Synthesis and Antibacterial properties of Copper Oxide Nanoparticles: A potential for combating Bacterial Resistance

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Abstract:

Co-precipitation technique were acquired to synthesize nanosized CuO particles. A series of nanoparticles of copper oxide were manufactured by varying precursor concentrations and pH. Absorption peaks of CuO NPs revealed the absorption spectra in the UV- region which clearly indicates the formation of CuO NPs. The NPs have been analyzed by X-ray diffraction pattern, Fourier Transformer Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). The disc diffusion method has been used to examine the antimicrobial effects of CuO NPs towards gram-positive and gram-negative pathogenic bacteria.

Keywords: copper oxide nanoparticles, ROS, bacterial resistance, antibacterial activity.

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1. Introduction:

Both (Gram-positive and negative) bacterial strains are considered to present a major public health problem. According to the *National institute of communicable disease [NICD],* there is a constant spreading of disease and reinfection caused by certain bacteria like *E. coli, S. Typhi, and K. Pneumonia [1].* Over the years, antibiotics have been used to control infections, but the major threat is the emergence of antibiotic-resistant strains of bacteria which has prompted a search for novel substitutes. Inorganic metal oxide nonmaterial of specific morphologies with a large surface-to-volume ratio provides a better substitute for new antibacterial agents. For example, metal oxides like CuO, Ag_2O, ZnO MgO CaO [2] are most commonly used as an anti-bacterial agent. The nanoparticles release reactive oxygen species [ROS] into the cell that are harmful to bacteria [3]. Other studies have shown that because of their small size, nanoparticles may enter into the bacterial cell wall and damage the protein bodies and cell organelles which causes cell death [4]. The *United- state environmental protection agency* states that copper is a recognized antibacterial substance having enhanced antimicrobial properties toward pathogenic microorganisms [5]. Chemically stable and biocompatible CuO NPs fulfill all the demanding criteria for being an antibacterial agent. The antibacterial effect of CuO NPs depends on several factors like size, shape, and surfactant [6]. Copper oxide nanomaterials have gained a large interest of researchers due to their unique optical, electrical, and biological properties. These properties are widely used in the number of applications in different fields from the formation of sensors, storage devices, supercapacitors, and infrared filters to the health care sector [7]. Copper oxide NPs are cheaper and easy to synthesize. Various methods of synthesis are available to give the desired feature to NPs. Among them, the coprecipitation method is acquired to synthesize CuO NPs due to its simplicity, cost-effectiveness, and also to get desired featured NPs.

The present work aims to synthesize copper oxide nanoparticles with the simple, facile co-precipitated method. The motive of the research is to analyse the anti-bacterial action of CuO NPs against E. coli and S. aureus (G. negative and G.

positive respectively) with varying concentrations of CuO NPs used.

2. Experimental:

2.1 Material and methods:

Cupric Nitrate, Distilled Water, Sodium Hydroxide Pellets, Magnetic Beads, Magnetic Stirrer, Buffer solutions.

2.2 Synthesis of CuO NPs.

The following procedure had been followed to conduct the experiment:[8]. CuO NPs were manufactured by the coprecipitation approach utilizing Cu (No3)2.3H2O as a precursor. 50 ml distilled water was used to dissolve 0.5 M cupric nitrate and the solution was stirred on a magnetic stirrer for 15 minutes. 1M of NaOH solution was added to the given solution dropwise till the pH did not reach 10. The above solution was then stirred magnetically for 2:30hr till the blackish-brown precipitate of Cu(ii) O nanoparticles have been obtained. The precipitate was then washed with the help of deionized water and ethanol so that excess impurities drained out. The precipitate was left to be dried to get its powdered form. The black powdered CuO nanoparticles were annealed at 450^{o}C for an hour in order to achieve ***"stabilized CuO nanoparticles".***

2.3 Detection and characterization of CuO NPs.

Firstly, we confirm the formation of copper oxide nanoparticles with our naked eye observation by changing the color of the precursor from blue to black. The use of ***"X-ray diffractometry"*** has been done to analyze the structural configuration and crystal size of CuO nanoparticles. The measurements obtained with the help of XRD have been used for crystal analysis of CuO nanoparticles. XRD measurements were taken with a Rigakumini flex II diffractometer in the 2θ range of 20^{o} - 60^{o} using Kα radiation of the wavelength λ = 1.5406. The particle size, its distribution, and its morphology has been investigated with the help of ***"Scanning electron microscopy"*** SEM. The use of JEOLJSM-6510 (Japan) SEM has been used in the experiment. The information about optical studies has been gained with the help of ***"diffuse reflectance spectrum"*** which was recorded with Shimadzu UV-visible 3600 plus spectrophotometer. Thishas been furnished with a sphere accessory in the 200-500 nm range***. FTIR- spectroscopic technique*** is used to find out the functional group attached to the CuO NPs surface by identifying their stretching and bending frequencies. The spectra was captured with ABB MB 3000 IR spectrophotometer (Japan) within the range of 600-4000 cm$^{-1.}$ The concentration of heavy metal ions after absorption was analyzed by atomic absorption spectroscopy (AAS 301 thermo-scientific, wavelength range 190-900 nm).

2.4 Antibacterial study:

Escherichia coli and *Staphylococcus aureus* were utilised to test the anti-microbial potential of CuO nanoparticles using the disc diffusion method. The CuO nanoparticles have demonstrated an enhanced antimicrobial activity. Agar from Hi media was used to perform the experiment.

2.5 Preparation of culture plates

The antibacterial properties of CuO NPs were examined by the 'disc diffusion method'as per the guidelines of the NCCL. For media preparation, 11.5 g of Muller Hinton powder was dissolved in 300 ml of distilled water and then disinfected by autoclave at 121 °C for 15 min. The pH of the medium is often keep close to the physiological pH of 7.4. After cooling, about 25-30 ml of the freshly prepared medium was added uniformly to sterilized 100mm x 15mm Petri dishes .

2.6 Inoculation of cultural plates

Using a sterile loop, G. negative strain of bacteria E. coli ATCC8739 and the G.-positive strain of bacteria S. aureus ATCC 6538 were evenly distributed on nutrient agar plates. Next, sterile well borers were used to create wells 6 mm in diameter, and NPs of Cu O at concentrations of 25 gml^{-1} and 50 gml^{-1} were added. After the plates were incubated at 37 °C for 24 h to provide typical conditions for the growth of the bacteria, the average diameter of the (zone of inhibition) ZOI surrounding the wells was measured to the nearest 0.5 mm resolution with a ruler. The measurement of standard deviations and mean deviation for the CuO NPs with both bacteriological strains were presented based on three replicates.

3. Results and analysis:

SEM images shown in figure (1) reveal the morphology and shape of nanosized particles of CuO synthesized by the coprecipitation method. Investigation through 1µm magnification shows the nano- rod-shaped structure of CuO NPs. The XRD pattern of as-synthesized CuO NPs is shown by using ***Debye- Scherer- formula.***

$$D = (0.89\lambda/\beta \cos \theta)$$

By using the above formula size of the nanocrystal was found 8.6 nm. By graphical representation and particle size, it has been observed that the sample does not contain any impurities and is highly stable.

FTIR spectroscopy was analyzed by scanning in the range of 600-4000 cm^{-1}. Figure (2) reveals the FTIR spectra of powdered CuO nanoparticles. Peaks at 640 cm^{-1} confirmed the monoclinic phase of pure copper oxide [9]. Band obtained at 1643 cm^{-1} shows a c=o stretching bond. A strong broad band observed at 3556- 3310 cm^{-1} indicates the bending and stretching vibration of absorbed water molecules [10].

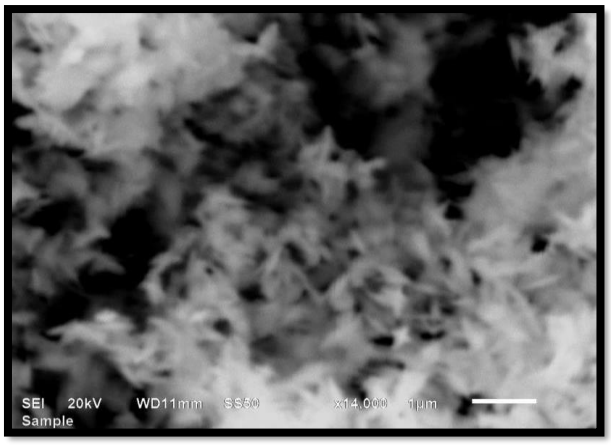

Figure.1 SEM images of CuO nanocrystals

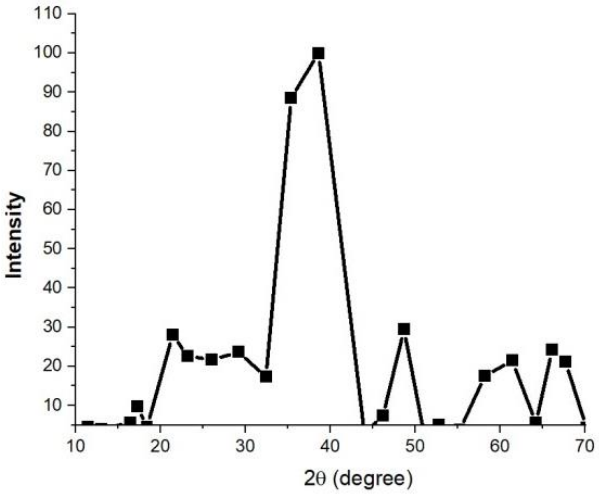

Figure .2 XRD pattern of CuO nanocrystals

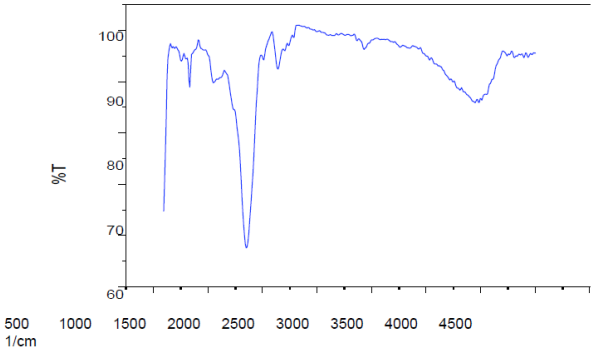

Figure 3. FTIR spectra of copper oxide

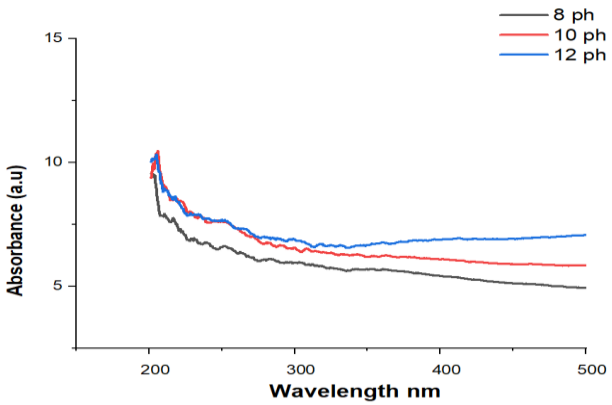

Figure.4 Absorbance spectra of CuO nanoparticles at different pH value.

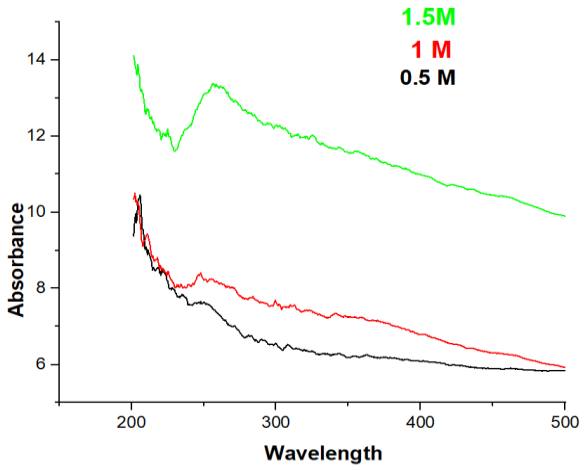

Figure 5. UV-vis spectra of CuO nanoparticles at varying concentration of precursors.

The optical characterization of the CuO nanoparticles has been done by absorption spectra at different pH and concentrations (fig. 4,5). The above figure 4 graphically represents the absorption spectra of the CuO nanoparticles in distinct pH. As the pH increased particle size decreased, and the surface-to-volume ratio increased with a decrease in particle size. The absorption spectra study of CuO nanoparticles shows a sharp peak at 220 nm for 1.5 M due to the direct transition of electrons. The absorptionband was at 230 for 1M and 240 nm for 0.5 M.

Antibacterial activity of CuO NPs:

(A) **(B)**

Figure6.Anti-bacterialpotential of CuO NPtowardE. coli (a) and against S. aureus (b).

Antibacterial potential:

By quantifying the zones of inhibition (ZoI) using the disk diffusion method, CuO nanoparticles' antibacterial activity was tested against both Gram-positive and Gram-negative bacterial strains (Table 1)

Table 1 In vitro Antimicrobial Assay Readings Against Gram-positive and Gram-negative bacterial strains

Name of Bacterial Strains		Zone of Inhibition (in mm) Results Expressed as Mean±SEM (SD)		Concentration (ug/ml)
Gram Positive bacteria	Staphylococcus Aureus	14 ± 1 16.67 ±1		25 50
Gram Negative bacteria	Escherichia coli	11.34 ±1 14.33 +_1		25 50

Figure 6 (a-b) clearly demonstrated that the zone of inhibition (ZOI) is positively correlated with the concentration of antibacterial agents means higher the concentration of CuO NPs, more inhibition in bacterial growth is seen. The diameter of ZOI clearly indicated that E. coli bacteria are more susceptible to CuO NPs than S. aureus.

It was observed that the cell membrane of "Gram-negative bacteria" is comparatively more permeable to NPs than "Gram-positive bacteria". The antibacterial action of CuO nanomaterial has been attributed to a wide range of mechanisms up to this point. One of these is the production of reactive oxygen species (ROS), which damages cells and eventually results in programmed cell death [11].Being small-sized CuO NPs can penetrate the bacterial cell and alter membrane shape that enhance cell permeability and affect transport by the release of Cu^{2+}which caused cell death. The release of Cu^{2+} ions results in the damage of the helical structure of DNA in bacterial cells as they bind with DNA molecules. The antibacterial activity of nanomaterials

depends on their shape and size also. In the present study large surface-to-volume ratio of nanoparticles plays an important role to inhibit the growth of microorganisms. The large particle size of NPs causes agglomeration and reduced antibacterial potential. Agglomeration can be reduced by adding suitable surfactants which prevent the aggregation of NPs. Surfactants' properties greatly affect the antimicrobial potential of CuO NPs. [12]. Results clearly indicate that copper oxide nanoparticles showed tremendous antibacterial potential against both strains of bacteria.

Conclusion:

It is concluded that NPs of copper oxide were synthesized by the co-precipitation method. CuO nanoparticles' crystalline structure was confirmed by XRD analysis, which also investigated their size in the 8.6 nm range. CuO NPs showed excellent antimicrobial against the bacteria E. coli ATCC 8739 and S. aureus ATCC 6538, with significant zones of inhibition measuring 14+_1 mm and 16.67+_1 mm for concentrations of 25µgml^{-1} and 50 µgml^{-1} of CuO NPS for E. coli and 11.34+_1 mm and 14.33+_1 mm for concentrations of 25 µ gml^{-1} and 50 µ gml^{-1}. This work recommends more research into the antibacterial response mechanism of CuO NPs in various bacterial species.

References:

1. National Institute of Communicable Disease (NICD), Antimicrobial Resistance Enterobacteriacea. 2019. Available online: **http://www.nicd.ac.za/diseases-a-z-index/enterobacteriaceae** (accessed on 18 February 2020
2. Ebrahimzadeh, M.A.; Mortazavi-Derazkola, S.; Zazouli, M.A. Eco-friendly green synthesis of novel magnetic Fe3O4/SiO2/ZnO-Pr6O1 nanocomposite for photocatalytic degradation of organic pollutants. *J. Rare Earths* 2020, 38, 13-20
3. R. Foldbjerg, et al., Toxicology Research', (2010, 2015, pp. 563–575, https:// doi.org/10.1039/c4tx00110a
4. 1. Azam, A.; Ahmed, A.S.; Oves, M.; Khan, M.S.; Memic, A. Size-dependent antimicrobial properties of CuO nanoparticles against Gram-positive and -negative bacterial strains. Int. J. Nanomed. 2012, 7, 3527–3535. [CrossRef]
5. . Meghana, S.; Kabra, P.; Chakraborty, S.; Padmavathy, N. Understanding the pathway of antibacterial activity of copper oxide nanoparticles. *RSC Adv.* **2015**, *5*, 12293–12299. [**Google Scholar**]
6. M. Sahooli, S. Sabbaghi, R. Saboori, Synthesis and Characterization of Mono Sized CuO Nanoparticles', Materials Letters, vol. 81, Elsevier B.V., 2012, pp. 169–172
7. 4 G. Borkow and J. Gabbay, Putting copper into action, copperimpregnated products with potent biocidal activities, FASEB J., 2004, 18, 1728–1730
8. K. Jhansi, S. Chandralingam, N. Manohar Reddy, Padma Suvarna, Ch. Ashok, K. Venkateswara Rao, CuO nanoparticles Synthesis and Characterisation for Humidity Sensor Application, journal of nanotechnology and material science, 3(1): 10-14.2016
9. . Nogueira, E.A.; Giroto, S.A.; Neto, B.S.A.; Ribeiro, C. CuO synthesized by solvothermal methods as a high capacity adsorbent for hexavalent chromium. Coooids Surf. A Phys. Eng.Asp., 498, 162–167,2016
10. Dhineshbabu, N.; Rajendran, V.; Nithyavathy, N.; Vetumperumal, R. Study of structural and optical properties of cupric oxide nanoparticle. Appl. Nanosci. 2016, 6, 933–939.
11. C. G. Rensing and G. Grass, Escherichia coli mechanisms of copper homeostasis in a changing environment, FEMS Microbiol. Rev., 2003, 27, 197–213
12. Nan-Fu Chen et.al. Investigation of the Characteristics and Antibacterial Activity of Polymer-Modified Copper Oxide Nanoparticles, s. Int. J. Mol. Sci. 2021, 22, 12913.