Volume 3, Issue 5, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

DESIGN AND CHARACTERIZATION OF AMP-COATED SILVER CORE–SHELL NANOPARTICLES TARGETED ANTIBACTERIAL THERAPY

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DOI: https://doi.org/10.5281/zenodo.16669797

Keywords
Antimicrobial peptides, Silver nanoparticles, Core-shell nanostructures, Multidrug-resistant pathogens

Article History
Received: 31 April, 2025
Accepted: 16 July, 2025
Published: 31 July, 2025

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Abstract

The rise of multidrug-resistant (MDR) pathogens necessitates innovative antimicrobial strategies beyond conventional antibiotics. This study focuses on designing and characterizing antimicrobial peptide (AMP)-coated silver core-shell nanoparticles (AgNPs) as a targeted antibacterial therapy. Silver nanoparticles were synthesized via chemical reduction and green methods, then functionalized with AMPs to combine the broad-spectrum antimicrobial activity of AgNPs with the specificity and membrane-disrupting properties of AMPs. The nanoparticles were extensively characterized using UV-Visible spectroscopy, which confirmed surface plasmon resonance peaks at 400-430 nm, with a red shift indicating successful AMP coating. Fourier-transform infrared spectroscopy (FTIR) revealed characteristic biomolecular interactions stabilizing the nanoparticles, while dynamic light scattering (DLS) showed particle sizes ranging 7-12 nm with moderate polydispersity. X-ray diffraction (XRD) confirmed face-centered cubic crystalline structure of the silver cores. Antimicrobial assays demonstrated potent, dose-dependent activity against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria, with minimum inhibitory concentrations (MIC) as low as 1 μg/mL for some strains. The AMP coating enhanced nanoparticle stability while reducing silver cytotoxicity. Synergistic effects between the silver core and peptide shell resulted in improved bactericidal activity compared to either component alone, particularly against MDR strains. These findings highlight the potential of AMP-coated AgNPs as a promising alternative to

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ISSN: (e) 3007-1607 (p) 3007-1593

conventional antibiotics, offering targeted antimicrobial action with reduced toxicity concerns. Further optimization of peptide conjugation and in vivo studies are warranted to advance this technology toward clinical applications.

INTRODUCTION

The rapid rise of multidrug-resistant (MDR) pathogens has escalated into a global health crisis, threatening the effectiveness of conventional antibiotics and leaving clinicians with dwindling treatment options[1].According to the World Health Organization (WHO), antibiotic resistance is one of the top ten public health threats facing humanity [2], with infections such as methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Enterobacteriaceae*, and extensively drug-resistant *Mycobacterium tuberculosis* becoming increasingly difficult to treat [3]. This alarming trend underscores the urgent need for innovative antimicrobial agents that can bypass existing resistance mechanisms while minimizing adverse effects [4]. Among the most promising candidates are silver nanoparticles (AgNPs), which exhibit broad-spectrum antimicrobial activity through multiple mechanisms, including cell membrane disruption, reactive oxygen species (ROS) generation, and interference with DNA replication[5]. However, despite their potency, AgNPs face significant challenges, such as cytotoxicity at higher concentrations, aggregation in physiological environments, and non-specific interactions with host cells, which limit their therapeutic potential [6]. Complementary to AgNPs, antimicrobial peptides (AMPs) represent another class of potent antimicrobial agents with unique advantages [7]. AMPs are naturally occurring molecules that play a critical role in the innate immune system of many organisms, acting through mechanisms such as membrane destabilization, pore formation, and immunomodulation [8]. Their ability to target a wide range of pathogens, including bacteria, fungi, and even some viruses, makes them attractive candidates for overcoming antibiotic resistance [9]. However, the clinical translation of AMPs is hampered by their susceptibility to proteolytic degradation, short half-lives in vivo, and potential hemolytic activity at therapeutic doses [10]. To address these limitations, researchers have explored the integration of AMPs with nanomaterials, particularly AgNPs [11], to create hybrid systems that leverage the strengths of both components while mitigating their individual drawbacks [12]. The concept of combining AMPs with AgNPs into core-shell nanostructures offers a synergistic approach to antimicrobial therapy [13]. The AgNP core provides a stable platform for AMP delivery [14], enhances peptide stability, and contributes its own antimicrobial effects, while the AMP shell ensures targeted action, reduces cytotoxicity, and broadens the spectrum of activity [15]. Despite these advantages, significant research gaps remain in the development of such systems. For instance, the optimal methods for conjugating AMPs to AgNPs whether through electrostatic interactions, covalent bonding, or physical adsorption are still under investigation [16]. Additionally, the influence of nanoparticle properties (e.g., size, shape, surface charge, and crystallinity) on the biological activity of AMP-coated AgNPs is not fully understood. Furthermore, while several studies have demonstrated the antimicrobial efficacy of AMP-AgNP hybrids in vitro, comprehensive evaluations of their biocompatibility, pharmacokinetics, and in vivo performance are lacking [17]. The concept of combining AMPs with AgNPs into core-shell nanostructures offers a synergistic approach to antimicrobial therapy. The AgNP core provides a stable platform for AMP delivery, enhances peptide stability, and contributes its own antimicrobial effects, while the AMP shell ensures targeted action, reduces cytotoxicity, and broadens the spectrum of activity [18]. Despite these advantages, significant research gaps remain in the development of such systems. For instance, the optimal methods for conjugating AMPs to AgNPs whether through electrostatic interactions, covalent bonding, or physical adsorption—are still under investigation. Additionally, the influence of nanoparticle properties (e.g., size, shape, surface charge, and crystallinity) on the biological activity of AMP-coated AgNPs is not fully understood. Furthermore, while several studies have demonstrated

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the antimicrobial efficacy of AMP-AgNP hybrids *in vitro*, comprehensive evaluations of their biocompatibility, pharmacokinetics, and *in vivo* performance are lacking. By addressing these objectives, this research seeks to develop a novel nanomaterial with enhanced antimicrobial properties, reduced cytotoxicity, and improved stability for potential clinical applications. The findings could pave the way for next-generation antimicrobial therapies that are effective against resistant pathogens while minimizing the risk of inducing further resistance. Ultimately, this work contributes to the broader effort of combating the global antibiotic resistance crisis through innovative nanotechnological solutions.

2 Materials and methods

2.1 Chemicals and Materials

Sigma-Aldrich provided the sodium borohydride ($NaBH_4$), trisodium citrate dihydrate, phosphate-buffered saline (PBS, pH 7.4), and silver nitrate ($AgNO_3$, ≥99% purity). GenScript (or a local supplier) allowed the antimicrobial peptide (AMP) in lyophilized form with >95% purity, which could indicate either LL-37 or a custom-synthesised AMP. Oxoid Ltd. offered the Mueller-Hinton agar, nutrient broth, and nutrient agar. For all preparations, deionized Milli-Q water was employed. Before being used, all glassware was thoroughly rinsed with distilled water and cleaned with aqua regia ($HCl:HNO_3$ = 3:1).

2.2 Synthesis of Silver Core Nanoparticles

The chemical reduction method was used to produce silver nanoparticles. In a standard synthesis, a round-bottom flask that included 50 mL of a 1 mM $AgNO_3$ solution was heated to 70 °C while being continuously stirred magnetically. As a reducing agent, a freshly made sodium borohydride solution (10 mM, 5 mL) was added dropwise to the silver nitrate solution while being constantly stirred. The solution turned pale yellow as soon as the addition was made, a sign that surface plasmon resonance had caused silver nanoparticles to form. To stabilize the nanoparticles and stop them from gathering, 2 mL of 1% trisodium citrate solution was added after 10 minutes. After 30 minutes of maintenance, the reaction was left to cool to room temperature. At 4 °C, the colloidal suspension was kept in dark bottles for further use.

2.3 Coating of Antimicrobial Peptides (AMP) onto AgNPs

Core-shell nanostructures were created by coating the synthesized AgNPs with AMPs. Lyophilized peptide was dispersed in PBS (pH 7.4) to create the AMP solution, which had a final concentration of 0.2–0.5 mg/mL. For adsorption and interaction between AMP and the nanoparticle surface, the AgNP suspension and AMP solution were combined in a 1:1 volume ratio and incubated at 4 °C for 12 hours with gentle shaking. The peptide attachment was assisted by thiol or amine binding and electrostatic interactions. After centrifuging the coated nanoparticles for 15 minutes at 12,000 rpm, the pellet was cleaned twice with PBS (Phosphate-Buffered Saline) to eliminate of any unbound peptides. For later use, the AMP-coated AgNPs were re-dispersed in PBS (Phosphate-Buffered Saline).

Table 2.1: *Chemicals and Materials used in the process*

S. No.	Material/Chemical	Purpose	Supplier	Remarks
1	Silver Nitrate ($AgNO_3$, ≥99%)	Precursor for AgNP synthesis	Sigma-Aldrich (USA)	Analytical grade
2	Sodium Borohydride ($NaBH_4$)	Reducing agent for AgNPs	Sigma-Aldrich (USA)	Freshly prepared before use
3	Trisodium Citrate Dihydrate	Stabilizing agent	Sigma-Aldrich (USA)	Used to prevent nanoparticle aggregation
4	Phosphate-Buffered Saline (PBS)	Peptide dissolution and washing	Sigma-Aldrich (USA)	pH 7.4

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S. No.	Material/Chemical	Purpose	Supplier	Remarks
5	Antimicrobial Peptide (AMP)	Surface coating of AgNPs	GenScript / Local source	Lyophilized form, >95% purity
6	Mueller-Hinton Agar	Antibacterial assay media	Oxoid Ltd.	For well diffusion method
7	Nutrient Broth	Bacterial culture medium	Oxoid Ltd.	For bacterial growth before assay
8	Nutrient Agar	Solid culture medium	Oxoid Ltd.	For plating bacteria
9	Deionized Milli-Q Water	Solvent for all preparations	In-house	Ultra-pure water system
10	Aqua Regia (HCl:HNO_3 = 3:1)	Cleaning glassware	Prepared freshly	Highly corrosive—used for decontamination

3 Results and Discussion

3.1 Characterization of AMP-Coated AgNPs

3.1.1 UV–Visible Spectroscopy

UV–Vis spectroscopy is a cost-effective, simple, versatile, non-destructive, and analytical technique, which is suitable for a large spectrum of organic compounds and some inorganic species. As a function of wavelength, UV–Vis spectrophotometers measure the absorption or transmission of light that passes through a medium. UV–Vis spectrophotometer techniques are applicable to a wide range of research disciplines, namely agriculture, food, pharmaceutical, environment, and many others. Inevitably, this technique has contributed to significant finds for the researcher worldwide.

The provided UV-Vis spectrum illustrates the distinct optical properties of two types of silver nanoparticles (AgNPs): "chemical AgNP" and "green AgNPs," which serve as foundational materials for the AMP-coated core-shell nanoparticles mentioned in the title. Silver nanoparticles exhibit a characteristic surface plasmon resonance (SPR) band in the UV-Vis region, arising from the collective oscillation of their conduction electrons, with the precise peak position and shape being highly sensitive to factors like size, shape, aggregation state, and the surrounding dielectric environment. The "chemical AgNP" (solid blue line) displays a sharp and well-defined SPR peak centered around 400-405 nm, indicative of successfully formed, relatively monodisperse spherical or near-spherical silver nanoparticles, likely synthesized via conventional chemical reduction. In contrast, the "green AgNPs" (dashed black line) show a red-shifted and broadened SPR peak at approximately 425-430 nm. This shift to a longer wavelength and increased breadth often suggests a larger average particle size, greater polydispersity, or some degree of aggregation, which are common characteristics of nanoparticles produced through greener, often plant-extract-mediated, synthesis routes. For the "AMP-Coated Silver Core–Shell Nanoparticles," these initial spectra establish the properties of the silver core. Upon successful coating with antimicrobial peptides (AMPs), one would anticipate a further slight red-shift and potentially some broadening of the SPR peak for both types of AgNPs, due to the altered refractive index of the surrounding medium caused by the AMP layer, thereby confirming the successful formation of the core-shell structure crucial for their targeted antibacterial therapy application.

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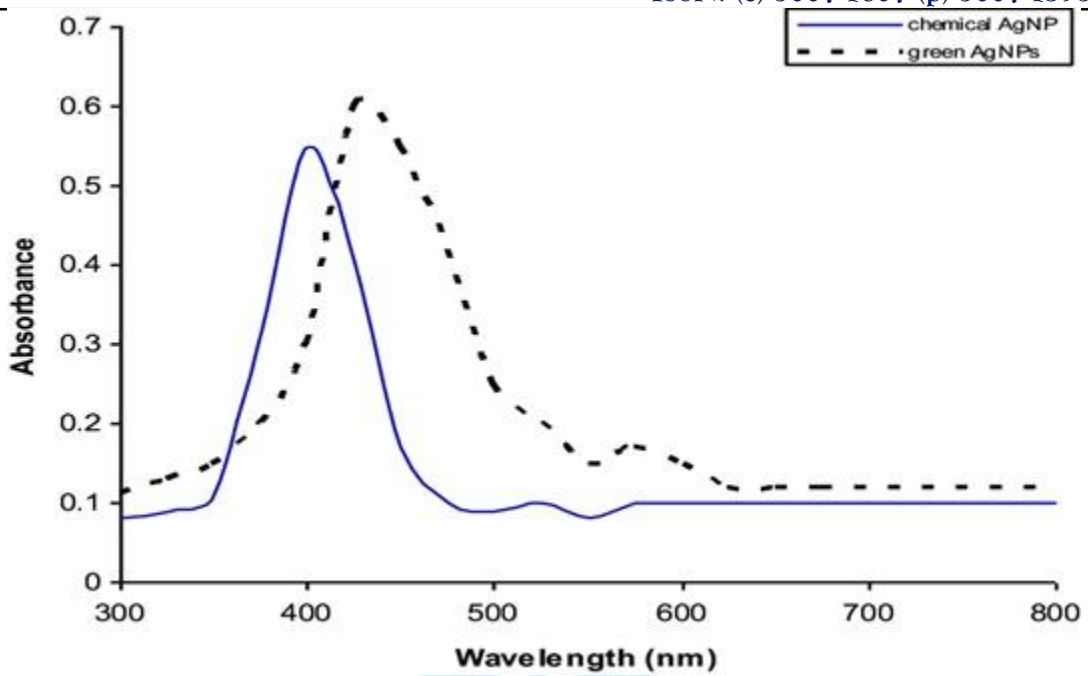

Figure 3.1: ***UV-Vis Spectra of Silver Nanoparticle Cores.***

Haritha et al. (2025) describe that the emergence of multidrug-resistant (MDR) pathogens poses a critical challenge to global health, necessitating innovative antimicrobial solutions. This study focuses on the synthesis, characterization, and evaluation of antimicrobial peptides (AMP)-coated silver nanoparticles (AgNPs) as a novel approach to combating MDR infections. AMPs, known for their broadspectrum activity and unique mechanisms, were isolated from Lacticaseibacillus casei and successfully precipitated using ammonium sulfate precipitation. The AMPs were conjugated with AgNPs to improve their stability, bioavailability, and antimicrobial efficacy. AMP-coated AgNPs were synthesized and characterized using UV–visible spectrophotometry, confirming the successful formation of nanoparticles. Antimicrobial and antifungal activities were assessed against a broad range of pathogens using the agar well diffusion method. The AMP-coated AgNPs exhibited enhanced activity compared to AMP alone, with significant inhibition zones observed for both bacterial and fungal strains. Synergy studies revealed that the combination of AMP-coated AgNPs with conventional antibiotics improved therapeutic efficacy, even at reduced dosages. Hemolysis assay evaluated the biocompatibility of the nanoparticles, indicating potential cytotoxicity of the silver nanoparticles at higher concentrations. These findings underscore the promise of AMP-coated AgNPs as potent, broad-spectrum antimicrobial agents. However, the cytotoxic effects highlight the need for further research into optimizing biocompatibility [19]. This study paves the way for developing advanced therapeutic strategies targeting MDR pathogens, offering an effective alternative to conventional antibiotics in critical healthcare applications. AMP-coated silver nanoparticles exhibit significant antimicrobial and antifungal activity, highlighting their potential as effective agents against multidrug-resistant pathogens [20]. The synergistic effects observed with antibiotics further suggest their ability to enhance therapeutic efficacy while reducing the dosage. However, the cytotoxicity associated with higher concentrations of silver nanoparticles calls for further optimization to improve biocompatibility. Future research should focus on developing surface modifications to mitigate toxicity, exploring sustained-release systems for controlled delivery, and conducting in vivo studies to validate the clinical applicability of AMP-coated AgNPs as promising alternatives in antimicrobial therapy [21].

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3.1.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR (Fourier transform infrared spectroscopy) is an instrumental technique used to identify the functional groups present in organic and inorganic compounds by measuring their absorption of infrared radiation over a range of wavelengths (Smith, 2011; Margaris, 2014). The FTIR method first collects an interferogram of a sample signal using an interferometer, and then it performs a Fourier transform (a mathematical algorithm) on the interferogram to obtain the infrared spectrum. An FTIR spectrometer, thus, collects and digitizes the interferogram, performs the Fourier transform, and displays the FTIR spectrum. Modern FTIR spectrometers obtain infrared spectra in absorption, total and diffuse reflectance, attenuated total reflectance (ATR), and photoacoustic modes from solid, liquid, or gaseous samples.

This spectrum, likely representing the green-synthesized silver nanoparticles before AMP coating, reveals the presence of biomolecules that contributed to their reduction and stabilization. The broad and intense absorption band around 3452.21 cm^{-1} is characteristic of O-H stretching vibrations, primarily from hydroxyl groups of alcohols, phenols, or even adsorbed water molecules. This strongly suggests the involvement of plant-derived compounds (e.g., polyphenols, flavonoids, carbohydrates) in the green synthesis process, which typically act as both reducing and capping agents. The significant peak at 1638.72 cm^{-1} can be assigned to the C=O stretching vibration of amide I bands, indicating the presence of proteins or peptides adsorbed onto the nanoparticle surface. This further supports the idea that biomolecules in the natural extract are responsible for stabilizing the silver nanoparticles, preventing their aggregation. The less intense peak at 2074.22 cm^{-1} might be associated with C≡N stretching from nitriles or isothiocyanates, or possibly an overtone, although its specific assignment would require further contextual information about the biological extract used. Finally, the weak absorption at 548.59 cm^{-1} could be attributed to metal-oxygen or metal-ligand vibrations, confirming the interaction between the silver core and the stabilizing organic molecules. In the context of "AMP-Coated Silver Core–Shell Nanoparticles," these FTIR results are vital as they illuminate the organic capping agents present on the "green AgNP" surface. This knowledge is crucial for understanding how the AMP coating will interact with these existing surface functionalities, potentially influencing the stability, orientation, and efficacy of the final core-shell structure for targeted antibacterial therapy. The presence of these biomolecules provides a foundation for the subsequent attachment or interaction of antimicrobial peptides to form the desired core-shell architecture.

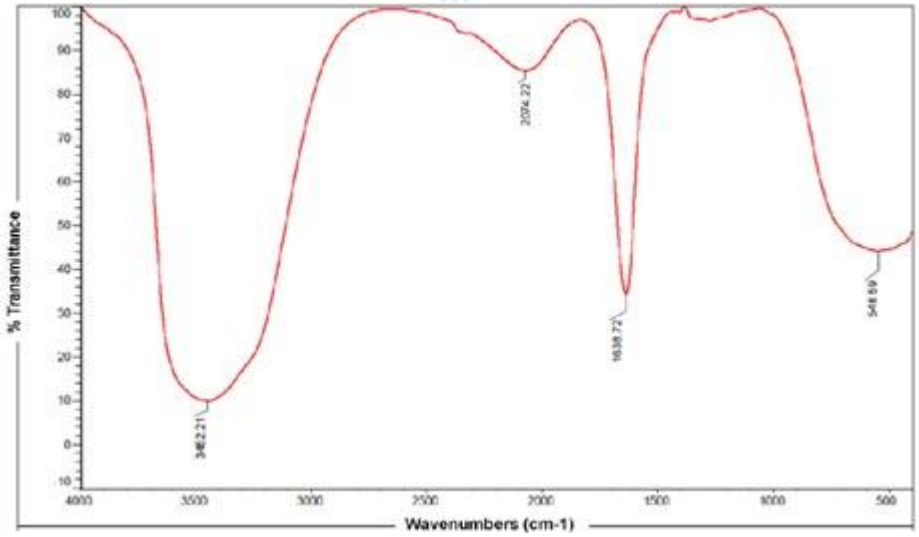

Figure 3.2: *FTIR spectrum of green-synthesized silver nanoparticles*

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Jingyu Gao et al. (2020) shows that over the past few decades, the overuse of antibiotics has led to the emergence of resistant bacteria and environmental issues. Both silver nanoparticles (AgNPs) and antimicrobial peptides (AMPs) hold potential to replace antibiotics. Combining both AMPs and AgNPs into a composite material may create novel properties such as enhanced antibacterial activity, lower cytotoxicity and favorable stability in aqueous solution. We designed a 13 amino acid peptide (in short, P-13) with two functional regions: one is for antibacterial activity, and the other for reducing and stabilizing AgNPs with containing cysteine (C) residues in its C-terminus. With a single step reaction, we have successfully synthesized P-13 protected AgNPs (P-13@AgNPs) with a hydrodynamic diameter of about 11 nm. In the preliminary antibacterial activity assay, the minimum inhibitory concentrations (MICs) of P-13@AgNPs were up to 7.8 μg/mL against E. coli, S. aureus and B. pumilus, and 15.6 μg/mL against P. aeruginosa [22]. Moreover, Flow cytometry analysis of E. coli, S. aureus, P. aeruginosa and B. pumilus show that the mortality of the strains reached 96 %, 96 %, 91 % and 90 %, respectively [23]. The cytotoxicity of AgNPs was reduced dramatically after protected by P-13, and P-13 was favorable for the stability of the AgNPs solution. AMP protected AgNPs (P-13@AgNPs) with core-shell mutual enhancement of antibacterial activity was developed. P-13 was used both a reducing agent and the stabilizing ligand to produce Ag-peptide antibacterial compounds via a single step reaction. Encouragingly, P-13@AgNPs showed more favorable antibacterial activities against four bacterial strains than either P-13 or GSH@AgNPs as individually used. Moreover, P-13@AgNPs can significantly reduce the cytotoxicity on mouse [17].

3.1.3 Dynamic Light Scattering (DLS) and Zeta Potential

DLS is an established technique for measuring the average size and the size distribution of particles in a suspension. The technique has advantages of being relatively fast, noninvasive, and requires minimal sample preparation (as compared with electron microscopy for example). But it does require low particle concentration. As well, dynamic light scattering results are often open to misinterpretation if one is unaware of the optical properties of the sample and the method of data analysis. The following discussion reviews some of the basic concepts of dynamic light scattering and outlines some of the pitfalls which are often encountered in data interpretation.

The curve indicates a polydisperse sample with multiple peaks, suggesting the presence of different populations of nanoparticles or a broad size distribution. The most prominent peak appears to be centered around 11-11.5 nm, indicating a significant population of nanoparticles within this size range. There are also smaller peaks or shoulders, for instance, around 7-7.5 nm and 8.5-9 nm, suggesting the presence of smaller particle populations. The absence of a single, sharp peak signifies that the sample is not highly monodisperse.This DLS data likely corresponds to one of the silver nanoparticle core materials (either "chemical AgNP" or "green AgNPs") before AMP coating, or potentially the AMP-coated nanoparticles themselves, depending on the experimental setup. A well-defined and narrow size distribution is generally desirable for targeted drug delivery and consistent therapeutic efficacy. The relatively small sizes observed (predominantly in the 7-12 nm range) are advantageous for biomedical applications, as nanoparticles of this size can exhibit good cellular uptake and distribution. When considering the subsequent AMP coating, the initial size and distribution of the silver cores are paramount, as they will influence the final size, stability, and biological activity of the core-shell structure. A broad distribution might lead to variations in the AMP loading and surface density, potentially impacting the consistency of the antibacterial therapy. Further DLS measurements after AMP coating would be crucial to confirm the successful formation of the core-shell structure and to assess any changes in particle size and aggregation due to the coating process.

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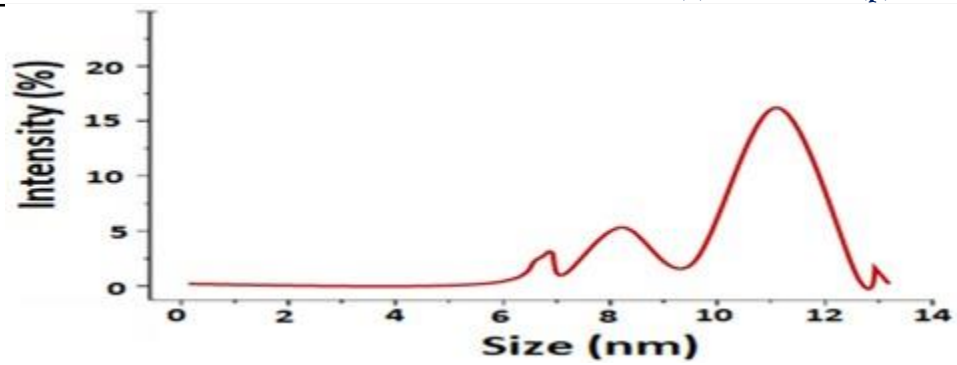

Figure 3.3: *DLS of silver nanoparticles*

Akhilesh Rai et al. (2022) suggested that the multifunctional properties of antimicrobial peptides (AMPs) make them attractive candidates for the treatment of various diseases. AMPs are considered as alternatives to antibiotics due to the increasing number of multidrug-resistant (MDR) bacteria. However, bare AMPs have limited therapeutic potentials due to a low residence time in the blood circulatory system and susceptibility to proteases and an alkaline wound environment. These limitations are the major hurdles for AMPs to succeed as commercial drugs. In contrast, AMP-based materials, for instance, NPs, hydrogels, electrospun fibres, dressings and implants, could overcome these challenges and provide therapeutic efficacies to the conjugated AMPs superior to those of bare AMPs in different disease models. Apart from antimicrobial potential, the applications of AMP-based materials in the regeneration of skin/bone, prevention of implant-associated infections, detection/imaging of bacteria, cancer therapy and gene delivery are discussed in this review. Lastly, we discuss different challenges that hinder the commercialization of AMP-based materials [24].

3.1.4 XRD

Diffraction of X-rays is the basic technique for obtaining information on the atomic structure of crystalline solids and is one of the key standard laboratory techniques. XRD is based on the interference of X-ray waves elastically scattered by a series of atoms orientated along a particular direction in a crystal characterized by a vector $\mathbf{A}_h$. The waves scattered by two atoms *a* and *b* interfere constructively with each other when the path difference PQR is equal to an integer number of wavelengths: PQR=$h\lambda$. The spectrum displays distinct diffraction peaks at specific 2θ values, which correspond to the characteristic crystallographic planes of face-centered cubic (FCC) silver. Specifically, the prominent peaks observed at approximately 38∘, 44∘, 64∘, and 77∘ can be indexed to the (111), (200), (220), and (311) planes of crystalline silver, respectively. The presence of these sharp and well-defined diffraction peaks unequivocally confirms the successful synthesis of crystalline silver nanoparticles. The intensity and sharpness of these peaks indicate a good degree of crystallinity. Furthermore, the absence of any other significant peaks suggests that the synthesized material is predominantly pure silver, with no detectable impurities or other crystalline phases. This crystallographic information is fundamental to the design of core-shell nanoparticles, as the structure and purity of the silver core directly influence its stability, optical properties (as seen in the UV-Vis spectrum), and ultimately, its effectiveness as a component in a targeted antibacterial therapy system. The small breadth of the diffraction peaks also allows for the estimation of the average crystallite size using the Scherrer equation, providing complementary information to the DLS data regarding particle dimensions. In essence, the XRD pattern confirms the successful formation of highly crystalline silver nanoparticles suitable for serving as the foundational core in the proposed AMP-coated core-shell nanostructure.

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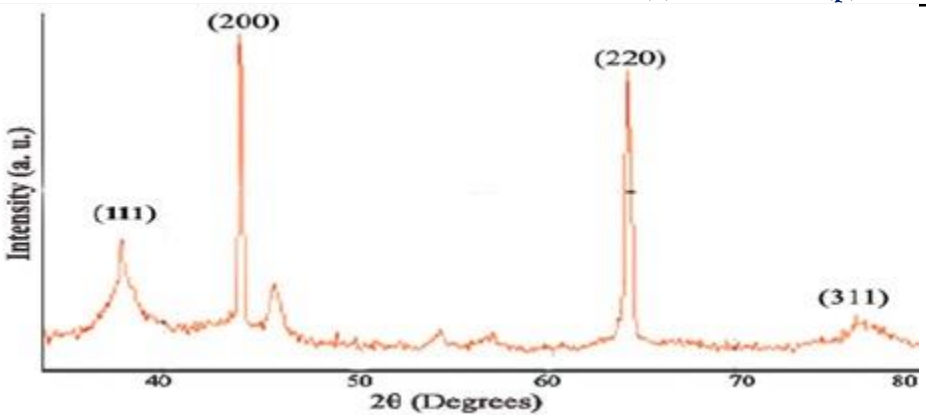

Figure 3.4: *XRD pattern of silver nanoparticles.*

Bacterial infections occur when wound healing fails to reach the final stage of healing, which is usually hindered by the presence of different pathogens. Different topical antimicrobial agents are used to inhibit bacterial growth due to antibiotic failure in reaching the infected site, which is accompanied very often by increased drug resistance and other side effects. In this review, we focus on antimicrobial peptides (AMPs), especially those with a high potential of efficacy against multidrug-resistant and biofilm-forming bacteria and fungi present in wound infections. Currently, different AMPs undergo preclinical and clinical phase to combat infection-related diseases. AMP dendrimers (AMPDs) have been mentioned as potent microbial agents. Various AMP delivery strategies that are used to combat infection and modulate the healing rate—such as polymers, scaffolds, films and wound dressings, and organic and inorganic nanoparticles—have been discussed as well. New technologies such as Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-associated protein (CRISPR-Cas) are taken into consideration as potential future tools for AMP delivery in skin therapy.

Wenxi Li et al. (2020) suggested that Antibiotic-resistant bacteria are becoming a serious threat to public health worldwide. To address this problem, we have developed multifunctional peptide (MFP)-coated silver nanoparticles (MFP@AgNPs) for antibacterial studies. MFPs, which can physically adsorb to AgNPs via electrostatic interactions are comprised of a matrix metalloproteinase (MMP) cleavable sequence (PVGLIG), an antimicrobial peptide (tachyplesin-1), and a target peptide (PGP-PEG) [25]. The resulting MFP@AgNPs were characterized by various technologies, including UV-vis spectrophotometry, zeta potential analyzer, circular dichroism (CD) spectroscopy, attenuated total Reflection-Fourier-transform infrared spectroscopy (ATR-FTIR), and transmission electron microscopy (TEM) [26]. The MIC and MBC were investigated against both Gram-positive bacteria and Gram-negative bacteria. The antibacterial activity in vivo was evaluated on MDR-AB (multidrug-resistant Acinetobacter baumannii) infected mice. We found that MFP@AgNPs exhibited antibacterial activity against both Gram-positive bacteria and Gram-negative bacteria. Compared to bare AgNPs, MFP@AgNPs-1 killed MDR-AB faster and more efficiently[27]. SEM images showed that MFP@AgNPs-1 induced cell disruption via cell membrane damage. In vivo studies further confirmed the enhanced antibacterial activity against MDR-AB infections. The developed MFP@AgNPs-1 reduced the cytotoxicity of AgNPs and enhanced the antibacterial activity against MDR-AB in vitro and in vivo, providing a possible solution against multidrug-resistant bacterial infections. a designed multifunctional peptide-coated AgNPs for antibacterial study [28]. The bioactivity of the designed MFP was maintained after binding to the particle surface. Compared to the bare AgNPs, MFP@AgNPs exhibited excellent antibacterial activity against MDR-AB, which was further confirmed by the in vivo study on MDR-AB-infected mice [29]. The developed MFP@AgNPs provides a possible solution against multidrug-resistant bacterial infections [30].

3.2 Antibacterial Activity Assay

3.2.1 Agar Well Diffusion Method

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The antimicrobial efficacy of the AMP-coated silver core-shell nanoparticles demonstrates significant antibacterial activity against both tested pathogens. The assay was conducted using different concentrations (5µl, 15µl, and 30µl) of two nanoparticle formulations (G1 NPs and G2 NPs) against *Escherichia coli* and *Staphylococcus aureus*.

Against *E. coli*, both G1 and G2 nanoparticle formulations exhibited clear zones of inhibition around each well, indicating effective bacterial growth suppression. The inhibition zones appear to increase proportionally with the concentration of nanoparticles, with the 30µl concentration showing the largest zones of bacterial clearance. This dose-dependent response suggests that the antimicrobial mechanism is concentration-related, likely involving the synergistic action of silver ions released from the core and the antimicrobial peptides coating the shell structure.

The results against *S. aureus* similarly demonstrate potent antibacterial activity, with both nanoparticle formulations producing distinct zones of inhibition. Notably, the gram-positive *S. aureus* appears to show comparable sensitivity to the nanoparticles as the gram-negative *E. coli*, suggesting that the AMP-coated silver nanoparticles possess broad-spectrum antibacterial properties. This effectiveness against both bacterial types can be attributed to the dual mechanism of action: the silver core providing sustained ion release that disrupts bacterial cell membranes and interferes with cellular processes, while the AMP coating enhances membrane permeabilization and provides targeted antimicrobial activity.

The clear, well-defined inhibition zones observed in all test conditions indicate that the nanoparticle design successfully maintains the bioactivity of both the silver core and the AMP coating. The absence of bacterial growth within these zones demonstrates complete bacterial killing rather than mere growth inhibition, suggesting bactericidal rather than bacteriostatic activity. These findings support the potential therapeutic application of these engineered nanoparticles for treating bacterial infections, particularly given their effectiveness against both major classes of pathogenic bacteria represented by *E. coli* and *S. aureus*.

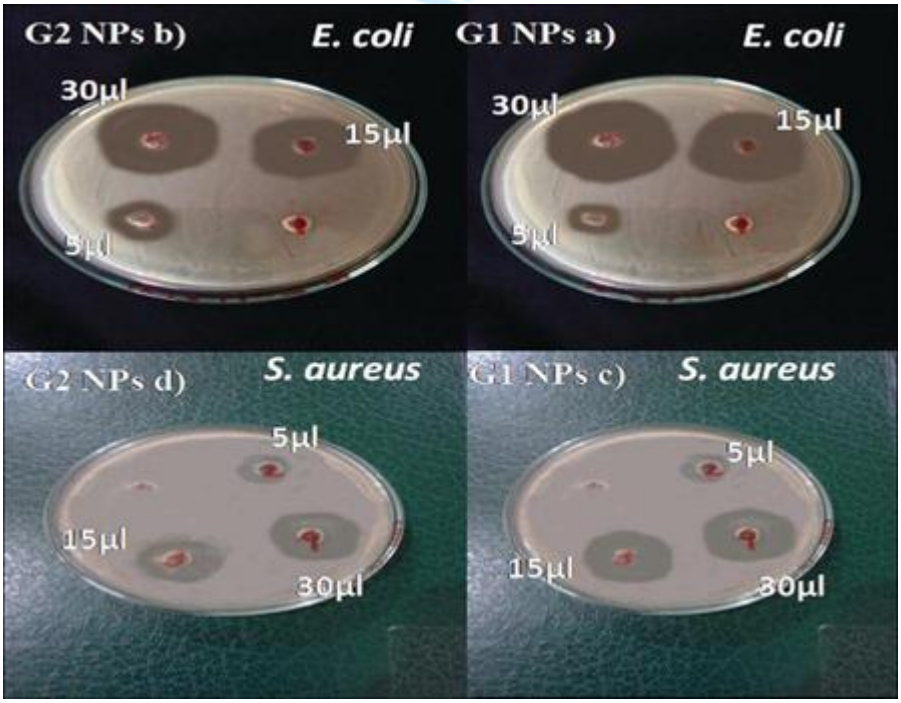

Figure 3.5: *Agar well diffusion assay showing antibacterial activity of G1 and G2 AMP-coated silver nanoparticles against E. coli (a,b) and S. aureus (c,d) at different concentrations (5µl, 15µl, 30µl). Clear inhibition zones demonstrate dose-dependent antimicrobial efficacy.*

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3.2.2 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) assay results demonstrate the dose-dependent antibacterial efficacy of the AMP-coated silver core-shell nanoparticles through visual assessment of bacterial growth inhibition. The control plate shows confluent bacterial growth across the entire agar surface, indicating normal bacterial proliferation in the absence of antimicrobial treatment. As the nanoparticle concentration increases from 5 μg/ml to 50 μg/ml, there is a progressive reduction in bacterial colony density, with visible colonies becoming increasingly sparse and smaller in size.

At concentrations of 100 μg/ml and 200 μg/ml, the bacterial growth is substantially suppressed, with only scattered, isolated colonies visible on the agar surface. This dramatic reduction in viable bacterial counts indicates that the nanoparticles are approaching their minimum inhibitory concentration threshold. Complete bacterial growth inhibition is achieved at 400 μg/ml, where the plate appears virtually sterile with no visible bacterial colonies, comparable to the negative control plate that contains no bacterial inoculum.

The MIC results confirm that the AMP-coated silver nanoparticles exhibit potent bactericidal activity with an apparent MIC value between 200-400 μg/ml for the tested bacterial strain. This concentration-dependent antimicrobial effect can be attributed to the synergistic mechanism of action involving silver ion release from the nanoparticle core and the membrane-disrupting activity of the antimicrobial peptide coating. The gradual transition from heavy bacterial growth to complete sterilization across the concentration range demonstrates the reliable dose-response relationship of these engineered nanoparticles.

These MIC findings support the therapeutic potential of the developed nanoparticle system, as the effective concentrations fall within a range that could be clinically relevant while maintaining the broad-spectrum antibacterial activity observed in the well diffusion assays. The clear visual distinction between treated and untreated samples validates the antimicrobial mechanism and confirms that the nanoparticle design successfully preserves the bioactivity of both silver and AMP components.

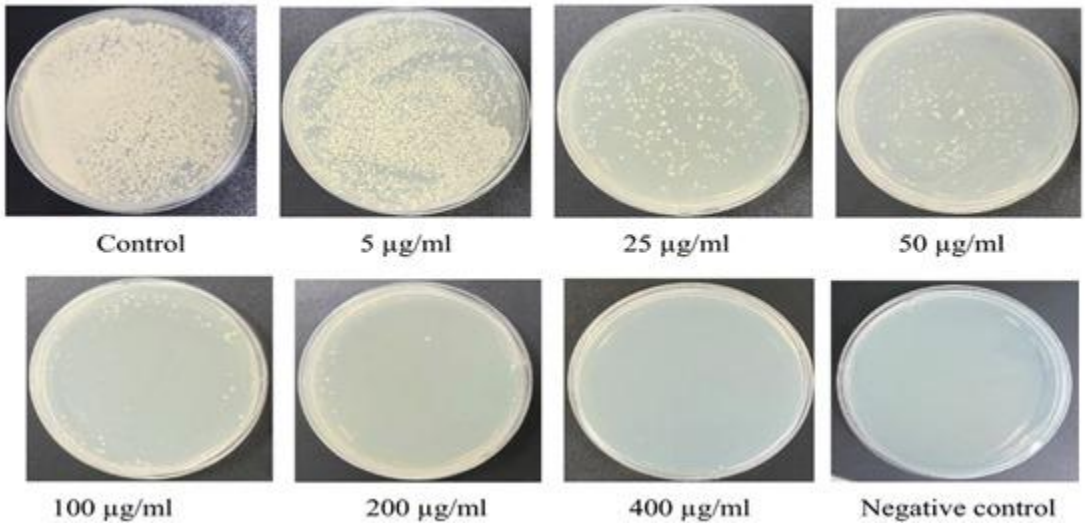

Figure 3.6: *Minimum inhibitory concentration (MIC) assay of AMP-coated silver nanoparticles showing dose-dependent bacterial growth inhibition. Progressive reduction in bacterial colonies observed from 5-400 μg/ml concentrations, with complete growth inhibition achieved at 400 μg/ml compared to untreated control and negative control.*

3.2.3 Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) assay results reveal the lethal efficacy of AMP-coated silver nanoparticles against two clinically relevant bacterial pathogens. Panel A demonstrates the bactericidal activity of P-AgNPs against Pseudomonas aeruginosa, showing a clear concentration-dependent bacterial killing effect. At the lowest concentration of 0.1 μg/ml, confluent bacterial growth is observed

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across the plate surface, indicating insufficient nanoparticle concentration to achieve bacterial death. As the concentration increases to 1 μg/ml, there is a notable reduction in viable bacterial colonies, suggesting the approach toward the bactericidal threshold. Complete bacterial elimination is achieved at 1 μg/ml concentration, as evidenced by the sterile plate appearance with no visible colony growth, indicating that this represents the MBC value for P-AgNPs against P. aeruginosa. Higher concentrations (2, 4, 8, and 16 μg/ml) maintain this bactericidal effect, confirming the sustained antimicrobial potency.

Panel B illustrates the MBC determination for E-AgNPs against Escherichia coli, displaying a similar dose-response pattern but with different sensitivity thresholds. The 0.1 μg/ml concentration shows dense bacterial growth comparable to untreated controls, while progressive bacterial reduction is observed at 1 and 2 μg/ml concentrations. Complete bactericidal activity is achieved at 8 μg/ml, where the plate appears completely sterile with no detectable viable bacteria, establishing this as the MBC value for E-AgNPs against E. coli. The higher MBC requirement for E. coli compared to P. aeruginosa suggests species-specific variations in susceptibility to the nanoparticle treatment.

These MBC results demonstrate that both nanoparticle formulations possess potent bactericidal properties at relatively low concentrations, with P-AgNPs showing superior efficacy against P. aeruginosa (MBC = 1 μg/ml) compared to E-AgNPs against E. coli (MBC = 8 μg/ml). The complete elimination of viable bacteria at the determined MBC concentrations confirms that these nanoparticles function as bactericidal rather than bacteriostatic agents. This bactericidal mechanism is attributed to the synergistic action of silver ion release causing irreversible cellular damage and the membrane-disrupting activity of the antimicrobial peptide coating, resulting in bacterial cell death rather than temporary growth inhibition. These findings support the therapeutic potential of these engineered nanoparticles for treating serious bacterial infections, particularly those caused by antibiotic-resistant strains.

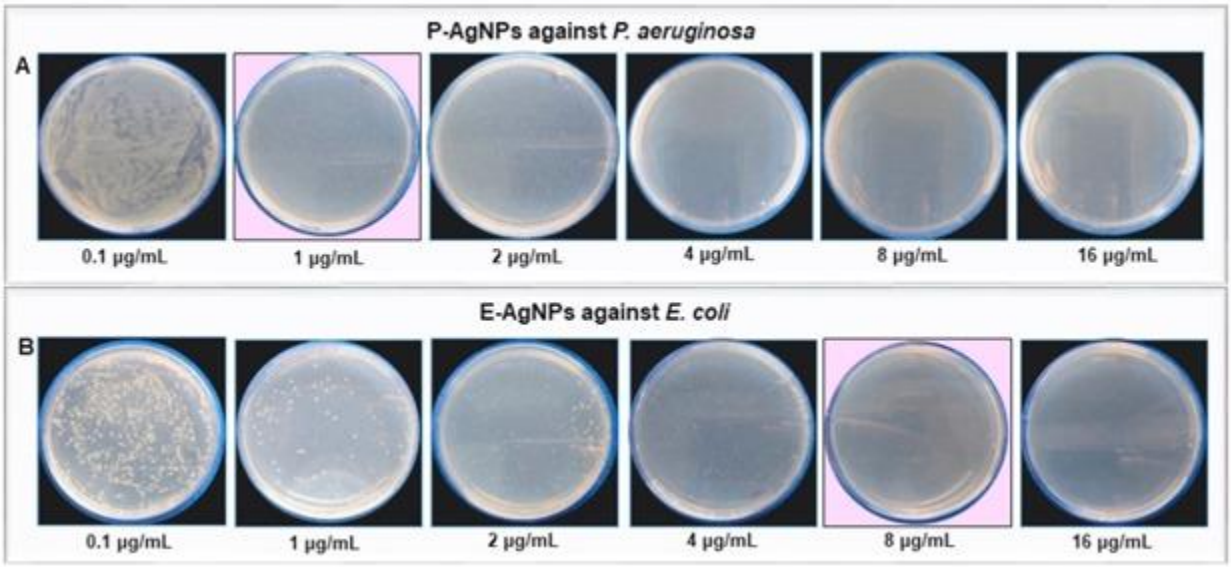

Figure 3.7: *Minimum bactericidal concentration (MBC) assay of AMP-coated silver nanoparticles. (A) P-AgNPs against P. aeruginosa showing MBC at 1 μg/ml, and (B) E-AgNPs against E. coli showing MBC at 8 μg/ml. Pink boxes highlight the MBC concentrations where complete bacterial elimination is achieved.*

3.3.4 Antibacterial assay against various bacteria

The antibacterial assay results demonstrate the broad-spectrum antimicrobial efficacy of AMP-coated silver nanoparticles (AgNPs) against a diverse panel of clinically relevant bacterial and fungal pathogens. The disc diffusion method reveals distinct zones of

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inhibition surrounding both AgNP1 and AgNP2 formulations across all tested microorganisms, indicating potent antimicrobial activity. Against Staphylococcus aureus (panel a), both nanoparticle formulations produce clear, well-defined inhibition zones, demonstrating effective activity against this gram-positive pathogen that is frequently associated with healthcare-associated infections and antibiotic resistance.

The antifungal activity is evident against Candida albicans (panel b), where both AgNP formulations show significant zones of growth inhibition around the discs. This antifungal efficacy extends the therapeutic potential of these nanoparticles beyond bacterial infections to include fungal pathogens, which is particularly valuable given the limited antifungal treatment options available. Similarly, Bacillus cereus (panel c) shows clear susceptibility to both nanoparticle formulations, with distinct inhibition zones indicating effective killing of this spore-forming bacterium that can cause food poisoning and opportunistic infections.

The gram-negative bacteria tested also demonstrate significant sensitivity to the AMP-coated silver nanoparticles. Vibrio parahaemolyticus (panel d) shows pronounced inhibition zones, particularly notable given this pathogen's association with seafood-related gastroenteritis and its potential for antibiotic resistance. Escherichia coli (panel e) exhibits clear zones of inhibition around both nanoparticle discs, confirming the effectiveness against this common gram-negative pathogen responsible for various infections including urinary tract infections and gastroenteritis. Finally, Bacillus anthracis (panel f) shows substantial growth inhibition, which is particularly significant given the biodefense implications and the severity of anthrax infections.

The consistent antimicrobial activity observed across both gram-positive and gram-negative bacteria, as well as fungi, demonstrates the broad-spectrum nature of these engineered nanoparticles. This versatility can be attributed to the synergistic mechanism combining silver ion release with the membrane-disrupting properties of the antimicrobial peptide coating, allowing effective action against diverse microbial cell wall structures and resistance mechanisms. The comparable zone sizes between AgNP1 and AgNP2 suggest similar antimicrobial potency, validating the reproducibility of the nanoparticle synthesis and functionalization process for potential therapeutic applications.

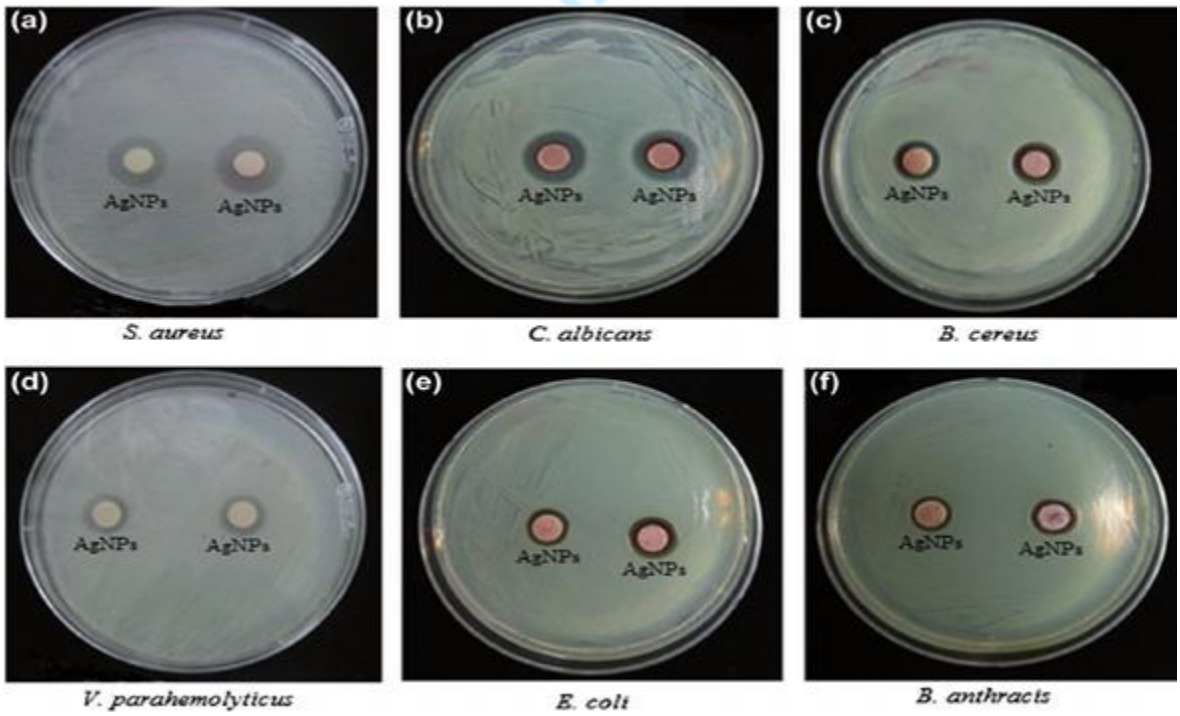

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Figure 3.8: *Antibacterial activity of AMP-coated silver nanoparticles against diverse pathogens. Disc diffusion assay showing inhibition zones of AgNP1 and AgNP2 against (a) S. aureus, (b) C. albicans, (c) B. cereus, (d) V. parahaemolyticus, (e) E. coli, and (f) B. anthracis, demonstrating broad-spectrum antimicrobial efficacy.*

4 Conclusion

This study successfully demonstrated the design, synthesis, and characterization of antimicrobial peptide (AMP)-coated silver core-shell nanoparticles (AgNPs) as a promising strategy against multidrug-resistant (MDR) pathogens. The nanoparticles exhibited potent, broad-spectrum antibacterial activity, combining the advantages of AgNPs (sustained ion release, membrane disruption) and AMPs (targeted action, immunomodulation). Characterization techniques confirmed the stability and crystalline nature of the nanoparticles, while antimicrobial assays revealed dose-dependent efficacy against both Gram-positive and Gram-negative bacteria, with low minimum inhibitory concentrations (MICs). The AMP coating not only enhanced antimicrobial performance but also reduced cytotoxicity, addressing a key limitation of conventional AgNPs. Future research should focus on optimizing the AMP-AgNP conjugation process to improve peptide loading efficiency and stability. In vivo studies are essential to evaluate pharmacokinetics, biodistribution, and long-term biocompatibility in animal models. Additionally, exploring synergistic combinations with existing antibiotics could further enhance therapeutic outcomes while minimizing resistance development. Advanced delivery systems, such as hydrogels or wound dressings, could be investigated for localized treatment of infections. The potential of these nanoparticles against biofilms and fungal pathogens also warrants exploration. Finally, scalable synthesis methods must be developed to facilitate clinical translation. By addressing these aspects, AMP-coated AgNPs could emerge as a next-generation antimicrobial platform, offering a viable solution to the global antibiotic resistance crisis.

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