A SYSTEMATIC ANTIBACTERIAL STUDY OF NANOSTRUCTURED SILVER PARTICLES IN THE CONTEXT OF BIOMEDICAL APPLICATIONS

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ABSTRACT

Numerous studies on silver nanoparticles and silver metal as a broad-spectrum bactericidal and virucidal agent, place silver nanoparticles as a potent biocidal candidate in the field of nanomedicine for eliminating bacteria and viruses, especially multidrug resistant ones[1]. Health and medicine are the sectors with the most research and development of technologies that take advantage of the antimicrobial activity of silver nanoparticles[2,3,4]. Commercial silver nanoparticles are extensively studied[5] here against both Gram-negative and Gram-positive bacteria. Well defined in vitro bacterial cell cultures were set up by using suspensions of silver nanoparticles as a function of silver nanoparticles concentration. Analytical techniques such as TEM, DLS, z-potential, XRD and Ultraviolet-visible (UV Vis) Spectroscopy were systematically performed in order to have a thorough characterization of the suspension of silver nanoparticles. The fully reproducible results of the in vitro cell culture experiments confirmed the bactericidal action against bacterial strains. The minimum inhibitory concentration was determined to be well below 25 ppm of silver nanoparticles. Based on this present study, well characterized silver nanoparticles are highly promising antimicrobial agents as they can be used in cases that require conditions of control of microbial load and particularly in the context of several biomedical devices that we are currently working on.

KEYWORDS: Silver nanoparticles, Gram positive bacteria, Gram negative bacteria, antibacterial properties, medical devices

Introduction

The long-term use of antibiotics results in the increasing resistance of bacteria[6,7]. This has forced scientists to investigate new antimicrobial agents in order to replace antibiotics[8]. Silver is an excellent bacteriostatic and disinfectant chemical substance. Greeks and Romans, were the first who observed the antimicrobial effect of silver, as they extended the potability of water by storing it in silver vessels. Silver ions provide an antimicrobial effect via a silver ion interaction with thiol groups of vital bacterial enzymes and proteins. This affects cellular respiration and transport of ions across membranes, resulting in cell death[9,10]. Silver nanoparticles is another more modern form of antibacterial silver[11,12,13,14]. They penetrate the bacterial cell wall, leading to damaging structural changes in the cell membrane[15]. Reactive oxygen species generated at the surface of the silver nanoparticles result in oxidative stress providing a further mechanism for cell damage[16], thus they have been reported to have an effect against both Gram-negative and Gram-positive bacteria[17]. The strong toxicity against bacteria, while maintaining low toxicity for human cells make silver nanoparticles interesting candidates in biomedical engineering. Here we characterise a commercial silver nanoparticle product both in terms of its physicochemical and in terms of its antibacterial

properties, with the view of incorporating it at the next stage in an informed manner, in the design of medical devices where the control of bacterial load is of prime importance.

Methods

The techniques that were used in order to determine the shape, size and the morphology of silver nanoparticles were Ultra violet Visible Spectroscopy(UV-Vis), X-ray Powder Diffraction (XRD), Dynamic Light Scattering (DLS), Transmission Electronic Microscopy (TEM) and z-potential. Aqueous suspensions of silver nanoparticles were analyzed by using two different UV-VIS spectrophotometers, models 120-02 and 1800 Swimadzu in a range of 390-800nm and a glass cuvette of 10mm with the pure solvent as the reference. X-Ray diffraction was used in order to define the crystal structure. The XRD measurement was performed in a D8 Advance model of Brüker. The source of x-ray generation was copper and the wavelength was regulated to λ = 1.54 Å. The range in which the XRD analysis was performed is between 20 and 90° under 40 kV voltage and 40 mA current. In order to obtain an accurate measurement of the nanoparticles' size, both TEM and DLS techniques were employed. The TEM measurements were performed on a HRTEM Jeol Jem model, which was operated at an accelerating voltage of 200 kV; silver nanoparticles were prepared by placing a drop of nanoparticle suspension on a carbon-coated copper grid and water was allowed to dry overnight. A Vasco DLS device (Cordouan Technologies, Pessac, France) was used to measure the hydrodynamic diameter of silver nanoparticles. NanoQ software package facilitated control of hardware and analysis of results. Finally, to determine the electrical charge of the particles, the z-potential was measured on the Cordouan Wallis Zeta Potential Analyzer instrument. The instrument was set up by taking into account the relevant values of the deionized water in temperature of 25°C with viscosity 0.889 cP and relative dielectric constant of water equal to 78.06.

Two different methods were used in order to study the antibacterial activity of silver nanoparticles. Firstly, we investigated the antibacterial effect of silver nanoparticles in liquid cell cultures using the broth dilution method. Liquid *E. coli* cultures in concentration of 10 and 10^4 CFU/ml, were used and 25-50-100 ppm of silver nanoparticles were added in them. After incubation at 37 °C, changes of cell population were measured as a function of time. The well diffusion method was also utilized to evaluate the antibacterial effect on solid cell cultures under the same cell culture conditions as above.

Results & Discussion

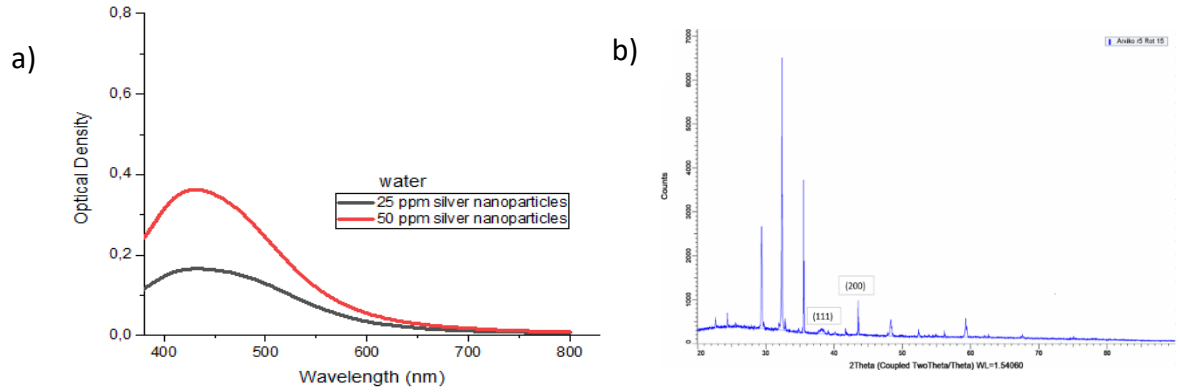

Figure 1: (a): UV absorption spectra of aqueous AgNPs suspensions of 25 and 50 ppm. (b): XRD pattern of AgNPs

In Figure 1 (a), a Surface Plasmon Resonance (SPR) peak at 430nm wavelength is observed for 25 and 50 ppm AgNPs suspensions. Various studies have shown that silver nanoparticles exhibit a characteristic peak due to Surface Plasmon resonance around 400 nm and by comparing with absorption spectra of AgNPs of known sizes and shapes, it appears that the spectrum is likely to refer to small size spherical AgNPs[13] . The absorption spectrum of 50 ppm concentration is identical to that of 25 ppm concentration but more intense, obeying well Lambert-Beer law. In Figure 1 (b), the XRD pattern indicates face centered-cubic (FCC) as a crystal structure[18] for the silver nanoparticles. Peaks in 2θ = 39.15° and 43.52° correspond to (111) and (200) hkl planes. The other peaks are overlooked, as they do not correspond to Ag patterns[JCPDS file No. 04-0783].

From the analysis of the XRD data above, the average nanoparticle size is determined in the range of 12.8 nm.

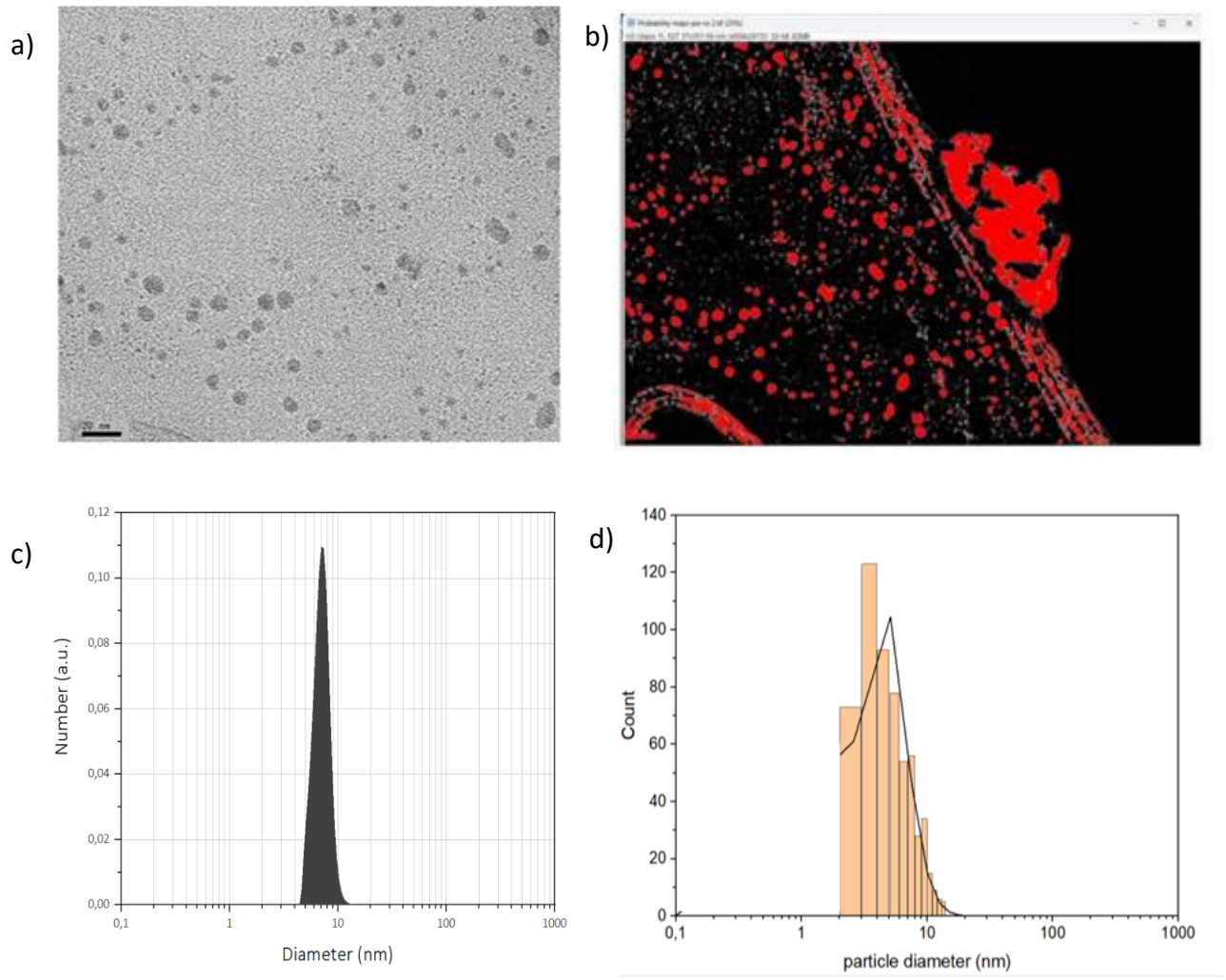

Figure 2: Transmission electron micrographs of silver nanoparticles: (a & b), size distribution based on DLS (c) and size distribution from the TEM analysis (d).

The TEM analysis shows the average particle diameter to be 5.9 ± 2.7 nm (Figure 2d). This value agrees with the DLS analysis, which found a diameter of 10.2 nm (Figure 2c), and does not differ significantly from the XRD measurement of 12.8nm. Also, z-potential was determined with a value of -35 mV which reveals the negative charge of silver nanoparticles which endows them with some significant suspension stability.

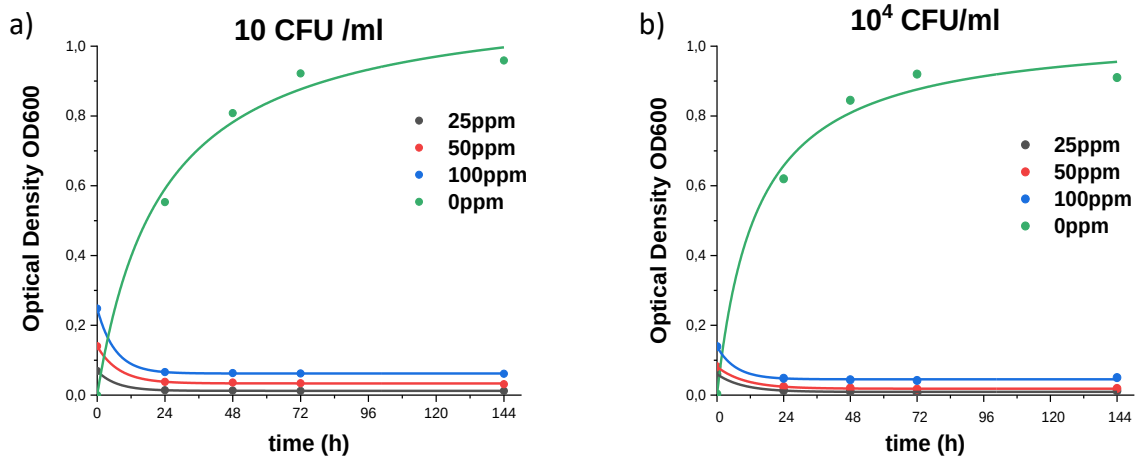

Figure 3: Variation in cell growth versus time for liquid bacterial cell culture of E. coli with population $X_0 \approx 10$ CFU/mL (a), and $X_0 \approx 10^4$ CFU/mL (b), in the presence of silver nanoparticles of concentration of 0, 25, 50 and 100ppm.

The kinetic studies in Figure 3 illustrate the strong antibacterial activity of silver nanoparticles on model liquid bacterial cell cultures. It is observed that in both concentrations of *E. coli*, the nanoparticles exhibit an intense antibacterial activity from the first 24 hours, after which a low plateau of virtually no cell growth appears. It is concluded that the Minimal inhibitory Concentration (MIC) is very much well below 25 ppm for an *E.coli* concentration up to 10^4 cfu/ml.

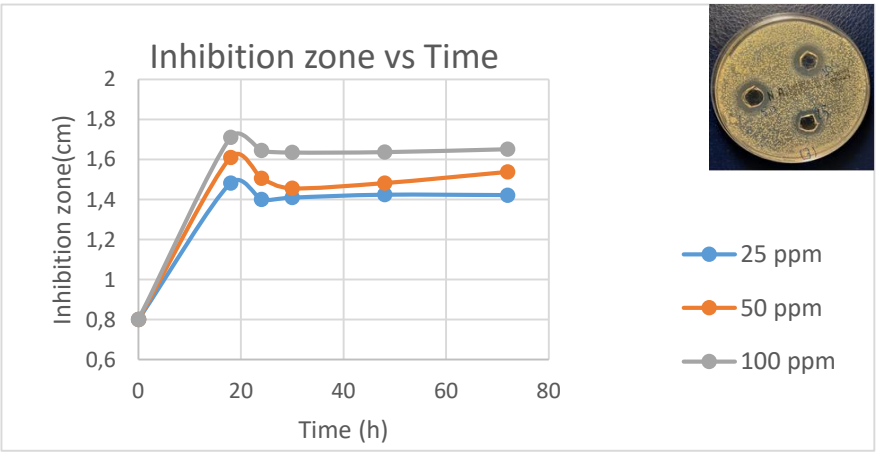

Figure 4: Kinetic of inhibition zone diameter versus time for silver nanoparticles suspensions of 25, 50, 100 ppm.

The kinetic studies in Figure 4 illustrate the antibacterial activity of silver nanoparticles in solid model bacterial cell cultures. A dependence of the inhibition zone with the concentration of silver nanoparticles is observed. The plateau in the inhibition zone is found after 24 hours, with a minimal increase up to 72 hours. Based on these studies in conjunction with complementary on going experiments, it may be concluded that the MIC is close to a silver nanoparticle concentration of 10 ppm.

Based on the above encouraging results, the silver nanoparticles are currently studied in the context of dental and other biomedical applications.

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