

Nanocomposites with antibacterial properties using CNTs with magnetic nanoparticles

B. Ortega García¹, O. V. Kharissova¹, H. V. Rasika Dias²,
F. Servando Aguirre T.³, J. Salinas Hernández⁴

¹Universidad Autónoma de Nuevo León, Facultad de Ciencias Físico-Matemáticas,
Monterrey, Nuevo León, México

²The University of Texas at Arlington, Department Chemistry and Biochemistry,
Arlington, Texas 76019, USA

³Centro de Investigación en Materiales Avanzados (CIMAV),
Monterrey, Nuevo León, México

⁴Centro de Investigación y Desarrollo de Educación Bilingüe (CIDEB),
Monterrey, Nuevo León, México

beatriz.ortega24@gmail.com, okhariss@mail.ru, dias@exchange.uta.edu,
servando.aguirre@cimav.edu.mx, jeannie05@hotmail.com

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In this work, multiwall carbon nanotubes (MWCNT) were functionalized with silver nanoparticles using two different methods of incorporation. Characterization of these composites was done using Raman spectroscopy and Transmission Electron Microscopy (TEM) and the antibacterial properties were measured using method of dilution and plating. The four-point probe method was used to measure the resistivity of the nanocomposite thin films made via the spin coating method.

Keywords: carbon nanotubes, functionalization, silver nanoparticles, polymers, nanocomposites.

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1. Introduction

It is well-known that diverse polymers possess antibacterial properties [1], however, this also applies to several nanoparticles (for example Ag-NPs [2]) or carbon nanotubes (CNTs) [3]. The CNTs can be magnetic if they contain iron nanoparticles inside as a result of the incorporation of Fe-containing catalysts during their production [4]. It is hoped that once the nanoparticles are integrated into the polymer matrix that all of the NPs properties will be transferred to the polymer, resulting in its antibacterial and magnetic properties. There are many applications reported for polymer-NP nanocomposites with antibacterial properties; however, in the case of also having magnetic properties, there also exists the potential for an alternative means for drug delivery or biosensor applications.

The natural polymer chitosan (CS) belongs to the group of antibacterial polymers [1]; it is compatible with tissues and is utilized in biomedicine. In this work, the functionalization of CNTs with Ag and Fe and their further uniform dispersion in chitosan polymeric matrix is studied.

2. Experimental

2.1. Materials

Multiwall carbon nanotubes (MWCNTs) were prepared by spray pyrolysis method in the presence of iron as a catalyst at two different temperatures, 760 °C and 800 °C. Chitosan (low molecular weight) from Aldrich, AgNO₃ from Fisher, Luperox® LP, Lauroyl peroxide, 97 % from Aldrich, Ethanol 99.2 % from Decon Labs Inc., Acetic Acid (2 %), cetyltrimethylammonium bromide (CTAB) from Aldrich, deionized water (DIW) were used as supplied.

2.2. Functionalization of MWCNTs

Two methods, used for functionalization of carbon nanotubes, were as follows:

For the first functionalization (1NP), the CNTs (0.025 g), synthesized at 800 °C, were ultrasonically dispersed in ethanol (10 mL). Another solution containing DMF (20 mL), silver nanoparticles (0.08 g), prepared by a more environmentally-benign method, and chitosan (2 wt.%), was added to the first solution under nitrogen flow and stirred for 48 hrs (see Fig. 1.1).

For the second functionalization (2NP), the CNTs (0.1 g), synthesized at 760 °C, were ultrasonically dispersed in ethanol (400 mL) and lauroyl peroxide (Luperox® LP, Lauroyl peroxide) (0.5 g) was added for 30 min. Next, the CTAB (0.2 g) was added and the mixture was subjected to ultrasonication for 15 min. After this step, one of previously selected silver compounds (silver(I) acetate, silver(II) oxide or AgNO₃) was added and ultrasonication was continued for an additional 60 min.

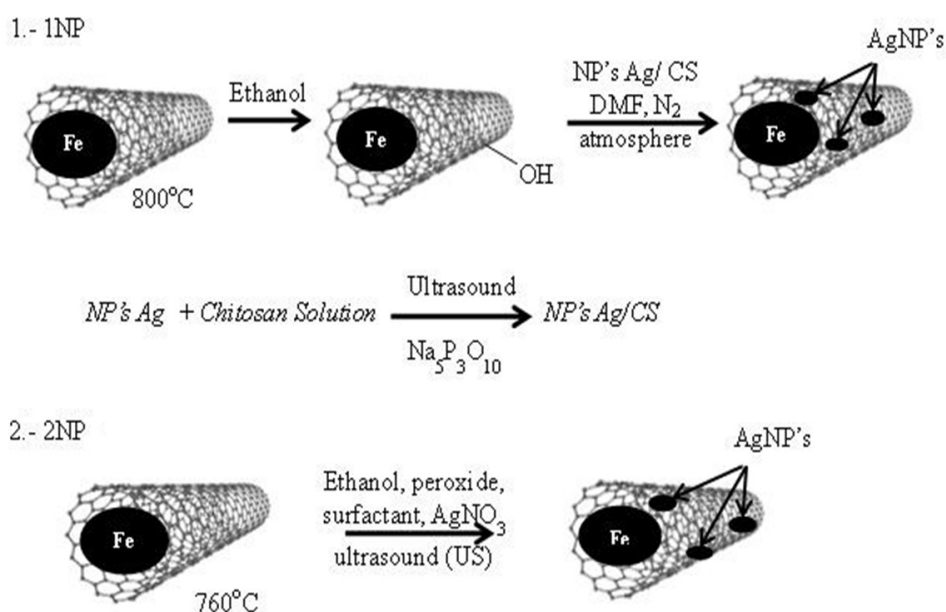


FIG. 1. Two methods for carbon nanotube functionalization with silver nanoparticles

2.3. Preparation of CS/Ag/MWCNT nanocomposite

Carbon nanotubes functionalized with silver nanoparticles were then used (0.02 % wt.%) for the preparation of nanocomposites with different chitosan contents (1 %, 3 % and 5 %) by mixing using ultrasonication for 1 and 5 min. Films of different nanocomposites were obtained by a spin coating technique onto glass slides.

2.4. Characterization

The samples were analyzed by Raman spectroscopy, Transmission Electron Microscopy (TEM); method of dilution and plating was used for measurement of antibacterial property. The four-point probe method was applied to measure the resistivity of the nanocomposites.

2.4.1. Method of dilution and plating:

1. A colony of the strain *Escherichia coli* DH5a was placed in 75 mL of liquid nutrient medium for 12 h at 37 °C (pre-culture).

2. The analyzed samples were Luperox or Lauroyl peroxides, CTAB, mixture of peroxide and CTAB, ethanol, silver nitrate solution as a reference, carbon nanotubes synthesized at 760 °C and carbon nanotubes containing silver nanoparticles. All reagents were introduced into an ultrasonic bath for 5 min for sample dispersion.

3. 1 mL of the pre-culture of *E. coli* DH5a was mixed in 15 – 20 mL of melted nutrient medium agar which was cooled to 40 °C, and allowed to solidify undisturbed on a flat table top.

4. 0.1 mL of the dispersed samples were taken and poured over the filter paper disks and placed in the center of the Petri dish with the bacteria, for this process, sterilization of equipment was done using a Bunsen burner (called “disk technique”).

5. 0.05 mL of the scattered samples were taken and poured into a hole made in the center of the agar plate placed in the center of the petri dish with the bacteria, for this process, sterilization of equipment was done using a Bunsen burner (called agar hole technique)

6. Both plates (paper disks and agar holes) were maintained at 37 °C for 12 h.

Note. The Luperox and CTAB were diluted in absolute ethanol and later dispersed by ultrasonic treatment for 25 min.

3. Results and discussions

Using Raman spectroscopy and TEM analyses, we confirmed the functionalization of the synthesized carbon nanotubes (760 °C). Fig. 2 shows the Raman spectra of MWCNT synthesized at 760 °C and two characteristic peaks are observed: one peak at a Raman shift of 1310 cm^{-1} , called the D-band, which is assigned to the disordered graphitic structure of MWCNTs. The other peak, at a Raman shift of 1600 cm^{-1} , called the G-band, is assigned to the C=C bond in the graphitic plane.

The Raman spectra of both pristine MWCNT and MWCNT/Ag, obtained with an excitation energy of 2.41 eV, are shown in Fig. 2 (an inset in this image corresponds to the reported data for SWCNTs/Ag [5]). The details of sample preparation and Raman experiment have been reported elsewhere [5]. The presence of silver in the Raman spectra is confirmed by the presence of characteristic peaks in the 1100 – 1600 cm^{-1} range. These peaks are slightly shifted and broadened in comparison to the Raman data of the samples without silver. It should be pointed out that the tangential band (at about 1590 cm^{-1}) is composed of several peaks. The downshift in the tangential G^+ -mode from 1600 to 1593 cm^{-1} indicates that electrons are transferred from the silver to the MWCNTs; a similar peak amplification was previously reported for SWCNT/Ag [5].

Figure 3 shows the TEM images of MWCNTs synthesized at 760 °C and MWCNT with silver nanoparticles. Carbon nanotubes synthesized using an iron-containing precursor and functionalized with silver nanoparticles demonstrate magnetic properties. As it is seen in Fig. 4 using a small magnet, the nanotubes are attracted to the magnet. This confirms that magnetic properties are not lost upon functionalization.

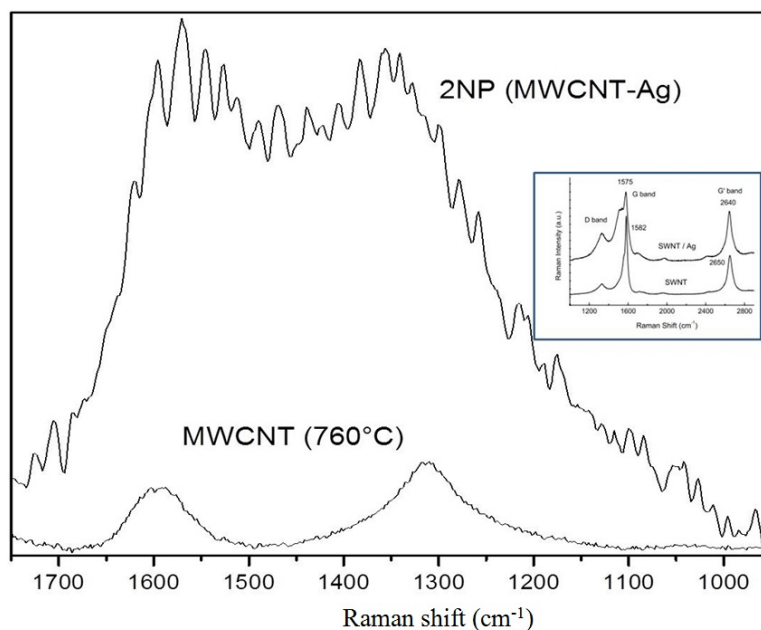


FIG. 2. Raman spectra of MWCNT: Raman spectra of MWCNTs made at 760 °C with Raman spectra of functionalized MWCNTs made at 760 °C and silver nanoparticles. An inset contains earlier reported data for SWCNTs/Ag

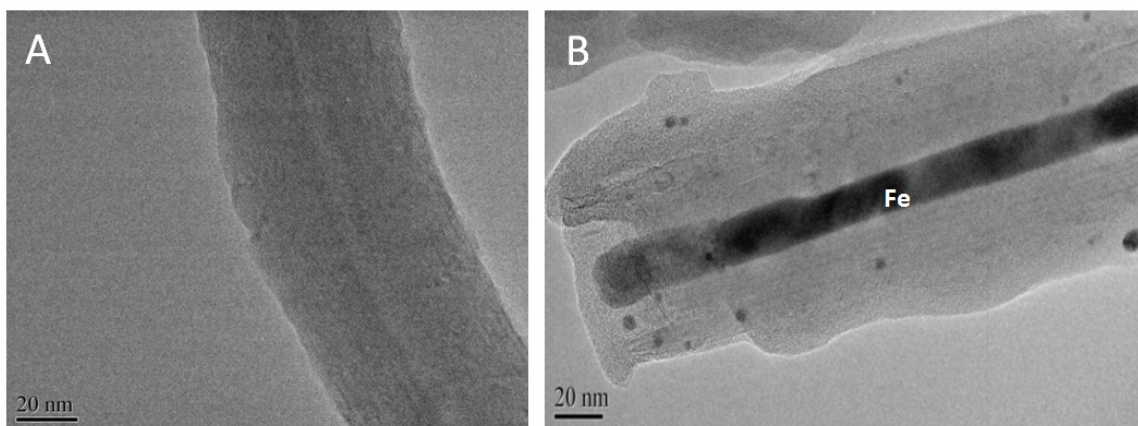


FIG. 3. TEM micrograph: A) MWCNT made at 760°C and B) functionalized MWCNT (760°C) with silver nanoparticles (2NP)

Figure 5 and Fig. 9 show an increase in the resistivity when you increase the number of layers using chitosan solution for 1 % and 3 % and 1NC and 2NC. In Fig. 7, high contents of CS (5 %) were used; one layer presented high resistivity to infinity but increasing other layer resistivity is decreased. In case of addition of nanoparticles (1NC and 2NC), only with 2NC the similar decrease of resistivity takes place.

Figures 8 and 9 show a decrease of resistivity when there is an increase of percentage of CS.

3.1. Antibacterial test of carbon nanotubes with silver

The CNT samples with silver nanoparticles at two concentrations (0.0199 g / 0.5 mL and 0.00199 g / 10 mL) were observed to generate homogeneous foams, while for the remaining



FIG. 4. Carbon nanotubes with magnet nearby

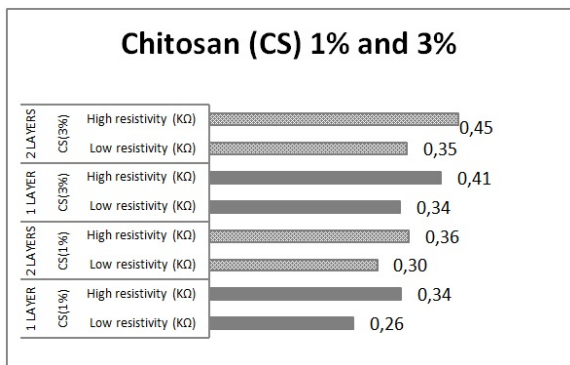


FIG. 5. Results for solutions with different percentages of chitosan

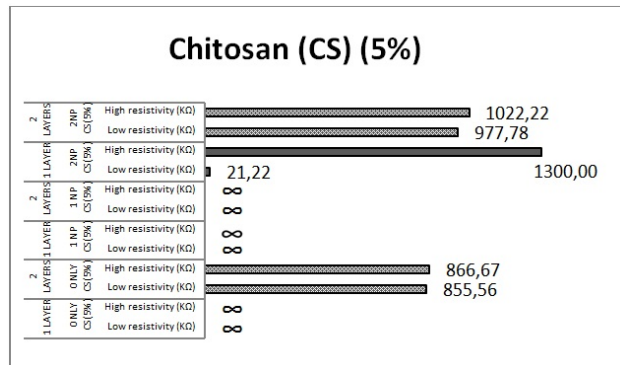


FIG. 6. Results for chitosan solution of 5 % using only chitosan and two different nanoparticles (1NP and 2NP)

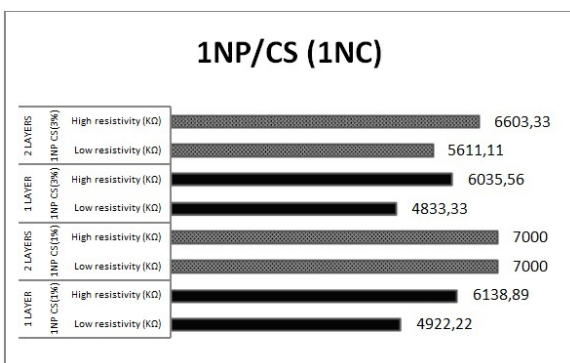


FIG. 7. Decrease in resistivity average (MΩ) of 1NC nanocomposite with CS solution (1 % and 3 %)

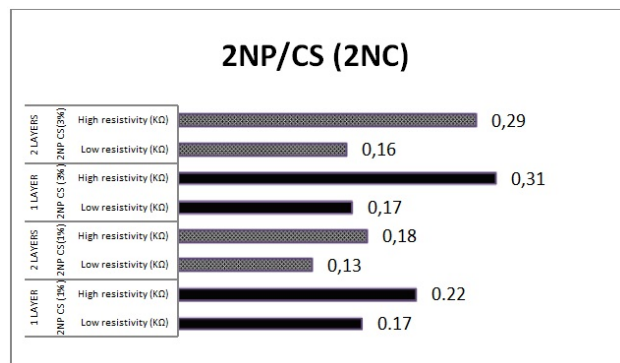


FIG. 8. Decrease in resistivity average (KΩ) of 2NC nanocomposite with CS solution (1 % and 3 %)

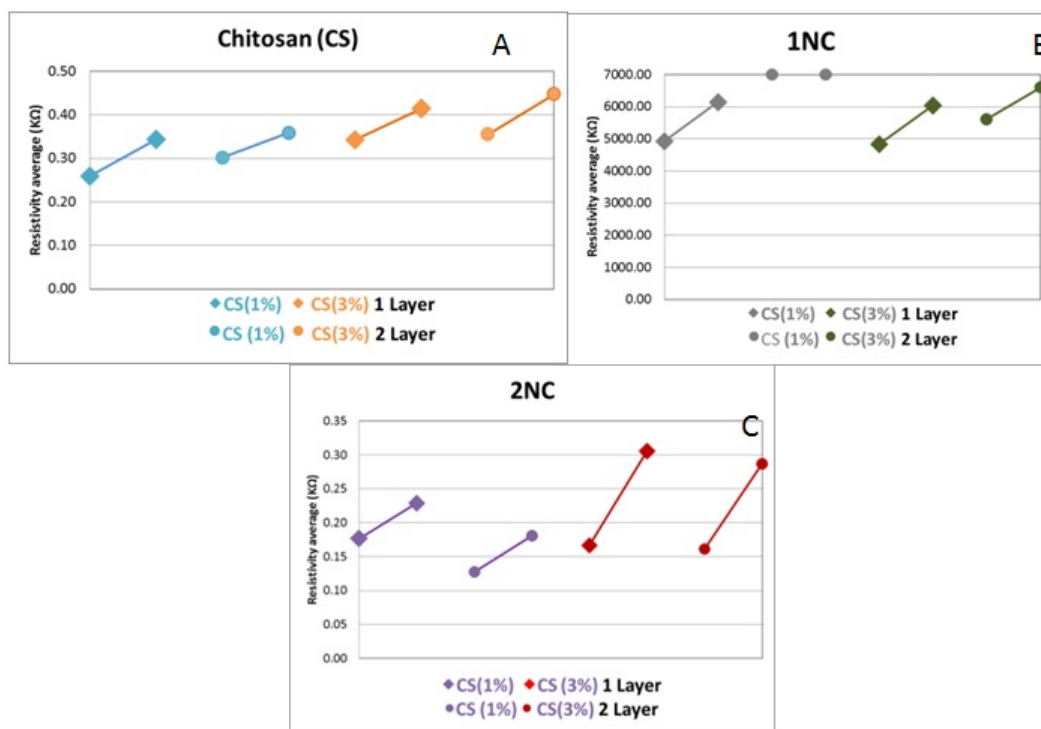


FIG. 9. Resistivity average for 1 layer and 2 layers of A) CS solution (1 % and 3 %) and nanocomposites B) 1NC and C) 2NC

samples, dispersion was difficult. Because this sample showed foaming due to the surfactant CTAB used in the reaction, an experimental design was utilized whereby all reagents were tested in order to rule out that the growth inhibitory activity of *Escherichia coli* DH5 was attributable to the effect of one of them.

The CTAB in agar hole in petri dish showed an inhibition halo of 3.0 cm of diameter. The CTAB in paper filter disk showed in petri dish an inhibition halo of 0.1 cm of diameter.

In Fig. 10, the sample with a concentration of 0.02 g / 0.5 mL in 0.05 mL poured in the agar holes of the sample MWCNT/Ag showed an inhibition halo of 2.3 cm of diameter in the *E. coli* DH5a culture. The samples with a concentration of 0.02 g /0.5 mL in 0.1 mL in disks, only the sample MWCNT/Ag showed an inhibition halo of 0.5 cm of diameter in the *E. coli* DH5a culture (see Table 1).

4. Conclusion

In the present work, we obtained nanoparticles with antibacterial and magnetic properties using an ultrasonic method for functionalization. We confirm the functionalization with Raman analysis by comparing the Raman spectra of MWCNT with and without functionalization. In addition to the antibacterial and magnetic properties of our nanoparticles, we measured the resistivity of chitosan and the nanocomposites. One of the nanocomposites (2NC) decreased the resistivity among 33 % (1 layer) and 58 % (2 layers) less for CS (1 %) and 36 % (1 layer) and 46.6 % (2 layers) less for CS (3 %). These mean that multiwall carbon nanotubes with silver nanoparticles allow increasing the conductivity of the nanocomposite.

TABLE 1. Antimicrobial growth inhibition

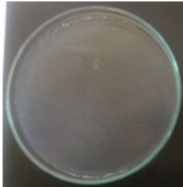



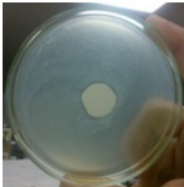
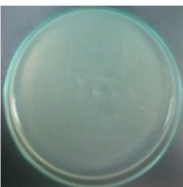
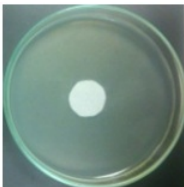

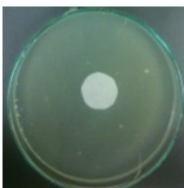
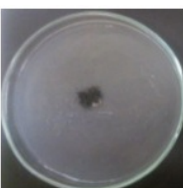


Sample	Antimicrobial growth inhibition		
	Technique:	Agar hole	Disk
Control (only agar with bacteria)	NO		
Peroxide	NO		
CTAB	YES		
Mix of peroxide and CTAB	NO		
Ethanol	NO		
CNT	NO		
CNT with Ag	YES		



FIG. 10. MWCNT with silver nanoparticles (2NP) with a concentration of 0.02 g / 0.5 mL in 0.05 mL poured in the agar holes with a halo of inhibition of 2.3 cm

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