Size-dependent Antibacterial of Gold Colloids

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The bacterial action of colloidal-gold particles on Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa , Candida albicans, were examined by the agar-well-diffusion method. The gold nanoparticles were chemically prepared by reaction of the Schiff base with gold and characterized by transmission electron microscope and UV -Vis spectroscopy. Best antibacterial activity was observed on using small gold colloids (2-5nm) with zone of inhibition 20mm against Staphilococcus aureus.

Keywords: antimicrobial activity, gold nanoparticles, pathogens, TEM

Gold nanoparticles have attracted scientific interest due to their unique optical properties[1], biomedical applications in chemical sensing [2], biological imaging, and cancer treatment [3,4]. Nanoscale materials with antimicrobial activity are emerging as a new class of biomedical materials, especially silver and gold nanoparticles have been used extensively in bactericidal field [5-7].

In literature there are studies on the toxicity of gold nanoparticles according to their size. Thus gold nanoparticles with size between 3-5.5nm and 100nm did not show harmful effect in mice, however gold nanoparticles ranging 8 to 37nm did severe sickness in mice [8]. Gold nanoparticles have demonstrated promise in biological applications. Gold clusters (0.8, 1.2, 1.4, 1.8, and 15 nm in diameter) were tested in several human cancer cell lines for cell toxicity (SK-Mel-28 human melanoma, HeLa human cervix carcinoma, L929 mouse fibroblasts, and J774A1 mouse macrophages) [9].

In the present study we evaluated the antibacterial and antifungal activity of spherical gold nanoparticles (AuNPs) against three bacteria and one fungal isolates using agar disk diffusion method. We used two sizes of gold nanoparticles. The experimental data revealed a higher inhibition Staphilococcus aureus and E.coli bacteria.

Experimental part

Chemicals

2-Hydroxy-3-methoxybenzaldehyde and 4-aminoantipyrine were purchased from Sigma-Aldrich. The (tripheylphosphinegold)oxoniumtetrafloroborate was provided by Stem Chemicals.

Instrumentations

The morphology of the samples was characterized by transmission electron microscopy (TEM). The TEM images were recorded on a using a JEOL-200 CX transmission electron microscope operated at an accelerating voltage of 200 kV. An ethanolic solution of the sample was drop cast on a 100 mesh carbon-coated copper grid. The ethanol was then evaporated under vacuum before the grid was imaged by TEM. The UV/Vis absorption spectra of the synthesized gold colloids were recorded using a Jasco UV/Vis V-540 spectrophotometer in the wavelength range of 190 – 900 nm. All the spectra were recorded in air at room temperature.

The antimicrobial activity of gold colloid

A modified Kirby Bauer technique [10], I replaced the medium Muller-Hilton with TSA (Tryptone Soya Agar)-Sharlau because it is a medium used for growing a variety of microorganisms, peptones containing casein, peptones soy, sodium chloride, lecithin, polysorbate 80 agar. It is prepared by dissolving 45.7 g in 1L TSA dehydrated distilled water, followed by autoclaving 15 min. at 121°C. After cooling to 50°C, pour into sterile Petri plates and wait at least 30 min before use to allow its solidification.

Pure colonies were used: Staphylococcus aureus ATCC; Pseudomonas aeruginosa ATTC; Echerichia coli ATTC , Candida albicans ATTC.

Results and discussions

The gold colloids were obtained by interacting the 1-phenyl-2,3-dimethyl-4-(N-2-hydroxy-3-methoxybenzaldehyde)-3-pyrazolin-5-one (0.1452g) with $[O(Au\ PPh_3)_3][BF_4]$ (0.0740g) in CH_3CN solvent at room temperature for 2-5nm AuNps and 60°C for large AuNps.

The Schiff base 1-phenyl-2,3-dimethyl-4-(N-2-hydroxy-3-methoxybenzaldehyde)-3-pyrazolin-5-one was prepared by refluxing an equimolar mixture of 4-amininoantipyrine with *o*-vanillin in ethanol using a method that is described detail elsewhere [11].

The spectrum (spectra 1) recorded shows a absorption band centered $\lambda = 525$ nm for gold nanoparticles with size dimmensions 2-5nm. The second spectrum (spectra 2) shows two characteristic absorption bands at 525nm respectively 680nm for gold nanoparticles with size dimensions 22-24nm, figure 1. The absorption properties are related to changes in the characteristic surface plasmon band of gold nanoparticles [12]. The color of the colloids is caused by the excitation of a collective oscillation of the valence electrons in the gold nanoparticles called surface plasmon resonance (SPR) [13]. The wavelength of the plasmon resonance depends on the size of the particles [12].

The actual structure of the particles is best characterized by TEM. Figure 2, 3 shows a typical TEM image of the synthesized samples. The image reveals that the prepared particles have a spherical shape with a size between 2-5nm and 22-24nm.

Pure microorganisms are grown on TSA and incubated for 24 h at 36°C. The technique used is to spread bacterial colonies on an agar plate with a sterile loop. Bacterial suspension obtained by inoculating 1-2 colonies isolated in suspension using a sterile loop. The concentration of all

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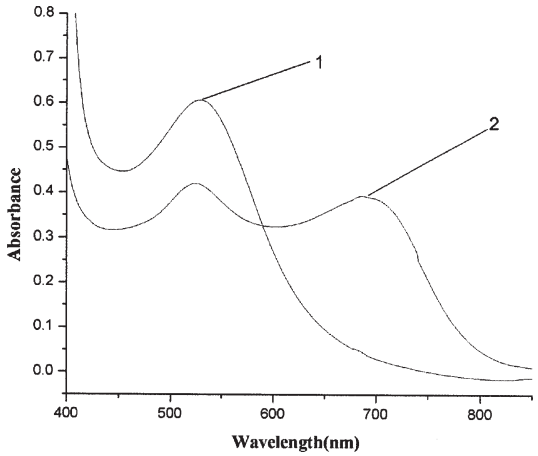

Fig. 1 UV-Vis spectra of gold nanoparticles (AuNps): 1- AuNps for size 2-5nm; 2 – AuNps for size 22-24nm

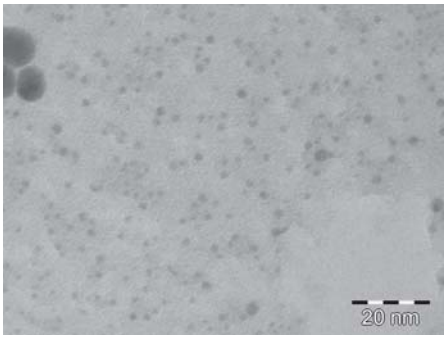

Fig. 2. Typical TEM image for AuNps 2-5nm.

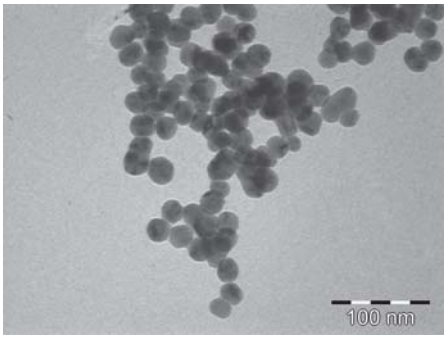

Fig. 3. Typical TEM image for AuNps 22-24nm

Staphilococcus aureus

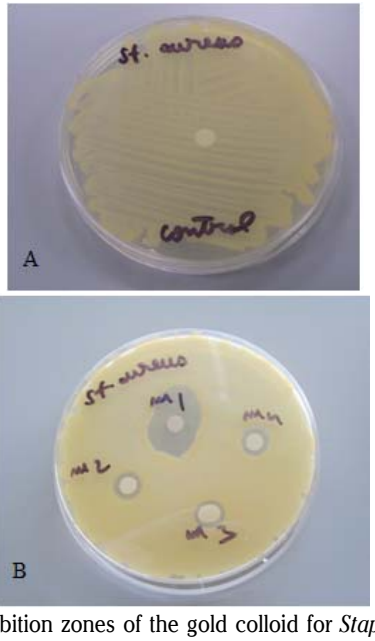

Fig. 4. The inhibition zones of the gold colloid for *Staphilococcus aureus*: A – control; B - gold colloids size 2-5nm

Fig. 4. The inhibition zones of the gold colloid for *Staphilococcus aureus*: C – gold colloids size 22-24nm.

Echerichia coli

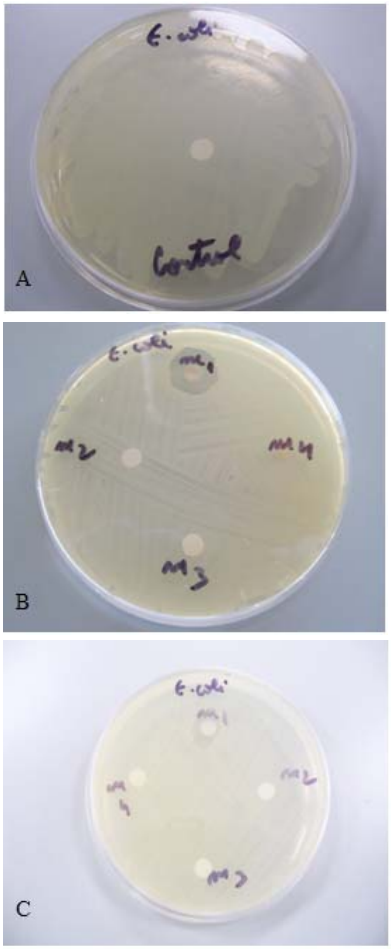

Fig. 5. The inhibition zones of the gold colloid for Echerichia coli: A – control; B - gold colloids size 2-5nm; C – gold colloids size 22-24nm.

the bacterial cell suspensions used for antibacterial activity was cca $1.8x10^8$ colony-forming unit (cfu)/mL.

Paper disks (diameter 6 mm, made from chromatographic paper) were impregnated in colloidal gold solutions of various concentrations (m_1 = 0.0724mg/mL; m_2 = 0.0362mg/mL; m_3 = 0.0181mg/mL; m_4 = 0.00905mg/mL). There were tested two sizes of gold colloids: 2-5nm and 15-20nm.

Disks were placed onto inoculated agar plates and left for 24 h at 36 °C. After incubation, plates were observed for antimicrobial activities by determining the diameters of the zones of inhibition for every sample. For an accurate analysis, tests were run in duplicate for each isolate to avoid any error.

Pseudomonas aeruginosa

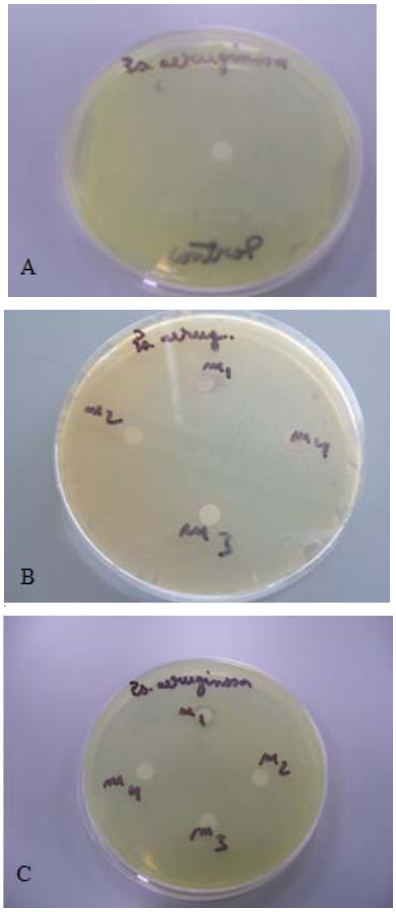

Fig. 6. The inhibition zones of the gold colloid for Pseudomonas aeruginosa: A – control; B - gold colloids size 2-5nm; C – gold colloids size 22-24nm.

Candida albicans

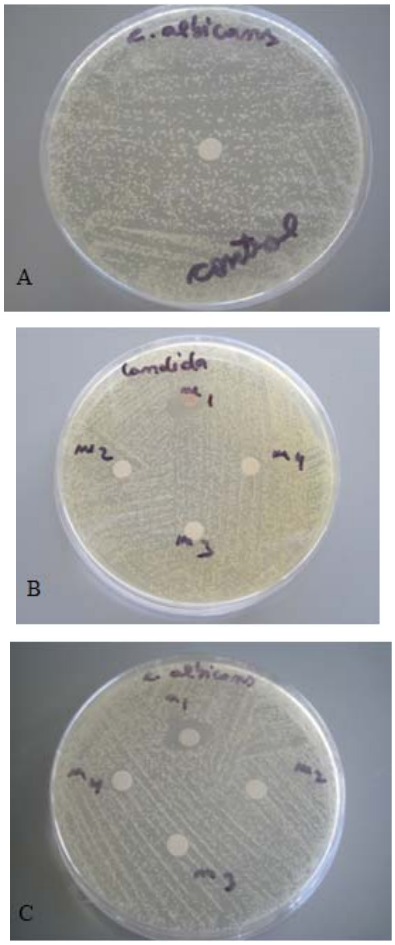

Fig. 7. The inhibition zones of the gold colloid for Candida albicans: A – control; B - gold colloids size 2-5nm; C – gold colloids size 22-24nm.

To determine the antibacterial activity by DDM, AuNps with four different concentrations of gold nanoparticles in the range of 0.00905, 0.0724mg/mL were tested against gram-positive bacteria (S. aureus), gram-negative bacteria (E. coli), non-ferment Gram-negative bacteria (P.aeruginosa), and fungi of Candida albicans. It was found that concentrations on 0.0724mg/mL exhibit bactericidal and fungic activity by the appearance of an inhibition zone in the range 7.5–20mm (fig. 4-7). Higher antibacterial activity was observed against both gram-positive S. aureus, and gram-negative E. coli. and lowest antibacterial activity was detected for P. aeruginosa.

Conclusions

Gold nanoparticles were prepared using chemical reduction by Schiff base, and their formation was proven by UV-Vis spectroscopy and TEM analysis.

Gram positive bacteria shows a higher sensitivity than Gram negative action of gold colloids. Sensitivity of E. coli bacteria and St. aureus is even greater as the colloidal particle size is smaller.

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