Interaction of silver doped carbon nanotubes-cyclodextrin nanocomposites with *Escherichia coli* bacteria during water purification

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**ABSTRACT**

Silver doped multi-walled carbon nanotube/β-cyclodextrin (1% Ag-MWCNTs/β-CD) nanocomposite was prepared by reductive pre-treatment of Ag⁺ on MWCNTs, followed by binding with β-CD. Crystalline structure, morphology and crystallite size estimation were carried out by X-ray diffraction (XRD), Fourier transform infrared spectroscopy, field emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM). The interaction between *Escherichia coli* (*E. coli*), ATCC 25922 bacteria and 1% Ag doped MWCNTs/β-CD nanocomposite was investigated by FE-SEM. The antibacterial activity of the nanocomposite was studied by monitoring the absorbance of contaminated water using a UV-vis spectrophotometer. The nanocomposites caused a growth delay in the growth rate of *E. coli* bacteria. The structural analysis of the bacteria by FE-SEM showed clusters of nanocomposite anchored to the bacterial cell wall during the initial stages of interaction. Results also indicate that treated bacteria show significant damage on the outer membranes, which were recognised by the formation of ‘pits’. The study shows that 1% Ag-MWCNTs/β-CD nanocomposite is bactericidal.

**Key words** | 1% Ag-MWCNTs/β-CD nanocomposite, absorbance, antibacterial, *E. coli*, field emission scanning electron microscopy, nanocomposites

**INTRODUCTION**

Safe drinking water is one of the most basic needs in society. Drinking polluted or contaminated water can cause serious health problems, e.g. cholera and other diarrhoeal diseases which cause many deaths, especially amongst children in developing countries (Havelaar & Bartram 1996). Water that does not meet accepted drinking water standards should be treated to ensure that the health of the consumer or community is not compromised through exposure to toxic pollutants (Reddy & Voortman 2005). Given the importance of clean water to people in developed and developing countries, numerous organisations are considering the potential application of nanoscience to solve technical challenges associated with the removal of water contaminants (Korom et al. 2000; Maynard 2007).

Carbon nanotubes exhibit unique characteristics, such as electrical properties, large specific surface areas, high stabilities and strong adsorption properties (Baughman et al. 2002). Studies have shown that multi-walled carbon nanotubes can remove 25-nanometer-sized polio viruses from water as well as larger pathogens such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* bacteria (Mamba et al. 2007). The same group investigated the adsorption properties of β-cyclodextrin and noted that this material has a greater binding to water contaminants of up to 100,000 (Mhlanga et al. 2007).

Lam et al. (2006) suggested that carbon nanotubes may interfere or damage DNA and this could cause harmful effects to organs if introduced into the body. Further *in vitro* studies indicate that multi-walled carbon nanotube
(MWCNTs) cause cytotoxicity and cytokine production (Hirano et al. 2010), but others indicate that MWCNTs did not cause any significant biological responses (Pulskamp et al. 2007). In a review by Yildirimer et al. (2011), it was also reported that exposure of animals to MWCNTs results in contradictory reports since some authors have reported asbestos and carbon nanotubes as posing the same health risk (Jaurand et al. 2009), while other studies reported MWCNTs to be biocompatible and far from cytotoxic (Takagi et al. 2008). Research on exposure to carbon nanotubes on human and environmental health risks is limited. Based on these few reports, results are conflicting and inconsistent making it difficult to draw any conclusions (Aillon et al. 2009).

Studies have demonstrated that metal nanoparticles have good antibacterial activities and are effective bactericides (Hamouda et al. 1999; Pal et al. 2007). The biocidal action of silver nanoparticles against E. coli bacteria was investigated using transmission electron microscopy (TEM) and scanning electron microscopy (SEM) (Sondi & Salopek-Sondi 2004). Their study has demonstrated that cell walls of E. coli bacteria are damaged in the presence of silver nanoparticles. Silver nanoparticles possess an effective antibacterial activity because of their massive high surface area (Park & Jang 2005). However, a recent review by Tran et al. (2013) has noted that silver nanoparticles alone have the potential to cause health hazards in humans and ecotoxicity. A review by Ahamed et al. (2010) has indicated that Ag nanoparticles are toxic to a variety of organs including the lung, liver, brain, vascular system and reproductive organs. They further suggested that Ag nanoparticles-induced toxicity includes induction of oxidative stress, DNA damage and apoptosis. Tran et al. (2013) suggested that a new approach would be to develop a hybrid nanostructure between carbon-based nanomaterials such as carbon nanotubes or graphene and Ag nanoparticles. By decorating Ag-NPs on carbon nanomaterials, the toxicity of Ag nanoparticles will be reduced, thus helping in preventing potential human hazards and contamination to the environment (Tran et al. 2013). Further incorporation of the Ag-MWCNT nanocomposite to β-cyclodextrin, a non-toxic starch derivative will reduce their toxicity.

The Ag-MWCNTs/β-CD nanocomposite will greatly reduce the toxicity which can be posed by Ag nanoparticles to humans and the environment, due to the fact that a strong interaction exists between Ag nanoparticles and functionalised MWCNTs (Ma et al. 2008). Thus will ultimately reduce Ag nanoparticles toxicity and help prevent environmental contamination. Herein, the study aimed at synthesising Ag-MWCNTs/β-CD nanocomposite, and investigates its effects and interaction on E. coli bacteria during drinking water disinfection.

**METHODS**

**Chemicals and reagents**

Sodium dodecyl sulphate (NaC12H25SO4), dimethyldiformamide (C3H6NO) (DMF), nitric acid (HNO3), sulphuric acid (H2SO4), silver nitrate (AgNO3), calcium hydride (CaH2), hydrazine hydrate (N2H4), trisodium citrate (Na3C6H5O7), β-cyclodextrin (C42H70O35), hexamethylene disiocyanate (C8H12N2O2) and MWCNTs (>95% purity) were purchased from Sigma-Aldrich (South Africa). Escherichia coli, ATCC 25922 bacteria, bacteriological agar and nutrient broth (NB) were also purchased from Sigma-Aldrich. Sterile distilled water was obtained from the Microbiology Department, University of Limpopo, Turfloop campus. DMF was dried over calcium hydride for 48 hours, followed by distillation over calcium sulphate under an argon atmosphere. MWCNTs were treated in acid media before use. All chemicals used were either of reagent or analytical grade.

**Apparatus and measurements**

All synthetic experiments were carried out in an argon atmosphere. The absorbance of the bacterial cultures in NB medium supplemented with Ag-MWCNTs/β-CD nanocomposite was measured in 10 mm optical path length quartz cuvettes using an Ultraspec 2000 UV-Vis spectrophotometer (Pharmacia Biotech, Cambridge, UK). The microstructure of the Ag-MWCNTs/β-CD was examined using TEM at 200 kV with a JEOL (Tokyo, Japan) JEM-1010 instrument.

SEM data were obtained using field emission scanning electron microscopy (FE-SEM), a Zeiss Supra 55VP (Carl Zeiss, Germany) operated between 2 and 3 kV. X-ray
powder diffraction (XRD) patterns of the nanocomposite materials were determined on a Bruker D2 Phaser X-ray diffractometer with high intensity of Cu-Kα radiation (\(\lambda = 1.54 \text{ Å}\)) and a graphite monochrometer at a scanning rate of 0.02 s\(^{-1}\) ranging from 10 to 120°. The diffractometer was operated at 30 kV and 10 mA current. An Agilent Cary 600 series Fourier transform infrared spectroscopy (FTIR) spectrophotometer was used to get the spectra of MWCNTs.

Preparation of silver doped multi-walled carbon nanotube/\(\beta\)-cyclodextrin (1% Ag-MWCNTs/\(\beta\)-CD) nanocomposites

Preparation of 1% Ag-MWCNTs

Multi-walled carbon nanotubes were functionalised following the method described elsewhere (Salipira et al. 2008). Briefly, MWCNTs were treated by refluxing in a mixture of concentrated nitric acid and sulphuric acid (1:3 V/V) for 30 min at 80 °C. After 30 min, the mixture was diluted with 500 mL of distilled water. The MWCNTs were thoroughly washed with distilled water until a pH 7 was achieved. Finally the MWCNTs were dried in an oven at 50 °C for 15 h.

The Ag-MWCNT nanocomposite was prepared by reductive pre-treatment of AgNO\(_3\) as follows: the Ag nanoparticles on MWCNTs were prepared by drop-wise addition, under stirring, of a mixture of 0.027 M hydrazine hydrate and 0.001 M sodium citrate as reducing agents into an aqueous solution containing sodium dodecyl sulphate (9%, w/w), MWCNTs and 0.0014 M AgNO\(_3\).

Polymerisation of 1% Ag-MWCNTs on \(\beta\)-CD

Approximately 0.02 g of Ag-MWCNTs was added while stirring into a solution of \(\beta\)-CD in 40 mL of DMF. The mixture was heated to 75 °C and a bi-functional linker, 4 mL of hexamethylene diisocyanate, was added. The reaction was allowed to continue for 24 h under argon atmosphere at 75 °C. The resultant nanocomposite materials were precipitated, washed with acetone and dried under vacuum (Mamba et al. 2007).

Killing kinetics in the presence of 1% Ag-MWCNTs/\(\beta\)-CD nanocomposites

Disinfection of water contaminated with \(E.\ coli\) bacteria

Approximately 20 mL of water contaminated with a known concentration of bacteria (\(E.\ coli\) bacteria (ATCC 25922)) were transferred into a vial containing magnetic stirring bars and about 100 mg of 1% Ag-MWCNTs/\(\beta\)-CD nanocomposites. The vial was closed securely to ensure that no further contamination occurred. The mixtures were allowed to stir for 2 min and about 1 μL of the mixture was drawn every 2 min and plated on agar plates. The plates were incubated for 24 hours to observe any possible bacterial growth. After incubation, the number of colonies observed on plates were counted and recorded. The colony forming units (cfu) and percentage inactivation of the bacteria were calculated from the visible number of colonies.

Effect of 1% Ag-MWCNTs/\(\beta\)-CD nanocomposite on the growth rate of \(E.\ coli\) bacteria in the NB medium

To examine the bacterial growth rate and to determine the growth curve in the presence of 1% Ag-MWCNTs/\(\beta\)-CD nanocomposites, \(E.\ coli\) bacteria were cultured in 100 mL NB medium supplemented with 5, 10, and 100 mg of these nanocomposites per ml of NB. The cylindrically shaped sample containers were placed horizontally on an orbital shaker platform and continuously agitated at 225 rpm. The absorbance was measured at 600 nm for every 30 min (absorbance of 1.1 corresponds to a concentration of 1.28 × 10\(^6\) cfu/ml).

Approximately 1 ml of a mixture of bacteria and the nanocomposite was drawn out of the reaction mixture. A solution of Karnovsky’s fixative (prepared in phosphate buffer at pH 7.4) was added to stop the reaction for further analysis with FE-SEM. Before FE-SEM could be carried out, 1 ml of the stopped reaction mixture was filtered, and rinsed in a phosphate buffer to remove the fixative. The filtrates were dehydrated with a range of 50 to 100% concentrations of ethanol. For complete dehydration, the bacteria were rinsed and evaporated using hexamethyldisilisane reagent. Once the bacteria were dry, filters were mounted directly onto aluminium pin-type stubs and sputter coated with...
thin layers of carbon. Samples of bacteria at 30, 60, 90, 120, and 150 intervals were subsequently investigated for morphological changes by viewing at 1.0 kV in a Supra 55VP FE-SEM (Carl Zeiss, Germany).

RESULTS AND DISCUSSION

Characterisation of 1% Ag-MWCNTs/β-CD nanocomposite

Figure 1 shows the FTIR spectra of raw and acid treated MWCNTs. The FTIR spectrum of raw MWCNTs (Figure 1(a)) appeared slightly different to the FTIR of acid treated MWCNTs (Figure 1(b)). Acid treated MWCNTs (Figure 1(b)) showed additional peaks at positions 1,217 and 1,340 cm\(^{-1}\) which are due to the C-O bond of the carboxylic acid.

The peak at 1,733 cm\(^{-1}\) is attributed to the C=O functional group, whereas the peak at 3,688 cm\(^{-1}\) is due to the O-H bending distortion mode of the carboxylic acid, as reported by Buang et al. (2012). The increase in the peak intensity of the C-H stretching mode at 2,341 cm\(^{-1}\) suggests that oxidation of the MWCNTs successfully introduced the carboxylic group (-COOH) on the walls of carbon nanotubes. The C-H signal also confirmed that the carbon atoms were now sp\(^3\) hybridised instead of being sp\(^2\) hybridised as in the case of raw MWCNTs (Salipira et al. 2008).

Figure 2 shows the energy-dispersive X-ray spectroscopy (EDX) of 1% Ag-MWCNTs/β-CD nanocomposite. The elemental composition by EDX confirms the presence of silver in the 1% Ag-MWCNTs/β-CD nanocomposite and in very low loading. It can be seen that the atomic ratios of Ag and carbon are nearly 1:100, which is in good agreement with the original inputs.

Figure 3 shows the XRD patterns of 1% Ag-MWCNTs/β-CD compared with the Ag-MWCNT nanocomposite material. In Figure 3(a), the peaks intensity of Ag on MWCNTs is related to the amount of Ag loaded (32 wt. % Ag on MWCNTs). This loading reduced the intensity of the (002) plane, corresponding to carbon reflection of both cyclodextrin polymer and carbon nanotubes. Similar results were observed by Ma et al. (2008). However, in Figure 3(b), a broad peak corresponding to (002) planes is observed, due
to lower concentration of Ag nanoparticles introduced. The low concentration of Ag on MWCNTs/β-CD is related to a highly reduced peak intensity of Ag (corresponding to (111) plane, Figure 3(b)). The estimated size of Ag crystallites using XRD was approximately 4.67 nm for Ag on MWCNTs and 2.93 nm for Ag on 1% Ag-MWCNTs/β-CD. This shows that these Ag particles are well dispersed throughout the organic frameworks of 1% Ag-MWCNTs/β-CD nanocomposite (Tran et al. 2013). However, the TEM data (Figure 4(b)) could not provide a good picture of Ag particle size, probably due to the presence of β-CD.

Figure 4 shows the FE-SEM and TEM of 1% Ag-MWCNTs/β-CD nanocomposites. Figure 4(a) shows MWCNTs attached to cyclodextrin and silver nanoparticles, which help prevent Ag nanoparticles from contaminating the environment (Yildirimer et al. 2011). The TEM in Figure 4(b) shows the presence of dark spots on the outer wall of the carbon nanotubes which is indicative of the presence of Ag nanoparticles on MWCNTs/β-CD nanocomposites.

**Disinfection of water contaminated with E. coli bacteria using 1% Ag-MWCNTs/β-CD nanocomposite**

Figure 5 shows a graphical representation of the E. coli bacterial activity in the presence of 1% Ag-MWCNTs/β-CD nanocomposite studied using the colony count method during water disinfection. It was noted that after 10 min of interaction, there were no colonies observed on the plates which were incubated. After 10 min, the 1% Ag-MWCNTs/β-CD nanocomposite acted as an effective bactericide, since no bacterial growth was observed on the plates which were incubated for 24 hours.

**Effect of 1% Ag-MWCNTs/β-CD nanocomposite on the growth rate of E. coli bacteria in the NB medium**

Figure 6 shows the absorbance measurements of E. coli bacteria in NB medium supplemented with 1% Ag-MWCNT/β-CD nanocomposite and without nanocomposites (Figure 6(insert)). The study focused on comparing the effect of different concentrations of 5, 10 and 100 mg of 1% Ag-MWCNT/β-CD nanocomposite in the NB medium. The data show that the bacterial growth was inhibited after 25 min, due to the presence of nanocomposite. The results also show that the absorbance of bacterial growth...
drastically decreased with increasing concentration of nanocomposite. The decrease in absorbance of contaminated water was enhanced compared to that viewed by Sondi & Salopek-Sondi ([111]2004) who were using Ag nanoparticles alone in the Luria broth (LB) medium. These findings are therefore a result of the combined effects of Ag nanoparticles, β-cyclodextrin and carbon nanotubes in 1% Ag-MWCNT/β-CD nanocomposite.

Investigation of the interaction between *E. coli* bacteria and 1% Ag-MWCNTs/β-CD nanocomposite over a period of 150 min by means of a FE-SEM

Figure 7 shows FE-SEM micrographs of *E. coli* bacteria, before and after interaction with the 1% Ag-MWCNTs/β-CD nanocomposite. Figure 7(a) shows the structure of *E. coli* bacteria, before interacting with the nanocomposite surrounded by a visible capsule, exhibiting the morphology of a normal rod-shaped bacterium. During the initial stages of interaction (Figure 7(b)), clusters of nanocomposite materials were found anchored to the bacterial cell wall. After 60 min of interaction (Figure 7(c)), the bacteria displayed changes in their cell wall structure, with *E. coli* losing its outer capsule. The nanocomposite, with clear images of MWCNTs, was ‘trapping’ *E. coli* (Figures 7(c) and 7(d)).

The bacterial structure in Figures 8(a) and 8(b) showed critical changes after 120 and 150 min. The damage had occurred on both the membrane and at the cellular level. After a long exposure of these nanocomposites to *E. coli* bacteria, pits were clearly visible on the external structure of the bacteria. This formation of pits was more visible compared to that reported by Sondi & Salopek-Sondi (2004).

![Figure 5](https://iwaponline.com/ws/article-pdf/14/3/367/415591/367.pdf)

*Figure 5* | Colony forming unit versus time of *E. coli* bacteria in the presence of 1% Ag-MWCNTs/β-CD nanocomposite. Initial cfu = 15,400.

![Figure 6](https://iwaponline.com/ws/article-pdf/14/3/367/415591/367.pdf)

*Figure 6* | Absorbance curves of *E. coli* bacteria supplemented with 1% Ag-MWCNT/β-CD nanocomposites in NB medium.
This clearly shows that the nanocomposite materials tend to penetrate inside the bacteria causing damage by interacting with phosphorus- and sulphur-containing compounds such as DNA (Morones et al. 2005). A similar effect was reported by Sondi & Salopek-Sondi (2004) when E. coli was treated with Ag nanoparticles.

Studies by Amro et al. (2000) suggest that when E. coli bacteria were treated with Ag nanoparticles alone; changes took place in its membrane morphology which produced a significant increase in permeability, thus ultimately affecting sufficient transport through the plasma membrane. This left the bacterial cells incapable of appropriately regulating transport through the plasma membrane, therefore resulting in cell death (Raffi et al. 2008). This finding suggests that the 1% Ag-MWCNTs/β-CD nanocomposite is effectively bactericidal.
CONCLUSIONS

The FTIR has confirmed that MWCNTs were successfully functionalised with carboxylic acids and hydroxyl groups. The EDX and XRD confirmed the doping of Ag nanoparticles on MWCNTs/β-CD nanocomposite. The study has shown that the Ag nanoparticles of crystallite size 2.93 nm were well dispersed throughout the organic frameworks of 1% Ag-MWCNTs/β-CD nanocomposite. FE-SEM has shown that Ag nanoparticles were attached to MWCNTs/β-CD, which helps prevent Ag nanoparticles from contaminating the environment. The absorbance curve showed that nanocomposite inhibited the bacterial growth and this was dependent on the concentration of nanocomposite. Results also indicated that treated bacteria show significant damage on the outer membranes, which were recognised by the formation of ‘pits’. Finally, the 1% Ag-MWCNTs/β-CD nanocomposite material has proven to be a good antibacterial material against E. coli.

The findings corroborate the reliability of 1% Ag-MWCNTs/β-CD nanocomposites in water purification, which may ultimately provide access to supplies of safe drinking water for regions of the world stricken by periodic drought or where water contamination is rife.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of this work by National Research Foundation (NRF) and University of Limpopo (Turfloop Campus), Department of Chemistry and the Department of Microbiology, Biochemistry and Biotechnology. We also acknowledge the assistance of Dr C. Baker (Director of Electron Microscopy unit, Medunsa Campus) and Prof. P. Masoko for assistance with the bacterial studies.

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First received 8 July 2013; accepted in revised form 7 October 2013. Available online 21 October 2013