Evalutation of Antimicrobial Activity of sliver nanoparticles on S. aureus in vitro study

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Abstract. Nowadays, the researchers search in their studies for new antimicrobial agents which have the unique physical and chemical properties. In this experiment, AgNPs solution was synthesized by chemical reduction, characterized and tested as the antibacterial effects of sliver nanoparticles (Ag-Nps) on S. aureus. Sliver nanoparticles revealed unique physical and chemical properties that suitable to understand to possess a broad spectrum of antimicrobial activities. Sliver nanoparticles (AgNPs) characterized by UV-Visible spectrophotometry by exhibiting the typical surface plasmon absorption maxima at 400 nm. The morphology of the sliver nanoparticles was measured by Scanning Electron Microscope (SEM). Investigating Antimicrobial activity of nanoparticles were evaluated by using Minimum Inhibitory Concentration (MIC) technique. The experiment results showed that the lowest MIC and MBC of Ag-NPs to S. aureus was 30 ppm, 300 ppm, respectively. The obtained results suggested that Ag-NPs exhibit bacteriostatic and bacteriocidal effect towards S.aureus. The present study indicates (AgNps) has considerable antibacterial activity.

Key words : Sliver nanoparticles, chemical reduction method, S. aureus

Introduction

Nanostructured materials are attracting a great deal of attention because of their potential of achieving specific processes. (1-3). Therefore, a confluence of nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine (4-5). (Ag-NPs have distinctive physical and chemical properties, such as, chemical stability, high thermal and electrical conductivity, nonlinear optical behavior, surface-enhanced Raman scattering and catalytic activity (6-7). These properties made this nanoparticles to the top of the priority list, to be used in inks, in electronics, and of course biology (8-12). (For a long time silver ion has been used as antimicrobial agent since time immemorial in the form of metallic sliver nitrate, sliver sulfadiazine for the treatment of wounds, burns due to its strong inhibiting effect on bacteria, Viruses, fungi, protozoa (13-22).

Due to their proven antimicrobial properties, Ag-NPs are widely used in the daily used commercial products, such as cosmetics, detergent, plastics, food packaging, soaps, pastes, food, and textiles, which has increased their market value to a great extent (23-25).
Experimental

*S. aureus* was isolated by rubbing a sterile cotton swab over oral mucosa from patients at the Department of Pediatric and preventive Dentistry, Faculty of Dentistry, University of Babylon. *S. aureus* were cultured on blood agar Difco, USA), then streaking on mannitol salt agar. In this study, isolated of bacteria. The bacteria were grown in the nutrient broth at 37°C, using Nutrient agar slants for sub culturing and preserved in refrigeration at 4°C.

preparation of silver nanoparticles:

synthesis of silver nanoparticles was carried out by using a volume of 100 ml silver nitrate solution with 1 x 10⁻⁴ mol/l was mixed with 300 ml of 2 x 10⁻² mol/l sodium borohydride both solution was immersed in ice bath. The reaction protected from light. After the addition of silver nitrate solution was finished, the mixture was than vigorously stirred during 15min.

Characterization of silver nanoparticles:

Sliver nanoparticles were characterized spectrophotometrically using vis spectroscopy analyses. Scanning electron microscopy (SEM) has been employed to characterize the shape and morphologies of formed synthesized of AgNPs. (26)

Antimicrobial activity of silver nanoparticles: Determination of Minimum inhibitory concentration and Minimum Bactericidal concentration.

*S. mutans* were prepared by picking colonies from nutrient agar (24h) and using sterile saline (0.85%) v/v Nacl to determine turbidity equivalent to 0.5 McFarland standard (1 x 10⁸) colony forming units/ml.

Broth dilution method was followed for measurement of MIC values. Different concentration of nanoparticles sliver solution (10,20, 30, 40, 50, 100, 200, 300, 400 ppm) were added to LB broth medium, the test tubes were incubated aerobically at 37°C. The MIC values (Bacteriostatic) were estimated the lowest concentration in the test tube that showed no turbidity (growth of isolate) after incubation. The minimum bactericidal concentration (MBC) was evaluated through sub culturing from each test tube showing no apparent growth on the agar after further 24hr incubation.

Results and Discussion

UV- visible analysis

The absorption spectrum was measured immediately after preparation. UV – visible spectroscopy is one of the most widely and sensitive techniques for structural characterization of silver nanoparticles synthesis. Figure (1) shows the maximum absorption peak was observed at about 413nm which is the characteristic absorption peak for Ag nanoparticles.
Figure (1) Uv – Vis spectra . The maximum absorbance was at 413 nm

SEM analysis :

SEM was used for observing the morphology of synthesized AgNPs. Fig.(2) shows the SEM images of AgNPs. As , It can been seen that the inner diameter of the AgNPs is ranging beteewn (65-72 )nm .

Figure (2):- SEM image of Ag nanoparticles.
Antimicrobial activity of sliver nanoparticles

In present investigation, antimicrobial activity of sliver nanoparticles of (AgNps) was evaluated against *S. aureus* using broth dilution technique with different concentration. Table (1) showed the antimicrobial properties of Ag-NPs were studied against *S. aureus*. The Minimum inhibitory concentration of Ag-NPs against *S. aureus* was found 30 ppm. While the Minimum bactericidal count of Ag-NPs against *S. aureus* was found 300 ppm.

**Table (1):** Determination of MIC and MBC for nanoparticles

| Isolate  | Con (ppm) | Visible growth in tubes | MIC (ppm) | MFC (ppm) |
|----------|-----------|-------------------------|-----------|-----------|
| *S. aureus* | 10        | Turbidity               | 30        | 300       |
|          | 20        | Turbidity               | 30        | 300       |
|          | 30        | No turbidity            | 30        | 300       |
|          | 40        | No turbidity            | 30        | 300       |
|          | 50        | No turbidity            | 30        | 300       |
|          | 100       | No turbidity            | 30        | 300       |
|          | 200       | No turbidity            | 30        | 300       |
|          | 300       | No turbidity            | 30        | 300       |
|          | 400       | No turbidity            | 30        | 300       |

Similar results have been reported on some bacteria and fungi (28-31).

Some authors demonstrate different values in MIC results for determining antimicrobial activity of sliver nanoparticles against bacteria and fungi due to general physiological differences in the cell wall membrane of microorganisms (31). Indeed to that other scientific studies referred to Ag NP activity depend on the their concentration, size and shape (32-35).

Also antimicrobial activity of sliver ions bind to the protein and nucleic acid negatively charged, causing structural changes and deformations in the (wall, membranes, nucleic acids). Sliver ions interact with a number of electron donor functional groups such as hydroxys, imidazoles, thiols, phosphates and indoles. Also AgNPs induce and release of reactive oxygen species (ROS), forming free radicals. AgNPs can enter the bacteria or fungi and damage of its cellular structures, inhibition of protein synthesis as a result of ribosomes denaturation, as well as translation and transcription will be blocked by the binding with genetic material of the bacteria (36-39).

**Conclusion:**

In this study, simple and effective method of synthesis of AgNPs solution using a chemical reduction method, was tested. The current interest in nano-materials is focused on the controllable properties of size and shape.

The study conclude that Ag-NPs have potent antimicrobial activities against tested *S. aureus*. Therefore, it was proposed as an alternative drug for antimicrobial activity and can inhibit the Gram positive bacteria growth.

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