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Chemical synthesis of metal and metal oxide nanoparticles and their antibacterial activity against pathogenic bacteria

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The increasing emergence of bacterial resistance against antibiotics or antibacterial drugs has challenged the development of antibacterial agents like nanoparticles which have received increased attention due to their antibacterial activity against pathogenic bacteria. In this investigation, zinc oxide, copper oxide, and silver (ZnO, CuO, and Ag) nanoparticles were chemically synthesized by sol-gel, wet chemical precipitation, and chemical reduction procedures, respectively. Characterization of nanoparticles was performed using UV-Visible and Fourier-Transform Infrared (FTIR) spectroscopy. Antibacterial activity of nanoparticles was analyzed against *Staphylococcus aureus* and *Salmonella typhi*. UV-Visible spectroscopy of ZnO, CuO, and Ag nanoparticles showed peaks at 375, 225, and 400 nm, respectively, and indicated optical properties of the nanoparticles, thereby confirming their formation. FTIR analysis showed presence of Zn-O and Cu-O bonds in ZnO and CuO nanoparticles, respectively, and various functional groups in Ag NPs. Antibacterial studies conducted against *S. aureus* and *S. typhi* reflected no antibacterial activity by ZnO and CuO nanoparticles individually, while Ag NPs showed good antibacterial effects against both pathogenic bacteria. Additionally, the antibacterial activity of ciprofloxacin impregnated with Ag NPs against *S. aureus* showed a synergistic effect.

Keywords: characterization, metal, nanoparticles, synthesis

1. Introduction

Nanotechnology is considered as the new paradigm for the control of pathogenic bacteria and is increasingly being studied for clinical applications (1). Nanoparticles (NPs) are colloidal solid particles of size ranging 1–100 nm. NPs have demonstrated different antibacterial activities against gram-positive (GPB) and gram-negative bacteria (GNB). Metal-based NP's toxicity mechanism is non-specific, making bacterial resistance difficult (2). NPs thus are better alternatives for the conventional antibiotics and are considered as next-generation antibiotics (1). The discovery of antibiotics in the 20th century was the biggest achievement in medicinal history (2). With the development of new antibiotics against different disease-causing bacteria, their efficacy and cost-effectiveness gave them widespread indiscreet usage in the late 20th century, which resulted in the development of antibacterial resistance.

Pathogenic bacteria like *Staphylococcus aureus* (GPB) and *Salmonella typhi* (GNB) are known to majorly cause Staphylococcal food-borne disease and typhoid fever, respectively. *Staphylococcus aureus* is known to have developed resistance against various antibiotics such as penicillin, methicillin, ciprofloxacin, vancomycin, oxacillin, etc. (3), while *S. typhi* has shown resistance against chloramphenicol, co-trimoxazole, fluoroquinolones, ampicillin, etc. (4). The abiding emergence of bacterial resistance against antibiotics or the antibacterial drugs highlights the critical demand for antibacterial agents, which have strong and promising antibacterial activity.

Synthesis of NPs is an important aspect of nanotechnology. Various applications of such nano-structured materials are possible with their desired morphology, shape, size, chemical composition, and crystal structure. Metal-based NPs such as silver (Ag) and metal oxide NPs such as zinc oxide (ZnO), copper oxide (CuO), and iron oxide (Fe_3O_4) are widely being studied for their strong antibacterial activity (5). They have easy and economical chemical synthesis routes (6). Chemical methods allow control on reaction parameters so as to get desired particle size and shape, and support bulk production of NPs due to high production rate (7).

Sol-Gel method for ZnO NP synthesis is advantageous over other approaches, as it is easy and requires low temperature for the synthesis (8). Moreover, it can control the morphology and size of the NPs through efficient monitoring of various reaction parameters (9). Wet chemical precipitation method for CuO NP synthesis has several advantages in comparison to other chemical synthesis methods like its cost-effectiveness, low reaction temperature, and reaction simplicity (10). Furthermore, chemical reduction method for Ag NP synthesis is most widely used (11). The method is known to consist of three major components: a metal precursor, stabilizing/capping agents, and reducing agents (12). Stabilization of Ag NPs is important to prevent oxidation process and agglomeration. For stabilization, gluconic acid, chitosan, polyvinylpyrrolidone (PVP), polyethylene glycol, or polyacrylamide can be used (13). Ag NPs synthesized using PVP and $NaBH_4$ are spherical in shape (14).

There are majorly three antibacterial mechanisms of NPs: oxidative stress, dissolved ions, and non-oxidative mechanisms. Oxidative stress is induced by the generation of several types of free radicals such as hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), singlet oxygen (O_2), and the superoxide radical (O_2^-) (15, 16).

Different NPs generate separate ROS by reduction of oxygen molecules. For example, CuO NPs can generate different ROS, while ZnO NPs can generate H_2O_2 and ·OH but not O_2^- (17). ROS plays a vital role in increasing the gene expression levels of oxidative proteins, interaction between DNA and bacterial cells, attacking proteins and reducing periplasmic enzyme activity that are necessary to regulate morphological and physiological activities in bacterial cells (18). In the second mechanism, NPs releases metal ions which enter the cell membrane resulting in further interaction with several functional groups of nucleic acid and proteins to induce damages such as impeding enzyme activity, affecting physiological processes and altering cell structure (19). The third mechanism of action, i.e., non-oxidative mechanism involves multiple related factors such as direct contact, release of metal ions, and effect on pH. These have been observed in the toxicity by magnesium oxide NPs (20).

ZnO NPs have shown to be effective antibacterial agents against methicillin-resistant *S. aureus,* methicillin-resistant *S. epidermidis*, and methicillin-sensitive *S. aureus* strains. The antibacterial mechanism of ZnO NPs includes generation of ROS (H_2O_2), release of Zn^{2+} ions, and cell wall damage by binding, resulting in leakage of intracellular components, leading to bacterial cell disruption (19). CuO NPs have shown to be effective against various pathogenic bacteria such as *P. aeruginosa*, *E. coli*, *S. typhimurium*, *S. aureus*, etc. (21). The mechanism of CuO NPs antibacterial activity involves production of ROS and membrane disruption of bacteria. Furthermore, studies have shown that release of Ag ions is the main mechanism of antibacterial activity of Ag NPs, as they inhibit the catalytic activity of the enzymes by interacting with sulfhydryl groups and by impeding cell wall synthesis of GPB (20).

Small-sized Ag NPs interact with cell envelope and alter its lipid layer, thereby increasing membrane permeability; resulting in cell death (19). Many variables affect antibacterial effects of NPs like size, shape, zeta potential, doping modifications, environmental conditions, and roughness (22). Therefore, it is important to analyze the NPs by various characterization procedures such as Scanning Electron Microscopy, Transmission Electron Microscopy, Fourier-Transform Infrared (FTIR) spectroscopy, UV-Vis spectroscopy, and Dynamic Light Scattering. Furthermore, presence of surfactants (such as cetyltrimethylammonium bromide) on the surface of NPs affects their activities resulting in either promoting or impeding cell proliferation (6).

There have been multiple studies on the synthesis of ZnO, CuO, and Ag NPs and their antibacterial studies against *S. aureus* and *S. typhi* mentioned in literature. As per our information, there is no study reflecting ZnO and CuO NPs antibacterial activity generated with the specific Sol-Gel and Wet chemical precipitation methods, respectively. In this study we aimed to chemically synthesize ZnO, CuO, and Ag NPs; characterized them using UV-Vis and FTIR spectroscopy; investigated the antibacterial activity of the NPs alone and with ciprofloxacin.

2. Methodology

2.1. Synthesis of ZnO NPs

The sol gel technique was applied to prepare ZnO NPs. The experimental technique was performed with modifications to the method reported in the literature by Hasnidawani et al. (9). 2.0 g of zinc acetate dehydrate and 8.0 g NaOH were mixed in 30 and 10 ml of distilled water, respectively. The solution was stirred with magnetic stirrer for 5 min. NaOH solution was incorporated drop wise to the solution having zinc acetate with constant stirring for 15–20 min. Subsequently, 10 ml of ethanol was mixed drop wise to the above solution. The resultant solution was left overnight. The solution was centrifuged at 5000 rpm for 10 min.

The sample was washed by centrifuging at 8000 rpm for 10 min, dried overnight, and stored in an incubator at room temperature.

2.2. Synthesis of CuO NPs

The wet chemical precipitation technique was used to prepare CuO NPs. The experimental technique was performed with modifications to the method reported in the literature by Mayekar et al. (23). The amount of the chemicals or reagents used was calculated accordingly. 0.7 g of copper nitrate and 0.3 g of PVP were added to 25 ml of distilled water. The solution was stirred and heated on a magnetic stirrer till it reached at a temperature of 60°C. On reaching the desired temperature, 1 M NaOH was added drop wise to the solution to maintain 10.5 pH (pH was checked at regular intervals with the help of pH paper) and the solution was further stirred at 60°C for approximately 1 h. The solution was centrifuged at 5000 rpm for 10 min. The sample was dried at 50°C for 2 h in an incubator and was stored at room temperature in an incubator.

2.3. Synthesis of Ag NPs

The chemical reduction procedure was used to generate Ag NPs. The Experimental part was performed with modifications to the method reported in the literature by Vazquez-Munoz et al. (24). 30 mM PVP (0.01 g) and 30 mM $NaBH_4$ (0.02 g) were mixed in 5 ml and 10 ml distilled water, respectively. 30 ml of distilled water was heated at 70 ± 5°C and 15 mM (0.08 g) of silver nitrate (AgNO3) was added with constant vigorous stirring. PVP solution was mixed to the above solution and then immediately 3.6 ml of 30 mM NaBH4 solution was added drop wise, and the solution was stirred for 1 h with temperature maintained at 70 ± 5°C until the solution turned grayish brown. The solution was cooled at room temperature and further was centrifuged at 5000 rpm for 20 min. The sample was covered with aluminum foil and stored at 4°C.

2.4. Characterization of NPs

The chemically synthesized NPs were characterized by UV-Vis and FTIR spectroscopy.

2.5. UV-Vis spectroscopy

UV-Vis spectroscopy analysis was performed on all the chemically generated NPs. Quartz cuvette was used for UV range (100–400 nm) and glass cuvette was used for visible range (400–700 nm). The scanning data was obtained in the range from 200 to 500 nm.

2.6. FTIR spectroscopy

The infrared scan was performed for the synthesized NPs within the range of 4000–500 cm^{-1}.

2.7. Antibacterial activity using disk diffusion method

2.7.1. Preparation of nutrient agar media (NAM)

Nutrient agar medium was prepared by addition of 0.25 g of peptone, 0.1 g of meat extract, and 0.25 g of NaCl in 50 ml of distilled water. After mixing, pH was checked and maintained at 7.0 by adding a few drops of 0.1 M NaOH solution. 0.75 g of agar was added. The medium was autoclaved at 121°C for 15–20 min and was then cooled at room temperature. The prepared medium was poured in previously sterilized petri plates in laminar flow chamber; they were allowed to solidify and kept under UV light for 10–15 min.

2.7.2. Disk diffusion method

Staphylococcus aureus and *Salmonella typhi* were used to test for antibacterial activity for the synthesized NPs. The plates containing the medium were marked to divide each plate in four sections for disk diffusion. Each plate was spread with the culture broth using a cotton swab. The paper disks were impregnated with the prepared concentration of the NPs (1 mg/ml each) and were placed in the marked sections on the agar plate. In one of the marked areas, a disk with antibiotic ciprofloxacin (5 μg) was also placed. The plates were incubated at 37°C overnight. Zones of inhibition around paper disk due to antibacterial activity of NPs were observed and measurements were taken with a millimeter scale and were analyzed accordingly. After the observation of the antibacterial activity results, the disk-diffusion procedure was performed again to check antibacterial activity of combination of NPs that reflected activity against both pathogenic bacteria and ciprofloxacin. Ag NPs and ciprofloxacin were taken in 1:1 concentration. The plates were kept at 37°C overnight and the zones of inhibition were analyzed.

3. Results

3.1. Synthesis of NPs

3.1.1. ZnO NPs

For chemical synthesis of ZnO NPs, distilled water was utilized as a solvent medium, zinc acetate dehydrate chemical was applied as a precursor, and ethanol as a reagent. In this experiment a cascade of reactions takes place, which include complete hydrolysis of zinc acetate with the help

of NaOH in the presence of ethanol which should lead to the formation colloidal ZnO because of equilibrium obtained between hydrolysis and condensation reaction as referred to by (9). The overall chemical reaction is shown below:

$$(Zn\ (CH_3COO)2.\ 2H_2O) + 2NaOH \rightarrow ZnO + 2NaCH_3COO + H_2O.$$

3.1.2. CuO NPs

The wet chemical precipitation technique was used to synthesize CuO NPs which involved the reaction of copper nitrate and sodium hydroxide. In the synthesis of CuO NPs, PVP (a polymer) was used as a stabilizing agent for stabilization of the aggregation of the metal ions. This method is easy, convenient, and efficient in comparison to other chemical synthesis methods. In the given reaction, the copper hydroxide decomposes to CuO and water on heating (23).

$$Cu\ (NO_3)_2 + 2NaOH \rightarrow Cu(OH)_2 + 2NaNO_3.$$
$$Cu\ (OH)_2 \rightarrow CuO + H_2O.$$

3.1.3. Ag NPs

In the chemical reduction method for Ag NPs generation, $AgNO_3$ was used as metal precursor, PVP was used as the coating/stabilizing agent, while $NaBH_4$ was used as the reducing agent (24). The role of PVP is the stabilization of the growth of NPs and protection of NPs from agglomeration and sedimentation by interacting with the surface of the NPs.

3.2. Visual observation

ZnO, CuO, and Ag NPs formed were white, brown, and grayish-brown, respectively, as shown in **Figure 1**.

3.3. UV–Vis spectroscopy

This is a technique for characterization of NPs, used to examine their stability. It is fast, sensitive, simple, and selective for different NPs. The absorption in UV-Vis is strongly dependent on the chemical environment, dielectric medium, and the particle size (8). The UV–Vis spectrum of absorbance vs. wavelength of chemically synthesized NPs showed absorption peaks at wavelengths of 375, 225 nm, and at 400 nm for ZnO, CuO, and Ag NPs, respectively as shown in **Figure 2**.

ZnO NPs showed an absorption peak at a wavelength of 375 nm (**Figure 2**), which can be attributed to intrinsic bandgap of ZnO absorption. Related results of absorption bands were observed in previous work (6), in which the range of absorption band was 355 to 380 nm. Further, CuO NPs showed an absorption peak at a wavelength of 225 nm (**Figure 2**), which can be due to interband transition (electron transition from a valence band to a conduction band) of core electrons of Cu metal. Related results of absorption peaks were observed in previous studies (6). Furthermore, Ag NPs showed an absorption peak at a wavelength of 400 nm (**Figure 2**). Similar results of absorption bands have been reported in literature (24). This may be because of surface plasmon resonance phenomenon of electrons in conduction band electrons of silver.

3.4. FTIR spectroscopy

FTIR spectra provide information about the nature of the NPs which is important for verifying the purity of the oxide. The infrared spectra of ZnO NPs are shown in **Figure 3**. According to Asamoah et al. (6), the peak reported at 855 cm^{-1} can be a marker of Zn–O vibration. Additional peaks correspond to the carboxylate impurities in the materials. Infrared spectra of CuO NPs are shown in **Figure 3**. The peak at 597 cm^{-1} could be related with stretching vibration of Cu–O bonds (6). The band at 1363 cm^{-1} could be ascribed to the C = O bond. The infrared spectra of PVP-Ag NPs are shown in **Figure 3**. Band absorption at 2926 cm^{-1} denotes the C–H stretching vibration with the alkene group. The bands obtained at 1654 and 1667 cm^{-1} show carbonyl C = O stretching bonds. The peak at 1155 cm^{-1} could be of C–O stretching. The peak at 1118 cm^{-1} represents the functional unit C–N, indicating the

FIGURE 1 | Synthesized **(A)** ZnO NPs; **(B)** CuO NPs; and **(C)** Ag NPs.

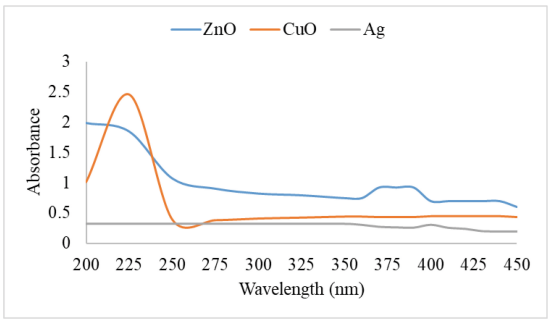

FIGURE 2 | UV-Vis profile showing absorption peaks of ZnO NPs, CuO NPs, and Ag NPs.

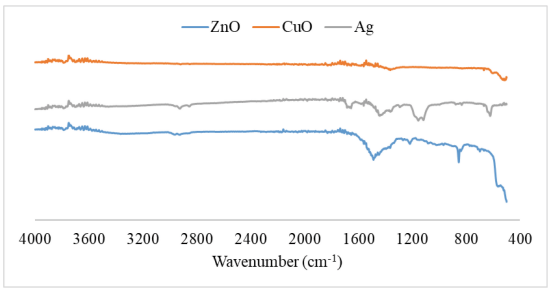

FIGURE 3 | Fourier-transform infrared (FTIR) spectra showing functional groups of ZnO NPs, CuO NPs, and Ag NPs.

interaction of the N or O atoms of the PVP molecules with the surface of Ag NPs by chemical absorption.

3.5. Antibacterial activity of synthesized NPS

ZnO and CuO NPs independently did not show any antibacterial activity against *S. aureus* (GPB) and *S. typhi* (GNB) as shown in **Table 1** and **Figure 4**. Ag NPs alone showed antibacterial activity against *S. aureus* and *S. typhi*. Ciprofloxacin too showed antibacterial activity as reflected in **Table 1** and **Figure 4**.

The combination of ciprofloxacin and Ag NPs showed a synergistic effect against *S. aureus* as compared to ciprofloxacin only. Whereas the combination of ciprofloxacin and Ag NPs was like antibacterial activity of Ag NPs alone against *S. typhi* as shown in **Table 1** and **Figure 5**. A comparative assessment of antibacterial activity of samples is given in **Figure 5**.

4. Discussion

Earlier study has demonstrated the antibacterial activity of ZnO NP against *S. aureus* and *S. typhi*. Dadi et al., (25) too have shown the antibacterial effect of thin film coating of ZnO NPs against *S. aureus*. Mohamed et al., (26) have shown the antibacterial activity of chemically synthesized CuO NP

TABLE 1 | Antibacterial activity shown against pathogenic bacteria.

Sample	Bacteria	
	Zone of inhibition for *S. aureus*	Zone of inhibition for *S. typhi*
ZnO NPs	No	No
CuO NPs	No	No
Ag NPs	10 ± 0.5 mm	15 mm
CIPROFLOXACIN	12 ± 0.5 mm	10 ± 0.5 mm
AgNPs + CIPROFLOXACIN	16 ± 0.5 mm	15 mm

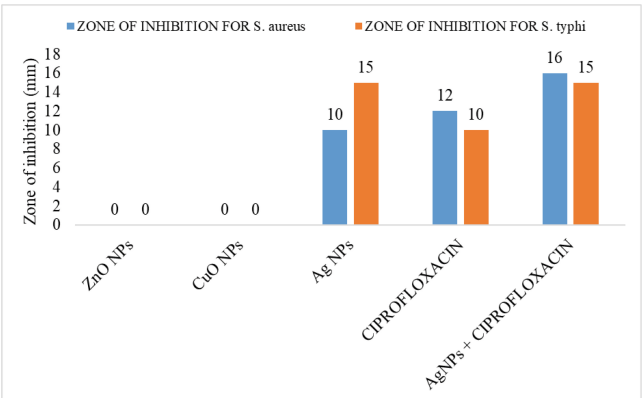

FIGURE 4 | A comparative assessment of antibacterial activity of samples.

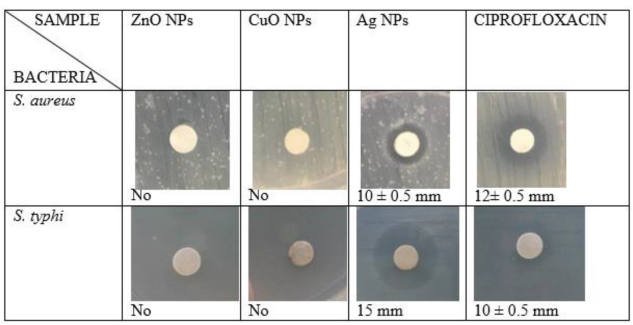

FIGURE 5 | Antibacterial activity of ZnO NPs, CuO NPs, Ag NPs (1 mg/ml each), and ciprofloxacin (5 μ g).

against *S. aureus*; and against *S. typhi*. We did not find any antibacterial activity of ZnO and CuO NPs against these pathogenic bacteria in our study, for two reasons. Firstly, we could not confirm the morphology of the NPs synthesized by our method, which plays a significant function in the bactericidal activity of NPs. Secondly, we did not vary the concentration of the NPs to assess the exact concentration at which the bactericidal activity is maximum (**Figure 6**). Inhibition of growth of pathogenic bacteria by Ag NP has been demonstrated by assorted studies (19, 21). A higher synergistic effect of Ag NPs with ciprofloxacin on GPB than on GNB has been shown.

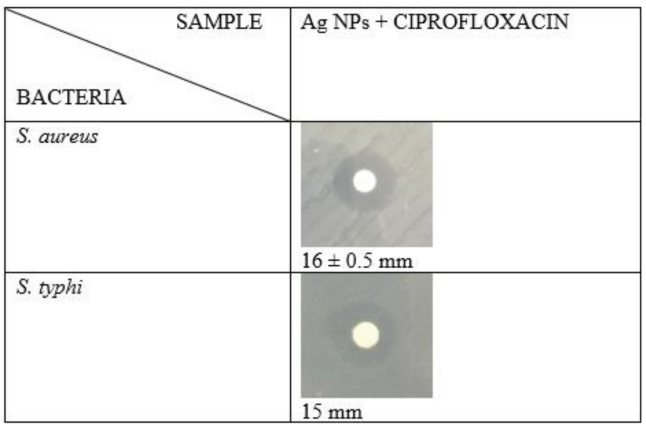

FIGURE 6 | Antibacterial activity of Ag NPs (1 mg/ml) impregnated with ciprofloxacin (5 μ g).

5. Conclusion

In this study, easy and inexpensive chemical methods were used to synthesize metal and metal oxide NPs for characterization and antibacterial activity. ZnO, CuO, and Ag NPs were successfully generated by sol-gel, wet chemical precipitation, and chemical reduction method, respectively. NPs were characterized by using UV-Vis and FTIR spectroscopy. The ZnO, CuO, and Ag NPs reflected absorption peaks at 375, 225, and 400 nm, respectively, indicating optical properties of the NPs. The supporting data from earlier literature confirm formation of ZnO, CuO, and Ag NPs. FTIR analysis showed presence of Zn–O and Cu–O bonds in ZnO and CuO NPs, respectively, and of various functional groups in Ag NPs. The antibacterial studies conducted in *S. aureus* and *S. typhi* showed that only Ag NPs presented a good antibacterial effect against both pathogenic bacteria. Additionally, the antibacterial activity of ciprofloxacin impregnated with Ag NPs against *S. aureus* showed a synergistic effect.

Author contributions

VG and RK contributed to conceptualization, data collection, and manuscript writing. SS contributed to proofreading of manuscript and refined the data presentation. All authors contributed to the article and approved the submitted version.

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