J Nanostruct 16(2): 2511-2517, Spring 2026

RESEARCH PAPER

Aminoglycoside-Bacteriogenic Nano-Silver Synergism: Synthesis, Characterization, and Biomedical Potential

Hussein H. Al-Turnachy

Department of Biology, Faculty of Science, University of Kufa, Iraq

ARTICLE INFO

Article History:
Received 14 December 2025
Accepted 23 March 2026
Published 01 April 2026

Keywords:
AgNPs
Bacteriogenic Nanoparticles
Enterobacter spp
Garamycin
Synergism
Zeta potential

ABSTRACT

Antimicrobial resistance exerts major global health concern, urging search for innovative therapeutic strategies and updating the weakened in use ones. Nanobiotechnology has emerged as a promising field for developing alternative antimicrobial agents. Silver Nanoparticles were Fabricated using cell-free extracts of *Enterobacter sp.* broth culture. Characterized using UV–visible spectroscopy, field emission scanning electron microscopy, and atomic force microscopy. Antimicrobial potential was assessed against antibiotic-resistant *Staphylococcus sp., Klebsiella sp.*, and *Pseudomonas sp*., Minimum inhibitory concentrations were determined, potential synergistic effects with Garamycin were evaluated, and polydispersity were assessed by Zeta potential. Bacteriogenic SNPs exhibited peak absorbance at 400–420 nm, with a mean particle size of 32.01 nm, spherical shaped, and homogeneity. MIC values were 0.7 mg/lm against *Staphylococcus sp. and Klebsiella sp*., and 1.8 mg/lm against *Pseudomonas sp.*, showing superior activity compared to conventional antibiotics. Synergy testing revealed weak to negligible interactions between SNPs and Garamycin. Bacteriogenic SNPs showed stable dispersion in liquid solution with potential 20.1 mV at scattering signal strength intensity 30000. This investigation demonstrates the potential of biosynthesized silver nanoparticles as effective antimicrobial agents against multidrug-resistant Bacteria. While SNPs showed significant independent activity, limited synergism with Garamycin suggests that their primary value lies in serving as standalone alternatives to Garamycin.

How to cite this article
H. Al-Turnachy H. Aminoglycoside-Bacteriogenic Nano-Silver Synergism: Synthesis, Characterization, and Biomedical Potential. J Nanostruct, 2026; 16(2):2511-2517. DOI: 10.22052/JNS.2026.02.091

INTRODUCTION

Antimicrobial resistance give raise to significant global concern, largely attributed to its uncontrolled assimilation. The rapid worldwide spread of resistant bacteria necessitates urgent action to control its spread [1]. In the United States of America (USA) alone, there was 2.8 million resistant infections were recorded annually, leading to 35,900 deaths. Additionally, *Clostridium difficile* infection, a consequence of antimicrobial disturbance of intestinal microbiome, reporting another 12,800 deaths annually [2]. Reports suggest that accurately monitoring the economic impact of AMR resistance remains challenging. AMR infections unquestionably indisputably impose increased costs on healthcare systems, stemming from extended treatment durations and prolonged hospitalization. The medications required for treatment can be costly and may have reduced tolerability. However, a standardized

* *Corresponding Author Email: husseinh.abdulla@uokufa.edu.iq*

methodology for estimating the economic burden of resistant infections is currently uncovered [3]. Direct treatment costs for six prevalent multidrug-resistant pathogens amount to $4.6 billion annually, with an additional $1 billion attributed to Clostridium difficile, and billions more for drug-resistant gonorrhea, totaling another $133.4 million [4]. Silver and its compounds have a long history of use in therapeutic applications and especially as antibacterial, spanning thousands of eras. Ancient Romans and Greeks utilized silver vessels for water and food storage to prevent deterioration. Hippocrates employed silver prescriptions for treating abscesses and promoting healing. $AgNO_3$ was used also for instrument disinfection and wound care. The early 19th century saw the development of silver preparations. Silver has historically been utilized for treating wound infections and managing burns. However, its medical applications were largely superseded by new clinical introductions in the 1940s [5]. Nano Silver have also served as antimicrobial agents since the 19^{th} century, and their applications have now expanded to encompass diverse physical, chemical, and biological uses in contemporary research [6]. Silver nanoparticles SNPs have proven their exceptional efficiency in their use as effective antimicrobial, anti-inflammatory and anti-tumor activity [7]. The use of combination therapy leads to the emergence of synergistic reactions to eliminate bacteria. Examples of these reactions include increasing membrane permeability, inhibiting the protein synthesis process, disrupting biofilms, and other activities, this is called the synergistic effect [8]. Although there are many synergistic treatments, there are treatments that show an antagonism effect, and this effect is often undesirable [9]. Research is still ongoing to understand the mechanism of action of antagonism, because logically antagonism, especially strong antagonism that limits the response, may also limit the effectiveness of the drug combination [10]. Present study aimed to prepare an antibiotic substitute targeting resistant bacteria and synergistic effect between Bacteriogenic SNPs and some antibiotics.

MATERIALS AND METHODS

Samples collection

The most resistant-bacteria to SNPs were isolated from soil and identified using built-in vitek2 system. Two sterile beakers containing 50 ml distilled water (DW). Each flask was supplied with 0.3 gms of soil sampled from two different areas separately and mixed well. 200ul from each flask were inoculated into SNPs – Enriched Brain Heart Infusion (BHI) agar plates were incubated at 37°C for a duration of 24 hours. Growing bacterial colonies were selected, Identified and used as a catalyst. One liter of BHI broth was prepared according to the manufacturer's instructions, inoculated with a colony of the screened silver resistant bacteria (*Enterobacter sp.*) and incubated for 24 hrs. at 37C, Cell free extract (CFE) harvested by filtration of the culture through fast filter paper θ11cm and used as a catalyst for synthesis of SNPs [11].

Preparation of chemicals

Preparation of $AgNO_3$ stock solution One molar (1M) silver nitrate stock solution prepared by dissolving 1.6987 g $AgNO_3$ in 10 ml of reagent-grade deionized distilled water. One liter of 10 mM $AgNO_3$ working solution was prepared by dilution of 10 ml of 1 M stock solution to 1000 ml with reagent-grade deionized distilled water. Preparation and storage of $AgNO_3$ was performed in dark condition (its preferred to prepare and use rather than storge of $AgNO_3$ solutions to avoid photolytic reactions) [11].

Preparation of NaOH Five ml of 2M NaOH have been prepared by dissolving 0.39997 g of pure NaOH in final volume 5 ml deionized water.

Preparation of cell-free extract CFE 1.4ml of 10mM $AgNO_3$ was added to 900ml of reagent-grade distilled water. The solution was placed on a magnetic stirrer (60 °C, 200 RPM). Then, 98.6ml of the CFE product was gradually added (dropwise addition) to the solution along one hour [10].

Preparation of gentamycin 900ml of distilled water was taken and 1.4ml of 10 mM $AgNO_3$ was added to it, and it was placed on a magnetic mixer, where 100ml of the antibiotic gentamicin was added to it, drop by drop for an hour, and then it was placed in the incubator for 4 days. Then, two substances were added gradually: (100 ml of CFE) and (100 ml of gentamicin) to the reaction mix, the PH adjusted for 8 by NaOH solution and they were incubated at 37 °C for 4 days a shaking incubator at 150 RPM [11].

Synthesis of SNPs

Cell free extract was titrated against 10 mM $AgNO_3$, in a ratio of 1:3 (v/v) to acquire 1L of

reaction solution on heated magnetic stirrer 200 rpm and monitored for trans coloration. During this period, for 1h intervals and examined spectrophotometrically at 420 nm using U.V.-visible spectrophotometer, after 4 days, reaction solution centrifuged at 16000 xg and washed three times using deionized distilled water, by repeating resuspension-centrifugation step. The precipitated SNPs was dehydrated (desiccated) in an oven at 60 °C. Finally, the dehydrated AgNPs, collected for further physicochemical and biological characterization [11].

Characterization of Bacteriogenic SNPs

Physicochemical characterization. Bacteriogenic SNPs was indicated by color change and U.V. – visible spectroscopy; FESEM for morphology, homogeneity, and size distribution; Zeta potential for dispersity in colloids [12,13].

Antimicrobial activity: Antimicrobial activity was examined using Kirby Bauer method. SNPs were used against three types of bacteria, and SNPs were used with the antibiotic (gentamicin) also against resistant bacteria [14,15].

Erythrocyte toxicity (Biocompatibility) of bacteriogenic silver nanoparticles (SNPs) was assessed through an erythrocyte lysis assay. Human blood was sampled into heparinized tubes, and RBCs were separated by centrifugation (Eppendorf Minispin, Germany) at 2200 rpm for 10 minutes at 4 °C. sediment was carefully washed three times with phosphate buffer saline (pH 7.4), and re-suspended in the same buffer, and subsequently utilized for the test. For experimental purposes, 500 µL of SNP colloidal dispersion or 1% Triton w/v used as (positive control) was added to 500 µL of RBCs suspension. The suspension then incubated at 37 °C for one hour with continuous shaking. Post-incubation, the tubes were centrifuged. Supernatant was spectrally analyzed at 540 nm by UV–visible spectrophotometry (Shimadzu, Japan) to quantify hemolysis [15]. The supernatant from untreated blood served as the blank. All assays were triplicated, and the ratio of RBCs cytotoxicity

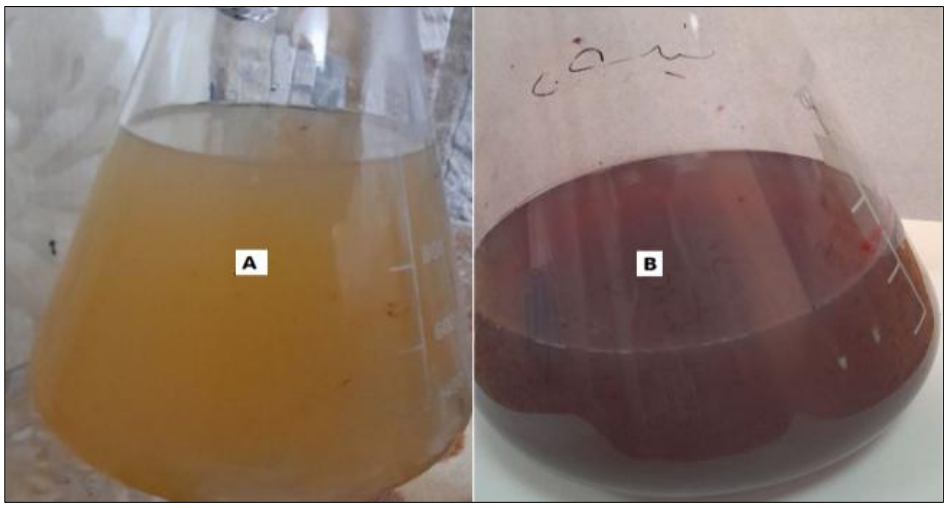

Fig. 1. Trans-coloration indicates synthesis of SNPs in the CFE reaction solution. (A) nucleation. (B) growth of SNPs.

Table 1. Change in absorbance at λ 420nm with time in the course of SNPs synthesis, reflecting nucleation, growing, and maturation of SNPs.

Absorbance (λ420 nm)	Time \ Hrs.
0.644	1.0
0.678	1.5
0.692	2.0
0.740	3.0
0.790	4.0

(cell viability index) was computed using the following formula:

$$\text{Heamolisis\%} = \frac{(\text{ODsample} - \text{ODnegative control})}{(\text{ODpositive control} - \text{ODnegative control})} \text{X } 100 \quad (1)$$

RESULTS AND DISCUSSION

Colorimetric evidence through reaction period trans coloration from yellowish to brown of the reaction solution indicate formation of silver nanoparticles (Fig. 1) and the gradient density of the brown color profiles the nucleation and growing of the nanoparticles.

Spectrophotometric (U.V.-Visible) analysis of the reaction mix, revealed an optical absorption peak at approximately 420nm. This finding aligns with previous research, specifically the work of Xu and coworkers, which found that absorbance at λ 420nm was linked with the reduction of ionic (Ag+) to atomic (Ag°). The brown color suggests formation of AgNPs this finding was in accordance

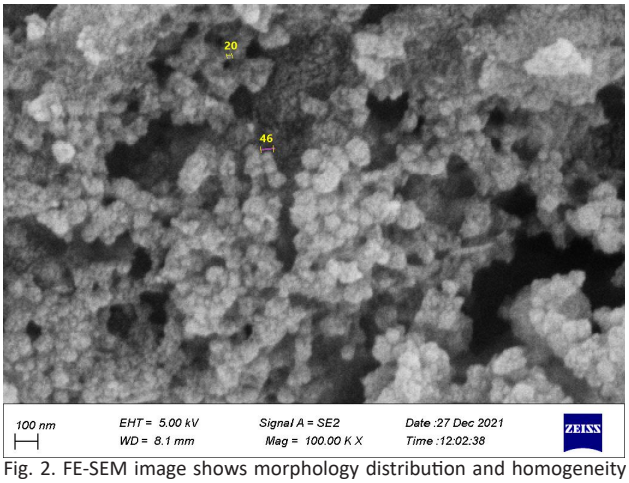

Fig. 2. FE-SEM image shows morphology distribution and homogeneity and size of bacteriogenic SNPs.

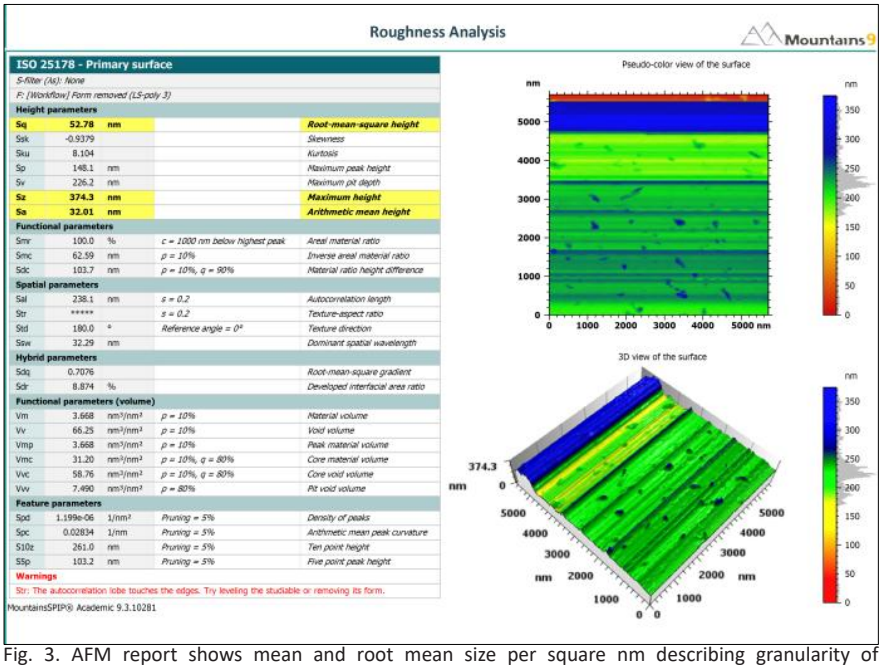

Fig. 3. AFM report shows mean and root mean size per square nm describing granularity of bacteriogenic SNPs.

wth that of Xu and colleagues in 2020 [13].

FE-SEM Results

Interpretation of FE-SEM image (Fig. 2) shows homogeneously shape distributed spherical SNPs with an average size of approximately 33 nm. These results are within the range consistent with the majority of researchers, as these properties obtained from bacteriogenic SNPs are considered ideal for biological applications and are consistent with the results of the researcher [12,16].

Results of Atomic Force Microscopy AFM

AFM analysis revealed size distribution indicated by arithmetic mean height RMH 32 nm (Fig. 3), and root means require height RMSH 52.78. Homogeneous mesh improves the biomedical application of bacteriogenic SNPs was supported by the work of Al-Turnachy 2018, Xu 2020, and Luna 2021 [12,13,15].

Antibacterial activity

Antibacterial activity of bacteriogenic SNPs produced by *Enterobacter sp.* has been assayed using agar well diffusion method (Kirby-Bauer). Five concentrations (4.5, 1.8, 0.7, 0.3 and 0.1) mg/ml were examined against antibiotic resistant members of both gram-negative and gram-positive isolates (*Staphylococcus sp. Klebsiella sp.*), and synergism between SNPs and garamycin. Results showed MIC (0.4.5mg/ml) for SNPs against both (*Staphylococcus sp. Klebsiella sp.*) and stronger activity against G+ bacteria represented by *staphylococcus sp*. For garamycin, MIC was (0.28 mg/ml) against *Staphylococcus sp.*, and (1.8 mg/ml) against *Klebsiella sp*. MIC for SNPs-Garamycin

combination revealed (1.8 mg/ml) for both G+ and G- represented by (*Staphylococcus sp. Klebsiella sp.*) respectively, stronger activity against G+ bacteria represented by *staphylococcus sp*. Antagonistic activity of SNPs against Garamycin was

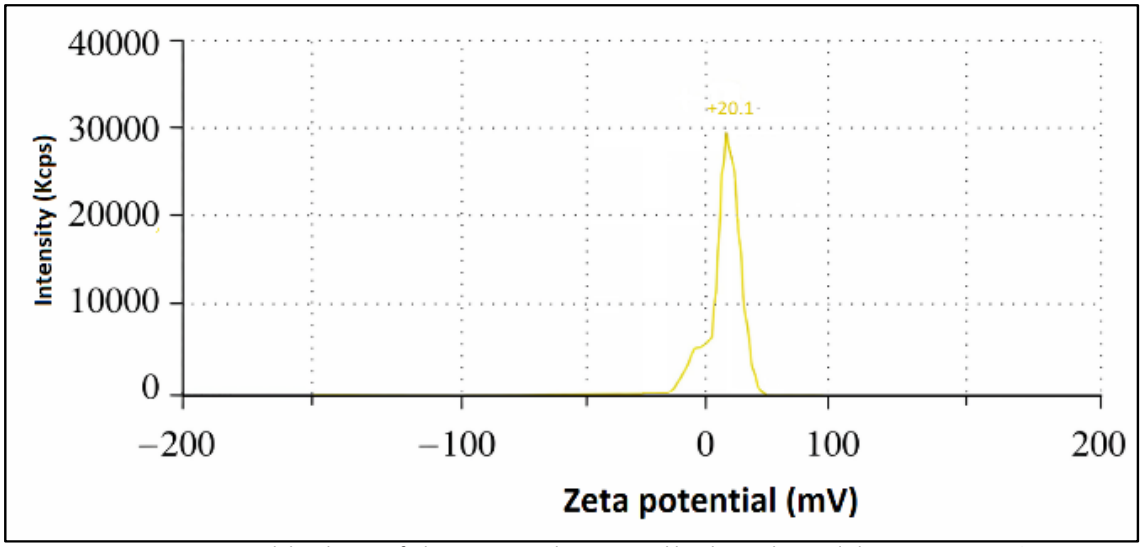

Fig. 4. Zeta potential distribution of silver nanoparticles measured by electrophoretic light scattering at 25 °C.

Table 2. Antibiogram of SNPs and Garamycin against *Klebsiella sp.*, and *Staphylococcus sp*.

Bacteria	Material	Concentration mg/ml				
		0.1	0.3	0.7	1.8	4.5
SNPs	*Staphylococcus*	R	R	R	R	20
	Klebsiella	R	R	R	R	10
Gentamycin	*Staphylococcus*	R	12	18	20	26
	Klebsiella	R	R	R	11	16
SNPs And Gentamycin	*Staphylococcus*	R	R	R	21	22
	Klebsiella	R	R	R	10	16

Table 3. Clarify Hemolysis index HI (Hemolysis%) at different AgNPs concentrations.

Concentration	HI (Hemolysis%)±SEM
1 mg/ml	5.84 ±0.425
0.5 mg/ml	4.59±0.425
0.1 mg/ml	4.24±0.425

recorded at concentration (0.3 mg/ml), (0.7 mg/ml) (Table 2). While weak synergism has recorded for staphylococcus between SNPs and Garamycin (4.50 mg/ml). For Klebsiella, neither synergism nor antagonism was recorded at concentration (4.50 mg/ml) these results found to be disagreed with the finding of Dove and coworkers their findings concluded that there was synergistic mechanism between AgNPs and Aminoglycosides [17-20].

Blood biocompatibility (RBCs toxicity Assay)

Cytotoxicity of silver nanoparticles (SNPs) was assessed in vitro using human erythrocytes. Hemolysis Index (HI) was quantified by measure the absorbance at 540 nm. Findings revealed that at concentration 1 mg/ml of bacteriogenic SNPs, hemolytic Index (HI) recorded a highest value (5.84±0.425), and at 0.5mg/ml (4.59±0.425), while at 0.1mg/ml it was recorded the lowest HI (4.24±0.425) (Table 3). However, hemolytic index found increased proportionally with AgNP concentrations, suggesting membrane disruption as an underlying mechanism. These findings highlight the importance of evaluating erythrocyte cytotoxicity alongside antibacterial activity. Luna and colleagues finding support this finding [21].

Zeta potential

Results of Zeta potential for SNPs revealed (+21.1 mV) (Figure 4). Zeta potential serves as a crucial technique for assessing the stability of dispersion in the colloidal solutions, indicating the balance between repulsive and attractive electrostatic forces among charged silver nanoparticles (SNPs). Particles exhibiting stronger repulsive forces establish more powerful Brownian motion these results supported by the findings of Ntolia and coworkers [22].

CONCLUSION

The results of this study concluded that the bacterial extract that stimulates the manufacture of nanoparticles supports the antimicrobial activity, as the bacterial biproducts present in the cell free extract protect and synergize the biomedical potential of the SNPs and cover the nanoparticles, but when silver nanoparticles are mixed with the antibiotic gentamycin, an antagonistic interaction appears, which reduces the effectiveness of the SNPs. Nano silver and also the antimicrobial activity of gentamycin [19].

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Bouafia A, Laouini SE, Ahmed ASA, Soldatov AV, Algarni H, Feng Chong K, et al. The Recent Progress on Silver Nanoparticles: Synthesis and Electronic Applications. Nanomaterials. 2021;11(9):2318.
2. Verma S, Verma S, Abbas M, Mahdi F. COMBATING THE ANTIMICROBIAL RESISTANCE BY PERSONALIZED MEDICINE: A MINI-REVIEW. Era's Journal of Medical Research. 2023;10(01):88-92.
3. Combating Antimicrobial Resistance and Protecting the Miracle of Modern Medicine: National Academies Press; 2022.
4. Nelson RE, Hatfield KM, Wolford H, Samore MH, Scott RD, Reddy SC, et al. National Estimates of Healthcare Costs Associated With Multidrug-Resistant Bacterial Infections Among Hospitalized Patients in the United States. Clin Infect Dis. 2021;72(Supplement_1):S17-S26.
5. Assessment of Silver-Nanoparticles-Induced Erythrocyte Cytotoxicity through Ion Transport Studies. Cellular Physiology and Biochemistry. 2019;53(3):532-549.
6. Gherasim O, Puiu RA, Bîrcă AC, Burduşel A-C, Grumezescu AM. An Updated Review on Silver Nanoparticles in Biomedicine. Nanomaterials. 2020;10(11):2318.
7. Morales-Durán N, León-Buitimea A, Morones-Ramírez JR. Unraveling resistance mechanisms in combination therapy: A comprehensive review of recent advances and future directions. Heliyon. 2024;10(6):e27984.
8. Pachori P, Gothalwal R, Gandhi P. Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. Genes and Diseases. 2019;6(2):109-119.
9. Richards R, Schwartz HR, Honeywell ME, Stewart MS, Cruz-Gordillo P, Joyce AJ, et al. Drug antagonism and single-agent dominance result from differences in death kinetics. Nat Chem Biol. 2020;16(7):791-800.
10. Savvidou MG, Kontari E, Kalantzi S, Mamma D. Green Synthesis of Silver Nanoparticles Using the Cell-Free Supernatant of Haematococcus pluvialis Culture. Materials. 2023;17(1):187.
11. Taheri-Araghi S. Synergistic action of antimicrobial

peptides and antibiotics: current understanding and future directions. Front Microbiol. 2024;15.
12. Al-Turnachy HHH, Aldujaili NH. Isolation of metallic Klebsiella Pneumoniae HHHF1 potent for AgNPs nanobiosynthesis from iron-rich soil samples. AIP Conference Proceedings: AIP Publishing; 2020. p. 020039.
13. Xu L, Wang Y-Y, Huang J, Chen C-Y, Wang Z-X, Xie H. Silver nanoparticles: Synthesis, medical applications and biosafety. Theranostics. 2020;10(20):8996-9031.
14. Wang H, Zhang G, Mia R, Wang W, Xie L, Lü S, et al. Bioreduction (Ag+ to Ag0) and stabilization of silver nanocatalyst using hyaluronate biopolymer for azo-contaminated wastewater treatment. J Alloys Compd. 2022;894:162502.
15. Luna-Vázquez-Gómez R, Arellano-García ME, García-Ramos JC, Radilla-Chávez P, Salas-Vargas DS, Casillas-Figueroa F, et al. Hemolysis of Human Erythrocytes by Argovit™ AgNPs from Healthy and Diabetic Donors: An In Vitro Study. Materials. 2021;14(11):2792.
16. Jaafer MD, Turnachy HHA, Al-Khazali SMS, Al-khayatt AHO. Comparative of silver and gold nanoparticles synthesized by the chemical reduction method for antimicrobial applications. AIP Conference Proceedings: AIP Publishing; 2020. p. 050026.
17. Tavan M, Hanachi P, Mirjalili MH, Dashtbani-Roozbehani A. Comparative assessment of the biological activity of the green synthesized silver nanoparticles and aqueous leaf extract of Perilla frutescens (L.). Sci Rep. 2023;13(1).
18. Dolai J, Mandal K, Jana NR. Nanoparticle Size Effects in Biomedical Applications. ACS Applied Nano Materials. 2021;4(7):6471-6496.
19. Yin N, Ma W, Pei J, Ouyang Q, Tang C, Lai L. Synergistic and Antagonistic Drug Combinations Depend on Network Topology. PLoS One. 2014;9(4):e93960.
20. Dove AS, Dzurny DI, Dees WR, Qin N, Nunez Rodriguez CC, Alt LA, et al. Silver nanoparticles enhance the efficacy of aminoglycosides against antibiotic-resistant bacteria. Front Microbiol. 2023;13.
21. Luna-Vázquez-Gómez R, Arellano-García ME, Toledano-Magaña Y, García-Ramos JC, Radilla-Chávez P, Salas-Vargas DS, et al. Bell Shape Curves of Hemolysis Induced by Silver Nanoparticles: Review and Experimental Assay. Nanomaterials. 2022;12(7):1066.
22. Ntolia A, Chatzigiannakou T, Michailidis N, Aggeli A. A Comprehensive Physicochemical Characterization of Silver Nanoparticles as a Prerequisite for Their Successful Biomedical Applications. Inorganics. 2025;13(10):341.