Journal of Basic and Environmental Sciences 3 (2016) 90-93

ISSN
Online: 2356-6388
Print: 2536-9202

Research Article Open Access

Antibacterial activity of cupric oxide nanoparticles against pathogenic bacteria

Ahmed M. Azzam[a1] ; Mahmoud M. Hazaa[b], Ashraf M. El Saeed[c] and Marwa R. Hamed[b]

[a] Environmental Researches Department, Theoder Bilharz Research Institute, Egypt.

[b] Microbiology Department, Faculty of Science, Banha University, Egypt.

[c] Petroleum Application Department, Egyptian Petroleum Research Institute, Egypt.

[1] Corresponding author : ah.azzam@tbri.gov.eg

Abstract

Nano materials have a wide range of applications due to their interesting size-dependent chemical and physical properties compared to particles of size in the range of micrometer. In this study, we studied the structural and antimicrobial properties of cupric oxide nanoparticles (CuO NPs) that synthesized by the sol–gel method and characterized by using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The antimicrobial activity of CuO NPs was evaluated against gram-negative bacteria (Escherichia coli) and gram-positive bacteria (Staphylococcus aureus) using agar-well diffusion method and minimum inhibition concentration (MIC). The average diameter of synthesized CuO NPs was 25.8 nm with approximately round-shape particles. CuO NPs showed excellent antimicrobial activity against both E. coli and S. aureus, but gram-positive bacteria is more sensitive to CuO NPs than gram-negative one, where the MIC of S. aureus and E. coli were 15 and 22 µg/ml, respectively. So, CuO NPs could be suggested as new effective agents of multidrug-resistant bacteria.

Keywords: Cupric oxide, Nanoparticles, Antibacterial activity, Bacteria

Received: 8 December, 2016, Accepted 11 December, 2016, Available online 1 Jan., 2017.

1. Introduction

Metal oxide nanoparticles have been receiving considerable attention for their potential applications in optoelectronics, nanodevices, nanoelectronics, nanosensors, information storage, and catalysis. Among various metal oxide NPs, CuO has attracted particular attention because it is the simplest member of the family of copper compounds and shows a range of useful physical properties such as high temperature superconductivity, electron correlation effects, and spin dynamics [1, 2]. CuO NPs are increasingly used in various applications such as in catalysis, batteries, gas sensors, heat transfer luids, and solar energy [3]. CuO crystal structures possess a narrowband gap, giving useful photocatalytic and photovoltaic properties [4]. Bacteria possess an extraordinary ability to adapt to environmental challenges like antimicrobials by both genetic and phenotypic means, which contributes to their evolutionary success. The gram-positive pathogen S. aureus provides a good example of how a microorganism can gradually become resistant to multiple antibiotics belonging to different classes. In subsequent year, the proportion of resistant strains as well as the determined MIC values increased dramatically. Indeed, around 50% of S. aureus isolates were already resistant to penicillin by 1950 [5]. The bactericidal property of nanoparticles depends on their size, stability, and concentration added to the growth medium, since this provides greater retention time for bacterium nanoparticles interaction. Generally bacterial cells are in the micron-sized range. Most bacterial cells have cellular membranes that contain pores in the nanometer range. A unique property of crossing the cell membrane can potentially be attributed to synthesized nanoparticles through such bacterial pores. However, to make this possible, it is important to overcome challenges and prepare/design nanoparticles which are stable enough to significantly restrict bacterial growth while crossing the cell membrane [6].

The present study aimed to determine the efficiency of synthesized CuO NPs against pathogenic gram-negative bacteria (Escherichia coli) and gram-positive one (Staphylococcus aureus).

2. Materials and Methodes

2.1. Prepration of copric oxide nanoparticles

A precursor solution was prepared using ethanol (99.9%) and deionized (DI) water as solvent (1:1). Then, copper nitrate [Cu (NO3)2•3H2O] was added. Citric acid and ethylene glycol were used as polymerization and complex agents, respectively. After 1 h of stirring at 40 °C, a green solution was obtained. The homogeneous mixture was maintained under reflux at 100–110 °C for 4 h. After vaporizing the excess solvents, a wet gel was attained. Finally, the black powder was calcined at 600 °C for 1 h and then milled [7].

2.2. Characterization of nanoparticles

The particles size of synthesized nanoparticles were determined 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).

2.3. Antibacterial activity

The tested bacterial species gram-negative bacteria (Escherichia coli) and gram-positive one (Staphylococcus aureus) were obtained from Theodor Bilharz Research Institute. Antibacterial activities of CuO NPs were determined using agar well-diffusion method [8]. Approximately, 25 ml of molten and cooled nutrient agar media were poured in the sterilized petri dishes. The plates were left over night at room temperature to check for any contamination to appear. The bacterial test organisms were grown in nutrient broth for 24 hours. A 100 ml nutrient broth culture of each bacterial organism was used to prepare bacterial lawns. Agar wells were prepared with the help of a sterilized stainless steel cork borer. The wells in each plate were loaded with 100ml of different concentration of nanoparticles. The plates containing the bacteria and solutions of nanoparticles were incubated at 37 0 C. All the tests were repeated in triplicates. The antibacterial activity was taken on the basis of diameter of inhibition zone, which was measured at cross-angles after 24 hours of incubation.

2.4. Determination of minimal inhibitory (MIC)

Both bacterial species were cultured overnight at 37 ºC in Mueller Hinton (MH) broth and adjusted to final density of 108 CFU/mL by 0.5 McFarland standards. Then in 96-well plate we added 90 µL of MH broth, 10µLof bacterial inoculum, and 10µL of NPs with different concentrations. Further, 96-well plate was incubated at 37 ºC for 12 hours. After incubation, the bacterial growth was visually inspected and the lowest concentration of NPs at which no observable bacterial growth was taken as the MIC value. The experiments were carried out in six replicates.

3. Results and Discussion

The scanning electron microscope images showed morphological surface of CuO NPs were round-shape and tended to form aggregates (Figure 1A). Transmission electron microscopy study was carried out to understand the crystalline characteristics of the nanoparticles. The particles were observed to be approximately spherical in shape and the average size of particles was found 25.8 nm (Figure 1B).

In this study, cupric oxide nanoparticles showed remarkable antibacterial activity against both gram-positive (S. aureus) and gram-negative (E. coli) bacteria. E. coli recorded the highest inhibition zone at 0.5 mg/ml of CuO NPs (21±2.3mm) and the lowest one (11±1.1mm) at 0.1 mg/ml, while S. aureus recorded the highest inhibition zone at 0.5 mg/ml of CuO NPs (23±1.4mm) and the lowest inhibition zone at concentrations 0.1 mg/ml was (13±1.8mm) (Figure 2). Moreover, the highest MIC concentration of CuO NPs was recorded with gram-negative E. coli (22 µg/ml) and the lowest one (15 µg/ml) was with S. aureus. So, gram-positive bacteria S. aureus are more sensitive for CuO NPs than gram-negative E. coli. A few studies have been performed to elucidate the mechanism of bactericidal action of nanoparticles. The exposure of gram-positive bacteria to carboxyfullerene nanoparticles resulted in the puncturing of the bacteria leading to cell death [9]. The concentration of released ions for 10 mg of cupric nanoparticles suspended in 100 mL nutrient media and distilled water [10]. Demonstrated significant antimicrobial act [9]. The variation in the sensitivity or resistance to both gram-positive and gram-negative bacteria populations could be due to the differences in the cell structure, physiology, metabolism, or degree of contact of organisms with nanoparticles. For example, greater sensitivity among gram-positive S. aurus to the CuO nanoparticles has been attributed to the greater abundance of amines and carboxyl groups on their cell surface and greater affinity of copper towards this group due to the release of ions [12]. Gram-negative bacteria like E. coli have a special cell membrane structure which possesses an important ability to resist antimicrobial agents [13]. Copper ions released subsequently may bind with DNA molecules and lead to disordering of the helical structure by cross-linking within and between the nucleic acid strands. Copper ions inside bacterial cells also disrupt the biochemical processes [14]. Cu^{+2} ions

were also small studied well to disrupt the bacterial cell membranes and gain entry in order to disrupt enzyme function. Indirect effects through changes in the surrounding charge environment also have an impact on the effectiveness of nanoparticulate metals against microorganisms [15]. Another proposed mechanism is there will be copper ions released from the nanoparticles that may attach to the negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cause cell death [16]. Our data shows that E. coli recorded the highest inhibition zone at concentrations 0.5 mg/ml of CuO NPs (21±2.3mm). Where, Radhakrishnan et al. [17] reported that the antibacterial activity of CuO nanoparticles against gram-negative E. Coli inhibition zone for E. coli was lower than our results. Moreover, data reveals that gram-negative E. coli recorded MIC with CuO NPs (22µg/ml). However, Ahmed et al. [18] revealed that the MIC concentration of E. coli was 31.25 µg/m) with CuO NPs. Present data showed that S. aureus recorded the highest inhibition zone (23±1.4mm) at concentrations 0.5 mg/ml of CuO NPs and recorded MIC (15 µg/ml). On the other hand, Azam et al. [6] reported that the MIC for S. aureus was 25±4 µg/ml with CuO NPs. Also, Radhakrishnan et al. [17] showed that inhibition zone of CuO for S. aureus was only 9 mm.

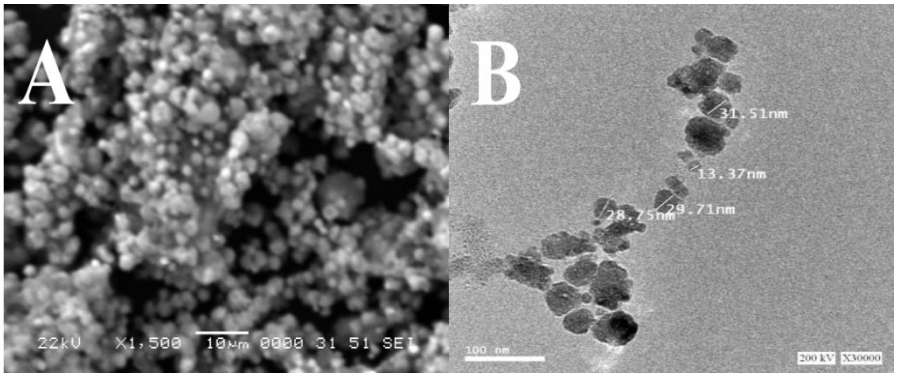

Fig (1): A: SEM and B: TEM of cupric oxide nanoparticles.

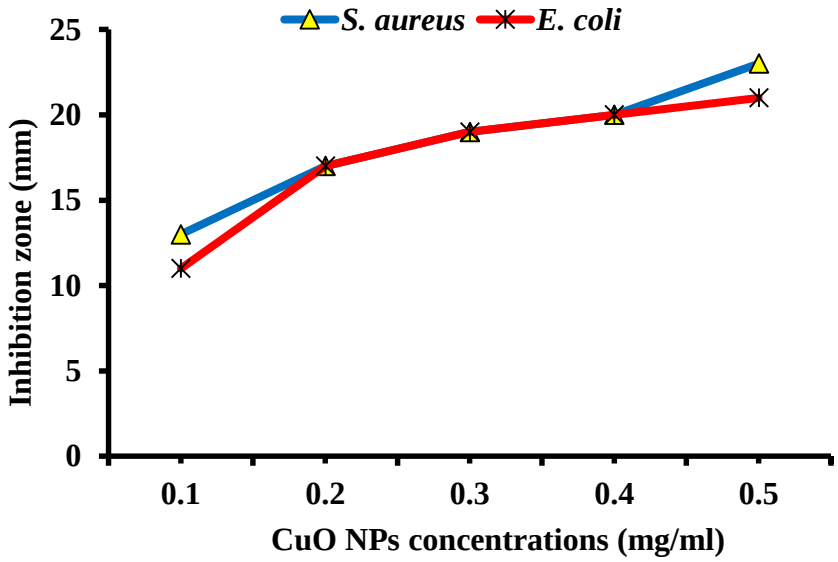

Fig (2): Inhibition zone of *E. coli* and *S. aureus*.

4. Conclusion

We have successfully synthesized cupric oxide nanoparticles by the sol–gel method with approximately average size 25.8 nm spherical in shape. CuO NPs showed excellent antimicrobial activity against gram-positive *S. aureus* and gram-negative *E. coli*. Consequently, CuO NPs have potential for uses as antibacterial agents against resistant bacterial species.

References

[1] F. Marabelli, G. B. Parravicini, and F. Salghetti-Drioli, Physical Review B, l52 (1995)1433–1436.

[2] A. El-Trass, H. Elshamy, I. El-Mehasseb, and M. El-Kemary,Applied Surface Science, 258(2012) 2997–3001.

[3] G. Filipic and U. Cvelbar, Nanotechnology, 23(2012).

[4] J. Li, F. Sun, K. Gu, T. Wu, W. Zhai, and W. Li, Applied Catalysis A, 406(2011) 51–58, 2011.

[5] L. Fernandez, E.B.M. Breidenstein, and R.E.W. Hancock, Drug Resistance Updates, 14 (2011) 1–21.

[6] A. Azam, A.S. Ahmed, M. Oves, M.S. Khan, A. Memic, Int. J.Nanomed. 7 (2012) 3527–3535.

[7] R. Etefagh, E. Azhir, N. Shahtahmasebi. Scientia Iranica F, 20 (2013) 1055–1058.

[8] P. Anandgaonker, G. Kulkarni, S. Gaikwad, A. Rajbhoj, Arabian Journal of Chemistry (2015) 1-8

[9] N. Tsao, T.Y. Luh, and C.K. Chou, J Antimicrob. Chemother, 49 (2002) 641–649.

[10] J.P. Ruparelia, A.K. Chatterjee, S.P. Duttagupta, and S. Mukherji, Acta. Biomater., 4(2008)707–716.

[11] N. Cioffi, N. Ditaranto, and L. Torsi, Anal. Bioanal. Chem., 381((2005) 607–616.

[12] T.J. Beveridge, R.G.E. and Murray, J. Bacteriol 141 (1980) 876–887.

[13] X. Liang, M. Sun, and L. Li, Dalton. Trans. 41(2012) 2804–2811

[14] J. Kim, H. Cho, S. Ryu, M. Choi, Arch. Biochem. Biophys. 382(2000) 72–80.

[15] S.J. Stohs, D. Bagchi, Free Radic. Biol. Med. 18 (1995) 321–336.

[16] I. Sondi, B.S. Sondi, J. Colloid Interface Sci. 275 (2004)177–182.

[17] A. Radhakrishnan, I.P. Rejan, and B. Beena, Int. J. Nano Dimens., 5(2014) 519-524.

[18] M. Ahmed, A.H Alhadlaq, M.A.M. Khan, P. Karuppiah, and N.A. Al-Dhabi, Journal of Nanomaterials, (2014) 1-4.