage of 200 kV. The elemental composition of the composite material was investigated by energy-dispersive X-ray attached to the TEM equipment. The infrared spectra of the samples were recorded in the range of 400-1000 cm\(^{-1}\) using a Perkin-Elmer instrument (Spectrum GX), made in USA.
Nitrogen adsorption measurements were performed at liquid nitrogen temperature with a Micromeretics ASAP 2020 surface area and porosity analyzer. Diffuse Reflectance Ultraviolet–visible spectroscopy (UV–vis DRS) of powder samples was carried out at room temperature using a PerkinElmer Lambda-900 spectrophotometer in the range of 200–800 nm. The edge energy \( E_g \) for allowed transitions was determined by finding the intercept of the straight line in the low-energy rise of the plot of \( [F(R_e \cdot h\nu)]^2 \), for the direct allowed transition, vs \( h\nu \), where \( h\nu \) is the incident photon energy. The PL spectra were recorded by photoluminescence (PL) spectrometer (Spectro Fluorescence JASCO fp-6200) using 290 nm as excitation wavelength at room temperature via pulsed YAG:Nd laser excitation.

2.3. Antibacterial Test

2.3.1. Bacterial Strains and Growth Conditions

Gram negative bacteria \( E. \text{coli} \) and gram positive bacteria \( S. \text{aureus} \) were grown and subcultured and maintained on nutrient agar and stored at 4°C. For the experiment, a single colony of each organism was inoculated into 10 ml of LB broth and incubated overnight at 37°C with shaking at 200 rpm. The optical density of the overnight culture was adjusted to that of a 0.5 spectrophotometer and diluted with LB broth to give a final working concentration of 1x10^6 CFU/ml.

2.4. Well Diffusion Method

Antibacterial activity of synthesized nanoparticles was tested against \( S. \text{aureus} \) and \( E. \text{coli} \) by using well diffusion method. Samples were doubly diluted in water (100µl) and bacteria (100µl;10^6 CFU/ml) were added in 5 mm diameter, which made on nutrient agar plates using gel puncture. 100µg/ml and 50µg/ml of the nanoparticles solution were poured onto each of the well at the core in all the plates. After incubation at 37°C for 24 h, the different level of zone was measured. Negative control sterile liquid broth, Positive control as well as Antibiotic disc test was tested in duplicate on each plate and each plate was analyzed in triplicate.

2.5. Bacterial Susceptibility To Nanoparticles

\( E. \text{coli} \) and \( S. \text{aureus} \) were grown in the presence of nanoparticles in liquid medium. The bacteria were grown in 2ml of the nutrient broth; overnight, via adding NB of the bacterial stock containing 0.12% glucose with and without nanoparticles. The bacteria were aerobically incubated at 37°C for 24 hr. Optical density (OD) measurements were taken at 600 nm to monitor the bacterial concentration with a Hitachi spectrophotometer (type 124). A high pressure Hg lamp (125 W) equipped with a special UV cut off filter \( \lambda > 450nm \); manufactured by Vilber Lourmat-France offering visible light source was used. It gives an average light intensity equal 60 mWcm
^2
 and the working distance was at 30 cm. A continuous cold water (16±1°C) supply was maintained during the experiment to control the temperature of the reaction mixture. For detecting the active species produced in the photocatalytic reaction, hydroxyl radicals (•OH), superoxide radical (•O
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) and holes (h\^+) were explored by adding 1.0 mM isopropanol (IPA-aquencher of •OH), p-benzoquinone (BQ-a quencher of •O
\_2
), and disodium ethylenediaminetetraacetic acid (Na
\_2
EDTA-a quencher of h\^+) respectively.

2.6. Isolation of Genomic DNA

Genomic DNA was isolated as described elsewhere [34]. Five random oligonucleotide primers OP9A, OPB7, OPB17, OPC13 and OPE19 were used in the experiment. Premiers were synthesized at Operon technologies, Alameda, USA (Table S see supporting information). The PCR products were separated on 1.4% (RAPD), Agarose gel in 1x TAE buffer containing 0.1 µg/ml of ethidium bromide for about 2 hrs at 80 V. Gel was photographed under UV light with Tracktel GDS-2 gel documentation system.

3. RESULTS AND DISCUSSION

3.1. XRD Investigation

The results of XRD analysis of synthesized titania nanopowders originated from polyethylene glycol and polyvinyl alcohol as hybrids (Tev) together with those of Ag ions (TevAg2-inset- Figure 1), confirm the findings of anatase (JCPDS 21-1272) as a main phase and rutile (JCPDS 21-1276) as a minor one. Incorporation of Ag at a loading of 2% decreases the anatase ratio to 90% in TevAg2. Very small line at 2θ =32° is also shown in this sample and ascribed to Ag\_2O [35-36]. Incorporating SWCNT at 20% loading relative to titania; synthesized via hybrid polymers, (Tev-SWCNT) did not alter the Tev structure but only affected the anatase 101 phase that exhibited a decrease in intensity and broadness only in TevAg2-SWCNT. This might be correlated to superimposing the anatase (1 0 1) peak over that of the CNT (0 0 2) one; assigned to graphitic basal plane reflection of CNTs. On the other hand, the
diffraction pattern of TevAg6-SWCNT shows characteristic peaks at 38°(111), 44°(200) and 64.4°(220); correlated to the standard peaks of AgO nanoparticles of face centred cubic (fcc) structure (JCPDS, File No. 4-0783), together with the tiny one depicted previously for Ag2O at 2θ=32°. The relative decrease in intensity of the TiO2 peaks in TevAg2-SWCNT than in TevAg2-MWCNT confirms the decrease in crystallites size of the former probably due to the strong interaction between TNT with SWCNT rather than with MWCNT. The TevAg2-MWCNT sample also motivates the appearance of rutile peaks; never existed in Tev-SWCNT, and AgO nanoparticles related to the 111 plane. This sample also shows peaks ascribed to Ag2O correlated to 200 and 220 phases together with the previously mentioned peak ascribed to same species at 2θ=32.0° due to the 111 plane. Retaining TiO2 structure following SWCNT (MWCNT) and Ag incorporations reflects the intact of the TiO2 structure.

3.2. Surface and Morphological Properties

The N2 adsorption isotherms of pure Tev-SWCNT and Ag containing nanocomposites are shown in Figure 2. According to IUPAC, it is realized that the adsorption isotherm belongs to Type II, while the desorption isotherm belongs to Type IV. Therefore, it can be concluded that the particle size of synthesized samples was mainly distributed in the mesopores range. Observing the hysteresis loops approaching P/Po = 1, suggests the presence of macropores (>50 nm) [32]. However, the pore size distribution (PSD) did not illustrate macroporosities recommending that it can be produced via aggregations of the titanate nanostructures with itself and with CNTs. Compared with the as-prepared Tev-SWCNT, the hysteresis loop of the TevAg2-SWCNT sample shows a wider relative pressure range (0.4–1.0) and larger hysteresis area proposing that Ag nanoparticles motivate the progression of microporosities following incorporation. This is confirmed from increasing the surface area and decreasing the pore volume values of TevAg2-SWCNT (97.5 m²/g, 0.132 cc/g) compared with that of Tev-SWCNT (86.2 m²/g, 0.152 cc/g). These later values are reduced in case of TevAg6-SWCNT (81.0 m²/g, 0.12 cc/g), suggesting deposition of Ag nanoparticles deep inside the TiO2 pores. The PSD of the Tev-SWCNT sample was in the 1.0–4.0 nm range whereas it was narrowed for Ag containing composites to be in the 1.4–3.0 nm range. The specific surface area of TevAg2-MWCNT (79.5 m²/g) revealed a lower value compared to TevAg2-SWCNT (97.5 m²/g) suggesting that MWCNTs were subjected to TiO2 pores rather than with SWCNTs. TevAg6-SWCNT shows narrowing of the PSD to be mainly around 2 nm; lower limit of

Figure 1: XRD patterns of TNT-SWCNT, TevAg2-SWCNT, TevAg2-MWCNT and TevAg6-SWCNT.

mesopores, where it was at 2 and 3 nm for TevAg2-SWCNT.

Figure 3 shows the TEM images of Ag free as well as Ag confined Tev-SWCNT(MWCNT) nanocomposites. The image of Tev-SWCNT established the existence of TiO$_2$ nanoparticles coating some parts of CNT surfaces. The diameter of the CNTs was 12–25 nm while that of TiO$_2$ nanoparticles was in the 7–18 nm range. The insets show the corresponding selected area electron diffraction (SAED) patterns. It shows that diffraction spots of the CNT were overlaid by the diffraction rings of TiO$_2$ nanoparticles existed as polycrystalline phases. The selected area electron diffraction patterns show that the spherical rings correspond to the planes of CNT (002), anatase (101), (004), (200), (105) and rutile (110) phases from inner to outer rings. This was consistent with the wide angle XRD results presented previously (Figure 1). The existence of elements Ti, O, C and Ag was detected by EDX analysis (not shown). The TEM image of the calcined TevAg2-SWCNT confirmed the presence of anchored crystalline TiO$_2$(Ag) nanoparticles via uniform coverage of the CNT surfaces. The image shows that CNT and TiO$_2$ in the nanocomposite indicate an average diameter equal 10 nm and 12 nm, respectively.

Figure 2: Adsorption-desorption isotherms of (A) TNT-SWCNT (B) TevAg2-SWCNT (C) TevAg6-SWCNT and (D) TevAg2-MWCNT, together with the corresponding pore size distributions as insets.
whereas it decreases to 5 nm for Ag nanoparticles. It can be seen that Ag nanoparticles were well encapsulated inside TiO$_2$ as a core-shell structure, based on the synthesized procedure, since no Ag nanoparticles were identified with its own in the micrograph as depicted from EDX. In TevAg6-SWCNT and TevAg2-MWCNT samples, it was hard defining the CNT as was in foregoing samples declaring sever interaction of TiO$_2$(Ag) with CNTs. Specifically, these later samples exhibited a significant decrease in CNT thickness, via displaying an outside diameter of ~ 8 nm, and breaking CNT into shorter tubes. These samples show lower average diameters for TiO$_2$ (7 nm) and Ag (3 nm) nanoparticles compared to rest of samples.

### 3.3. Raman Spectroscopy

The Raman spectra of as-synthesized samples are shown in Figure S1. The Raman spectrum of Tev-SWCNT depicted bands for TiO$_2$, via revealing vibrations at 150 cm$^{-1}$ (very strong, E$_g$), 392 cm$^{-1}$ (B$_{1g}$), 515 cm$^{-1}$ (A$_{1g}$) and 634 cm$^{-1}$ (E$_g$) due to anatase as well as a band at 260 cm$^{-1}$ (A$_{1g}$) attributed to rutile [37]. This confirms that the synthesized TiO$_2$ is composed of a mixture of anatase and rutile where anatase phase is the most predominant, as confirmed by XRD results. This spectrum also indicates a band at 1582 cm$^{-1}$ indicative of the G band (Raman-allowed E$_{2g}$ mode) characterizing the crystalline nature of the CNTs together with a band at 1332 cm$^{-1}$ (D band, assigned to A$_{1g}$ phonon mode) that measures the distortion on the CNT structures [38]. All the bands correlated to TiO$_2$ functional groups were decreased in intensity in TevAg2-MWCNT whereas that at 1582 cm$^{-1}$ of CNT showed an enhancement. This reflects the extent of the interaction exhibited between Ag nanoparticles within TiO$_2$ structures exceeds that with CNT, as emphasized...
from TEM results. Comparing the Raman spectrum of TevAg2-SWCNT relative to that of TevAg2-MWCNT elaborates different interaction themes depending on the CNTs type. In conformity, TevAg2-SWCNT that retains considerable fraction of the 1582 cm\(^{-1}\) band as well as the 2645 cm\(^{-1}\) one; an overtone mode of the D band, indicates that SWCNT is the least affected and distorted either by incorporating Ag or TiO\(_2\). The presence of small broad band at 1432 cm\(^{-1}\) in the later sample imposes the presence of an amorphous carbon. On the other hand, the broadness of the Raman band at 1332 cm\(^{-1}\) in TevAg2-MWCNT and TevAg6-SWCNT as compared to Tev-SWCNT may due to the varied adherence of TiO\(_2\) particles to SWCNT(MWCNT) surfaces; as also confirmed via TEM observation. In TevAg6-SWCNT, the hardly presence of CNT bands compared with those of TiO\(_2\) ones dictates that the excess of Ag nanoparticles affected the former structure as well.

3.4. Ftir Spectroscopy

The FTIR spectrum of Tev-SWCNT (Figure S2) indicates bands at 3423, 1627, 1384, 722, and 530 cm\(^{-1}\). The strong and broad characteristic band occurred in the 3100-3700 cm\(^{-1}\) (of max. at 3423 cm\(^{-1}\)) range constitutes with the one at 1627 cm\(^{-1}\) [39] the presence of O-H stretching vibrations and the OH bending vibration of chemisorbed or physisorbed H\(_2\)O molecule on the photocatalyst surface, respectively. The obtained IR prominent band at 1384 cm\(^{-1}\) is mainly attributed to the aliphatic stretching frequencies of CH\(_x\) groups [40]. Furthermore, the broad band at 722 cm\(^{-1}\) is assigned to the combination effects of Ti-O and Ti-O-C bands in CNT/TiO\(_2\) nanocomposite [39]. Where that at 530 cm\(^{-1}\) describes Ti-O of anatase structure. Insertion of Ag nanoparticles causes some changes such as shifting the 722 cm\(^{-1}\) band to 630 cm\(^{-1}\); as in TevAg6-SWCNT, and to 619 cm\(^{-1}\); as in TevAg2-SWCNT, together with evoking a new band at 1126 cm\(^{-1}\) ascribed to the presence an ester localized group. In TevAg6-SWCNT, a small broad band at 1725 cm\(^{-1}\) due to C=O stretching vibrations in carboxyl groups is observed. The disappearance of the 530 cm\(^{-1}\) band following Ag incorporation probably depicts the interaction of Ag with Ti-O bonds; as proposed previously using TEM-EDX and Raman. The spectrum of TevAg2-MWCNT presented the same bands as those shown in all Ag containing samples but with a decrease in intensity. This admits that the surface groups of TiO\(_2\) form stronger chemical bonding with MWCNTs than with SWCNTs.

3.5. Photoluminescence Spectroscopy

PL Curves of Tev-SWCNT(MWCNT) containing Ag nanoparticles (Figure S3) showed a significantly enhanced photoemission spectra of ratio comprised of 6-10 compared to Ag free Tev-SWCNT sample. Increasing PL intensities of Ag containing nanocomposites is accounted for increasing rate of recombination of electrons and holes. Increasing the PL of Ag containing composites is due to the intensive coupling between Tev and localized surface plasmon of Ag nanoparticles [41]. The PL spectra of Tev-SWCNT showed an emission peak centred at 361 nm due to fine crystalline TiO\(_2\) together with a distinct broad emission peak at 335 nm attributed to CNTs. These emission peaks decreased in intensity in all Ag containing composites probably due to low crystallinity. A dramatic enhancement in photoluminescence intensity of TevAg2-MWCNT relative to other Ag containing composites was established. Indeed, this verifies that the rate of recombination of electrons and holes is high in this specific sample, comparatively. Although silver content on the surface of TevAg6-SWCNT was optimum, it effectively traps the photoinduced electrons to act as a charge separator, thereby increasing the life time of electron and holes compared with TevAg2-MWCNT. The PL intensity of TevAg2-SWCNT was the lowest between all Ag containing composites. This indicates that the CNTs can act as electron reservoirs to trap or shuttle the photogenerated electrons from semiconductors based on its type [SWCNT(MWCNT)], nature of CNT interaction with TiO\(_2\) and loading of Ag nanoparticles [42].

3.6. Optical Properties

In order to clarify the optical response and to determine the band gaps of the nanocomposite samples, UV–visible diffuse reflectance spectroscopy (UV–vis DRS) was carried out (Figure 4). Although the spectral shapes are generally same, there are prominent differences in the absorption edges. The Tev-SWCNT composite exhibits a band at 286 nm together with a broad one at 353 nm. Generally, anatase-type TiO\(_2\) has a strong absorption edge below ca. 350 nm whereas that of CNT exhibits a band at ca. 280 nm due to \(\pi-\pi^*\)transition of the c-c aromatic ring. In comparison, the absorption edge of TevAg2-SWCNT displays the similar absorption edge of Tev-SWCNT, but an apparent enhancement of absorption throughout the visible-light region is observed with diminishing that at 280 nm. The adsorption intensity of the TevAg2-
MWCNT sample was extremely enhanced compared with TevAg2-SWCNT owing to the good dispersion and to the strong interaction between Ag(TiO$_2$) and MWCNT exceeding that with SWCNT; as committed previously using XRD, Raman and IR results. In addition, the TevAg2-MWCNT sample exhibits a band at 216 nm never seen in nanocomposites involved SWCNT probably correlated to the exhibited strong interaction between TiO$_2$ and MWCNTs [43]. Interestingly, TevAg2-MWCNT displayed the strongest absorption among those of carbon/TiO$_2$ composites; as confirmed from both shifting the band at 352 to 357 nm and enhancing the broadness that extends to 800 nm, proposing an intense electronic interaction at TiO$_2$/MWCNT interfaces. Besides, the MWCNTs/TiO$_2$ (Ag) composite exhibited enhanced visible-light absorption and highest emission as confirmed from PL measurements, exceeding those of free Ag and Ag containing SWCNT/TiO$_2$. All Ag containing composites showed a narrow surface plasmon resonance band at 440 nm reflecting the small size distribution of Ag nanoparticles.

To determine $E_g$, we have analyzed the measured absorption spectra of the studied nanocomposites, by calculating the absorption edge based on the following approximation$^{44}$: $a(h\nu) \propto (h\nu - E_g)n/2$ (Figure 5). Particularly, the $E_g$ decreases in the following sequence: Tev-SWCNT (2.6)$>$ TevAg2-SWCNT (2.25)$>$ TevAg6-SWCNT (1.9) $=$TevAg2-MWCNT (1.75). Smaller $E_g$ values were clearly observed for TevAg6-SWCNT and TevAg2-MWCNT composites. This proposes an intense electronic interaction between Ag(Tev) and SWCNT(MWCNT). Additionally, decreasing the $E_g$ for TevAg6-SWCNT and TevAg2-MWCNT; those showed the highest absorption, proposes that CNT acted as a photosensitizer when coupled with TiO$_2$, then it can be excited to inject electrons into the conduction band of TiO$_2$. It seems that the incorporation of MWCNTs stimulate the absorption to be higher than TiO$_2$ alone in the visible region, as they act as good electron acceptors capable of capturing electrons from light irradiation [44-45]. This exhibits that TevAg6-SWCNT and TevAg2-MWCNT composites will be more excellent photoactive than rest of samples based on dispersion of CNTs in TiO$_2$.

### 3.7. Antibacterial Activity

The antibacterial activity of silver nanoparticles was investigated against pathogenic Gram negative strain (E. coli) and gram positive strain S. aureus using a well diffusion technique (See supporting information, Figure S4). Negative control was maintained in which no zone of inhibition was observed. Positive control using antibiotic test disc was also obtained (Figure S5). The diameter of inhibition zones around each well in presence of nano samples is represented in Table 1. The highest antimicrobial activity observed against E. coli was 40 mm for TevAg6-SWCNT, and the least was 25 mm for TevAg2-SWCNT(MWCNT) samples. The positive control highest activity was 30 mm and the least was 10 mm. On the other hand, the highest
antimicrobial activity observed against \textit{S. aureus} was 30 for TevAg6-SWCNT and the least was 22 and 23 mm for Tev Ag2-SWCNT and TevAg2-MWCNT, respectively. The antibacterial effects of nanocomposites towards \textit{E. coli} and \textit{S. aureus} in liquid media for different time intervals indicated the high efficient inhibition for \textit{E. coli} and \textit{S. aureus} growth as compared to control groups. For \textit{E. coli} and at a concentration of 100µg/ml, a 20 time decrease in the optical density of the bacterial culture (Figure S6) was depicted similar to that seen for \textit{S. aureus} (Figure S7), as compared to the control experiment. On the other hand, exposure of the TevAg6-SWCNT catalyst to visible light irradiation reduces the inhibition time of \textit{E. coli} from 18 h in the dark (Figure S7) to only 85 min (Figure S8). This indeed highlights the photocatalytic action of this material. It seems also that the trend of the curves for the latter sample was almost same in the dark and under irradiation reflecting probably the same mechanism or same exposed species.

3.8. Effect of Nanoparticles on Bacterial DNA

The effect of nanoparticles; specifically sample TevAg6-SWCNT that gives the maximum inhibition towards both types of bacteria, was conducted using RAPD PCR. This is the first report to study the effect of nanoparticles on the bacterial DNA; at least on similar

![Figure 5: E_g values of TNT-SWCNT, TevAg2-SWCNT, TevAg2-MWCNT and TevAg6-SWCNT.](image)

<table>
<thead>
<tr>
<th>Zone of Inhibition mm (\textit{E. coli}) 100ug/ml</th>
<th>Zone of Inhibition mm (\textit{S. aureus}) 100ug/ml</th>
<th>Sample Name</th>
</tr>
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<td>25</td>
<td>23</td>
<td>Tev Ag_2 + MWCNT</td>
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<td>9</td>
<td>Tev+ SWCNT</td>
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<tr>
<td>45</td>
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<td>25</td>
<td>22</td>
<td>Tev Ag_2 + SWCNT</td>
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</tbody>
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Table 1: Zone Inhibitions of Different Nanoparticle Samples Against \textit{S. Aureus} and \textit{E. Coli}
Figure 6: RAPD PCR profile of *E. coli* by using 10 mer primer lane M: 100 pb plus marker, panel A, B, C, and D: lane 1: bacterial growth after 10 hr without nanoparticles, lane 2: bacterial growth after 10 hr with nanoparticles, lane 3: bacterial growth after 14 hr without nanoparticles, lane 4: bacterial growth after 14 hr with nanoparticles, lane 5: bacterial growth after 18 hr without nanoparticles, lane 6: bacterial growth after 18 hr with nanoparticles.

samples. In Figure 6A, primer OPA-09 is used and after 10 h of growth with nanoparticles, there were 6 new additional bands at 2.1, 0.77, 0.4, 0.295, 0.233 and 0.146 kbp. On contrast, there were 3 missing bands at 3, 2.2, and 0.8. While after 14 h of culture growth with nanoparticles, three bands of 1.9, 1.5 and 0.78 kbp were disappeared. On the other hand, two additional bands of 2.5 and 1.7 kbp were observed. After 18 hr of growth, only on band was missed at 1.7 kbp whereas one band appeared at 0.752 kbp. Figure 6B shows OPB-07 primer results after different periods. It can be observed that after 10 h of growth, 4 bands having 2, 1.4, 1.3 and 0.89 kbp lengths disappeared, while 6 new bands appeared at 3.1, 2.7, 2.5, 2.2, 1.99 and 0.98 kbp. Surprisingly, after 14 h of growth there were no difference between control and treated sample however, after 18 h of growth there were 2 missing bands at 0.7 and 2.5 Kbp. Whilst there were 2 bands detected at 2.2 and 0.818 kbp. In Figure 6C, primer OPB-17 was used, and after 10 h of growth 2 bands at 0.4 and 0.295 kbp were disappeared, whereas 3 additional bands at 1.39, 0.37 and 0.3 kbp were depicted. In addition, after 14 h of growth there was no difference between control and treated one. After 18 h, there was one unseen band at 1.69 kbp and one additional band at 1.6 kbp. In Figure 6D, the absence
of 3 bands at 2.3, 1.26 and 0.2 kbp were noticed after 10 h of growth together with an additional band at 0.85 kbp. In addition, after 14 h of growth, compared to control, there were 6 vanish bands at 1.6, 0.99, 0.77, 0.6, 0.4, 0.2 kbp length, with evolution of three new bands at 2.6, 1.5, and 0.7 kbp. Furthermore, after 18 h of growth, there were two missing bands at 2 and 0.67 kbp and two new bands appearing at 0.64 and 0.2 kbp. Using primer OPC-13. Conclusively, there were 27 missing bands and 27 new bands were observed instead.

3.9. Disinfection Activities of Composites under Visible Illumination and Stabilities

Generally, the illumination is appeared to markedly enhance the antibacterial activity of our nanocomposites to complete inhibition. Careful analysis showed that *E. coli* was more susceptible to nanoparticles than *S. aureus* either in dark or in light. The differences in response between 2 species are unclear and may reflect differences in physiology, metabolism, or degree of contact. In addition, it suggests that the mechanisms of toxicity might also differ depending on the type of nanoparticles. Appreciably, a more pronounced effect on the *E. coli* strands was obtained comparatively so an excessive work was performed on it. Exposure of the TevAg6-SWCNT catalyst to visible light irradiation reduces the inhibition time of E-Coli from 18 h in the dark (Figure S6) to only 70 min (Figure S8). In the light control experiment, the viable bacterial population did not decrease within the disinfection process suggesting that visible light irradiation has no toxic effect on the bacterial cells (Figure 7). TevAg2-MWCNT and TevAg2-SWCNT suspensions could completely deactivate E-Coli cells within 110 and 120 min respectively, whereas the disinfection efficiency of TevAg6-SWCNT takes only 70 min. This indeed highlights the photocatalytic action of Ag containing composites in enhancing the bactericidal effect during light irradiation. To check whether there any Ag⁺ ions could have been released during the disinfection process and thus affected the bactericidal activities, we measured Ag⁺ ions concentration using ICP-AES after visible irradiation of the TevAg6-SWCNT sample during performing the experiment. Accordingly, we cannot detect any Ag⁺ ions (of detection limit – 20 ppb) i.e. its contribution in the inactivation process is insignificant. Figure 8 summarizes the photocatalytic dosage effect of TevAg6-SWCNT on the growth inhibition of *E. coli* under Vis. irradiation. The mentioned photocatalyst showed an enhanced antimicrobial activity against *E. coli* bacteria at a dose of 100 mg L⁻¹, killing 100% of CFU in 30 min whereas increasing the dose into 150 mg/l showed a lower efficiency (100% in 50 min) comparatively. In addition, although the exhibited interaction between TiO₂ and MWCNT exceeded that with SWCNT; as devoted from XRD, Raman, FTIR and TEM measurements, they almost show (TevAg2-SWCNT and TevAg2-MWCNT) similar antibacterial behaviour toward *E. coli* and *S. Aureus* strands. This intensifies the importance of the interaction between Ti and C that attained via exposed surface oxygen irrespective of CNTs type. Accordingly, the TiO₂-CNT heterojunction could minimize recombination of photoinduced electrons and holes; as elaborated via PL and UV-diffuse reflectance measurements, and had a more effective light absorption when Ag nanoparticles are incorporated at a loading of 6%. Appreciable activity was also gained for Ag free Tev-SWCNT photocatalyst specifically toward gram positive *S.
Aureus (Table 1) due to Ti-C and Ti-O-C bonds formed at the heterojunction responsible for visible light absorption and photocatalytic oxidation processes.

It was shown that OH radicals was exhibited and affected bacterial degradation. Accordingly, as a diagnostic tool for OH radical diffusion, a small amount of isopropanol (0.05 ml of 5x10^{-3} M) was added as a scavenger while performing the agar well diffusion method. The presence of this agent significantly lowered the photocatalytic activity of the system verifying the lethal effect of OH radicals (Figure 9). On the other hand, the absorbed oxygen on CNT/TiO2 interface reacts with electrons to mainly form superoxide radicals (O_{2}^{-·}), which effectively oxidized bacteria as evaluated via using 0.05 mM p-benzoquinone radical scavenger. This indeed elaborates the absorption capability of our sample to sunlight. Upon using Na2-EDTA, as a hole scavenger, no any inhibitory effect on the bacterial growth was noticed (Figure 9). Although TevAg6-SWCNT showed higher E_{g} than TevAg2-MWCNT, it presented higher antimicrobial action probably due to increasing the recombination rate of electrons and holes on the later sample comparatively, as confirmed from PL measurements. The recovery and reuse of TevAg6-SWCNT for cell inactivation throughout six consecutive cycles; under visible irradiation, was examined and the results were provided in Figure 10. Although the bactericidal efficiencies of the photocatalyst slightly decreased (from 100% to 96% within 180 min) with the increase of reused cycles, complete inactivation of 1 x 10^{6} CFU/mL of E.coli cells was easily obtained by TevAg6-SWCNT in 30 min (Figure 10). This result indicated that TevAg6-SWCNT owns a good potential for repeated use.

Although the exact mode of action of AgNPs to bacterial membrane is still not very clear, possible mechanism of action has been suggested according to the morphological and structural changes in the bacterial cells. Deposition of silver NPs in the bacterial cell surface can affect cell membrane permeability as well as the bacterial cell wall. It may lead to disturbances in ions transportation and other substances between bacterial cell and its surrounding [46-47]. It has been acknowledged that K^{+} is an important component practically existing in bacteria and involved in the regulation of polysome content as well as protein synthesis. Its leakage was regarded as a symbol of membrane destruction [48]. To confirm the hurt of cell membrane, the concentrations of K^{+} were measured during the experimental duration and the
results were provided in Figure 11. In the control experiments, K⁺ concentrations were below 25 ppb in the presence of bacteria and TevAg6-SWCNT; in 50 min, under dark conditions. Similarly, less than 10 ppb of K⁺ were detected in cell suspensions without nanocomposites, under light conditions. The K⁺ concentration did not obviously increase with increasing time, indicating negligible release of K⁺ from cells in the absence of TevAg6-SWCNT. In contrast, in the disinfection system, the concentrations of K⁺ increased rapidly with increasing disinfection process, indicating that K⁺ quickly leaked from cells due to the cell membrane damage by TevAg6-SWCNT reaching 270 PPb in 50 min. This observation confirmed that TevAg6-SWCNT has damage capabilities.

3.10. Expected Mechanism of the Cell Membrane Damage

Several studies demonstrated the high reactivity of high density silver [111] facets [49]. These previous studies are in accord with our analysis of the Ag containing catalysts in which most probably bacteria corroborates the faceting of the particles and a direct interaction of the [111] facets can be presumed; as confirmed from XRD. Metal particles of sizes 3 nm presented local electronic structure of the surface due to size effects. These effects are reported to enhance the reactivity of the nanoparticle surfaces, [50] and thus increase surface area values that by its turn increase the binding strength of the particles to the bacterial cell wall. The mechanism by which the nanoparticles are able to penetrate the bacteria is not totally understood, but a previous report suggests that in the case of E. Coli treated with silver nanoparticles, the changes created in the membrane morphology may produce a significant increase in its permeability and thus affects proper transport through the plasma membrane [50]. To explore the damage of bacteria cells, TEM images obtained via using a negative staining technique was used to examine the morphology changes of S. Aureus and E.coli cells during the inactivation process (Figure 12). Prior to the light irradiation, S. aureus cells contained an intact and smooth elliptical structure that changed when contacted with TevAg6-SWCNT (Figure 12c) into cylindrical shape via a mutation mechanism. However, after exposure to visible irradiation to only 30 min, the nanocomposites are seen inside the cell in a form like cluster aggregates and in another cell it appeared that cell contents are unoccupied. It can be seen that the part of Ag encapsulated inside TiO₂ is the one diffused inside the cell while CNT is extended outside the bacterial cell. On the other hand, 10 min in the dark for E.coli is like a control exhibiting nothing however, many pits appeared on the cell wall and the cell gradually lost its morphology after 30 min exposure to visible light (Figure 12f). The cell was deformed to be cataplastic after 30 min treatment, indicating ROS generated by TevAg6-SWCNT damaged the cell membrane. Similar observation has also been reported previously [51]. In our case, this mechanism could explain that our silver containing nanocomposites can be found inside bacterial cell (as depicted via DNA mutation as well as via K⁺ leakage). As established by the theory of hard and soft acids and bases, silver nanoparticles will tend
Figure 12: TEM images of S. aureus (a-d) and E. coli (e-f) cells under dark and photocatalytic disinfection time when exposed to TevAg6-SWCNT catalyst at a dose of 100 mg/l: (A) S. Aureus after 10 min in dark (B) 10 h dark (C), 18 h dark (D), and 30 min under visible irradiation (E) E. Coli after 10 min in dark (F) and after 30 min visible light irradiation.

to have a superior affinity to react with phosphorus and sulfur compounds [52]. The membrane of the bacteria is well known to contain many sulfur-containing proteins; [52] these might be preferential sites for the silver nanocomposites to react inside the cell such as with DNA [53]. Electrostatic forces might be an additional reason for the interaction of the nanoparticles with the bacteria. It has been acknowledged from literatures that, at biological pH values, the overall surface of the bacteria are used to expose negative charges due to dissociation of carboxylic and other groups in the membrane [54]. On the other hand, the Ag nanoparticles embedded in carbon nanotubes (insulator) and TiO$_2$ matrix, will stimulate the existence of unlike charges on its surface. For these reasons it is possible to expect an electrostatic attraction of the nanocomposites and bacteria. Accordingly, based on the configured reactive species and the exposure of O$_2$$^\cdot$$^-$ and $^\cdot$OH on the nanocomposite surfaces, an invasion of either reactive species and/or nanocomposites inside the bacterial cell wall is expected and consequently mutation in DNA and K$^+$ leakage has been attained. As a further confirmation, protein separation on SDS-PAGE was performed. The SDS PAGE showed significant differences in protein profile of S. aureus (as an
example) with silver containing nanocomposite in the dark and visible light conditions (Figure 13). For example, lane 3 exhibited under visible light showed the disappearance of bands at 35, 45 and 135 KDa when compared with lane 2 at dark. In addition, lane 5 produced as a result of exposure to visible light illustrated the absence of the band at 45 KDa and presence of a band at 20 KDa when compared with lane 4. Finally, lane 7 depicted the absence of bands at 135 and 45 KDa when compared with lane 6 at dark.

**Figure 13:** Comassie blue SDS-PAGE showing protein profile of *S. aureus* with TevAg6-SWCNT at 100 µg/ml concentration, lane 1: control sample without photocatalyst, lane 2: sample TevAg6-SWCNT in dark after 10 h of growth, lane 3: after exposure to Visible light to 10 min, lane 4: sample TevAg6-SWCNT in dark after 14 h of growth, lane 5: after exposure to Visible light for 30 min, lane 6: sample TevAg6-SWCNT in dark after 18 h of growth, lane 7: after exposure to Visible light for 40 min, lane 8: broad range protein marker from promega.

**CONCLUSION**

The synthesized Ag/TiO$_2$-CNTs have presented magnificent photodegradation toward *E. coli* and *S. Aureus* bacterial strands under visible irradiation, in only 30 min. The inactivation mechanism was established based on performing physicochemical measurements including DNA and protein fragmentation, K$^+$ leakage, TEM of the bacterial strands in presence of photocatalyst, Ag$^+$ effect, catalyst weight % and expected electrostatic interaction between bacteria and catalyst surface. These measurements illustrated that due to unlike charges, the catalyst was found to stick onto bacterial cell wall and thus invade inside it. As a result, the leakage of K$^+$ ions along with DNA and protein mutations were explored as consequences to bacterial deterioration. The characterization results; carried out using UV-Vis diffuse reflectance, PL, FTIR and N$_2$ sorptiometry, have shown that the inactivation was assisted by the created ·OH and O$_2$* reactive species upon irradiation of catalyst surface together with high surface area and low E$_g$ and visible light capabilities of Ag/TiO$_2$ enlarged by CNTs.

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