



SYNTHESIS, CHARACTERIZATION, AND ANTIBACTERIAL/FUNGAL ACTIVITY OF COLLOIDAL SILVER NANOPARTICLES PREPARED BY TWO-ELECTRODE ELECTROLYTIC CELL TECHNIQUE

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Article history:	Abstract:
Received: 26 th October 2023 Accepted: 24 th November 2023 Published: 28 ^h December 2023	High-purity metallic silver was employed in the electrochemical synthesis of silver nanoparticles (AgNP). The nanoparticles created were subjected to characterization through UV-Vis spectroscopy and Field Emission Scanning Electron Microscopy (FESSEM). The nanoparticles that were synthesized resulted in a unimodal distribution, featuring an average size below 100 nanometers. The silver nanoparticles that were prepared were employed for their antibacterial properties against microorganisms such as <i>Bacillus subtilis</i> , <i>Pseudomonas pneumonia</i> , and also exhibited antifungal activity against <i>Alternaria alternata</i> .

Keywords:

1. INTRODUCTION

Microbial disease presents an imposing challenge in healthcare, especially within the setting of surgeries [1]. These treacherous microorganisms, regularly imperceptible to the exposed eye, have the ability to penetrate the foremost crucial frameworks of the body, counting the circulation system and lungs [2]. After surgical mediation, patients have a high chance of falling prey to these infinitesimal assailants. Such a disease, in the event that cleared out unchecked, can lead to genuine complications, a long healing centre remains, and indeed life-threatening results [3].

Silver nanoparticles (AgNPs) have garnered significant attention as potent antimicrobial agents, particularly in the context of preventing infections post-surgical procedures [4]. Their small size allows for effective penetration into various bodily tissues, making them valuable in combating infections not only in the bloodstream but also in the lungs and other body areas [5]. AgNPs exhibit broad-spectrum antimicrobial properties, inhibiting the growth of a wide range of bacteria and fungi [6]. This versatility makes them a promising candidate for medical applications, reducing the risk of post-operative infections and enhancing patient recovery [7]. Ongoing research explores the controlled use of AgNPs to optimize their effectiveness while minimizing potential side effects [8]. Apart from silver (Ag), various metallic nanomaterials, including copper, zinc, titanium [9], and gold [10], exhibit antibacterial properties. However, silver stands out not only because it boasts the most potent antimicrobial effects but also due to several processing benefits, such as exceptional high-temperature stability and minimal volatility [11]. Additionally, silver possesses the unique ability to absorb and break down ethylene gas, further enhancing its desirability in various applications [12]. Until now, diverse techniques have been utilized to create silver nanoparticles (Ag NPs) of various sizes and shapes because AgNP properties are size-dependent. These techniques encompass spray pyrolysis synthesis [13], microwave irradiation synthesis [14], chemical synthesis [15], hydrothermal synthesis [16], UV irradiation synthesis [17], sonochemical synthesis [18], thermal decomposition synthesis [19], electrochemical [20], and more.

The electrochemical method stands out as the preferred approach for Ag NP synthesis among various techniques due to its simplicity, affordability, and capacity to generate substantial quantities of Ag NPs [21]. However, the precise mode of action of silver nanoparticles (AgNPs) as antibacterial agents remains unclear. Several mechanisms have been suggested, including their impact on microbial cell walls, the formation of deposits, and the induction of toxicity by inhibiting key enzymes through the formation of complexes with the catalytic sulfur found in thiol groups within cysteine deposits [22]. Additionally, AgNPs have the capability to generate harmful radicals, such as singlet oxygen (¹O₂), superoxide anions (O²⁻), and hydroxyl radicals (OH^{*}), through the generation of reactive oxygen species (ROS) [23]. Another proposed mechanism is that AgNPs can cause DNA damage by altering the size of the cytoplasmic

membrane or detaching it from the cell wall, leading to the condensation of DNA molecules and a reduction in their replication capacity [24].

In the present study, numerous types of bacteria, such as *Bacillus subtilis* and *Pseudomonas pneumonia*, can lead to infections in the bloodstream, respiratory system, or other parts of the body after a surgical operation. Furthermore, the fungus *Alternaria alternata* can provoke upper respiratory infections and exacerbate asthma in people with compromised immune systems. These microbial issues were addressed using AgNPs.

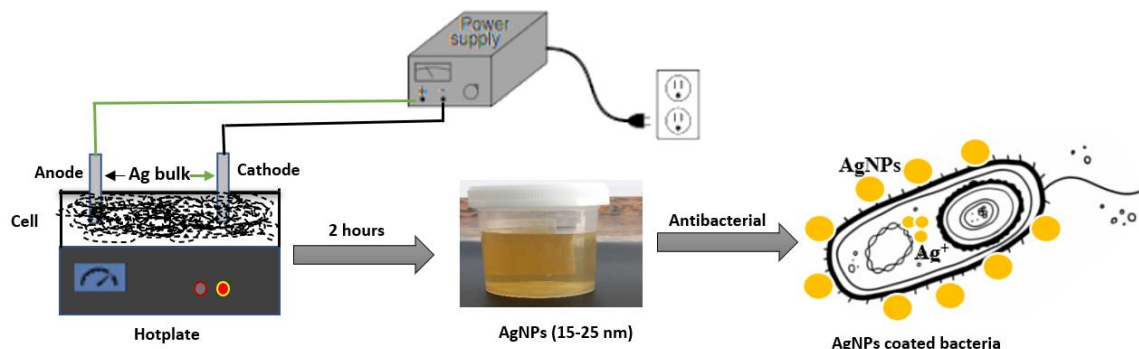


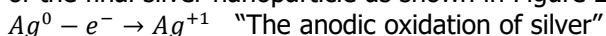
Fig.1: Schematic diagram of AgNPs-bacteria activities

2. EXPERIMENT SECTION

The electrochemical synthesis of AgNPs allows for precise control over the size and properties of the nanoparticles and is commonly used in research and industrial applications, this method used for the preparation of silver nanoparticles (AgNPs) involves using an electrochemical cell to reduce silver ions (Ag^+) to form AgNPs as followed. Two commercially accessible silver metal plates, both highly refined with a 99.9% purity, were utilized as the cathode and anode working electrodes. These plates had a circular shape with a diameter of 1.85 mm and a length of 70 mm. They were placed vertically, maintaining a 5 cm separation from each other, and submerged in 50 ml of distilled water (DW). A direct current (DC) voltage source supplying 20 volts was connected to these electrodes. The entire process of preparation spanned a duration of 2 hours, conducted under room temperature conditions. Following the completion of this meticulous procedure, a solution with a subtle, yellowish color was successfully produced. This resulting solution was carefully stored in a dark, secure location, ensuring its preservation for future applications.

3. MECHANISM PRODUCES OF COLLOIDAL SILVER NANOPARTICLES

In summary, the electrochemical procedure commences by dissolving the silver anode oxidatively. Subsequently, silver ions travel through the solution towards the cathode area. On the cathode's surface, these ions undergo reduction, leading to the creation of silver nanoparticle seeds with zero-valent properties. Meanwhile, in the bulk solution, the stabilized nanoclusters go through the Ostwald ripening process, ultimately resulting in the formation of the final silver nanoparticle as shown in Figure 2.



- Emission of oxygen gas resulting from water electrolysis.
- Simultaneously, Ag_2O is being deposited onto the anode's surface.
- The migration of silver ions toward the cathode.
- The cathode induces the conversion of ions into silver atoms through a reduction process.
- Formation of the silver particles via the nucleation and the growth due to Van der vales attraction.



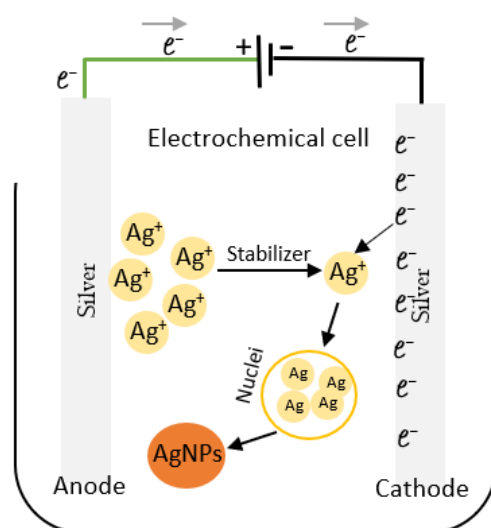


Fig.2: AgNPs formed from two-electrode electrolytic cell

4. RESULTS

4.1. MORPHOLOGY ANALYSIS

The shapes and structures of AgNPs were examined using a field emission scanning electron microscope (FE-SEM) and X-ray diffraction (XRD), respectively. As depicted in Figure 3a, it is evident that particles-Ag were uniformly grown dispersion within deionized water. The data presented in Figure 3b illustrates that approximately 60% of the synthesized particles exhibit an average diameter ranging between 15 to 25 nm, which is the predominant size range for AgNPs in this sample.

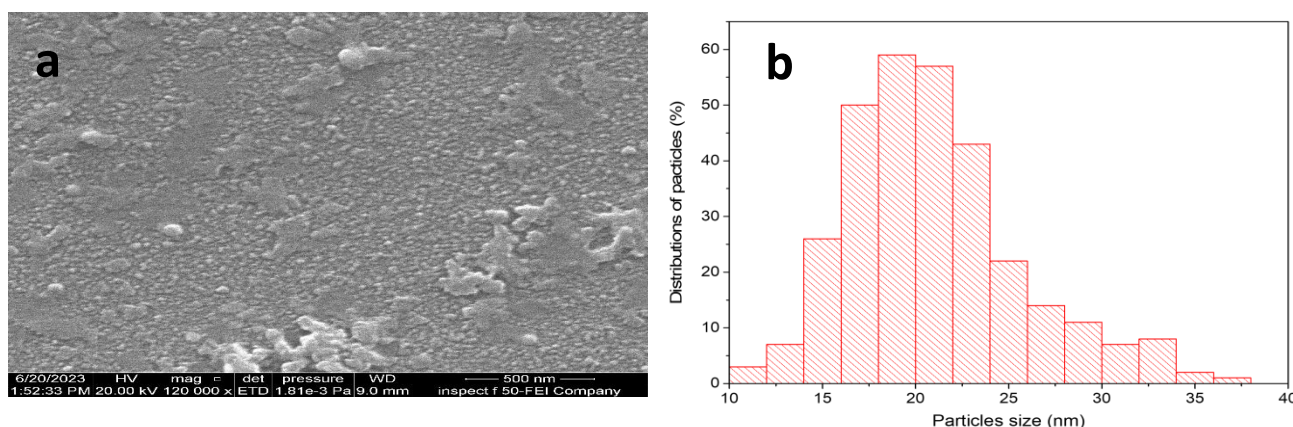


Fig. 3: FE-SEM of AgNPs and distribution particles

4.2. Optical properties

This technique was employed to ascertain the plasmonic surface resonance, and absorbance readings within the range of 300 to 900 nm were obtained using a Shimadzu UV spectrophotometer model 1800. The solution takes on a faint yellow hue, and the spectrum shows a broad absorption peak at 418 nm, indicating the presence of AgNPs formation as shown in Figure 4. The particles-Ag do not exhibit any tendency to come together and form larger clusters. S. Rahmah et al. [25] harnessed electrochemical techniques to synthesize AgNPs, and the results demonstrated that employing 0.2 M sodium citrate yielded AgNPs with specific optical properties. The presence of an absorption peak have a wavelength of 416-418nm and an absorbance of 0.825 in the UV-Vis spectra, attributed to surface plasmon resonance, confirmed the formation of silver nanoparticles. This transformation was visually validated by the change in color from colorless to yellow, and the assessment of silver nanoparticle uptake was conducted through ultraviolet ray characterizations, indicating absorption within the 400-500nm range for the silver-colored nanoparticles.

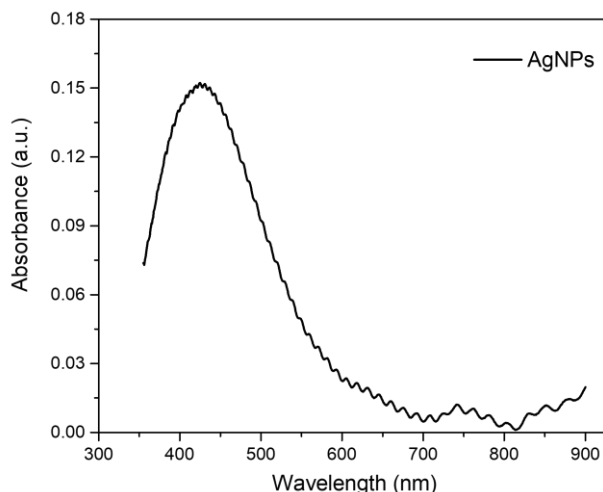


Fig.4: Surface plasmon effect of prepared AgNPs

5. ANTIBACTERIAL/FUNGAL ACTIVITIES

In the initial phase, both bacterial and fungal cultures were cultivated on nutrient and potato dextrose agar at a temperature of 37°C for a duration of 24 hours. The results showed that the prepared AgNPs exhibited a positive effect on the inhibition of the growth of microorganisms, whether the selected microorganisms were bacteria or fungi. The results from the experiments revealed significant data regarding the inhibitory effects of the synthesized compound. At a lower concentration of 0.1 ml, it was observed that the bacterium *Basillus sptillus* displayed a modest inhibitory zone with a diameter measuring 25 mm. However, as the concentration increased to 1 ml, AgNPs inhibitory potential became more pronounced, resulting in a substantial inhibitory zone with a diameter of 31, and 33 mm for *Basillus sptillus* and *Pseudomonas pneumonia*, respectively. In addition, The results also indicated that the prepared AgNPs had a greater inhibitory effect on bacteria compared to its effect on fungi. For the fungus *Alternaria alternata*, the smallest inhibition diameter was 10 mm at a concentration of 0.1 ml, and the largest inhibition diameter was 19 mm at a concentration of 1 ml as shown in Table (1) and the numbered images (a, b, c and d) in the Figure (5).

Table (1): Size of inhibition zone (mm)

Concentrations (ml)	<i>Basillus sptillus</i>	<i>Pseudomonas pneumonia</i>	<i>Alternaia alternata</i>
0.1	25	3	10
0.3	25	31	15
0.5	26	32	17
0.8	27	32	19
1	31	33	19

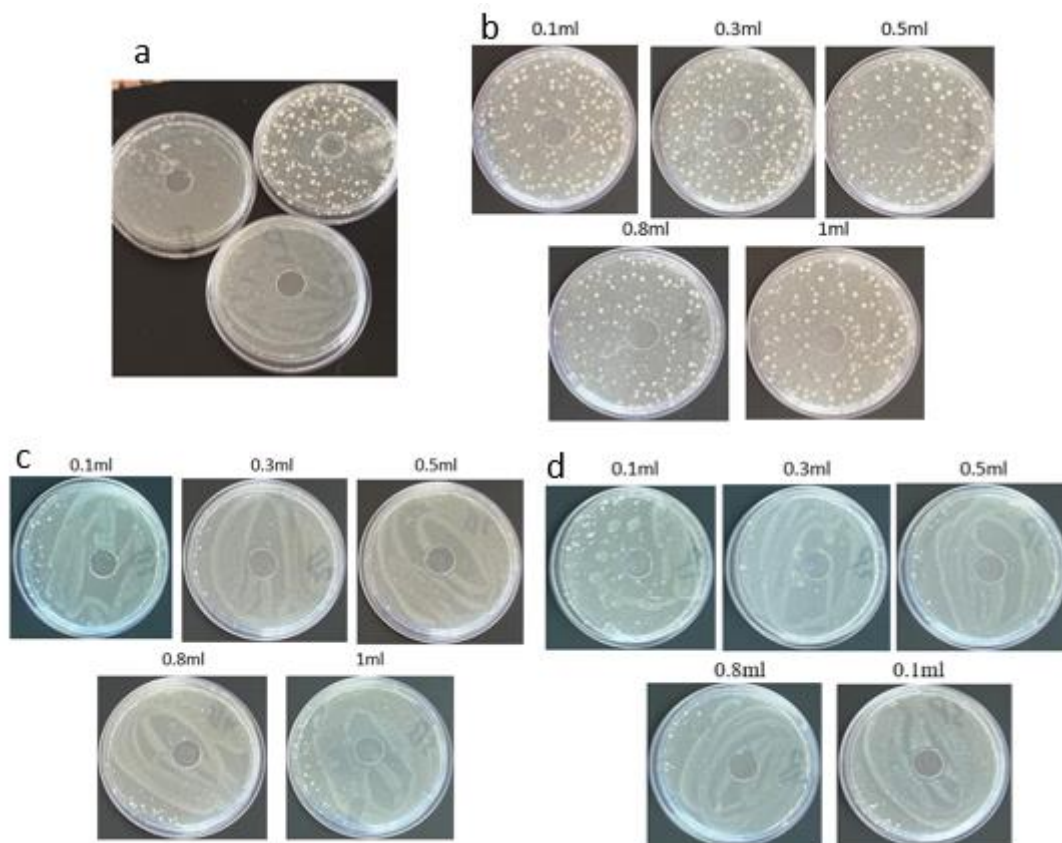


Fig. 5: Antibacterial/fungal activity images of AgNPs against a) *Basillus spittillus*, b) *Pseudomonus pneumonia*, and c) *Alternaia alternata*. a) Controller

CONCLUSIONS

It's important to highlight that despite the simplicity of the electrochemical synthesis method and its minimal reliance on complex equipment, it still enables the production of silver nanoparticles with a favorable distribution and an average size. The silver nanoparticles (AgNP) exhibit effective antimicrobial properties with a favorable minimal inhibitory concentration. The experimental findings provide valuable insights for the development of a system in which immobilized AgNP could be utilized as an antimicrobial agent

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