Green Biosynthesis of Silver Functionalized Multi-Walled Carbon Nanotubes, Using Satureja Hortensis L Water Extract and Its Bactericidal Activity

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ABSTRACT

Green biosynthesis of silver - functionalized multiwalled carbon nanotube (Ag/FMWCNT) nanohybride from silver nitrate solution using water extract of Satureja hortensis L as reducing agent and FMWCNT as anchoring agent at room condition was successfully carried out. Water-soluble organics present in the plant materials were mainly responsible for the reduction of silver ions to nano-sized Ag particles. The synthesized nanocomposite were characterized using ultraviolet-visible spectroscopy (UV-Vis), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and powder X-ray diffraction (XRD). The UV-Vis spectra of the Ag/FMWCNT nanocomposite showed absorption peak at around 401 nm. SEM and TEM studies showed that the average particles size of Ag/FMWCNT nanocomposite were about 16.05 nm and also XRD patterns indicated that the structure of the nanocomposite is face-centered cubic. The antibacterial effects of Ag/FMWCNT on Gram positive and Gram negative bacteria were also performed.

Keywords: Green bio synthesis; Water extract; Ag nanoparticles; TEM; CNTs

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INTRODUCTION

Both single-wall carbon nanotubes (SWCNTs) and multi-wall carbon nanotubes (MWCNTs) have been used widely these days. In order to take full advantage of these two kinds of nanomaterials, it is desirable to create novel CNT/Ag nanohybrids, so that the unique properties of each material can be integrated and the interactions between the two components may bring out novel properties. So carbon based Ag composites have attracted much attention and have become a very interesting field of research [1-3] Several techniques have been developed to fill CNTs with metals, e.g. in situ filling during arc discharge growth of CNTs, molten salt sorption and wet chemistry methods [4]. Silver nanoparticles (Ag-NPs) are a new kind of nanomaterials expands rapidly, since the obtained Ag-NPs have unique electrical, optical and biological properties. Ag-NPs are normally short lived in aqueous solution as they agglomerate quickly [3-7]. For avoiding this problem, nanoparticles can be anchored by functionalized carbon nanotubes. Several kinds of chemical reagents, irradiations and template were used in the synthesis of FMWCNT/Ag nanohybrids. Recent reports reveal the green synthesis of silver nanoparticles with the use of plant extracts as reducing agents, biological
methods provide various advantages including cost effective, simple and green biocompatibility. Biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step [8-12]. The biosynthesis of nanoparticles has been proposed as a cost-effective, environmental friendly alternative to chemical and physical methods [13, 14]. The green synthesis of Ag-NPs using methanol extract of Vitex Negundo L leaves and Callicarpa maingayi stem bark were reported [15-17]. In another study, Ag-NPs were synthesized by reduction of aqueous Ag+ with the culture supernatants of Aspergillus terreus at room temperature [18]. To fabricate Ag-NPs coated onto FMWCNTs, electrochemical, chemical and physical methods have been employed. In this work, the green biosynthesis and characterization of Ag/ FMWCNT nanocomposite at room temperature is reported using water extract of Satureja hortensis L as reducing and stabilizing agent.

MATERIAL AND METHODS

Materials used

Fresh leaves of Satureja hortensis L were collected from the shahryar region of Iran in November 2015. AgNO₃ (99.80%) were used as the silver precursor, which were purchased from Merck and the MWNT-COOHs (FMWCNT) used in this work are purchased from nanocarbon Co., Ltd. used as solid support. Double distilled water was used for all the aqueous solutions.

Preparing the water extract of Satureja hortensis L

Fresh leaves of Satureja hortensis L were washed and dried for a week in shade and were ground into a fine powder (figure 1). The Satureja hortensis L dried leaves were water extracted and used for reduction of Ag+ ions to Ag⁰, for this purpose, (10 g) of dried leaves of plant were mixed in 100 ml of double distilled water and the mixture were boiled for 10 minutes in a water bath. The extract was then filtered and centrifuged at 4000 rpm for 10 minutes to remove all proteins and other residues from the extract.

Synthesis of silver-functionalized multiwalled carbon nanotube

0.003 g of FMWCNT powder was dispersed with vigorous stirring in certain amount of double distilled water for 1 hour. FMWCNT suspension was added to 100 ml of 0.01 M AgNO₃ solution for the synthesis of Ag/ FMWCNT nano composite, The mixture was then added to 20 ml of Satureja hortensis L water extract as prepared in sec.2.2, at room temperature while soninating and then were mixed for 48 hours with vigorous stirring.

Methods

The structure and morphology of the synthesized Ag/ FMWCNT nanocomposites were characterized by using X-ray diffraction (XRD, Inel, EQUINX 3000), transmission electron microscopy (TEM), Zeiss, EM 10 C-100KV, scanning electron microscopy (SEM), Zeiss SIGMA VP. The UV-visible spectra were also recorded using UV Bio-TEK UV-visible spectrophotometer from 325 nm to 800 nm.

RESULT AND DISCUSSION

Biosynthesis of silver nanoparticle was
observed based on the color developed in the solution during the course of the reaction. The change of the color of the reaction solution can be visually observed for the formation of Ag/ FMWCNT nanocomposite in Fig. 2.

**UV-absorption measurements**

The UV-visible absorption spectra of FMWCNT anchored by Ag-NPs prepared by water extract of *Satureja hortensis* L and plant extract are shown in Fig. 3. The characteristic of the silver SPR bands were detected in the range of 360-415 nm. These absorption bands were presumably corresponding to the Ag-NPs smaller than 30 nm. The absorption peak in the wavelength of 401 nm was confirmed the formation of Ag/ FMWCNT nanocomposite in nanometer size in correlation to TEM results as 16.05 nm.

**X-ray diffraction (XRD) patterns**

The X-ray diffraction (XRD) patterns of the synthesized Ag/FMWCNT nanocomposite is shown in Fig. 4. In the Ag/FMWCNT nanocomposite, the XRD peaks at 2θ of 38.14°, 44.35°, 64.77°, 77.37° and 81.25° were characteristics to the (111), (200), (220), (311) and (222) planes of the face-centered cubic (FCC) of nanocomposite, respectively. The mean particles size of nanocomposite was evaluated using the Debye-Scherer Equation (1):

$$d = \frac{K\lambda}{\beta \cos \theta}$$

That λ is the X-ray wavelength (1.540560 Å), β is the width of the XRD peak, θ is the Bragg angle, d is the particles size and K is the Scherer constant with value 0.9. The average sizes of nanocomposite were calculated almost 16.05 nm.

**SEM and TEM observations**

In Fig. 5 and 6, SEM images of pure FMWCNT
and also Ag/FMWCNT nanocomposite, prepared by green reduction method are shown. SEM images were used for study the morphology of Ag/MMT nanocomposite. The size and shape of particles were evaluated that were spherical and average size of them were about 16.05 nm.

The formation of Ag/FMWCNT nanocomposite was confirmed by the TEM image. Fig. 7 TEM image of nanocomposite and size distributions of the Ag/FMWCNT nanocomposite suspension are also shown. TEM image and their size distribution of Ag/FMWCNT nanocomposite suspension show the mean diameter and standard deviation of the nanocomposite 16.05±9.807 nm for Ag-NPs anchored onto FMWCNT. Also the TEM image was confirmed a layer surrounding around of nanocomposite that was prevented from agglomeration of particles.

Antibacterial activity of synthesized silver nanoparticles and selected antibiotics against *Escherichia coli* and *Staphylococcus aureus* bacteria, are shown in in Table 1. The result showed effective concentration of kanamycin antibiotic (MBC) against *Escherichia coli*, effective concentration of vancomycin antibiotic (MBC) against *Staphylococcus aureus* and effective concentration of silver nanoparticles (MBC) against *Staphylococcus aureus* and *Escherichia coli* bacteria were 32.55 μg/ml, 3 μg/ml, 0.16 μg/ml and 0.25 μg/ml respectively.
Fig. 7. TEM images and size distributions of Ag/FMWCNT nanocomposite after 48 hours.

Table 1. The value of initial and effective concentration of antibiotics and silver nanoparticles against Escherichia coli and Staphylococcus aureus bacteria

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Initial concentration</th>
<th>Effective concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanamycin antibiotic against <em>E. coli</em></td>
<td>1:32 dilution</td>
<td>1000</td>
</tr>
<tr>
<td>Vancomycin antibiotic against <em>S. aureus</em></td>
<td>1:64 dilution</td>
<td>256</td>
</tr>
<tr>
<td>Silver nanoparticles against <em>E. coli</em></td>
<td>1:1024 dilution</td>
<td>375</td>
</tr>
<tr>
<td>Silver nanoparticles against <em>S. aureus</em></td>
<td>1:2048 dilution</td>
<td>375</td>
</tr>
</tbody>
</table>
CONCLUSION

The results showed, green synthesis of Ag/FMWCNT nanocomposite was have successfully carried out from the AgNO₃ solution using water extract of Satureja hortensis L at room temperature. The XRD pattern showed that the Ag/FMWCNT nanocomposite formed were crystalline in nature. Also, the SEM and TEM results showed that spherical silver nanoparticles and anchoring of silver nanoparticles onto FMWCNT and average particles size were about 16.05 nm. Also study found that the Ag/FMWCNT shows antibacterial activity on both Gram positive and Gram negative bacteria.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES