Journal of Optoelectronic and Biomedical Materials Vol. 12, No. 4, October-December 2020, p. 95-99

RED SAND SYNTHESIZED SILVER NANOPARTICLES: CHARACTERIZATION AND THEIR BIOMEDICAL POTENTIAL

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Since the ability of bacteria to acquire resistance is increasing, it is important to find alternative therapeutics. One possible way to deal with this problem is the use of nanoparticles as possible alternatives to antibiotic therapy. Silver nanoparticles (AgNPs) are viewed as a novel type of antibacterial agents. AgNPs can be synthesized using raw materials, such as red sand that found in nature. Characterization of the AgNPs was achieved using variety spectroscopic and microscopic devises. AgNPs showed antibacterial activity and large effect when combined with different antibiotics against *Staphylococcus aureus, Acinetobacter baumannii, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa* and *Proteus vulgaris.*

Keywords: Silver nanoparticles, Red sand, Characterization, Antibacterial, Antibiotics

(Received January 12, 2020; Accepted October 2, 2020)

1. Introduction

Recently, researchers have paid special attention to the unique properties and applications of nanotechnology. Basavegowda and Lee [2] reported that silver nanoparticles (AgNPs) can be synthesized in various shapes with sizes ranging from 1 to 100nm. The effectiveness of nanoparticles can be attributed to a combination of their small size and their high surface-to-volume ratio[8]. These properties make AgNPs applicable in many fields. One application is using nanoparticles as therapeutic alternative to antibiotics. Khameneh *et al.* [4] stated that as a result of the misuse of antibiotics, multi-drug resistant bacteria have become a worldwide problem. Therefore, it is urgent to find novel approaches to overcoming issues associated with bacterial resistance[4,9,14]. Our aims were to synthesize AgNPs that could be applied simply and had high stability, and to study their antibacterial activity and their ability to enhance the effectiveness of antibiotics against various resistant bacteria. To our knowledge, this is the first study describing the preparation of AgNPs using red sand (RS) suspension.

2. Preparation of suspension

145.45g of RS was weighted and added to 100ml distilled water. The supernatant was collected and centrifuged at 20 rpm/2 min.

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2.1. Synthesis of AgNPs

A magnetic stirrer combined 40ml of the suspension and 10ml of sodium hydroxide (2g) at 45ºC/110rpm. Distilled water (20ml) with 20mg of dissolved silver nitrate ($AgNO_3$) was dripped into the mixture of sodium hydroxide and the suspension. The product was centrifuged then dried at 35ºC for 24 hours.

2.2. Characterization of AgNPs

The initial characteristics of the formed AgNPs were observed visually. Average diameter size and size distribution were obtained using dynamic light scattering (DLS). For the shape and morphology, a transmission electron microscope (TEM) was used. Energy dispersive X-rays (EDX) from the TEM were used to analyze the elemental composition, and X-ray diffraction (XRD) determined the crystalline structure.

2.3. Antibacterial activity

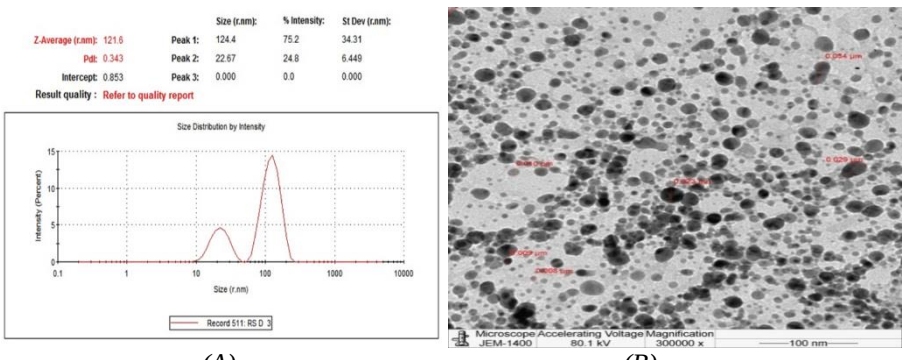
Antibacterial activity was tested against *Acinetobacter baumannii* (ATCC 19606*)*, *Salmonella typhimurium* (ATCC 14028), *E. coli* (ATCC 35218*)*, *Pseudomonas aeruginosa* (ATCC 27853*)*, *Staphylococcus aureus* (ATCC 25923*)* and *Proteus vulgaris*(ATCC 49132). Bacteria were grown on blood agar at 37ºC for 18 hours, colonies were suspended in saline (0.85% NaCl). Turbidity was adjusted to 0.5 MacFarland standards. The inoculum of bacterial suspension was swabbed on Muller-Hinton agar (MHA) plates. Wells were cut and 70µl of suspension were loaded. Plates were incubated at 37°C for 18-24 hours. Standard antibiotic discs like Gentamycin (CN 10µg), Augmantin (AMC 30µg) and Ciprofloxacin (CIP 5µg) were used as positive controls and for comparison of inhibition zones with the synthesized AgNPs from RS.

2.4. Combination of AgNPs and Antibiotics

The antibacterial effect of combining AgNPs with antibiotics was achieved by again adjusting the turbidity of bacteria to 0.5 MacFarland standards, then swapped on MHA plates. Antibiotic were used as a control. AgNPs (30µl) were loaded on the antibiotics discs placed on the swabbed medium. Plates were incubated for 24 hours at 37ºC.

3. Results and discussion

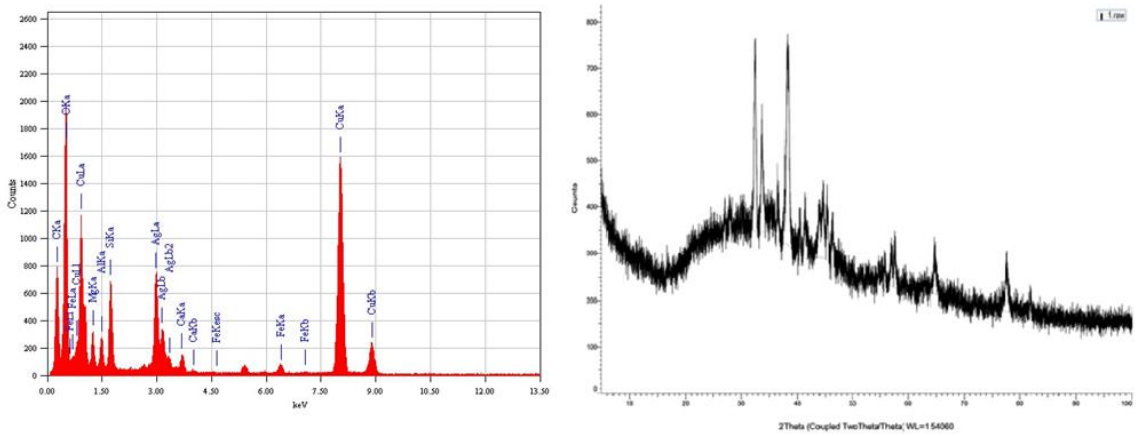
AgNPs were synthesized using RS suspension reacting with 2.5mM of $AgNO_3$, a yellowish color gradually changed to brown under continuous stirring after 1 hour indicating the reduction of silver and the formation AgNPs[15]. DLS analysis gave the average particle size as 121.6nm, PDI was 0.3 (Figure1-A), According to Stetefeld *et al.* [18], the results reveal a poly-dispersed nanoparticles, of diverse sizes with few agglomerations. TEM (Figure1-B) confirmed spherical shaped nanoparticles, with agglomeration as a product of the capping layer of the RS suspension used in the synthesis of AgNPs. The results agreed with the DLS analysis.



(A) *(B)*

Fig. 1. (A,B). (A) DLS spectrum, (B) TEM micrograph.

EDX (Fig. 2-A) showed peak absorption at 3 keV confirming silver in the suspension. Copper in the range 7.5- 9.0 keV, may have come from the grid used for the analysis. Other elements like carbon, iron, magnesium, aluminum, silica and calcium were also observed, and could be components of the RS suspension[19]. To confirm the crystalline nature of the AgNPs, XRD analysis was performed. (Fig. 2-B) showing 2θ ranging from 10° to 90°, with peak values of 32.5°,38°, 46°,55.5°, 58° and 64° confirming the presence of silver with AgNPs a face-centered cubic structure[13,1,6,7]. 2θ values of 32°, 38° and 58° may be correspond to the presence of copper as previously reported[10].Values of 37°,47° may show zinc as indicated by Kolekar *et al.* [5]. These results agreed with the EDX analysis.



(A) *(B)*

Fig. 2. (A,B): (A)EDX analysis, (B)XRD pattern.

The RS suspension showed no antibacterial activity, while the AgNPs synthesized from RS showed a maximum inhibition zone in *E. coli* of 14 mm, followed by *P. aeruginosa*, *S. typhimurium*, *P. vulgaris*, *A. baumannii* and *S. aureus* 13.5,13,12,11 and 9.5mm, respectively (Fig. 3).

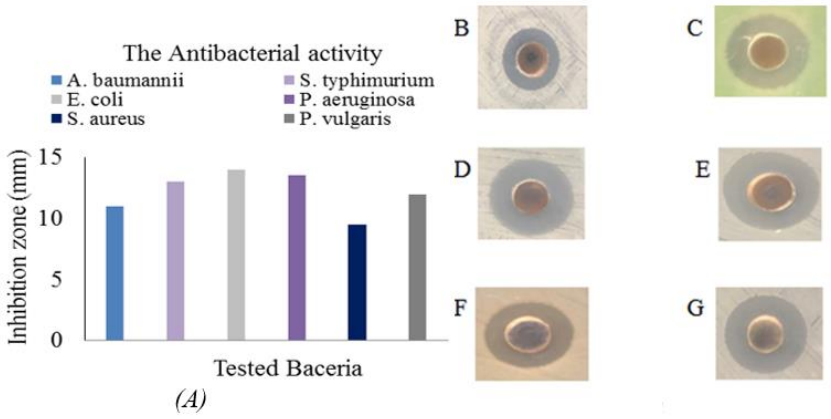


Fig. 3. (A-G): (A)antibacterial activity chart, (B)antibacterial activity against S. aureus, (C)P. aeruginosa, (D)A. baumannii, (E)S. typhimurium, (F)P. vulgaris, (G)E. coli.

Meanwhile, antibiotic-nanoparticles combinations displayed an enhanced effect against bacteria compared to the antibiotics alone. AgNPs and antibiotics may collaborate in the destruction of the cell wall, or may facilitate the penetration of antibiotics into the bacterial cell by

changing membrane permeability[17]. The greatest effect of the antibiotics combined with AgNPs was on *S. typhimurium*. Comparing the results from Figures3 and 4 the effect of AgNPs on *S. typhimurium* was 13mm, after combining AgNPs with Fosfomycin, the diameter of the inhibition zone increased to 25.5mm. Moxifloxacin displayed the strongest effect on the tested Gram-negative bacteria, and Tobramycin had the greatest effect on *S. aureus* as shown in Figure4-B.

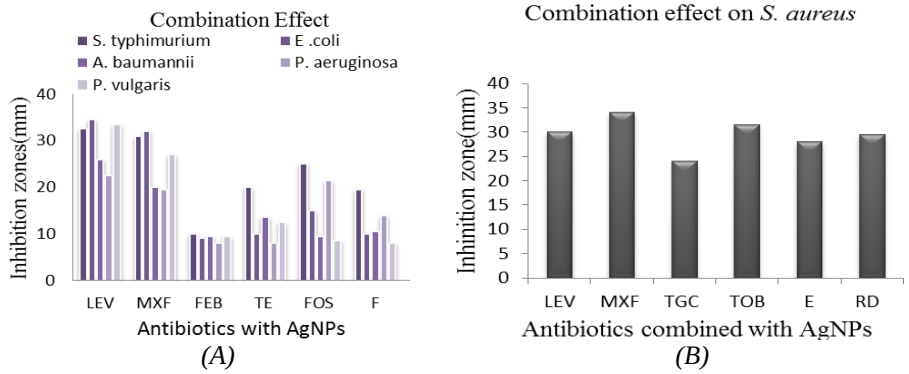


Fig. 4. (A,B): (A)combination with antibiotics, (B)combination effect on S. aureus.

Many factors contribute to the antibacterial activity of AgNPs, including surface charge, size and shape, bacterial type, species tolerance to AgNPs, and concentration and exposure time[3,12]. It could be hypothesized that the antibacterial effect a product of the ability of AgNPs to prevent bacterial DNA replication, through interaction with biomolecules, prevention of biofilm formation, affect on cellular signaling, free radicals, reactive oxygen species formation or interaction with thiol groups[11,16].

4. Conclusion

AgNPs were produced from RS suspension with an economical, efficient and eco-friendly approach. Reduction of $AgNO_3$ to AgNPs was confirmed by DLS, XRD, TEM and EDX. The antibacterial effect of AgNPs was best on *E. coli*. The resistant organisms exhibited higher sensitivity to a combination of antibiotics with AgNPs than to antibiotics alone. Finally, according to the finding results AgNPs synthesized from RS suspension is a promising therapeutic agent.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through the Undergraduate Student's Research Support Program, Project no. (URSP–3-18-107).The authors are also thankful to the Deanship of Scientific Research and RSSU at King Saud University for their technical support.

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