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Silver nanoparticles: characterization and antibacterial properties

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Abstract
In this study, two aqueous suspensions of silver nanoparticles, nano silver powder (Sigma Ag-NPs) and nano silver ink (Ink Ag-NPs), were characterized for studying their antibacterial activity. The X-ray diffraction (XRD) and high resolution transmission electron microscopy (HRTEM) were used to determine the crystal structure, shape and size of Ag-NPs. The bactericidal effects of these nanoparticles were investigated against gram positive and gram negative bacteria at two different concentrations, 10 and 50 µg/disc, by the disk diffusion method using Mueller–Hinton Agar to display good microbial culture growth. Antibacterial experiments results revealed that *Escherichia coli* (ATCC25922) and *Bacillus subtilis* (ATCC6633) inhibited by Ink Ag-NPs more than Sigma Ag-NPs. Both *Salmonella typhimurium* (ATCC14028) and *Staphylococcus aureus* (ATCC25923) were resistant to both sources of Ag-NPs, while *Pseudomonas aeruginosa* (ATCC27853) was resistant to Ink Ag-NPs and inhibited by Sigma Ag-NPs. This experiment showed that characteristics and suspension stability of Ag-NPs affect its antibacterial activity against some pathogenic bacterial strains.

Keywords Silver nanoparticles · Antibacterial activity · XRD · TEM · Suspension stability

1 Introduction

Nanoparticles have assumed a great importance because of their unique physical, chemical and biological properties as compared to bulk materials (Fayaz et al. 2011). Their applications ranging from optics, electronic devices, biomedicine, cosmetic products to catalysis, food and environment (Du et al. 2007). As antibacterial complementary to antibiotics, nanoparticles are highly promising and have large concern as might fill the gaps where antibiotics frequently fail (Gopinath et al. 2013; Sondi and Salopek-Sondi 2004). Among all, silver nanoparticles (Ag-NPs) are of interest because they have been utilized in many applications in plastics, textile, water treatment, paint industries, biosensors and antimicrobials (Yuranova et al. 2003; Jeong et al. 2005; Chou et al. 2005). Thanks to their high electrical conductivity, thermal stability, less resistivity to oxidation and interesting antibacterial properties.

In several research studies, Ag-NPs have been widely used in producing antimicrobial effect against bacteria, fungi, and viruses (Rai et al. 2009). Their effect was known already in ancient times. Silver and its compounds have been used from long time for the purification of water and disinfection of medical devices. In medicine, Ag compounds are generally applicable for using to treat burns, wounds, and various of infectious diseases (Avalos et al. 2014; Aditya et al. 2013; Elliott 2010). Multi-drug resistance is a growing problem in the field of infectious diseases treatment and the prevalent use of antibiotics has produced antibiotic resistance for many human bacterial pathogens. Advances in nanotechnology have opened new potential applications in the field of nano-medicine. Several studies and technologies are devoted to understand the mechanisms of diseases for the design of new drug, but unfortunately, infectious diseases continue to be a main health problem worldwide (Rai et al. 2009). In low concentrations, silver nanoparticles are a non-toxic and safe antibacterial agent for the human body (Castanon et al. 2008; Pal et al. 2007). In addition, Ag-NPs are also reported to possess antifungal activity (Kim

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et al. 2009), anti-inflammatory properties (Nadworny et al. 2008), antiviral activity (Rogers et al. 2008) and anti-angiogenic activity (Gurunathan et al. 2009). Ag-NPs can be applied safely in therapy when the effective concentrations against various types of organisms have been determined. In this experiment, Sigma Ag-NPs and Ink Ag-NPs were selected due to their small size and different shape because the antibacterial activity is size and shape dependent (Raza et al. 2016; Castanon et al. 2008). Both samples of silver nanoparticles were characterized and tested against pathogenic type culture bacteria. Ink Ag-NPs mainly used to print electronic components on flexible thin layers leading to the fabrication of unique electronic devices and circuit boards. The final ink Ag-NPs are always employed in suspension. Their dimension and geometry are really important to be controlled because the specific properties, electronic, optical and magnetic, are sensitive to size and shape (Rajan et al. 2016).

2 Materials and methods

2.1 Bacterial strains and culture conditions

Silver nanoparticles used in this study were purchased from Sigma-Aldrich and Metalon. The antibiotic discs used were Oxoid. The American type culture strains of *Bacillus subtilis* (ATCC6633), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhimurium* (ATCC14028) and *Staphylococcus aureus* (ATCC25923), were obtained from Center of Environmental Studies and Consultations (CESC) at Suez Canal University in Egypt. Cells were grown in Mueller–Hinton Agar (Oxoid) at 37 °C and cell growth was monitored by measuring the optical density at 600 nm. The two samples of silver nanoparticles were characterized by high resolution transmission electron microscope, X-ray Diffraction and Fourier transform infrared spectroscopy.

2.2 Sample preparation

In this context, two samples of Ag-NPs were prepared. The first one was nano silver powder from Sigma-Aldrich (0.076 g) dissolved in 4 ml of distilled water then the solution diluted by adding 250 ml of distilled water to have final concentration of colloidal silver nanoparticles 300 ppm. The second sample was nano silver ink from Metalon with very high concentration 3590 ppm (ICP Spectrophotometer, Perkin Elmer, Optima 7300DV). It is a dispersion of silver nanoparticles in aqueous that stabilized by adding capping agent to be suited to inkjet printing of electronic components. Both solutions were diluted by adding distilled water before using to study the antibacterial activity.

2.3 Antibacterial test

To examine the bactericidal effect of single silver nanoparticles (Ink Ag-NPs) and aggregated silver nanoparticles (Sigma Ag-NPs) on different bacterial strains, 100×10^7 colony-forming unit (CFU) of each bacterial strain were spread on Mueller–Hinton agar medium. Nanosized silver particles (Sigma, Metalon) and Ag^+ ($Ag\,NO_3$) were loaded on discs in concentrations of 10 and 50 µg/disc. The loaded discs were distributed on the surface of inoculated plates and the plates were incubated at 37 °C for 24 h. Tetracycline (TE, 30 µg/disc) was used as positive control, a blank disc was used as negative control as shown in Fig. 4. After incubation, the diameter of inhibition zones were measured in range of millimeter.

2.4 Determination of MIC

The Aliquots of 10 ml of Nutrient Broth (NB) were distributed in tubes, supplemented with Ag-NPs in concentrations of 10, 20, 30, 40 and 50 µg/ml to determine minimal inhibitory concentration (MIC). 10^7 CFU of *E. coli* cells (ATCC25922) was then inoculated in the tubes. Blank NB containing tubes without Ag-NPs or bacterial cells were used as a control. The growth of bacterial cells was monitored by means of turbidity after incubation at 37 °C for 24 h.

3 Results and discussion

3.1 Characteristics of silver nanoparticles

The same silver nanoparticles can have different characteristics and different suspension stability in medium. All Ag-NPs must be treated case by case to study their bactericidal effect. This work aims to explore the role of morphology, composition and suspension stability of Ag-NPs on tested bacteria strain. The characteristics of two different sources of silver nanoparticles, nano silver powder (Sigma Aldrich, 576832) and nano silver ink (Metalon, JS-B25HV) investigated. X-ray diffraction pattern was recorded for nano silver powder in the wide angle range from 10° to 90° as shown in (Fig. 1a). It showed four distinct diffraction peaks at 38.184°, 44.381°, 64.571° and 77.560° belong to the planes (111), (200), (220), and (311) for the cubic structure (Ag XRD Ref. No. 96-901-1608). The peaks declared that the main composition of powder was silver and there was not any peak indicated impurities in the XRD patterns. This result is consistent with the previously published reports of silver nanoparticles (Kamyar et al. 2012; Majid et al. 2011).

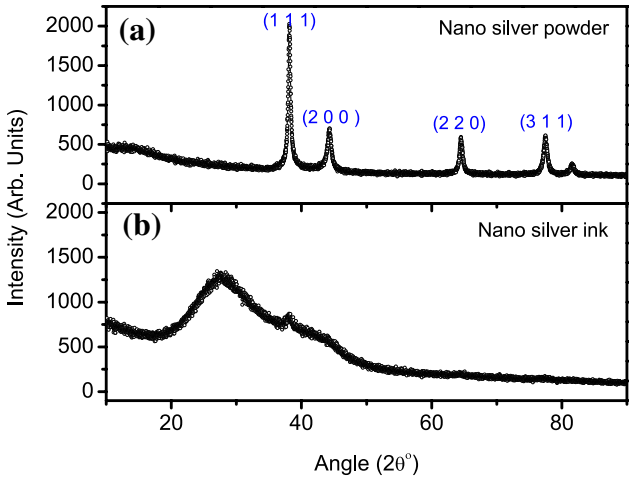

Fig. 1 X-ray diffraction patterns. **a** XRD of nano silver powder (Sigma Ag-NPs) and **b** XRD of nano silver ink (Ink Ag-NPs)

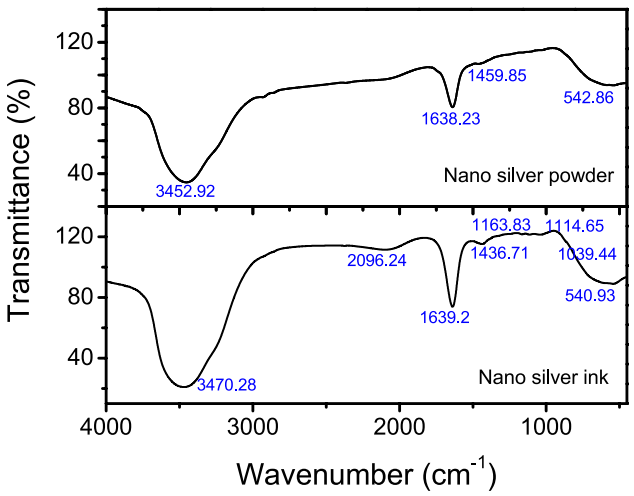

Fig. 2 FTIR spectra of silver nanoparticles. **a** FTIR of nano silver powder and **b** FTIR of nano silver ink

XRD of nano silver ink showed a bump instead of peak at 27.008°, this broad peak indicated that nano silver ink is amorphous as in (Fig. 1b).

Figure 2 showed the Fourier transform infrared spectra (FTIR) of silver nanoparticles, which represented the absorption bands of IR radiation by the two samples. The presence of various functional groups in each sample determined by comparing the observed absorption bands with standards values. The measurements are carried out from 4000 to 450 cm^{-1} at a resolution of 0.96 cm^{-1}. FTIR spectra of nano silver powder represented in (Fig. 2a), showed absorption bands at 3452.92, 1638.23 and 1459.85 cm^{-1}. The broad band at 3452.92 cm^{-1} attributed to O–H stretching vibration indicating the presence of alcohol. The peak at 1638.23 cm^{-1} corresponds to C=C stretching and the absorption band located at 1459.85 cm^{-1} assigned for N–H stretch vibration. Figure 2b represented FTIR spectra of nano silver ink, three bands located at 3470.28, 1639.2, 1436.71 cm^{-1} related to O–H stretch, C=C stretch and N–H stretch vibration mode, respectively. Four different characteristic peaks of nano silver ink observed at 2096.24, 1163.83, 1114.65 and 1039.44 cm^{-1} were assigned for C≡C, C–O, C–O and C–N stretch vibration mode, respectively (Kumari et al. 2016; Preetha et al. 2013).

Suspensions of two samples were tested using high resolution transmission electron microscopy to determine the particle size, shape and morphology as shown in (Fig. 3). HRTEM operated at 200 keV revealed that nano silver ink particles are single Ag-NPs and they have spherical shape and uniform size as in (Fig. 3b), their average size and standard deviation are 16.523 ± 7.792 nm. Also, they have excellent dispersive properties and their size and shape are controlled. The particle size distribution of Ink Ag-NPs is shown in (Fig. 3d). The nano silver powder particles, prepared by dispersing in distilled water using stirring and sonication, seem to be aggregated as in Fig. 3a and their average size and standard deviation are 18.936 ± 7.789 nm. The corresponding particle size distribution of Sigma Ag-NPs is shown in (Fig. 3c).

3.2 Antibacterial properties of silver nanoparticles

Nano-biotechnology is an approach in which biological and physical sciences share a common interest in new materials, tools and new phenomena of small structures, especially in the fight against socially and economically significant bacterial species. In previous study (Agnihotri et al. 2014), several pathogenic bacterial strains were treated by Ag-NPs and examined by SEM. Noticeably, all of the treated cells displayed some deep craters on their membrane surface, showing a damage of membrane structure. The treated cells appear to be shorter and more compact, suggesting there could be some leakage of the cellular contents caused by the treatment. Since the colloidal stability of Ag-NPs has an important role in their biological activity (Mac Cuspie 2011), the effect of single Ag-NPs (nano silver ink) and the effect of aggregation of Ag-NPs (nano silver powder) were investigated against tested bacteria. In our experiment, we measured the diameter of inhibition zones by the tested Ag-NPs in compare to Ag^+ (silver nitrate) at two concentrations (10 and 50 µg/disc) and TE (30) against the tested bacterial strains as shown in Table 1. All the tested bacterial strains were inhibited by the antibiotic (TE 30) with differences in the inhibition zones, also, the strains were inhibited by the Ag^+ and the diameter of inhibition zones increased by increasing concentration, except for *E. coli* (ATCC25922), the diameters were the same for both concentrations of Ag^+.

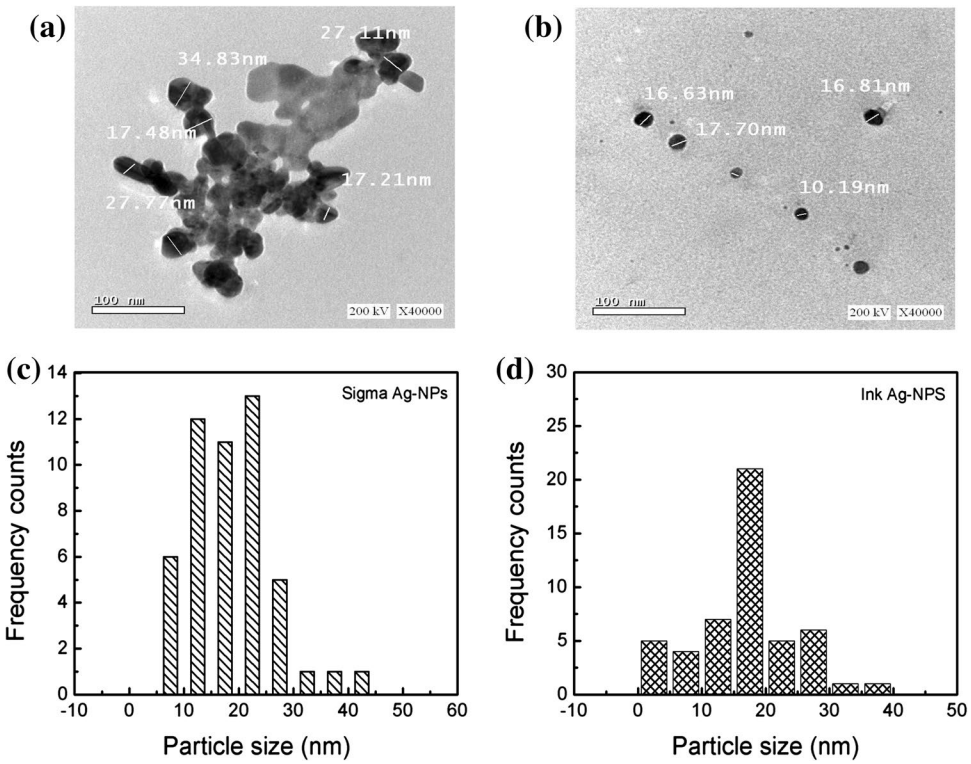

Fig. 3 Transmission electron microscopy analysis. **a** Image of Sigma Ag-NPs, **b** image of Ink Ag-NPs, **c** the particle size distribution of Sigma Ag-NPs and **d** the particle size distribution of Ink Ag-NPs (the total numbers of nanoparticles counted for each particle size distribution were 50 particles)

Table 1 Inhibitory behaviour of silver nanoparticles towards the tested bacterial strains

Tested bacterial strains	Sigma Ag-NPs		Ink Ag-NPs		Ag^+		Antibiotic (TE)
	10 µg/disc	50 µg/disc	10 µg/disc	50 µg/disc	10 µg/disc	50 µg/disc	30 µg/disc
Escherichia coli (ATCC25922)	9	11	11	12	13	13	25
Bacillus subtilis (ATCC6633)	6	8	7	9	8	9	15
Salmonella typhimurium (ATCC14028)	–	–	–	–	13	16	27
Staphylococcus aureus (ATCC25923)	–	–	–	–	11	14	19
Pseudomonas aeruginosa (ATCC27853)	11	6	–	–	12	14	20

Both *Salmonella typhimurium* (ATCC14028) and *Staphylococcus aureus* (ATCC25923) were resistant to both sources of Ag-NPs. These results in contrast with previous study reported by (Agnihotri et al. 2014), who synthesized silver nanoparticles, have 33.6 nm average mean size, and showed antibacterial activity against *Salmonella typhimurium*. The transmission electron microscopy analysis was performed in previous study (Saxena et al. 2010) to evaluate the interaction between Ag-NPs and different *Salmonella* strains. Their findings revealed that, the interaction with the Ag-NPs were strain dependent and time limited. In this study, the tested Ag-NPs size are larger than 15 nm while *Staphylococcus aureus* were sensitive to smaller sizes 5–7 nm (Agnihotri et al. 2014).

Bacteriostatic/bactericidal effects of Ag-NPs are size and dose-dependent (Agnihotri et al. 2014). *E. coli* (ATCC25922) and *B. subtilis* (ATCC6633) inhibited by both Ag-NPs and their inhibition zones increased in the diameter with increasing concentration. *E. coli* was more sensitive than *B. subtilis* for both samples of Ag-NPs; but the Ink Ag-NPs affected *E. coli* and *B. subtilis* more than Sigma Ag-NPs (Fig. 4a) because ink has morphology and suspension stability better than sigma. Ink contains single Ag-NPs which can easily move across the membrane surface. It was observed that the antibacterial activity of Ag-NPs against *E. coli* started at the effective concentration of 0.11 ppm (Liu et al. 2010). *E. coli* was the most sensitive bacteria against Ag-NPs (Ruparelia et al. 2008). In contrast, for *B. subtilis*, the discs showed a significantly smaller inhibition zone. In addition, Ag-NPs at concentration 50 µg/ml were showed complete antibacterial activity against *E. coli* (ATCC25922).

The behavior of *Pseudomonas aeruginosa* (ATCC27853) was different, it was resistant to Ink Ag-NPs and inhibited by Sigma Ag-NPs (Fig. 5a). Uncommon inhibition results

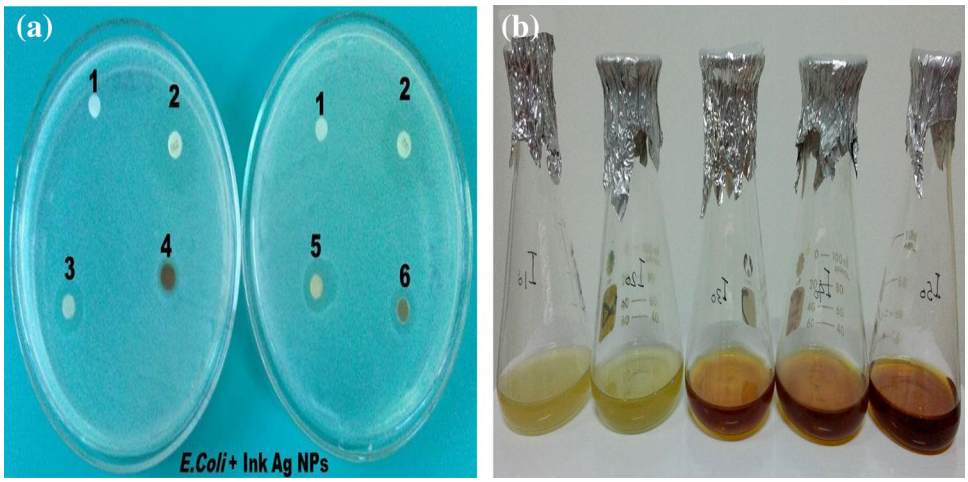

Fig. 4 Representative image of agar plates displays inhibition zone for *E. coli* (ATCC25922). **a** 1: negative control, 2: positive control, 3: Ag^{+} (Ag NO_3) at 10 µg, 4: ink Ag-NPs at 10 µg, 5: Ag^{+} (Ag NO_3) at 50 µg, 6: ink Ag-NPs at 50 µg and **b** MIC of ink Ag-NPs for *E. coli* bacteria (ATCC25922)

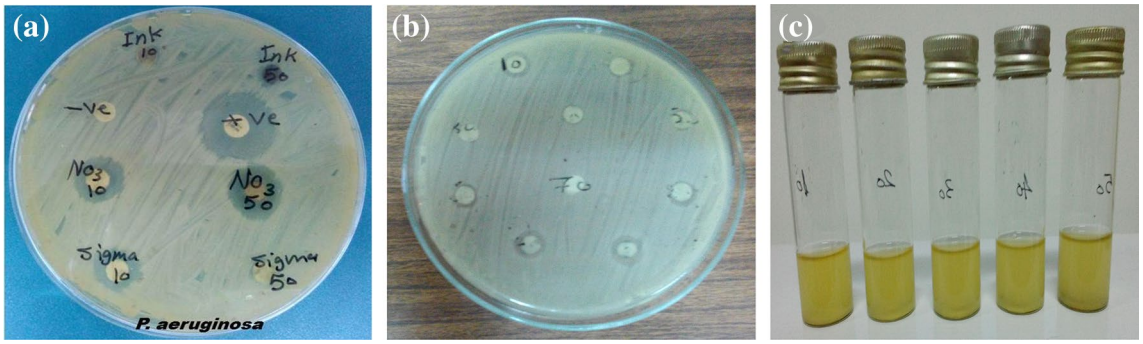

Fig. 5 Images of tested *Pseudomonas aeruginosa* (ATCC27853). **a** Inhibition zone for *P. aeruginosa* (ATCC27853), **b** different concentrations of Sigma Ag-NPs loaded on discs and **c** different concentrations of Sigma Ag-NPs loaded on broth

by the nano silver powder from Sigma Aldrich for *P. aeruginosa*, that the diameter of inhibition zone decreased by increasing the concentration of Ag-NPs. For confirmation, sigma Ag-NPs was tested against *P. aeruginosa* in agar and broth media. In broth, *P. aeruginosa* grown well in the tested concentrations (10–50 µg/ml) (Fig. 5c), while in agar medium, the inhibition zones formed around the tested concentrations were irregular. It does not increase with concentration; the larger zones were around 100, 90, 80, 60 and 10 µg/disc (Fig. 5b). It was observed that the sigma Ag-NPs was clumped in broth medium by shaking which may be the reason of its inefficiency. In a previous study (Singh et al. 2014), antibacterial activity of Ag-NPs against multidrug-resistant strains of *P. aeruginosa* showed inhibition zone of ranges from 10 to 21 and the MIC of Ag-NPs was found to be 6.25–200 µg/ml against *P. aeruginosa* strains. Although the Ag nanoparticles mechanism is still not clear, small diameter Ag-NPs have an excellent antimicrobial effect comparing to those of a larger diameter (Kim et al. 2009). The MIC of ink Ag-NPs is 30 µg/ml as shown in (Fig. 4b), below this concentration (10 and 20 µg/ml) *E. coli* grew well. This result is in a good agreement with (Ruparelia et al. 2008).

4 Conclusions

To conclude, this experiment clearly showed that the silver nanoparticles exhibit significant antimicrobial behaviour toward the tested gram positive and gram negative bacteria leading to a wide range of applications in medical field. The antibacterial activity of Ag-NPs is size, shape, morphology and suspension stability dependent. In this study, single Ag-NPs and aggregation of Ag-NPs are characterized and used against tested bacteria. Agar disk diffusion method is used because it is commercial and easy to use.

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