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Synthesis, Characterization and Antibacterial Assessment of Ciprofloxacin Conjugated Silver Nanoparticles

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Abstract

Antibiotic resistance remains a major global health concern, limiting the effectiveness of conventional antibiotic therapies. Recent advancement in nanomedicine, offer a promising solution using nano conjugated antibiotics to tackle AMR problem. In this study, silver nanoparticles and ciprofloxacin loaded silver nanoparticles were synthesized by chemical reduction process and further characterized by scanning electron microscope, zeta potential analysis, and dynamic light scattering technique. The combined effect of CIP-AgNPs demonstrated enhanced antimicrobial efficacy, in comparison with pure AgNPs and ciprofloxacin, thus highlighting their ability to combat antimicrobial resistance.

Keywords: Antimicrobial resistance, Silver nanoparticles, Ciprofloxacin, Antibacterial activity, Conjugate synthesis

1. Introduction

In recent decades, the increasing resistance of microorganisms to antimicrobials has resulted in serious health issues, and it is still a major concern worldwide regarding the use of the majority of antibiotics (Banin Ehud, et al., 2017). Antimicrobials are the chemicals, drugs or the substances used for killing or suppressing the growth and multiplication of microorganisms such as bacteria, fungi, viruses, and parasites. It is a broader term that includes antibiotics, antifungals, antivirals, and antiparasitic (Patrick Di Martino, 2022). Discovery of antibiotic is one of the greatest milestones in modern medicines (Julian Davies, et al., 2010). However, overuse and misuse of antimicrobial drugs over the time had led to the development of antimicrobial resistance. Antimicrobial resistance is an evolutionary process that occurs when microbes become resistant to the given drugs or it has developed the ability to survive in the presence of drugs designed to kill or inhibit them. As a result, the drugs become ineffective and infections become difficult to treat (World Health organization: WHO, 2023). Antibiotic resistance is most common than any other class of the antimicrobials. The four major mechanisms exhibited by bacterial cells to protect itself from antibiotics and contributing to the development of antibiotic resistance are: a) inactivation of antibiotic by enzymatic modification or chemical alteration of antibiotic molecule, b) altering the cellular permeability of outer membrane to avoid the entry of antibiotics into the cells, c) increasing efflux pump to pump out antibiotics from cell interior, d) modifying antibacterial target region so that antibiotic cannot

bind or bind poorly to the modified target (Syeda Fatima Nadeema, et al., 2020). Human activities like widespread use of antibiotics in humans, agriculture, livestock, and aquaculture, inadequate and overuse of antimicrobial drugs, inappropriate prescription of antibiotics, poor hospital environment are responsible for the development of antibiotic resistance (Manar Ali Abushaheen, et al., 2020). Serval clinically significant microorganisms such *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli,* and *Klebsiella pneumoniae* have developed resistance to multiple antibiotics (Giuseppe Mancuso, et al., 2021).

Numerous strategies have been explored to overcome the challenge of antibiotic resistance, with a focus on developing safer and more effective treatment. One such approach involves use of nanomedicine, where antibiotics are conjugated with nanoparticles, thereby enhancing their antimicrobial activity (Mohamed J Saadh, 2022). Nanoparticles are defined as particles ranging 1 to 100nm in size. They exhibit unique physiochemical properties due to their large surface area and nanoscale size (Tomy Muringayii Joseph, et al., 2023). Among these, metal nanoparticles have gained particular interest because of their surface properties, electrical structure, and antibacterial properties (Shreya Modi, et al., 2022). Metal nanoparticles exert their bactericidal action through two proposed routes: (a) surface dissolution and release of metal ions from the nanoparticle surface that disrupts bacterial cellular function, and (b) production of reactive oxygen species (ROS), which induces oxidative damage and cell death (Domenico Franco, et al., 2022).

Silver nanoparticles are among the most prominent and widely used nanomaterial for antimicrobial applications due to their broad-spectrum antimicrobial activity against Gram- positive and Gram-negative bacteria. They are known for their chemical stability, strong binding affinity for therapeutic agents, and relatively low toxicity. Silver nanoparticles are synthesized by chemical, physical, and biological methods. Silver is positively charged metal ion and react with negatively charged components of microbial cells such as phosphorous and sulphur containing groups found in protein, cell membrane, and nucleic acids, thus inhibiting DNA replication process and protein synthesis (Pragati Rajendra More, et al., 2023). In recent years, nanomaterials have been investigated as “antibiotic nanocarriers”, vehicles that transport antibiotics directly to targeted bacterial site. Encapsulating antibiotics within nanomaterials enhances their stability, improves pharmacokinetics, and increase bioavailability. Furthermore, combination of antibiotics with nanoparticles has been shown to reduce drug toxicity and required dosage, thereby allowing maximum tolerated dose with lower antibiotic concentrations (Nayanika Chakraborty, et al., 2022). Notably, silver nanoparticles have demonstrated synergistic interactions with many antibiotics such as amoxicillin, vancomycin, ciprofloxacin, and rifamycin. For example, citrate capped silver nanoparticle conjugated with vancomycin have been studied for their antibacterial activity against Gram-negative (*E. coli*) and Gram-positive (*S.aureus*) bacteria. The nanoconjugate exhibited remarkable enhanced antibacterial efficacy compared to the pure vancomycin, indicating a synergistic effect between antibiotic and the silver nanoparticles (Amritpal Kaur, et al., 2019).

Ciprofloxacin belongs to fluoroquinolone antibiotic family, used for treating serval bacterial infections including upper and lower respiratory tract, and some skin, bone, soft tissue infections. It also plays a significant role in the management of community acquired pneumonia. The World Health Organization (WHO) has recommended ciprofloxacin as second line agent for treatment of multidrug resistant tuberculosis. Chemically, ciprofloxacin is known as One-cyclopropyl-6-fluoro-4-oxo-7-(piperazine-1-yl)-1, 4-dihydroquinoline-3-carboxylic acid (Gui-Fu Zhang, et al., 2018). It has a molecular weight is 331.34 g/mol and its chemical formula is $C_{17}H_{18}FN_3O_3$ (Gui-Fu Zhang, et al., 2018). The presence of fluorine atom at position 6 and a piperazinyl group at position 7 are important for its broad-spectrum antibacterial

activity. Ciprofloxacin is known to inhibit bacterial growth by targeting DNA gyrase, an enzyme that belongs to the DNA topoisomerases family. DNA gyrase is essential for supercoiling of bacterial DNA, a process critical during DNA replication (Marc LeBel, 1988).

Despite its effectiveness, the clinical utility of ciprofloxacin has been compromised by the emergence of resistant bacterial strains, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. Multiple mechanisms have been identified that confers resistance to ciprofloxacin. These include mutation in DNA gyrase or DNA topoisomerase IV, reduced drug accumulation due to efflux pump or antibiotic degrading enzymes, and plasmid mediated quinolone resistance (Aref Shariati, et al., 2022). In Gram- negative bacteria like *E.coli,* resistance is mainly due to point mutation in the target enzymes DNA gyrase and DNA topoisomerase. These mutations reduce the binding affinity of ciprofloxacin, significantly lowering its bactericidal efficacy (Sandhya Bansal, et al., 2010). In clinical isolates of *S. aureus*, resistance to ciprofloxacin is caused by both mutations in topoisomerases and overexpression of efflux pump NorA, NorB, and NorC that reduces effect of ciprofloxacin (David C. Hopper, et al., 2015). The conjugation of ciprofloxacin with silver nanoparticles demonstrated increased antibacterial potency. This conjugate not only improves the stability of ciprofloxacin but also support its gradual release at the infection site (Ibtissam Laib, et al., 2025).

This study aims to synthesize ciprofloxacin conjugated silver nanoparticles via a chemical reduction process using silver nitrate as a precursor salt and trisodium citrate as a capping agent. The synthesized nanoparticles were characterized to determine their physiochemical properties. The antibacterial activity of conjugate was evaluated against gram positive as well as gram negative bacteria, demonstrating their use as an effective antimicrobial agent for combating antibiotic resistance among the pathogens.

2. Materials and Methods

2.1 Materials

Analytical grade chemicals such as silver nitrate ($AgNO_3$, MW:169.87 g moL^{-1}), trisodium citrate di-hydrate ($Na_3C_6H_5O_7$, MW:294.10 g moL^{-1}), and bacteriological media Muller Hilton agar were used in this experiment. All chemicals were obtained from Sigma Aldrich. The drug ciprofloxacin ($C_{17}H_{18}FN_3O_3$, MW: 367.9 g moL^{-1}) was purchased from local pharmacy. The bacterial strains used in this study include gram positive bacteria *S.aureus* and gram-negative bacteria *E.coli.*

2.2 Preparation of silver nanoparticles (AgNPs)

The silver colloid was prepared by using chemical reduction method. Briefly, 1M $AgNO_3$ solution was prepared in 100ml distilled water and heated to boil with continuous stirring for 10 min. To this solution, 10 ml of 1% trisodium citrate was added gradually. During this process, solutions were mixed vigorously for 20 min and heated on a heating plate, until its color changed from transparent to pale yellow and then dark brown, indicating the reduction of silver ions and formation of silver nanoparticles (AgNPs) (Rashid Mamun, et al., 2013). Later, the solution was cooled to room temperature. The obtained silver nanoparticle solution was divided into two parts, one part of the solution was centrifuged at 10,000 rpm, washed serval times with distilled water and dried at 85℃ for 4 hours. The dried AgNPs powder was further used for analysis. The other part of the solution was used for the preparation of ciprofloxacin conjugate silver nanoparticles.

2.3 Preparation of ciprofloxacin loaded silver nanoparticle (CIP-AgNPs)

An aqueous solution of 1M ciprofloxacin (CIP) in 10ml distilled water was prepared. Ciprofloxacin loaded silver nanoparticle was prepared by adding above solution of ciprofloxacin drop by drop to pre-synthesized silver nanoparticle solution with continuous stirring for 30 min at room temperature. The resulted conjugate CIP- AgNPs solution was stored at -4℃ to ensure its stability.

2.4 Characterization of AgNPs and CIP- AgNPs

To assess physiochemical properties of synthesized silver nanoparticle and ciprofloxacin conjugate silver nanoparticle, characterization techniques were performed. All the samples use for the analysis were in dried powder form. The morphology of nanoparticles was examined by scanning electron microscope (SEM FEI QUANTA 250 FEG). The particle size and zeta potential were measured using dynamic light scattering instrument (LITESIZER DLS). The optical properties were characterized by UV Visible spectrophotometer (V-730 UV-VISIBLE SPECTROPHOTOMETER) in the range of 200-800nm.

2.5 Antibacterial activity of synthesized AgNPs and CIP- AgNPs

The antibacterial activity of AgNPs and CIP-AgNPs was performed by disc diffusion technique. The antibacterial activity was tested against gram -positive bacteria *S.aureus* and a gram- negative bacteria *E.coli.* In this experiment, fresh overnight bacterial culture of each strain was used. The bacterial cultures were swabbed uniformly onto Muller Hinton agar plates under aseptic condition. Sterile disc with different concentrations (50mg/ml to 200 mg/ml) were impregnated onto the agar plates. Pure antibiotic ciprofloxacin and AgNPs were placed as control. The plates were incubated at 37℃ for 24 hr. After incubation period, zone of inhibition around each disc were observed and measured to determine the antibacterial activity of nanoparticles (J. Santoshkumar et al., 2017).

3. Result and discussion

3.1 Synthesis of silver nanoparticles and ciprofloxacin conjugated silver nanoparticles

In this study, silver nitrate was used as a precursor material while tri sodium citrate act as a reducing, capping and stabilizing agent. Here, citrate reduced metal ion Ag^{+} to neutral Ag^{0} atoms. The produced Ag atoms act as nucleation center and catalysed the reduction of remaining metal ions present in the bulk solution. The shape and size of the nanoparticle depends on the process parameters like pH, temperature, stirring speed, and concentration of the chemicals. The mechanism of the reaction is as follows:

$$4Ag^{+} + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2\uparrow$$ (G.Suriati, et al., 2014)

The colour of the AgNP colloid produced depends on the concentration of $AgNO_3$ in the solution. The colour change of silver nanoparticles from colourless to pale yellow and then dark brown is due to the change in surface plasmon resonance of silver nanoparticles during its formation. Addition of ciprofloxacin to synthesized AgNP solution resulted in deep red brown colour solution indicating formation of CIP-AgNPs (Fig.1) (Sutapa.Roy, et al., 2020).

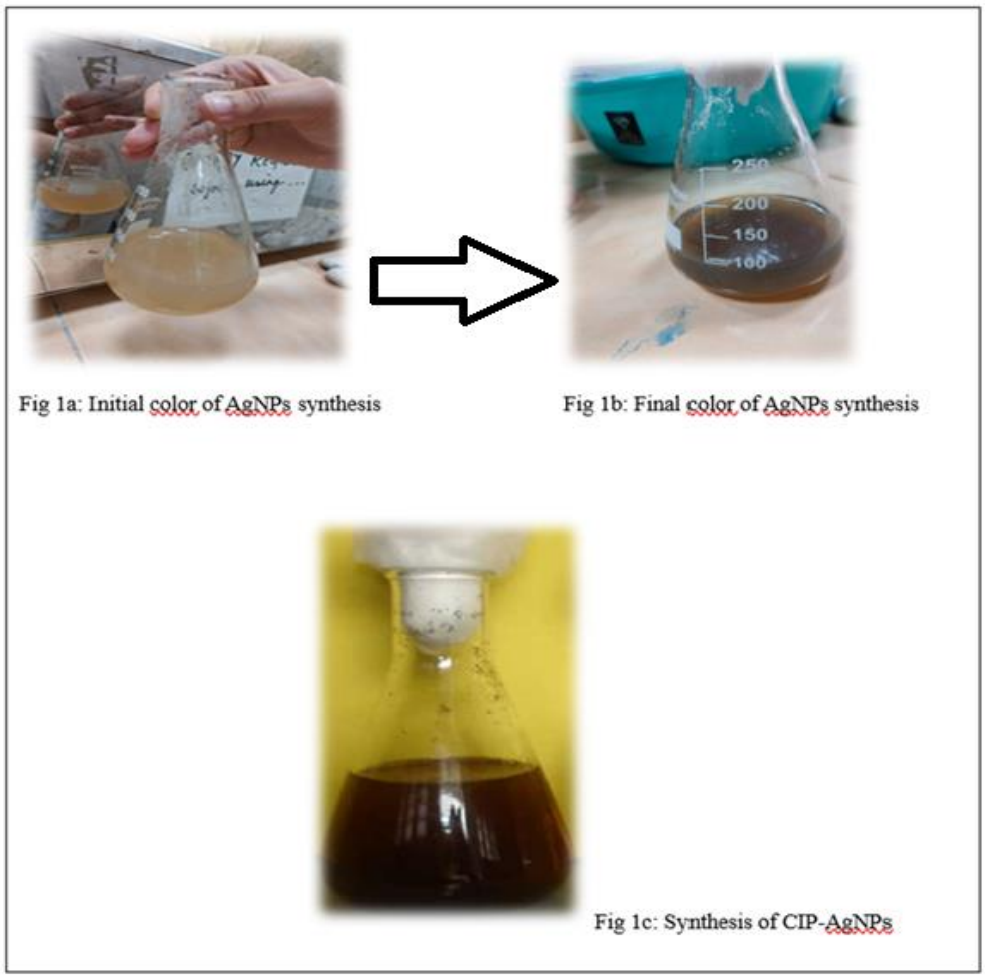

Fig 1a: Initial color of AgNPs synthesis

Fig 1b: Final color of AgNPs synthesis

Fig 1c: Synthesis of CIP-AgNPs

Figure 1: Synthesis of silver nanoparticles and ciprofloxacin conjugate silver nanoparticles

3.2 UV spectrophotometer analysis

The synthesized AgNPs and CIP-AgNPs were subjected to UV spectrophotometer analysis in the wavelength range of 200-800 nm. As illustrated in Fig.2a, the AgNPs exhibited a prominent absorbance peak at 413 nm, which is a characteristic of silver nanoparticles. This peak is attributed to surface plasmon resonance (SPR) phenomenon, arising from collective oscillation of electrons. The absorption spectrum consists of single sharp SPR band, indicating the presence of spherical silver nanoparticles.

The absorbance peak for CIP AgNPs is observed at 450 nm (Fig. 2b). The red shift is observed suggesting conjugation of ciprofloxacin with silver nanoparticles. The difference in peak positions between AgNPs and CIP-AgNPs also implies an increase in particle size or aggregation due to the presence of the drug molecules on the surface of nanoparticle. This observation is consistent with previous studies where surface functionalization or ligand binding has led to a similar bathochromic shift in the UV–Visible spectra of metal nanoparticles (Duaa Ibraheem, et al., 2022).

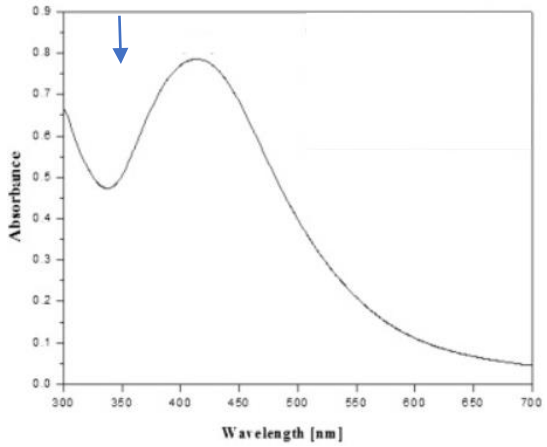

Figure 2a: UV spectrum of AgNPs

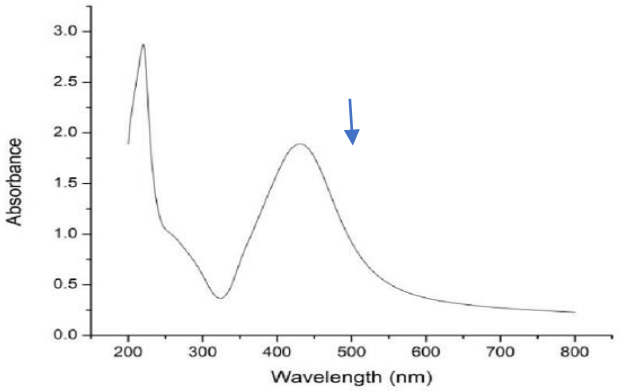

Figure 2b: UV spectrum of CIP-AgNPs

3.3 SEM Analysis

Scanning electron microscope is used to determine surface morphology, size and elemental composition of nanoparticles. It uses focused beam of electrons to create high resolution image. The (Fig 3a.) represents a SEM image of the silver nanoparticles, indicating spherical morphology and particle agglomeration. The energy dispersive X ray (EDX) spectrum (Fig 3b.) associated with SEM reveals silver (Ag), carbon (C), and oxygen (O), these components were part of the chemicals used for synthesis of Ag-NPs. In comparison, the SEM image of CIP-AgNPs (Fig 3c.) exhibits irregular cluster form, indicating structural changes after conjugation.

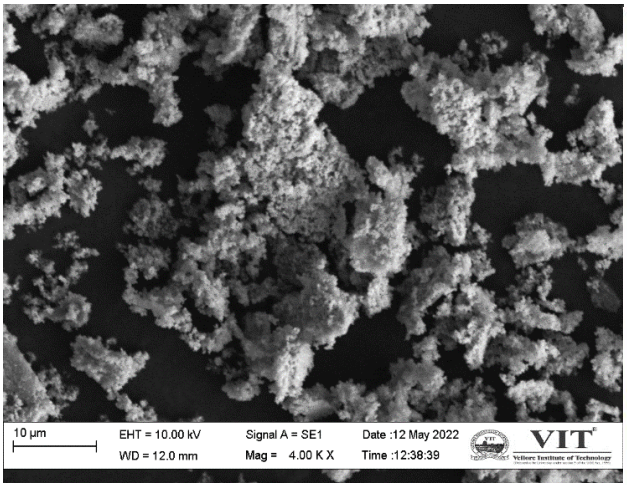

Figure 3a: SEM image of AgNPs

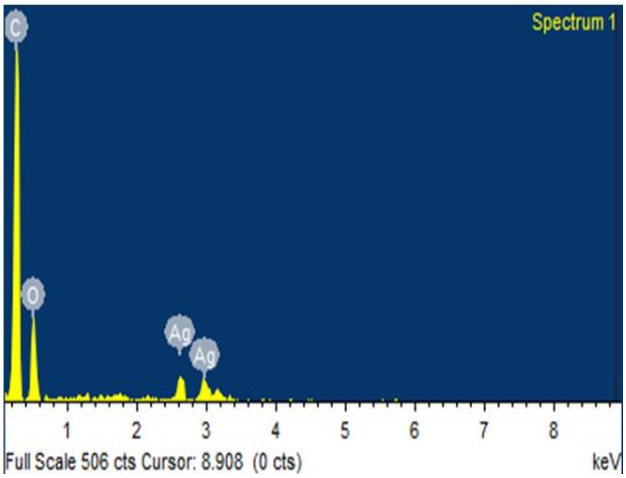

Figure 3b: EDAX spectrum of AgNPs

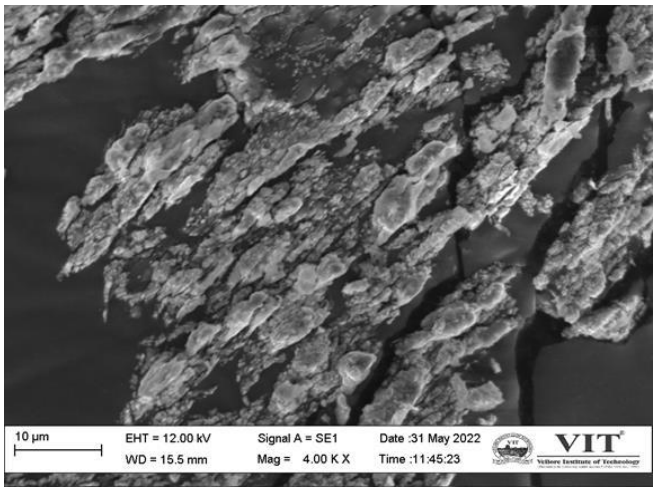

Figure 3c: SEM image of CIP-AgNPs

3.4 Size and zeta potential

Zeta potential represents the surface charge and stability of prepared nanoparticles in colloidal solution. It measures the magnitude of electric repulsion/ attraction between particles. Nanoparticles are in a stable state when zeta potential value is either greater than +30 mV or less than -30 mV. The zeta potential value of synthesized silver nanoparticle and ciprofloxacin conjugated silver nanoparticle are found to be -44.1 mV and – 53.7 mV (Fig. 4a &4b) respectively. The negative zeta potential may result from adsorption of citrate ions on surface of nanoparticles. The zeta potential values indicate that the nanoparticles possess good stability, and this stability is attributed to electrostatic repulsion between the particles, which prevents aggregation and ensures long term stability (Gospodinka Gicheva, et al., 2013).

The average particle size of synthesized silver nanoparticles was found to be 23.38 nm (Fig. 4c), as measured by dynamic scattering light technique. A secondary peak was also observed, following conjugation with ciprofloxacin, indicating increase in particle size. Increase in particle size supports the physical interaction between silver nanoparticles and ciprofloxacin.

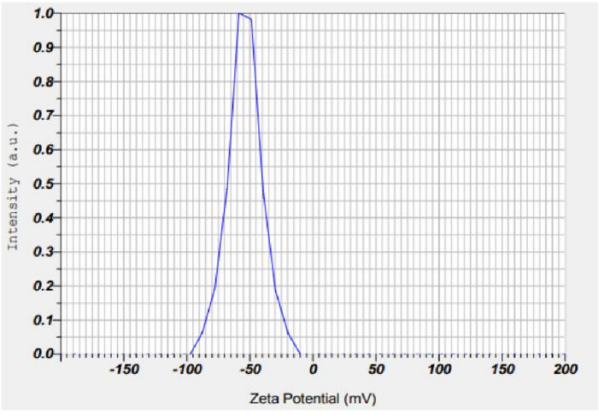

Figure 4a: Zeta potential AgNPs

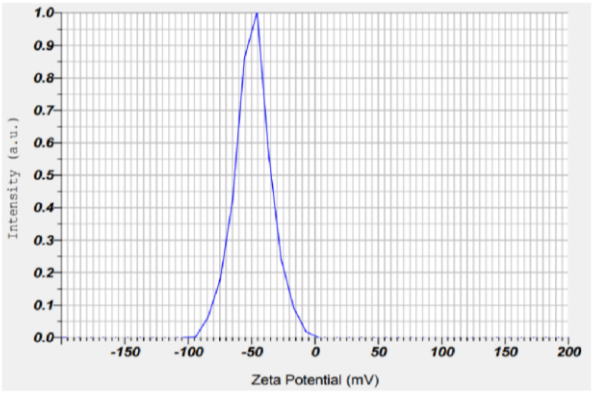

Figure 4b: Zeta potential CIP-AgNPs

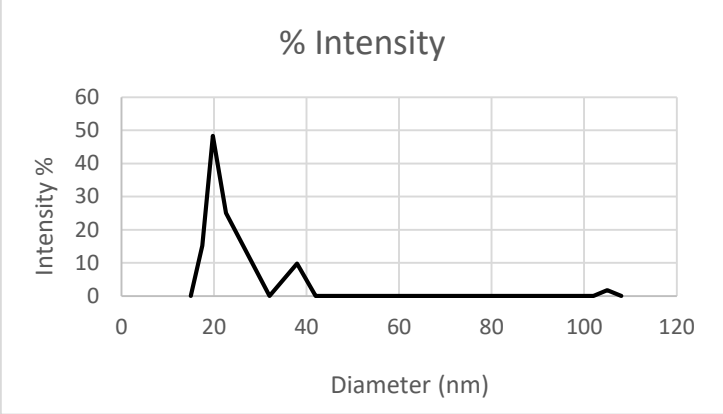

Figure 4c: Particle size distribution

3.5 Antibacterial activity

The synergistic antibacterial activity of conjugate CIP- AgNPs was studied and compared with that of pure silver nanoparticles and crude ciprofloxacin. The antibacterial activity of synthesized nanoparticles was assessed against pathogenic microbes *Escherichia coli* and *Staphylococcus aureus* as illustrated in Fig.5a &5b. The CIP-AgNPs demonstrated a concentration dependent antibacterial activity across different concentration ranging from 50mg/ml -200 mg/ml. Notably, the zone of inhibition increases with increasing concentration of the conjugate as show in Table 1.

Pure AgNPs demonstrated remarkable antibacterial activity with inhibition zones of 24mm for *E.coli* and 23mm for *S.aureus,* proving its bactericidal action. However, ciprofloxacin alone exhibited lower antibacterial activity with inhibition zones of 12mm and 9mm against *E.coli,* and *S.aureus* respectively. This reduced antibacterial efficacy is likely due to presence of bacterial resistance mechanism, limiting the effectiveness of antibiotic.

In contrast, the CIP-AgNP conjugate showed significantly larger zones of inhibition compared to either component alone, suggesting a synergistic interaction between ciprofloxacin and AgNPs. This interaction may improve permeability and cellular uptake of the antibiotic through bacterial cell wall. This nano sized carrier can be used in drug delivery system. Another advantage of such nanoconjugates is the potential reduction in the required antibiotic dosage thereby minimizing adverse effects. Additionally, the use of such nano conjugate antibiotics could help mitigate the growing issue of antimicrobial resistance (AMR), as the formulation can more effectively penetrate the bacterial cell walls. Nanoparticles also protect the antibiotics from enzymatic degradation and facilitate controlled drug release at targeted site.

Sample	**Zone of inhibition diameter (mm)**	
	E.coli (P1)	*S.aureus* (P2)
CIP-AgNPs		
50mg/ml	24mm	21mm
100mg/ml	27 mm	24mm
150mg/ml	30 mm	28mm

200mg/ml	32 mm	31mm
AgNPs	24mm	23mm
CIP	12mm	9mm

Table 1: Antibacterial activity of synthesized nanoparticles in terms of zone of inhibition

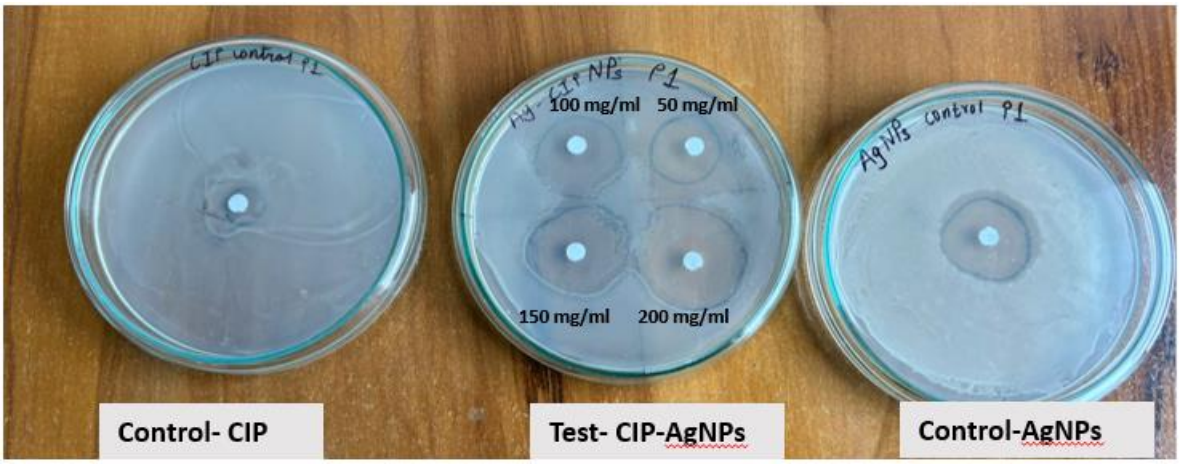

Figure 5a: Antibacterial activity against *E.coli*

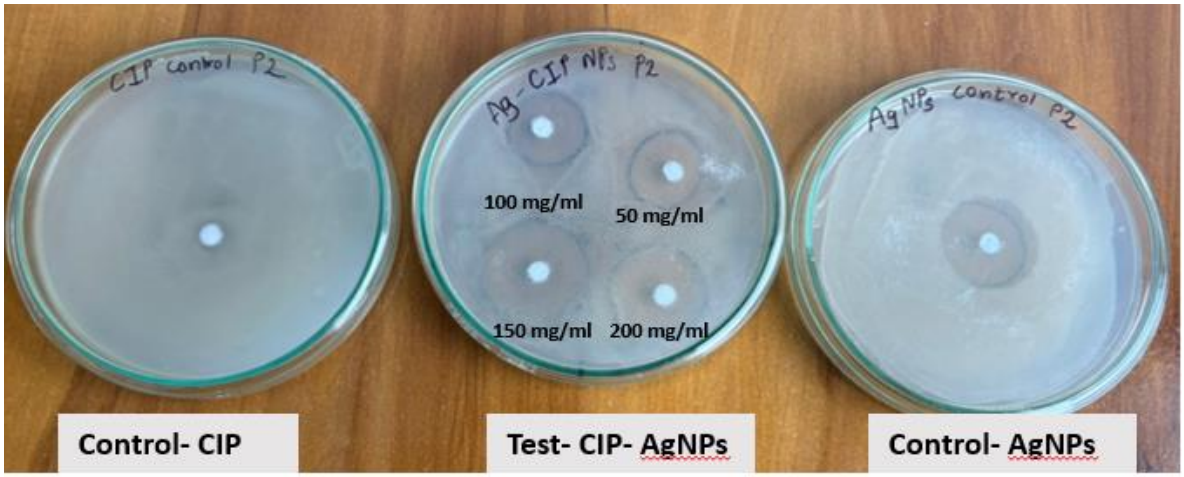

Figure 5b: Antibacterial activity against *S.aureus*

4. Conclusion

In present study, ciprofloxacin-conjugate silver nanoparticles were successfully prepared using a chemical reduction method. These nanoparticles were characterized to study their physiochemical properties such as morphology, size, and stability. The loading of ciprofloxacin antibiotic onto silver nanoparticle enhanced overall antibacterial efficiency, highlighting their potential as an effective nano-antibiotic formulation for mitigating the impact of antimicrobial resistance. The improved antibacterial activity is likely due to synergistic effect of silver ions and ciprofloxacin. These findings pave the way for future in vivo studies and the development of advanced nano-based drug delivery systems for clinical use.

Conflict of Interest

The authors declare that they are no conflicts of interest related to this publication.

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