Characterization of Silver-coated Carbon Nanotubes Dispersed in a Specifically Formulated Dispersant - NanoSperse AQ®

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ABSTRACT

Silver-coated carbon nanotubes (AgCNTs) are well documented for their antibacterial properties. However, aqueous dispersions of AgCNTs could be less effective against bacteria due to their tendency to agglomerate when re-suspended in water. This may be controlled by adopting different dispersion techniques for AgCNTs such as using a specifically formulated dispersant solution. In this study we have characterized AgCNTs dispersed in Nanosperse AQ® (dAgCNTs) from NanoLab Inc. and water dispersed AgCNTs (wAgCNTs), and compared their bactericidal activity against *Salmonella enterica* serovar Typhimurium and *Escherichia coli*. AgCNTs were characterized using zeta potential and fourier transform infrared spectroscopy (FT-IR) analysis. dAgCNTs were homogenously dispersed as compared to wAgCNTs which formed agglomerates even after repeated sonication. The zeta potential value of dAgCNTs was -41.9mv compared to wAgCNTs (-30.7mv) indicating a higher degree of stability for dAgCNTs. FT-IR analysis of dAgCNTs showed the presence of additional peaks specific for dAgCNTs as compared to wAgCNTs. The minimum inhibitory concentrations (MIC) of dAgCNTs (31-15 µg/ml) were approximately three folds lower compared to wAgCNTs (100-62.5 µg /ml) against both *Salmonella enterica* serovar Typhimurium and *Escherichia coli*. Live/dead staining revealed that exposure to 12.5 µg /ml of dAgCNTs resulted in at least twice the number of dead bacteria as compared to exposure to similar concentrations of wAgCNTs. NanoSperse AQ® did not have any antibacterial effect by itself. Our results indicate that dispersion of AgCNTs using an appropriate dispersion technique may result in a relatively stable and homogenous solution of AgCNTs, thereby improving their anti-bacterial activity.

Keywords: Silver-coated carbon nanotubes, dispersant, characterization, antibacterial, live and dead count

1 INTRODUCTION

Resistance to antimicrobials is a significantly growing problem and there is a pressing need for development of antibacterial products. For example, the incidence of methicillin and vancomycin resistant *S. aureus* (MRSA/VRSA) and extended-spectrum-lactamase (ESBL)–producing *E. coli*, *N. gonorrheae* and *H. influenzae* strains continues to grow [1]. Previous research has shown the ability of nanomaterials to alleviate the gradual resistance phenomenon of various strains [2-7]. Specifically, silver-coated carbon nanotubes (AgCNTs) have been shown to possess antibacterial activity [3, 4].

Due to their high surface area to volume ratio, high mechanical strength, unique chemico-physical properties, and the ability to target various biological pathways, AgCNTs are emerging as a suitable tool for several biomedical applications [3, 7, 8]. They have been shown to be effective against multidrug-resistant bacteria such as MRSA (ref?). However, AgCNTs are not well dispersed in water and tend to agglomerate in water, which could affect their antibacterial activity. AgCNTs are less dispersivein water and could adopt different functionalities and so have an undiscovered potential in the disinfection area [8, 9]. Most of the properties of AgCNTs depend upon good dispersion techniques and a choice of a suitable dispersant [10].

In the present study we used the commercially available Nanosperse AQ® dispersant from NanoLab Inc. to disperse AgCNTs. AgCNTs dispersed in water (wAgCNTs) or dispersant (dAgCNTs) were characterized by zeta potential measurements and Fourier transform infrared spectroscopy (FT-IR) analysis. The antibacterial activity of dAgCNTs was compared with that of wAgCNTs against pathogenic strains of *Salmonella enterica* serovar Typhimurium and *Escherichia coli*.

2 MATERIALS AND METHODS

2.1 Preparation of AgCNTs suspensions

AgCNTs of 1-5 nm diameter, produced by catalytic chemical vapor deposition (purity greater than 95%), were purchased from NanoLab, Inc. (Waltham, MA, USA). 1mg of AgCNTs were suspended in 1ml of water or 1ml of NanoSperse AQ® dispersant solution (according to manufacturer's instructions), sonicated for 1-2h and shaken for 30 min to obtain 1mg/ml suspensions of AgCNTs in water (wAgCNTS) or dispersant (dAgCNTs)

2.2 Determination of zeta potential

The zeta potential of wAgCNTs and dAgCNTs was measured using a Zetasizer (Nano-ZS; Malvern Instruments Ltd, Malvern, UK). The samples were diluted in distilled water to 1/10 (v/v), sonicated, and placed in a disposable cuvette for zeta potential measurements. All the measurements were carried out in triplicates for each sample. The values are reported as the mean of triplicate samples.

2.3 FTIR spectroscopy

FTIR spectra were recorded for wAgCNTs and dAgCNTs in attenuated total reflectance (ATR) mode using an infrared (IR) spectrophotometer (Nicolet 380 FT-IR; Thermo Fisher Scientific). The spectra were obtained with 64 scans per sample, ranging from 400 to 4000 cm^{-1} and a resolution of 4 cm^{-1}. The sample chamber was purged with dry N_2 gas.

2.4 Minimum inhibitory concentrations (MIC)

Salmonella enterica serovar Typhimurium and *Escherichia coli* from **American Type Culture Collection** (ATCC® , VA USA) were grown at 37°C in Luria-Bertani (LB) broth (Difco, Sparks, MD, USA) with continuous shaking until the optical density (OD) was 0.6–0.8 (at 600 nm). The MIC values of wAgCNTs and dAgCNTs were evaluated using the broth micro-dilution assay in sterile 96-well microtiter plates in quadruplicates. *Salmonella* Typhimurium and *Escherichia coli* (1×10^5 colony forming units/ml) were exposed to doubling concentrations of wAgCNTs and dAgCNTs starting at 1.9 µg/ml. Two-fold serial dilutions of wAgCNTs and dAgCNTs were performed in sterile nuclease free water and NanoSperse AQ dispersant solution, respectively. All plates were sealed and then incubated at 37°C for 24 hours. After incubation, the MIC level was determined by the turbidity of the culture media in the wells. Concentration of the first well without turbidity was considered as MIC.

2.5 Live/dead staining of bacteria

The viability of bacteria was examined by live/dead staining of bacteria using Baclight bacterial viability kit L13152 (Molecular probes, USA) according to manufacturer's instructions. Briefly, 1×10^5 cfu/ml bacteria were treated with wAgCNTs and dAgCNTs for 16h at 37°C. Post treatment, the bacterial cells were incubated in the dark for 30-45mins with an equal amount of 2X stock solution of the LIVE/DEAD *Bac*Light staining reagent containing the final concentration of 6 µM SYTO 9 dye and 30 µM propidium iodide. The stained bacterial suspensions were trapped (5µl) between a slide and an 18 mm square coverslip and the images were captured by Nikon Eclipse TE200 microscope (Nikon, Melville, NY, USA) using FITC-HYQ (Ex 450-500) and TRITC HYQ (Ex 530-550) filters. Viable cells were fluorescent green, while non-viable cells were fluorescent red.

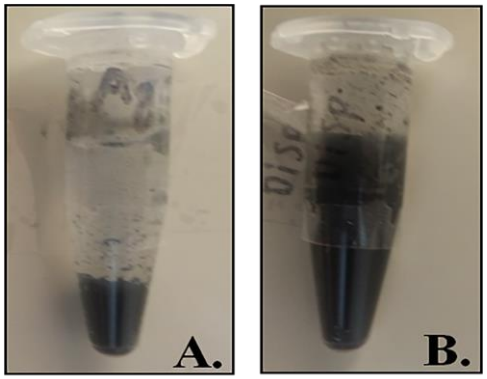

Figure 1: (A) AgCNTs in water; (B) AgCNTs in dispersant.

3 RESULTS AND DISCUSSION

When dispersed in the dispersant, AgCNTs were homogenously dispersed whereas wAgCNTs appeared as agglomerated suspension even after repeated sonication (Figure 1).

3.1 Zeta potential and FT-IR analysis

The zeta potential value of dAgCNTs was -41.9 mv compared to wAgCNTs (-30.7mv) indicating a higher degree of stability for dAgCNTs with less of agglomeration (Figure 2). FT-IR analysis of dAgCNTs showed the presence of additional peaks (indicated by arrows) specific for dAgCNTs as compared to wAgCNTs (Figure 3). Our results thus suggested that dAgCNTs were well dispersed in

dispersant resulting in more stable suspension compared to wAgCNTs. The distinct patterns of FT-IR for dAgCNTs and wAgCNTs were indicative of chemical interaction between the dispersant components with AgCNTs which ultimately changes the negative charge and results in the formation of more homogenous suspension.

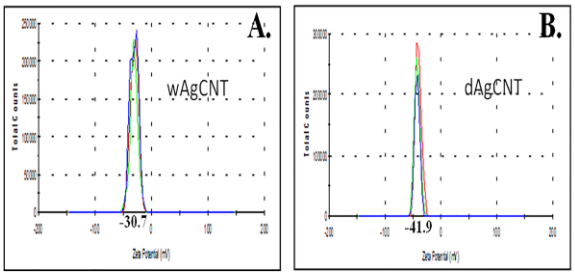

Figure 2: Zeta potential analysis. (A) AgCNTs in water; (B) AgCNTs in dispersant.

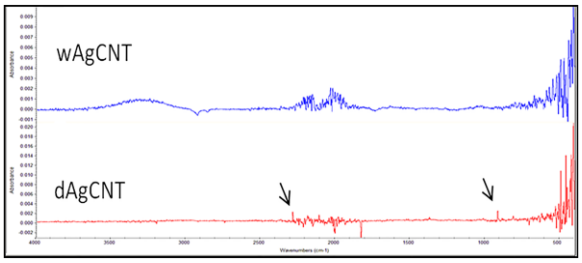

Figure 3: FT-IR analysis. (A) AgCNTs in water; (B) AgCNTs in dispersant (Arrow indicates peaks).

3.2 MIC

The antibacterial activity of dAgCNTs was more efficient compared to wAgCNTs against gram negative pathogens such as *Salmonella* Typhimurium and *Escherichia coli.* The MIC value of dAgCNTs against both the pathogens was 31-15 µg/ml which was approximately three folds lower compared to wAgCNTs (100-62.5 µg /ml, Figure 4 & 5).

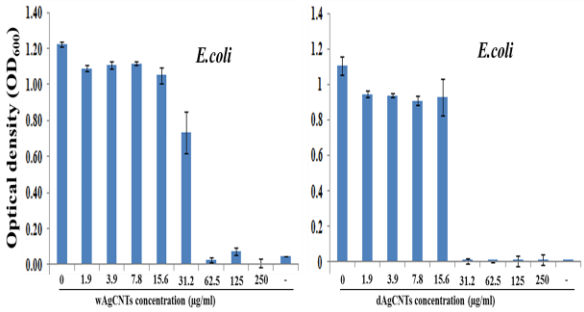

Figure 4: Minimum inhibitory concentrations of AgCNTs in water; AgCNTs in dispersant against *E. coli.*

NanoSperse AQ® did not have any antibacterial effect by itself. It has been postulated earlier that the electrostatic interaction between the Ag/MWCNTs and bacteria in the presence of natural organic matter was more repulsive due to the more negative zeta potential of NPs resulting in reduced antibacterial activity [11]. In the present study, although

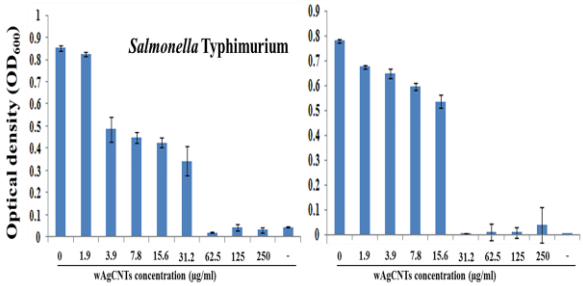

Figure 5: Minimum inhibitory concentrations of AgCNTs in water; AgCNTs in dispersant against *Salmonella* Typhimurium.

dAgCNTs possess more negative charge than wAgCNTs, it had better antibacterial activity on its counterparts. Our results thus indicate that homogenous suspension of dAgCNTs is more effective than the agglomerated form (wAgCNTs) irrespective of more negative zeta potential charge.

3.3 Live/dead staining

We further confirmed the efficient antibacterial activity of dAgCNTs compared to wAgCNTs by performing live/dead staining of bacteria. This assay provided clear evidence showing a higher number of dead bacteria upon exposure to dAgCNTs compared to wAgCNTs. As shown in Figure 5, exposure to 12.5 µg /ml of dAgCNTs resulted in at least twice the number of dead bacteria as compared to exposure to similar concentrations of wAgCNTs.

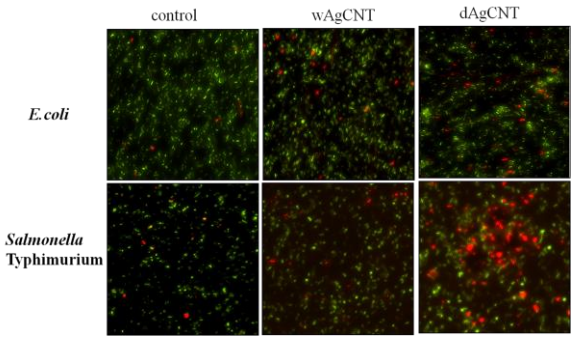

Figure 6: Live/dead staining of bacteria upon exposure to AgCNTs in water and AgCNTs in dispersant.

As stated earlier, dispersion techniques and choice of a suitable dispersant may control the physiochemical properties of AgCNTs [10]. Overall, our current findings indicate that that dispersion of AgCNTs using an appropriate dispersion technique may result in a relatively stable and homogenous solution of AgCNTs, thereby improving their anti-bacterial activity.

Acknowledgement:

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