



Antibacterial activity of Cu@Ag nanocomposites against water bacterial pollution

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Abstract

Nanomaterials have a great potential in purifying water and wastewater treatment due to their tremendous antibacterial activity. In the present study, Cu@Ag nanocomposite (NC) was synthesized and characterized using scanning electron microscopy (SEM) and transmission electron microscope. The antibacterial activity of Cu@Ag NC was tested against water Gram-positive bacteria (*Staphylococcus sp.*) and Gram-negative one (*Escherichia coli*) using well diffusion method and minimum inhibition concentration (MIC). Transmission electron microscopy images of fabricated Cu@Ag NC showed that particles are spherical and with average diameter ranged from 25-45 nm. The highest inhibition zones for *E. coli* and *Staphylococcus sp.* were 25.6 ± 1.6 and 28.3 ± 1.6 mm, respectively at 0.4 mg/ml of Cu@Ag NC. The MIC concentrations for *E. coli* and *Staphylococcus sp.* were 21 ± 2.2 and 16 ± 1.4 $\mu\text{g/ml}$, respectively. So, the Cu@Ag NC was more effective for Gram-positive bacteria than Gram-negative one. Accordingly, the Cu@Ag NC could be suggested as an effective antibacterial agent for treatment of water bacterial pollution.

Keywords: Nanocomposites, Cu@Ag, antibacterial, nanoparticles, water pollution, bacteria.

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1. Introduction

Water is the most essential substance for all life on earth and a precious resource for human civilization. Reliable access to clean and affordable water is considered one of the most basic humanitarian goals, and remains a major global challenge for the 21st century [1]. One of the most important factors of water pollution is the microbial contamination; especially with pathogenic microorganisms, where enteric pathogens are typically responsible for waterborne diseases. Also, contamination of water is a serious environmental problem as it adversely affects the human health and the biodiversity in the aquatic ecosystem [2]. The present well documented technologies used in water treatment such as reverse osmosis, ion exchange, UV-sterilization, aluminum sulphate and chlorine are becoming unsustainable, unecological, expensive to run, managed and maintained, particularly in Africa [3]. For example, chlorine is known to produce trichloromethane, a cancer precursor [4], while aluminum sulphate has been linked to Alzheimer's disease [5]. Furthermore, the cost of purchasing synthetic coagulants

and disinfectants is in hard currency leading to high pricing for treated water in Africa [6]. The rapid growth in nanotechnology has spurred significant interest in the environmental applications of nanomaterials. In particular, its potential to revolutionize century-old conventional water treatment processes has been enunciated recently [7,8]. Recently, several natural and engineered nanomaterials have also been shown to have strong antibacterial properties, including Ag@Cu nanoparticles [9]. Adhikary *et al.* [10] studied the antibacterial activity of Ag@CuO NC against *E. coli* and *S. aureus*. The NPs showed potent anti-microbial activity evidenced from the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values.

The present work aimed to study the antibacterial activity of the Cu@Ag nanocomposite against the two of the most prevalent bacterial species *Escherichia coli* and *Staphylococcus sp.* as indicator for using this nano-form in treatment of water bacterial pollution.

Materials and methods:

1.1. Preparation of Cu@Ag nanocomposite:

For the synthesis of copper and silver nanocomposite, silver nitrate and copper nitrate solution (0.01 M) was used as a metal salt precursor and 1% (w/v) aqueous starch solution was the stabilizing agent. Ascorbic acid (10% w/v) was used as a reducing agent. To the starch solution equal volume of metal salt solution and ascorbic acid (10%, v/v, of starch) were added and reaction was carried out under microwave at full power for 90 seconds. The transparent colourless solution was converted to the characteristic pale yellow colour, indicating the formation of silver nanoparticles. In case of copper, the light blue coloured transparent solution first changed to pale yellow and after MW heating to characteristic red colour, indicating the formation of copper nanoparticles. Bimetallic nanoparticles were prepared following the above mentioned procedure, using same ratio of copper and silver ion concentrations and ascorbic acid as a reducing agent. The solutions were kept under microwave for 90 seconds. The change in the colour of the solution indicated the formation of nanocomposite [9].

1.2. Characterization of nanoparticles:

Fabricated nanoparticles were characterized with the help of multiple techniques. The particles size of the resulting nanoparticles were analyzed using transmission electron microscope (TEM) (EM 208S Philips, Netherlands) connected to a high resolution imaging system. Samples for TEM studies were prepared by placing drops of nanoparticles solutions on carbon-coated TEM copper grids. The surface morphology of nanoparticles was characterized by a scanning electron microscopy (SEM) (JEOL JSM-5600).

1.3. Water samples:

The water samples were collected from two canals (El-Sharkawya and Ismailia canals) in Qualiobyia Governorate as standard methods according to Sabae and Rabeh [11]. The cover of the sterile sample bottle was aseptically removed and the mouth of the bottle was faced upstream (i.e. towards the flow of the water). The neck was plunged the neck downwards about 30 cm below the water surface and then tilted slightly upwards to let it fill completely before carefully replacing the cover. Samples were transported in an ice box to the laboratory for analysis within 12 hours.

1.4. Identification of bacteria present in water samples:

Water samples were cultivated on MacConkey agar medium (Himedia company) for Gram-negative bacteria and Barid-paker agar (Himedia company) for Gram-positive one (*Staphylococcus* sp.). For Gram-negative isolates, after incubation time based on morphological differences, colonies were separated and purified and recultured. By conventional methods Gram-negative bacterial isolates were identified by testing reactions of

isolated bacteria with: triple sugar iron agar (TSI), Lysine iron agar (LIA), motility indole Ornithine agar (MIO), citrate agar, urea agar and oxidase test [12].

2.5. Antibacterial activity:

The antibacterial activity was done by modified Kirby-Bauer well diffusion method [13]. The pure cultures of organisms were subcultured in Müller-Hinton broth at $35\pm 2^\circ\text{C}$ on a rotary shaker at 160 rpm. For bacterial growth, a lawn of culture was prepared by spreading the 100 μL fresh culture having 10^6 colony-forming units (CFU)/mL of each test organism on nutrient agar plates with the help of a sterile glass-rod spreader. Plates were left standing for 10 minutes to let the culture get absorbed. Wells were punched into the nutrient agar plates using sterile cork borers for testing nanomaterial antibacterial activity. Wells were sealed with one drop of molten agar (0.8% agar) to prevent leakage of nanocomposite solutions from the bottom of the well. Using a micropipette, 100 μL of the concentrations (0.05, 0.1, 0.2, 0.3 and 0.4 mg/ml) of nanocomposite suspension was poured onto each well on all plates. After overnight incubation at $35\pm 2^\circ\text{C}$, the different levels of zone of inhibition were measured.

2.6. Determination of minimum inhibitory concentrations (MIC):

All the newly synthesized compounds were screened in vitro for their antibacterial activities by broth dilution method to determine the lowest concentration inhibiting growth of the organism that recorded as the MIC. Dimethyl sulfoxide DMSO was used as diluent. The stock 1000 $\mu\text{g}/\text{ml}$ was prepared. Serial dilutions were prepared in screening. Mueller Hinton Broth was used as nutrient medium to grow and dilute the drug suspension for the tested bacteria. Inoculum size for test strain was adjusted to 1×10^8 cfu/ml by comparing the turbidity according to McFarland scale. For the broth microdilution test, 2 mL of each microbial suspension in suitable growth medium was added to tubes containing 2 mL of two-fold serially diluted tested compound. Control wells were prepared with culture medium, microbial suspension only, tested compound only and DMSO in amounts corresponding to the highest quantity present. The contents of each tube were mixed on a shaker incubator (Eppendorf, Hamburg, Germany) at 900 rpm for 1 min prior to incubation for 24-48 h in the cultivation conditions. The MIC was the lowest concentration where no viability was observed after 24-48 h on the basis of metabolic activity. To indicate respiratory activity the presence of colour was determined after adding 100 $\mu\text{L}/\text{well}$ of TTC (2,3,5- triphenyl tetrazolium chloride, Sigma) dissolved in water (20 mg/mL) and incubated under appropriate cultivation conditions for 30 min in the dark. After incubation, the optical density was measured by spectrophotometer (Spectronic 20D, USA). Positive controls were tubes with a microbial suspension in an appropriate growth medium in amounts corresponding to the highest quantity present in the broth microdilution assay. Negative controls were tubes with growth medium and tested nanocomposites. All

measurements of MIC values were repeated in triplicate [14].

3. Results

The scanning electron microscopy images showed that Cu@Ag NC were spherical shaped and tended to form aggregates (Figure 1A). Transmission electron microscopy showed that Cu@Ag NC are spherical and its total average diameter ranged from 25-45 nm, where the core ranged from 20-30 nm and the shell ranged from 6-10 nm (Figure 1B).

Positive water samples for bacterial growth were isolated and identified bacterial genera included Gram-positive bacteria (*Staphylococcus sp.*) that showed black colonies on Barid-paker agar and confirmed by gram stain and tested for the presence of *E. coli* Gram-negative bacteria, where its reactions were positive to lysine iron agar (LIA), motility, indole and ornithine. However, reactions gave negative results with citrate and urease. Also, fermentation of triple sugar iron agar (TSI) showed production of acid/acid (A/A) without H₂S.

In this study, Cu@Ag nanocomposite showed remarkable antibacterial activity against both Gram-positive (*Staphylococcus sp.*) and Gram-negative (*E. coli*) bacteria. *Staphylococcus sp.* recorded the highest inhibition zone at 0.4 mg/ml of Cu@Ag NC (28.3±1.6 mm) and recorded the lowest inhibition zone at conc. 0.05 mg/ml (13.1±1.2 mm). Also, inhibition zones of 0.1, 0.2 and 0.3 mg/ml of Cu@Ag NC reached (18.3±1.3, 21.4±1.1 and 25.2±1.2 mm) respectively. *E. coli* recorded the highest inhibition zone at 0.4 mg/ml of Cu@Ag NC (25.6±1.4mm) and recorded the lowest inhibition zone at 0.05 mg/ml (12.1±1.2 mm). However, inhibition zone of 0.1, 0.2 and 0.3 mg/ml of Cu@Ag NC reached (14.4±1.2, 17.1±1.6 and 23.7±1.8mm) respectively (Figure 2). Also, the highest MIC concentration of Cu@Ag NC (21±2.2 µg/ml) reported by Gram-negative *E. coli*, but Gram-positive *S. aureus* recorded the lowest one (16±1.4 µg/ml). So, Gram-positive bacteria *Staphylococcus sp.* is more sensitive to Cu@Ag NC than gram-negative *E. coli*.

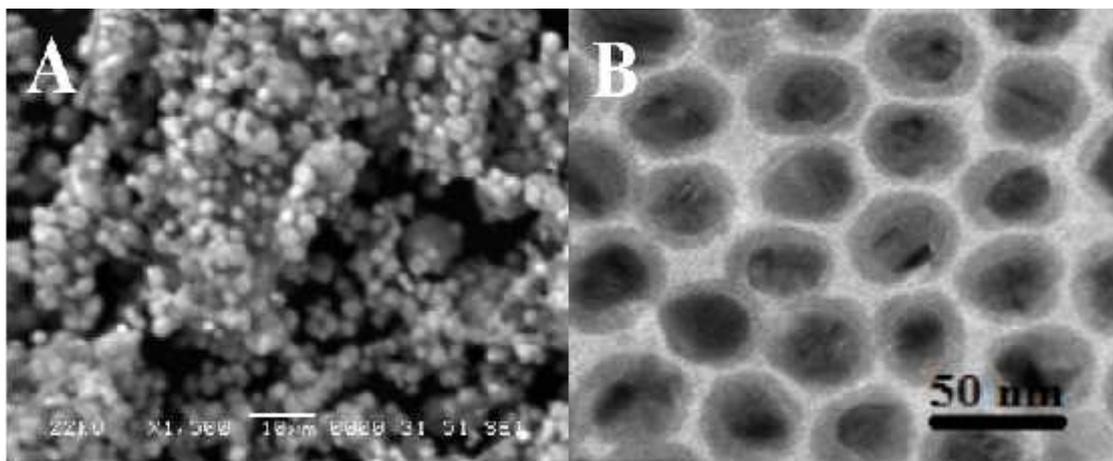


Fig (1): A) SEM B) TEM of Cu@Ag nanocomposite.

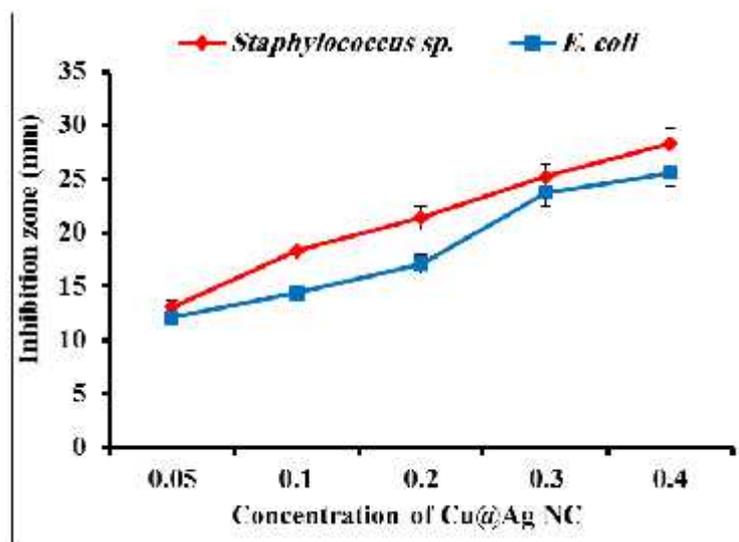


Fig (2): Inhibition zones of *Staphylococcus sp.* and *Escherichia coli* bacteria.

4. Discussion

The diverse impact of Ag@Cu nanocomposite on Gram-positive and Gram-negative bacteria is probably caused by a different structure of their cell walls [15]. In contrast to Gram-negative bacteria, Gram-positive cells have a thicker peptidoglycan layer which makes them more resistant to nanoparticles [16]. Yallappa et al. [17] studied the precise mechanisms of bacterial growth inhibition by Cu NPs and Ag NPs. For instance, Sondi and Salopek-Sondi [18] have proposed the formation of 'pits' in the cell wall, leading to cell death due to increased membrane permeability of *E. coli* cells when treated with Ag NPs and Cu NPs. Since Cu and Ag NPs are very reactive and can easily bind to tissue proteins, this induces structural changes in the bacterial cell wall and nuclear membrane that leads to cell death. Moreover, Zain et al. [19] illustrated the mode of action of silver and copper nanoparticles as bacteria bear a negative charge due to the excess number of carboxylic and other groups which make the cell surface negative. The nanoparticle suspensions produced have positive charge as revealed by zeta potential measurements. Electrostatic forces between positively charged nanoparticles and negatively charged bacteria cells will enhance the effect of antibacterial activity. Adhesion of nanoparticles to the surface of a bacterium alters its membrane properties ultimately causing death. Our data revealed that TEM showed that Cu/Ag NC are spherical and its total average diameter ranged from 25- 45 nm. Also, Paszkiewicz et al. [20] showed that the average size of Ag@Cu NC differs from 45 to 50 nm was close to spherical shape for antibacterial and antifungal

applications. Moreover, Valodkar et al. [9] indicated that the particle size of the Cu@Ag nanoparticles were in the range of 30–55 nm in diameter with narrow size distribution for anti-bacterial activity. Present data reported that *E. coli* recorded the highest inhibition zone at concentration 0.4 mg/ml of Cu@Ag NC (25.6±1.4mm), while Jaidev and Narasimha [21] revealed that the zone of inhibition of Ag nanoparticles at a concentration of 200µl for *E. coli* was 8 mm. Also, Ramyadevi et al. [22] showed the diameter of inhibition zone of the copper nanoparticles towards *E. coli* was 26±0.985 mm. On the other hand, the MIC value recorded with Cu/Ag NC for *E. coli* was (21±2.2 µg/ml). Mallick et al. [23] reported that the MIC of the core-shell Cu@Ag nanocomposite against *E. coli* was found to be 63.4 µg/ml. Our data revealed that *Staphylococcus sp.* recorded the highest inhibition zone at conc. 0.4 mg/ml of Cu/Ag NPs (28.3±1.6 mm). Also, Jaidev and Narasimha [21] revealed that the zone of inhibition of Ag NPs at a concentration of 200µl for *Staphylococcus sp.* was 9 mm. However, Ramyadevi et al. [22] showed the diameter of inhibition zone of the copper nanoparticles towards *S. aureus* was (21±1.612mm) On the other hand, the MIC value recorded with Cu/Ag NC for *Staphylococcus sp.* was (16±1.4 µg/ml). Moreover, Ahamed et al. [24] showed that the MIC concentration of CuO NPs against *S. aureus* was 62.5 µg/ml. Ruparelia et al. [25] revealed that the MIC of the silver and copper nanoparticles for *S. aureus* were 120 and 140 µg/ml.

5. Conclusion

We have successfully synthesized Cu@Ag nanocomposite and tested its antibacterial activity against the most water bacterial species prevalent *E. coli* and *Staphylococcus sp.* TEM images of Cu@Ag NC revealed that the average diameter ranged from 25-45 nm, while SEM images showed spherical shape and tended to form aggregates. Agar-well diffusion method and MIC reported

the excellent antibacterial performance of the Cu@Ag nanocomposite for both Gram-positive and Gram-negative bacteria which could be helpful in the water treatment and in wastewater reuse.

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