



Evaluation of Gold Nanoparticles in Terms of Their Use in Biomedical Applications

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ABSTRACT

Recently nanotechnology has become an integral part of modern biomedical applications. Accordingly, nanoparticles are considered as promising components for the development of innovative tags, probes, biosensors and carrier molecules for drug delivery. Spherical colloidal gold nanoparticles (AuNPs) are prime candidates to be utilized for these purposes due to their useful physical properties. However, in order for the gold nanoparticles to be used in nanomedicine, their biological properties should be extensively studied as well. Therefore, in this paper we chemically synthesized gold nanoparticles and studied their physical and biological characteristics to determine their potential use in medicine. Gold nanoparticles were synthesized by the reduction of chloroauric acid (HAuCl₄) solution with sodium citrate. The physical properties of the AuNPs were determined by UV-vis spectrophotometer and Zetasizer readings. The antimicrobial activity of the newly synthesized gold nanoparticles on *Escherichia coli*, *Salmonella infantis*, *Salmonella kentucky*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacillus subtilis* and *Candida albicans* were investigated via disk diffusion method. We found that the AuNPs were monodisperse, stable and not prone to aggregation with an average size of 22.12 nm and an emission band at 522 nm. The disk diffusion tests revealed that the gold nanoparticles did not have a significant growth inhibitory effect on the pathogens tested. In conclusion, here we showed the successful synthesis of gold nanoparticles by a safe and non-toxic method. Furthermore, our evaluation of the antimicrobial activity of these nanoparticles suggests that these molecules could be considered as biologically safe molecules for future medical applications.

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Introduction

Advances in biotechnology and molecular medicine have revolutionized the approaches and tools used for disease diagnosis and treatment. It has become more important within the last decades to develop novel therapeutic approaches in order to achieve high treatment efficiency with least possible side effects, which highlighted the significance of targeted drug designs. In line with this, use of nanotechnology and nanoparticles in medicine has rapidly progressed, leading to the emergence of new research fields, namely nanobiotechnology and nanomedicine (Whitesides, 2003). The small size of nanoparticles, which is comparable to that of cellular components such as proteins, has allowed them to be utilized as tags, probes, biosensors and carrier molecules for drug delivery (Nam et al., 2003, Salata, 2004, Jong and Borm, 2008).

One of the most popular nano-sized particles that are widely used for biomedical purposes is colloidal gold nanoparticles, abbreviated as AuNPs. Gold is the most stable metal for synthesis of nanoparticles, since it is chemically inert and does not react with other substances (Versiani et al., 2016). Gold nanoparticles can be synthesized in various shapes and sizes; spherical, rod-like and core-shell shaped AuNPs can range between 1 nm to more than 100 nm in size (Yeh et al., 2012). Spherical AuNPs are often preferred in nanomedicine over others due to their useful properties such as high surface-volume ratio, exceptional biocompatibility, low toxicity and cost-effective synthesis. (Murphy et al., 2008, Khlebtsov and Dykman, 2011). Furthermore, as AuNPs can absorb light with shorter wavelengths, they provide a minimally invasive, non-ionizing diagnostic imaging alternative utilizing near infrared light (Altinoğlu and Adair, 2010).

Pathogenic bacteria are disease-causing microorganisms. Even though most bacteria do not cause any harm and even provide the host with several benefits, some can cause infections when consumed in contaminated water or food, or via exposure to other environmental contaminants (Katas et al., 2019). Studies have shown that some metal-based nanoparticles such as zinc, gold and silver possess antimicrobial properties (Zhou et al., 2012). Since treatment approaches against antibiotic resistant bacteria are limited and costly, bactericidal activities of these nanoparticles are studied for their potential as cost-effective novel therapeutics (Soo- Hwan et al., 2011). In this respect, gold ions and nanoparticles have attracted great attention in the field. It was shown that some the organic complexes consisting of Au^+ and Au^{3+} ions have antibacterial effect, while gold nanoparticles were attributed antifungal activities and data on their effect on bacteria were inconclusive (Zhang et al., 2015). Therefore, in this study we aimed to comparatively investigate the antimicrobial activity of gold both as nanoparticles (AuNPs) we chemically synthesized and in ionic form (Au^{3+}) that we used during synthesis against several pathogenic bacteria, to be able to assess whether these nanoparticles would be appropriate to be used in further biomedical applications.

Materials and methods

Gold Nanoparticle (AuNP) Synthesis

AuNPs were synthetically synthesized as described by Turkevich et al. in 1951. Briefly, 20 ml of Chloroauric Acid (HAuCl_4) (10 mM) was boiled on a magnetic stirrer and 2 ml sodium citrate (1%) was rapidly added (Figure 1). After 10 minutes of boiling, the color of the solution started to change into a dark purple. After the solution cooled down to room temperature, it was centrifuged at 3500 rpm for 10 minutes. The supernatant was taken into a fresh tube and the pH was adjusted to 8.0 using 1N NaOH. Solution containing AuNPs was kept at 4°C.

Characterization of the AuNPs

In order to determine the physical characteristics of the chemically synthesized AuNPs, absorption bands of the particles were measured using a UV-vis spectrophotometer (Shimadzu, Kyoto, Japan) by scanning the samples at a range of 300-700 nm wavelength. Baseline correction of the spectrophotometer was performed using a blank reference. The average particle size (Z-average) and the polydispersity index (PDI) were determined using ZS-90 Zetasizer. All experiments were performed in triplicates at 25°C.

Strains and Growth Culture

Widely encountered pathogenic bacterial strains of *Escherichia coli* ATCC 25922, *Salmonella infantis*, *Salmonella kentucky*, *Salmonella typhimurium* SL1344, *Salmonella enteritidis* ATCC 13075, *Pseudomonas aeruginosa* DSMZ 50071, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 1971, *Enterococcus faecalis* ATCC 29212 and *Bacillus subtilis* DSMZ 1971, as well as *Candida albicans* ATCC 10231, a fungal isolate, were obtained from Niğde Ömer Halisdemir University, Department of Biotechnology. Activated microorganisms from the stock cultures were inoculated in LB medium and grown for 24 hours at 35°C. The turbidity of the microbial cultures was determined in accordance with 0.5 McFarland standard.

Determination of Antimicrobial Activity of AuNPs

The antibacterial tests were performed by the agar well diffusion method. 100 μL of different strains of pathogenic microorganisms (10^6 cells/mL) were plated on LB agar using sterile drigalski spatula. Next, 20 μL of AuNP-containing solution (synthesized using 0.5 and 1 mM chloroauric acid) was applied on 6 mm sterile discs, placed on agar plates and incubated at 35°C for 24 hours. Antibiotic discs containing gentamycin (20 $\mu\text{g}/\text{disc}$) was used as a positive control, while distilled water containing discs served as negative controls. After incubation, the diameter of the inhibition zones for each sample was measured using a digital compass. All experiments were performed in triplicates and the average value for the diameter of the inhibition zones were reported in mm units.

Results

Analyzing the physical properties of the AuNPs revealed first of all that a deep red color was formed after the solution was cooled down to room temperature following the chemical reaction, which is an indicator of colloidal gold nanoparticle formation. UV-vis readings indicated that the maximum absorbance value for the newly synthesized AuNPs was 522 nm (Figure 2.a). Zetasizer analysis showed that the average size of the gold nanoparticles was 22.12 nm (Figure 2.b). The polydispersity index, which measures the heterogeneity of molecules within a polymer, was determined as 0.457; suggesting that the nanoparticles was monodisperse, stable and not prone to aggregation.

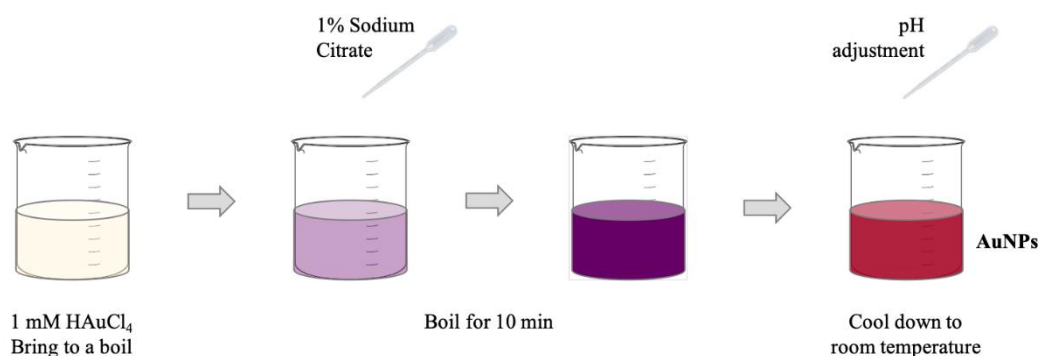


Figure 1 Chemical synthesis of gold nanoparticles (AuNPs) by the reduction of chloroauric acid method

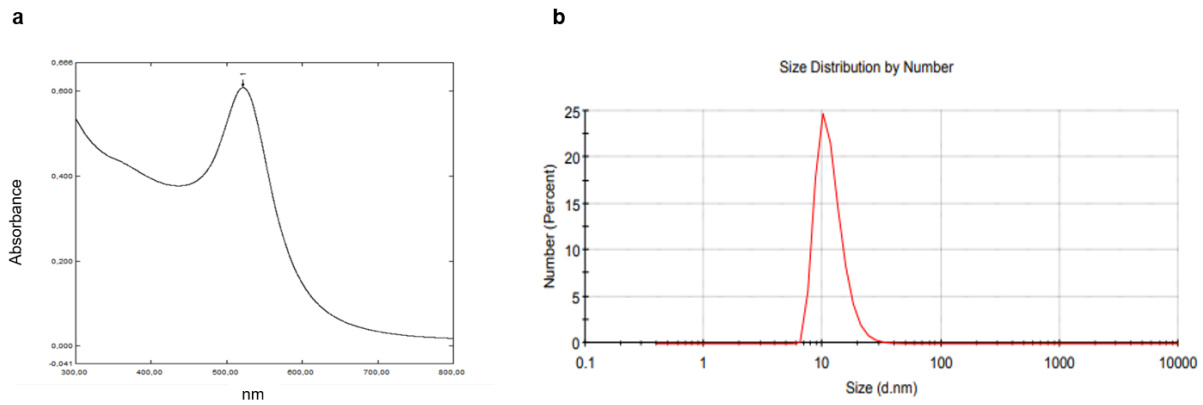


Figure 2 Characterization of the physical properties of AuNPs. UV-vis absorbance spectrum readings (a) and size distribution (b) of the synthesized nanoparticles.

In order to determine the antimicrobial activity of the gold nanoparticles that we synthesized against a variety of pathogenic microorganisms, disk diffusion method was employed using AuNPs synthesized with 0.5 and 1 mM chloroauric acid, as well as 1 mM chloroauric acid solution (Figure 3). The test results indicated that the gold nanoparticles had a slight growth inhibitory effect only on *Staphylococcus aureus* and did not significantly prevent the growth of other pathogens tested. On the contrary, we detected the formation of growth inhibitory zones for chloroauric acid (HAuCl₄) solution in *S. aureus*, *S. typhimurium* and *E. coli* inoculated plates. Gentamycin that we used as a positive control also successfully inhibited pathogenic growth (not shown).

Discussion

Gold nanoparticles have a great potential for the development of future biomedical approaches. However, in order for the gold nanoparticles to be used in medicine, their biological properties should be extensively studied. Gold nanoparticles can either be utilized in medicine as antimicrobial agents, as suggested by some studies in the literature. Conversely, if the aim is to develop more advanced medical approaches where gold nanoparticles are used as biosensors or vehicles for drug delivery, biosafety becomes an important issue as well. At this point, being biologically inert with little to no antimicrobial effect is an

advantage for AuNPs in order not to harm the beneficial bacteria in the human body. Therefore, in this paper we chemically synthesized gold nanoparticles and studied their physical and biological characteristics to determine their potential use in medicine.

In order to ensure that we successfully synthesized gold nanoparticles with appropriate size via the chemical reaction we performed, we analyzed the physical characteristics of the nanoparticle containing solution. Spherical AuNPs can be synthesized in several sizes, ranging from 1 nm to 100 nm, which gives rise to differentially colored solutions including red, purple, brown and orange. These nanoparticles, depending on their size, usually show an absorbance peak between 500-550 nm (Jain et al., 2006). In line with this, we observed the absorption bands of the AuNPs at 522nm, which demonstrates their successful formation. Furthermore, the average size of the newly synthesized gold nanoparticles was detected as 22.12 nm, which is within acceptable range as the Turkevich method produces AuNPs that are 20 nm in diameter (Herizchi et al., 2016). Using Zetasizer analysis, we also determined the polydispersity index, for which the PDI values below 0.7 are accepted as monodisperse with similar particle size distribution (Danaei et al., 2018). Therefore, we were able to conclude that our AuNPs, which had a PDI value of 0.457, were monodisperse, pointing towards their stability and lack of aggregation.

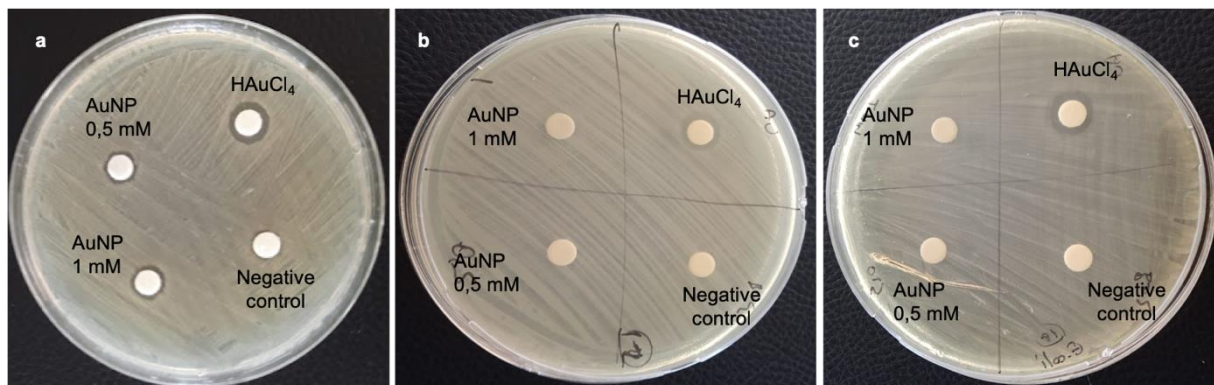


Figure 3 Disk diffusion tests using HAuCl₄ (1 mM) and AuNPs (synthesized with 0.5 and 1 mM chloroauric acid). (a) *Staphylococcus aureus* ATCC 25923, (b) *Salmonella typhimurium* SL 1344, (c) *Escherichia coli* ATCC 25922 strains

There are several studies in the literature investigating the antimicrobial activities of gold nanoparticles. Although the antibacterial effect of Au⁺ and Au³⁺ ions have been established, gold nanoparticles are suggested to have a weak bactericidal activity even at high concentrations (Zhang et al., 2015). The generally accepted view in the literature towards gold nanoparticles is that they are biologically inert molecules with almost no antimicrobial effect, which is evident by the formation of small growth inhibition zones and their high minimal inhibitory concentration (MIC) in comparison to other metal-derived nanoparticles such as AgNPs (Hernández-Sierra et al., 2008, Sreelakshmi et al., 2011, Shankar et al., 2014). Furthermore, various factors such as the size, shape and concentration of the nanoparticles, as well as the coating agent used during synthesis affect the antimicrobial activity of AuNPs. Previous studies have suggested that AuNPs synthesized via chemical methods, coated and stabilized with citrate do not display antimicrobial effect (Amin et al., 2009). Turkevich method relies on the reduction of chloroauric acid (HAuCl₄) with sodium citrate for the synthesis of gold nanoparticles; therefore, it can be considered as a safe and non-toxic method for AuNP synthesis. In accordance with the findings in the literature, we found that the AuNPs we synthesized via the Turkevich method has almost no antibacterial effect and the Au³⁺ ions within chloroauric acid (HAuCl₄) solution had a more pronounced effect on pathogenic growth when compared with the gold nanoparticles.

On the other hand, there are numerous studies showing that gold nanoparticles have efficient growth inhibitory activity against several pathogens including but not limited to *P. aeruginosa*, *B. cereus*, *L. monocytogenes*, *S. aureus*, *S. Typhimurium* and *C. albicans* (Zawrah et al., 2011, Geethalakshmi and Sarada, 2012, Liny et al., 2012, Emam et al., 2017). These studies use similar approaches for synthesizing gold nanoparticles, which are considered “green” or “eco-friendly” as they make use of natural compounds as coating agents during synthesis (Zhang et al., 2015). Since chemically synthesized AuNPs do not have antibacterial activity against these pathogens, the antimicrobial activity is thought to have originated from either residual particles with antimicrobial activity such as the plant extract, gold ions or surface coating agents which were not removed after synthesis or the synergistic effect of the antimicrobial plant extract and the gold nanoparticles (Annamalai et al., 2013).

In summary, here in the presented paper we showed the successful synthesis of gold nanoparticles and evaluated their antimicrobial activity, which collectively allowed us to conclude that these molecules are safe to be tested and utilized in future medical applications.

Conflict of Interest Statement

The authors declare no financial or non-financial conflict of interest.

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