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The Production of Gold Nanoparticles via Chemical Reduction Methods and Their Antimicrobial Characteristics

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Abstract

Due to their remarkable chemical and physical properties gold nanoparticles have demonstrated potential antibacterial efficacy in an attempt to address this problem. Many variations of these gold-based nanostructures have been created. With an emphasis on a temperature of 60 C⁰, a number of experiments have been conducted to create stable gold nanoparticles utilizing chemical reducing agents such as sodium trinitrate. Scanning electron microscopy (FESEM) and ultraviolet spectroscopy were used to examine the resultant gold nanoparticles. These analyses yielded findings between 27.69 and 53.26 nm. The red gold nanoparticle solution's spectrum started at 530 nm. Although the forms and sizes of gold nanoparticles produced by various reactions vary, spheres are the most prevalent. The existence of FCC cubic gold was indicated by the four primary peaks in the gold nanoparticles' XRD pattern, which were located at (111), (200), (220), and (311).

Keywords

Antimicrobial,
Staphylococcus
Aureus,
Moraxellaceae ,
Au Nanoparticles ,
Reduction Methods.



1-INTRODUCTION

The development of easy-to-implement methods for generating metallic nanoparticles has gained significant importance as a crucial area in nanotechnology. [1]. There has been a remarkable increase in interest surrounding the synthesis of gold nanoparticles, mainly due to their exceptional electrical, optical, magnetic and thermal properties driven by the quantum effect [2]. These properties have made them applicable in various fields, including electronics, sensing and detection systems, medicine, chemistry and nanobiotechnology [3]. A large number of scientists have focused their research on these materials.

The distinctive features of these phenomena arise from the confinement of the electronic wave function to three or fewer physical dimensions. The confinement phenomenon leads to variations in the surface area and electron concentration, thus modifying the properties of the material. It is worth noting that the dimensions of the particles greatly affect properties such as chemical response, electrical and magnetic mobility, fluorescence and boiling point. [4,5]. In the past ten years, a variety of methods have emerged for the synthesis of nanoparticles, especially those involving chemical approaches. [6,7]. Electrochemical methods [8]. Photochemical strategies [9], non-chemical processes [10]. X-ray irradiation [11,12], and laser ablation [13]. The properties of gold nanoparticles have been investigated, revealing that their unique properties are closely related to their size.

The prevailing approach to the synthesis of gold nanoparticles focuses on the production of nanostructures with a small and uniform size distribution. Due to its ease of use, the

Tonkovitch technique is recognized as the most common synthesis method for gold nanoparticles. This method uses sodium citrate as a reducing agent to reduce chloroauric acid ($\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$). In about 10 min, colloidal gold nanoparticles are produced by adding sodium citrate to a hot HAuCl_4 solution by this procedure.

A major drawback of this approach is that the reactants do not receive sufficient rapid and uniform thermal distribution from the conductive heating provided by a hot plate [6]. As a result, the sizes and shapes of gold nanoparticles produced using this method exhibit a scattered pattern. Due to their large surface area-to-volume ratio, which makes it easy to coat their surfaces with a variety of molecules, including ligands, antibodies, therapeutic agents, diagnostic agents, antigens, and targeting agents, gold nanoparticles are used in healthcare applications to deliver therapeutic agents [14,15] .

The aim of this research is the continuous development of integrated approaches as well as in medical applications by taking two types of bacteria, one of which is a positive-walled gram and the other is negative-walled. Its effect on positive gram was greater than negative gram.

2- EXPERIMENTAL METHODS

Materials

The chemical ingredients employed in this investigation were of GR quality. Merck and Sigma-Aldrich provided the sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$) and chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$). All of the trials were conducted using deionized water.

2-1 The initiation of reduction process for tetra chloroauric acid (HAuCl_4) was achieved by adding trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$). This step involved the precise injection of a specific volume of preheated trisodium citrate solution into the boiling gold solution within a double-walled reactor, which was kept at a constant temperature by a thermostatic bath.

A temperature gradient was created to prevent liquid formation. A simple reaction apparatus, consisting of multiple beakers, was arranged, with the jacket ensuring uniform temperature distribution in the reaction mixture. A temperature controller was employed to manage the water bath's temperature. A Teflon-coated magnetic rod was used to agitate the liquid extensively. As the gold nanoparticles were formed, the color of the solution gradually changed from a transparent light yellow to a deep black and then to a distinctive winter gray. The heating jacket was taken off around twenty minutes. Different particle size distributions were obtained by varying the quantities of trisodium citrate and tetra chloroauric acid. After the allotted amount of time, the liquid was cleaned and allowed to cool to room temperature. Aqua regia was used to properly clean all of the equipment used in the experiment during this process.

3- RESULTS AND DISCUSSION

3-1- UV-Vis Spectroscopy

The characteristics of gold nanoparticles made with a certain concentration of sodium citrate by a reduction process are depicted in the spectrum of ultraviolet light in Figure 1. It is clear that these nanoparticles demonstrate a peak absorption wavelength of 530 nm and have sizes ranging from 27.69 nm to 53.26 nm. A number of variables, including their size,

physical characteristics, and the surrounding environment, affect how they interact with light. Plasmons are defined by the Fermi liquid model as a cloud of negatively charged electrons that is coherently moved out of its equilibrium position with respect to a lattice of positively charged ions. [16]. When exposed to light, a field of electricity that oscillate causes the conduction electrons in tiny spherical gold nanoparticles to oscillate coherently.

The electron cloud is displaced as a result of this coherent behavior, and the Coulomb force between the electrons and their corresponding nuclei creates a restoring force. Consequently, the electron mist fluctuates in connection to the nuclei's configuration [17]. Localized surface plasmons (LSPs), which are non-traveling excitations, can be used to characterize interactions with nanoparticles (NPs) that are substantially smaller than a photon's wavelength. This occurrence can be explained by the plasmon oscillations being distributed across the particle's volume. The positively charged lattice propels the electrons' coherent motion, creating a restoring force that pulls the polarizing electrons back toward the lattice's surface.

On the other hand, synchronized electron oscillations result from a uniform oscillating electric field created if the wavelength is larger than the size of the NPs. The tiny size of the NPs, however, limits this collective oscillation by confining the electrons and causing a considerable absorption of wavelengths close to the green zone. As a result, gold nanoparticles (AuNPs) have a crimson appearance. These results we obtained are in good agreement with the results of studies. [18,19,20,21].

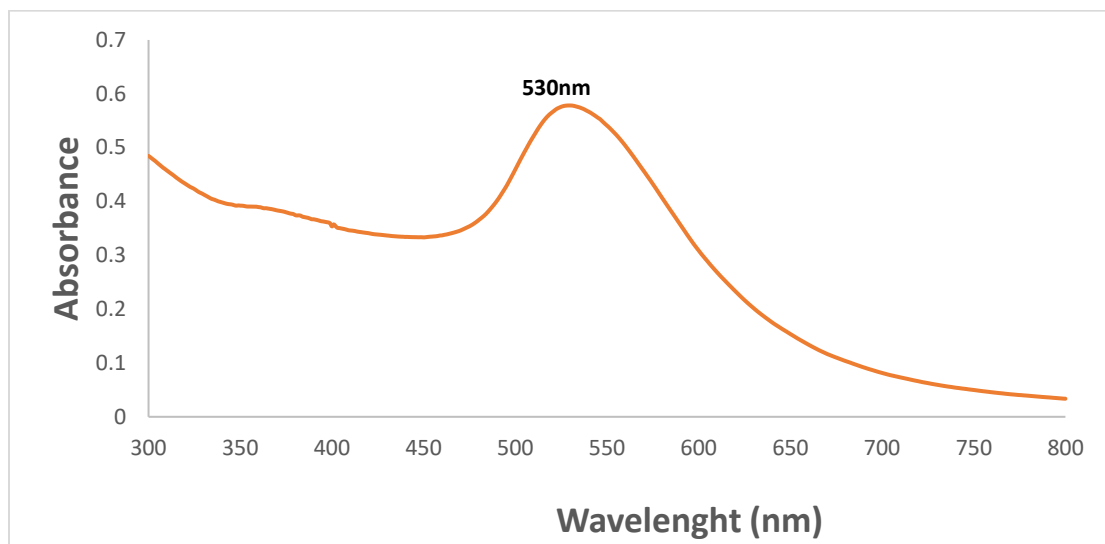


Figure 1. UV-vis absorption spectrum of gold nanoparticles

3-2- X-ray diffraction (XRD)

The gold nanoparticle reference map (89-3697) is contrasted with the X-ray diffraction (XRD) pattern displayed in Figure (2). Identified by indices (111), (200), (220), and (311), the diffraction peaks validate the gold nanoparticles' face-centered rectangular structure. There were also further peaks found at 2θ values of 37.172, 43.408, 63.558, and 598.76. The usual diffraction pattern for cubic gold metal was used to calculate the lattice constants. There was an unknown peak

at 30.596, which was probably caused by an organic substance used in the production of sodium citrate. However, the gold nanoparticles' reflection peaks were unaffected by this external peak [22]. The X-ray diffraction (XRD) analysis's results offered strong proof that gold nanoparticles were produced from NaAuCl_4 . To determine the average crystal size, the Scherrer equation was used. From the Scherrer equation to calculate the average crystal size for each of the four peaks, the lattice constant was calculated as in Table 1

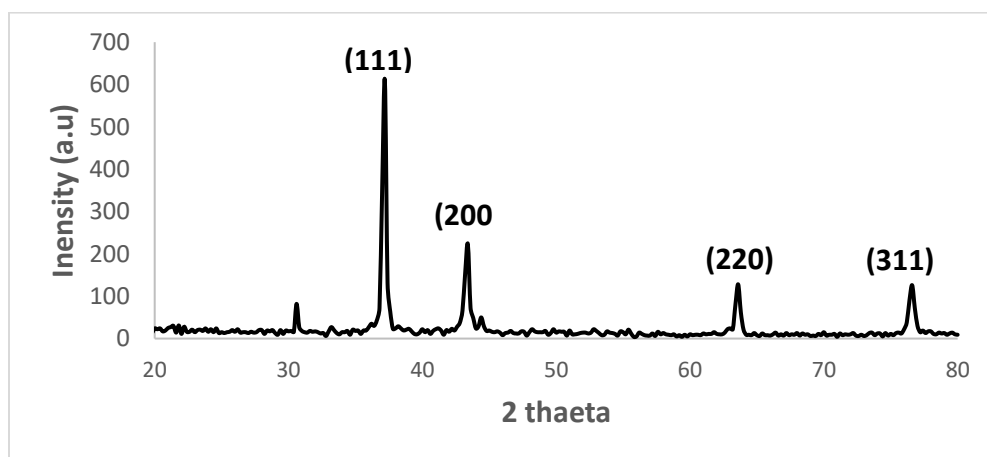


Figure 2. X-ray diffraction pattern of AuNPs

Table (1): (a) Gold nanoparticle's XRD pattern (b) the lattice constant (c) crystallite size obtained from each peak.

2 θ / degree	37.172	43.408	63.558	76.598
a / °A	4.1858	4.1658	4.1368	4.12223
Size / nm	24	18	19	19

3-3-Scanning electron microscope (FESEM)
The FESEM micrographs shown in Fig. 3 provide an in-depth examination of the structure, size, and aggregation of nanoparticles. Utilizing scanning electron microscopy (FESEM) effectively showcases a high concentration of gold nanoparticles, which are uniformly spread across the surface. The FESEM images indicate the presence of both mono-dispersed and poly-dispersed nanoparticles, with variations in size ranging

27.69 to 53.26 nm. The SEM images reveal the distinct morphologies of the nanoparticles. Both low and high magnification images confirm that (Au NPs) exhibit a spherical form, with diameters consistently under 100 nm (specifically 27.69, 33.82, 37.07, 45.17, and 53.26 nm). The results of the FESEM analysis are in agreement with earlier studies documented in the literature concerning gold nanoparticles [23].

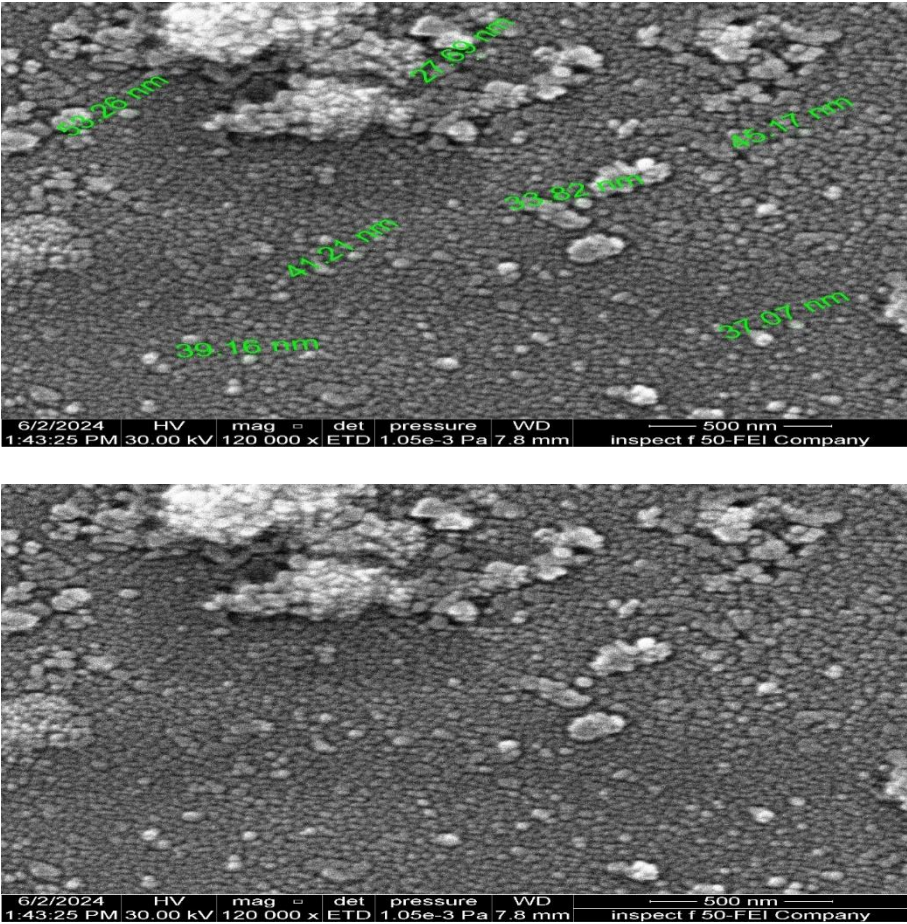


Figure3. FESEM micrograph of AuNPs

3-3 Antibacterial activity of Au NPs

The antibacterial properties of AuNPs nanoparticles against selected bacterial strains are shown in Figure 4 (E-C). The results confirm that AuNPs nanoparticles were harmful to all bacterial strains studied, with the level of effectiveness influenced by the dosage used. Among the strains studied, the most significant inhibitory effect was observed against *Staphylococcus aureus* and *Moraxellaceae*. 4 serial concentrations of nanoparticles (Au NPs) (1000, 500, 250, 125) $\mu\text{g/ml}$ were used by the agar inhibition method. The results were given at an inhibition diameter of 24 mm at a concentration of 1000 $\mu\text{g/ml}$ and at 500 $\mu\text{g/ml}$ the inhibition diameter is 18 mm. The inhibition diameter at 250 $\mu\text{g/ml}$ is 13 mm and at 125 $\mu\text{g/ml}$ there is no effect on the bacteria.

These results are at the *Staphylococcus aureus*. As for the bacteria (*Moraxellaceae*) (A.B), we notice from the panels that the inhibition at an inhibition diameter of 20 mm at a concentration of 1000 $\mu\text{g/ml}$ and at 500 $\mu\text{g/ml}$ the inhibition diameter is 16 mm. As is the case at 250 $\mu\text{g/ml}$ the inhibition diameter is 11 mm and at 125 $\mu\text{g/ml}$ there is no effect on the bacteria. The interaction of metal ions in solution with a bacterial cell results in a general dispersion of these ions in the surrounding environment, without any defined localization. In contrast, nanoparticles that engage with the bacterial cell wall generate a localized source of ions that continuously release ions, thereby elevating the toxicity to the cell [24]. The results obtained from our investigation reveal that gold nanoparticles possess a more pronounced antibacterial

effect against Gram-positive bacteria. This can be explained by the fact that Gram-positive bacteria have a relatively thin cell wall, whereas Gram-negative bacteria are protected by a thicker and more formidable cell wall.

Thus, gold nanoparticles are able to penetrate the cell membrane of Gram-positive bacteria more effectively, leading to damage within the cell [25,26]. Gold nanoparticles exhibited antibacterial properties through two key processes. Initially, they inhibited metabolic processes by altering the membrane potential and diminishing the activity of adenosine triphosphate (ATP) synthase. Subsequently, they interfered with the binding of tRNA to the ribosomal subunit, effectively disrupting its biological mechanisms. Notably, these nanoparticles were also found to be less detrimental to mammalian cells [27].

According to a recent literature review, gold nanoparticles, which come in a variety of shapes and sizes, are the most thoroughly researched nanomaterials for their antibacterial and anti-biofilm applications [28]. Gold nanoparticles (NPs) produce electronic effects as a result of their small dimensions and increased surface area, which are advantageous for enhancing their surface reactivity. Furthermore, the high percentage of surface area facilitates extensive interaction with bacteria, leading to improved bacterial contact. Consequently, these two fundamental features greatly enhance the antibacterial activity of NPs with a large surface area. [29].

Table (2): inhibition zone of nanostructures (Au NPs) as antibacterial agents in mm at different concentration

Kind of used Bacteria	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml
Staph. aureus	24	18	13	Zero
moraxellaceae	20	16	11	zero

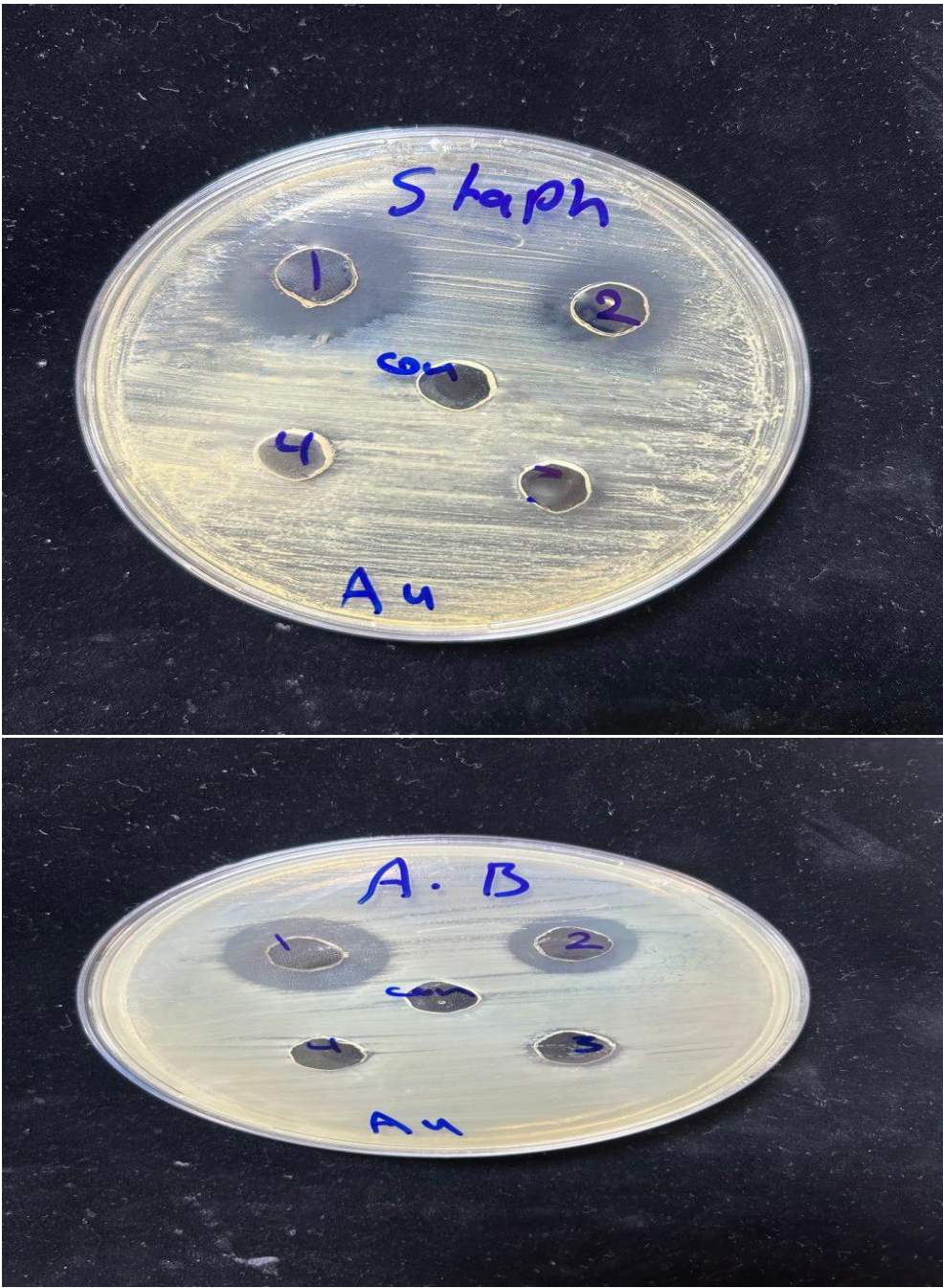


Fig. 4. Antibacterial activity of AuNPs against gram-positive and gram-negative organisms at 1000 µg/ml (1), 500 µg/ml (2), 250µg/ml (3) and 125 µg/ml (4). Staphylococcus aureus and Moraxellaceae

4. Conclusions

We have demonstrated a simple technique to generate spherical gold nanoparticles at room temperature using sodium trinitrate, with diameters ranging from 27.69 to 53.26 nm. The antibacterial properties of the nanoparticle surface were investigated by using sodium trinitrate as a reducing and capping agent. In this study, we have demonstrated that gold nanoparticles reduced with sodium trinitrate have strong antibacterial capabilities against Gram-negative bacteria belonging to the Moraxellaceae family as well as Gram-positive bacteria like *Staphylococcus aureus*. Due mainly to improved interactions with the negatively charged walls of bacterial cells, prior research has repeatedly demonstrated that positively charged gold nanoparticles are more reactive to bacteria than their negatively charged counterparts.

The size and shape of the nanoparticles also affect their antimicrobial effectiveness. The size of gold nanoparticles was examined in our review; some research suggests that smaller particles work better in antibacterial applications, while other research suggests the contrary. Furthermore, we assessed the influence of nanoparticle shape on antibacterial performance, finding that gold nanoparticles with rough surfaces, such as those with flower-like or star-like features, demonstrate superior antibacterial activity compared to smooth, spherical nanoparticles.

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