



Antibacterial Estimation of PEG-Lated Silver Nanoparticles (Chemical Synthesis) Against Multidrug-Resistant *Escherichia coli*

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Abstract

Background: The nano state of silver nanoparticles (Ag Nps), have made it possible to enhance the antimicrobial properties of silver ions, which are widely used in various mediations specially antimicrobials. **Objectives:** The study was aimed to synthesize Ag Nps using chemical coating with PEG, characterize it and assess their antimicrobial activity against MDR *Escherichia coli*. **Methodology:** An experimental study was carried out in the Department of Applied Biotechnology, College of Biotechnology, Al-Qasim Green University, Iraq. From October 2024 to February 2025. Ag Nps have been prepared using the chemical reduction method and coated with PEG, the resulted Ag Nps was confirmed using SEM and Uv-Vis Spectrophotometer, a Multiple Drug Resistance *E. coli* have been used to assess the inhibitory effect of the obtained Ag Nps using an in vitro hole diffusion approach. **Results:** A golden color was formed after adding the reducing agent to the silver salt, confirming the formation of Ag Nps, Uv-Vis Spectrophotometer obtained at 410, SEM have shown spherical shape with about 50 nm in diameter. Compared with various antibiotics, Ag Nps coated PEG had shown an enhanced ability to inhibit the Gram-negative MDR *Escherichia coli*. **Conclusion:** Due to its ultrafine size, Ag Nps coated PEG can provide an enhanced inhibitory effect against MDR *Escherichia coli*, compared with conventional antibiotics, which may attract the attention of researchers to use it in various medical applications. [Bangladesh Journal of Infectious Diseases, June 2026;13(1):203-207]

Keywords: Silver; Nanoparticles; PEG; MDR; *Escherichia coli*

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Introduction

Due to their novel properties, metallic nanoparticles have attracted the attention of many scientists to use that novelty in medical fields. Researchers have improved, over the last few decades, the unique properties of nanomaterials in medical applications, where size-dependent properties are the key issue that nanoscience depends on¹. Scientists have manufactured various nanomaterials and applied them in various medical applications, including

drug delivery², cancer treatment, diagnosis, wound healing, regenerative medicine³, biosensors, and antimicrobials⁴. Myriad researchers have focused on nano-antimicrobials due to their promising result to fight multiple drug resistant bacteria (MDR). Metal nanoparticles among these nanomaterials have been highly recommended by researchers due to their biocompatibility, easy to synthesis, and the ability to produce high quantity with cost effective. Silver ions have been implemented as an

FDA-approved antimicrobial, where it have been used in various medications as a therapeutic molecules⁵. Ag Nps have been applied as therapeutic molecules to fight the notorious phenomenon of MDR. Because of the increasing surface area compared with the volume ratio, silver ions are concentrated on the very end surface of Ag Nps⁶. This has given the nano state of silver the privilege to produce higher concentrations of silver ions to interact with the bacterial cell membrane and inhibit it through many mechanisms, including the production of Reactive Oxygen Species (ROS), which cause oxidative stress that leads to damage of cellular lipids, proteins, and nucleic acids⁷. DNA damage and inhibition of replication: Ag Nps can enter bacterial cells and interact with DNA. This interaction affects the central dogma and leads to cell death. Inhibition of protein synthesis: AgNPs can bind to ribosomal molecules and impair translation and stop the manufacture of important proteins vital for bacterial survival⁸.

Interference with cellular respiration, the existence of AgNPs plays a critical role in disturbing the electron transport chain and respiratory enzymes of the bacterial cell, which reduces ATP assembly and disturbs energy metabolism, which consequently leads to cell apoptosis⁹. Inhibition of biofilm formation, where AgNPs prevent bacterial aggregation on surfaces, and they inhibit biofilm formation. This may improve their effect against MDR bacterial infections¹⁰. We aimed in this research to chemically synthesize AgNPs coated with PEG, characterize them, and estimate their effect against MDR *Escherichia coli*.

Methodology

Study design: Experimental work had been carried out in the Department of Applied Biotechnology, College of Biotechnology, Al-Qasim Green University, Iraq, from October 2024 to February 2025. Bacterial samples had been collected from Al-Hashimia hospital and used for further experiments and used for further experiments.

Synthesis of Silver Nanoparticles: AgNPs coated with PEG have been prepared in this study using a chemical reduction method called the borohydride method¹¹. Where 150 ml aqueous of NaBH₄ 0.002 mM was added drop wise to 150 ml of 0.02 mM AgNO₃ with ambient conditions in magnet steering instrument, once the color began to change 2.5 mM of 400-PEG have been added to the interaction, until yellowish or pale gold, in room temperature., followed by continuing steering for one hour in

room temperature, then used further in the research. The above-obtained PEG-Lated AgNPs were characterized using a UV-Vis spectrophotometer and SEM.

***Escherichia coli* MDR Evaluation:** After collecting *Escherichia coli* from Al-Hashimia, it was resuspended in blood agar media via Petri Dish. Using three different antibiotics, 20 mg of tobramycin, 20 mg of amoxicillin, and 20 mg of cefotaxim were placed on the media and kept overnight at 37°C in the incubator.

Antimicrobial Assessment of the PEG-Lated AgNPs: Using well diffusion method three holes have been made and three different concentrations had been prepared 100% , 50% and 25% of the obtained PEG-Lated AgNPs were placed in the holes, the plate was kept overnight in the incubator at 37°C and the results were measured to estimate the inhibitory concentration of the PEG-Lated AgNPs.

Ethical Consideration: Ethical approval for the study was obtained from the Institutional Review Board. All methods were performed in accordance with the relevant guidelines and regulations.

Results

PEG-Lated AgNPs Synthesis and Characterization: The golden color formation of AgNPs indicated the synthesis of AgNPs, a peak at 410 nm obtained using a UV-visible spectrophotometer also confirmed the synthesis of AgNPs (Figure I).

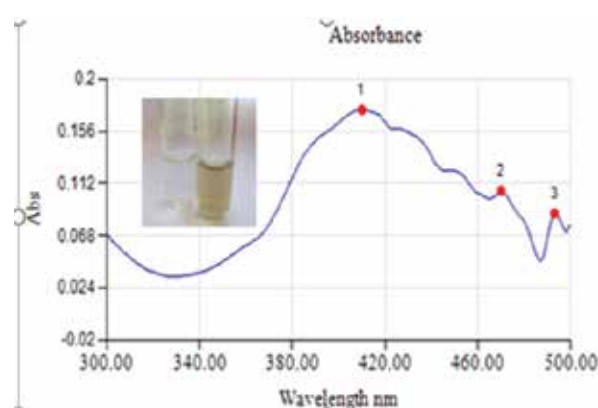


Figure I: Color change of the PEG-Lated AgNPs to the golden yellow and absorbance peak at 410 as indicator the formation of AgNPs

SEM was also used to measure the size and understand the morphology, which shows a size of about 60 nm in diameter with spherical morphology (Figure II).

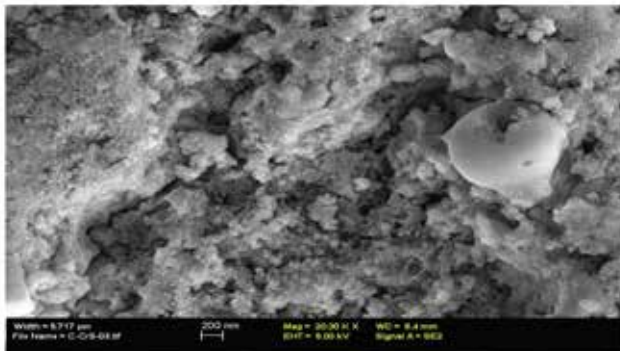


Figure II: SEM, Scanning Electron Microscopy, showing the size and morphology of PEG-Lated AgNPs

MDR Evaluation: After 24 hrs incubation at 37°C, *Escherichia coli* was tested with three different antibiotics such as 20 mg of tobramycin, 20 mg of amoxicillin, and 20 mg of cefotaxime. No zone of inhibition has been observed (Figure III).



Figure III: MDR of *Escherichia coli*

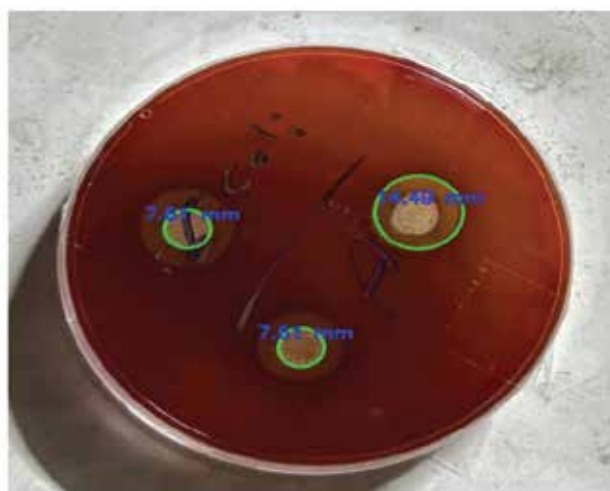


Figure IV: Zones of inhibition of PEG-Lated AgNPs in different concentrations

Antimicrobial Assessment Against *Escherichia coli*: With three different concentrations, the PEG-Lated AgNPs had shown clear different zones of inhibition, such as 7.61 mm, 7.61 mm, and 14.49 mm, as they are clearly measured (Figure IV). by MIC testing were resistant to nalidixic acid, whereas all ciprofloxacin-sensitive isolates were susceptible to nalidixic acid. These findings demonstrate that nalidixic acid disk testing serves as a reliable surrogate marker for detecting reduced ciprofloxacin susceptibility and may be particularly useful in resource-limited laboratories where routine MIC determination is not feasible (Table 3).

Discussion

Metal nanoparticle synthesis using the reduction method have been recommended by a lot of researchers because of its easy to synthesize, cheap, controllable, and the capability to produce large quantities¹². Various methods have been reviewed and innovated to synthesize metal nanoparticles, including the chemical reduction method. When the interaction proceeds between any ionic state and its salt with a reducing agent, and by controlling PH, temperature, and time agent the result is metallic nanoparticles. The reducing agents vary; they can be chemicals, plant extracts, or bacterial or fungal metabolites; in other words, any biological materials that has reduction molecules to reduce the ionic state to the metallic state¹³.

Based on the above derivative silver nitrate have been reduced using sodium borohydride in aqueous media in room temperature, the solution of silver nitrate is colorless, but when sodium borohydrae have been added drop wise a color of pale yellow have been formed, which indicated the reduction of the ionic state of silver nitrate to the nano state of silver which was the same result found by Padole et al 2022¹⁴. A stabilizing and capping agent PEG, has been added to coat AgNp. The absorption peak at 410 of the UV-visible spectrophotometer has indicated the total conversion of silver nitrate to AgNp¹⁵, SEM have confirmed the formation of PEG-Lated AgNPs as spherical shapes with size 60 nm in diameter. The size of the nanoparticle is crucial to achieve mechanisms to overcome the notorious phenomenon of MDR¹⁶.

Tobramycin, Amoxicillin, and cefotaxime discs have been used to test the MDR of the E-coli, no zone of inhibition has been noticed, confirming the MDR of the *Escherichia coli* strain, due to the existence of virulence factors¹⁷. The whole diffusion method has been used to test the ability of the PEG-Lated AgNPs to inhibit the MDR of *Escherichia coli* at different

concentrations, where three different concentrations have been prepared and used 100.0%, 50.0% and 25.0% of the obtained PEG-Lated AgNPs. The biggest zone of inhibition was noticed in 100.0% concentration 14.89 mm while both concentration 50.0% and 25.0% had the same zone of inhibition 7.61 mm, that could be contributed to threshold effect where the concentration of 25.0% and 50.0% didn't produce enough silver ions, while at 100.0% concentration have enough silver ions to obtain significant zone of inhibition. The ultrafine size of the PEG-Lated AgNPs has made it possible to penetrate and overcome the defense mechanisms that MDR *Escherichia coli* have developed. There are a few mechanisms that explain the ability of the PEG-Lated AgNPs to inhibit the MDR of *Escherichia coli* cells. The interaction of the NP with the cell membrane through electrostatic force¹⁸. This will cause rupture to the cell membrane and leakage of the intercellular contents, cell denaturation, and instability of the cell membrane¹⁹. ROS, or generation of Reactive Oxygen Species²⁰, which stimulate to produce free radicals such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals²¹. The high condensates of silver ions on the surface of silver nanoparticles enabled the release of silver ions, which are highly reactive molecule which bind to the thiol group SH in cell membrane proteins²², disturb ATP production and deactivate essential enzymes deactivation²³. The small size of the of the PEG-Lated AgNPs could penetrate into the bacterial cell and interact with DNA, causing damage to the central dogma, DNA condensation, and disturbing gene expression²⁴. Due to the small size of the AgNPs it can destruct the biofilm formation causing penetration to the existing biofilm, extracellular polymeric substances disruption²⁵.

Conclusion

Silver nanoparticles have been successfully synthesized and coated with PEG using the reduction method. The characterization of the PEG-Lated AgNPs have shown 60 nm in diameter and an absorbance peak at 410 which confirm the successful synthesis if the NP. The cytotoxic effect of the PEG-Lated AgNPs have been estimated against MDR *Escherichia coli*. After confirming the MDR of *E-coli* with various antibiotics, the PEG-Lated AgNPs have shown significant zone of inhibition after 24 hours of incubation at different concentrations. The results showed good ability of the obtained PEG-Lated AgNPs to inhibit the growth of *E-coli* as we recommend using this formula in various biomedical applications.

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Conflict of interest:

The Author has declared no conflict of interest in this work.

Financial Disclosure

No funding was received for this work

Authors' contributions

Mohammed Shawket Bairman has supervised the work, including protocol development and protocol finalization. Mustafa Abed AL-Jabber and Abdulrazzaq Muthanna Adeeb have collected the bacterial samples and synthesized the nanoparticles. All authors approved the final manuscript.

Data Availability

Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate

Ethical approval for the study was obtained from the Institutional Review Board. All methods were performed in accordance with the relevant guidelines and regulations.

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