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Research Paper

Synthesis, characterization and antibacterial effects of Silver nanoparticles

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Abstract

Silver nanoparticles are particularly attractive in a number of areas, particularly health and food. Their optical and physicochemical properties give them enormous potential. In this article, we have studied the antibacterial effect of silver nanoparticles. These nanoparticles were synthesized chemically by reducing the $AgNO_3$ precursor. Colloidal solutions of nanoparticles are obtained at different molar concentrations (0.0015, 0.0025, 0.0030 and 0.0035 moles). Morphological and optical characterization, by TEM, DLS and spectroscopy, revealed nanoparticles of spheroidal shapes, and sizes around 10 nm, and surface plasmon resonance wavelengths around 440 nm. The antibacterial activities of AgNPs on hospital strains of *Coagulase-negative staphylococci* (CoNS), resistant to several antibiotics was evaluated.

Keywords: Silver nanoparticles, synthesis, characterization, antimicrobial activity, Coagulase-negative staphylococci (CoNS).

Introduction

Silver nanoparticles (AgNPs) have interesting physico-chemical properties; indeed, they are non-toxic, they have a high electrical and thermal conductivity, in addition to their low cost of production. Silver nanoparticles are particularly attractive because of their unique optical properties. For example, they have a strong absorption band in the UV-visible region. However, NPs can also be manufactured with great precision in a variety of clearly defined structures, such as spheres and stems. In addition, NPs can be chemically functionalized to selectively bind to tumor cells or bacterial ones[1,2]. These properties have given increasing interest to the study of these nanoparticles. They are used in many applications such as chemical detection, biology and imaging or fluorescence enhancement[3]. In addition, AgNPs can be antibacterial agent that can replace antibiotics and have the ability to overcome bacterial resistance to antibiotics. AgNPs have this confirmed antibacterial action, because of their high surface ratios[4-7].

Several studies have shown that the antibacterial activity properties of AgNP are strongly influenced by their shapes, sizes, concentrations and colloidal solutions[8]. It has been found that reducing the size of AgNPs improves their stability and biocompatibility[9]. Therefore, it is necessary to design nanoparticles of suitable sizes, shaped with the desirable surface properties, for use in various clinical and therapeutic interventions[10,11]. The first objective of this work focuses on the synthesis of silver nanoparticles by chemical reduction of the $AgNO_3$ precursor, for different molar masses (0.0015, 0.0025, 0.0030, 0.0035). The second one is the evaluation of the size and shape of synthesized nanoparticles, particularly experimentally using characterization techniques such as transmission electron microscopy (TEM) and dynamic light scattering (DLS) and UV spectroscopy –Visible. For the third objective, we evaluated the antimicrobial activity of silver nanoparticles against the pathogenic bacteria *Coagulase-negative staphylococci* (CoNS) for different molar concentrations of AgNP.

Materials and Methods

The silver nanoparticles were synthesized by chemical reduction of silver nitrate with benzoic acid and stabilized with polyvinylpyrrolidone (PVP K30, molar mass 40000) in an ethanol solvent medium. For this purpose, two 10 ml ethanoic solutions, containing respectively 1 g of PVP and 0.5 g of benzoic acid are added to a third 10 ml ethanoic solution, containing 0.0015 mol $AgNO_3$. The mixture is stirred vigorously by magnetic stirring for 20 minutes at room temperature then samples are collected in opaque glass vials and labeled accordingly. The operation is repeated for the quantities 0.0025M, 0.0030M and 0.0035M $AgNO_3$[12].

Transmission electron microscopy (TEM) was used to obtain detailed structural and morphological information on the samples and was performed using a JEM-2100Plus microscope, operating at an accelerating voltage 200 kV, with high resolution (0.4 nm). The hydrodynamic diameters of the nanoparticles studied were measured using a DLS, Zetasizer NanoS PCS dynamic light scattering system of a Malvern instrument equipped with a red laser (532 nm). UV-Vis spectra of silver nanoparticles were recorded at room temperature using an Ultrospec 2100 spectrophotometer PRO with a standard quartz cuve of 10 mm optical path. To predict the geometric parameters and optical properties of silver nanoparticles considered here, we used a 3D simulation based on the finite element method using COMSOL Multiphysics[13]. The numerical model is described elsewhere[14].

Results and Discussion

Experimental characterization

Transmission Electron Microscopy (TEM) was used to characterize the shape and the size of synthesized AgNPs. The micrographs recorded are from the TEM grid where drops of the colloidal solution of silver nanoparticles were deposited. The results show a spheroidal shape of AgNP with an average size of 8 nm. Figure 1 presents the morphologies and the sizes of the silver nanoparticles for the concentration of 0.0025 mol.

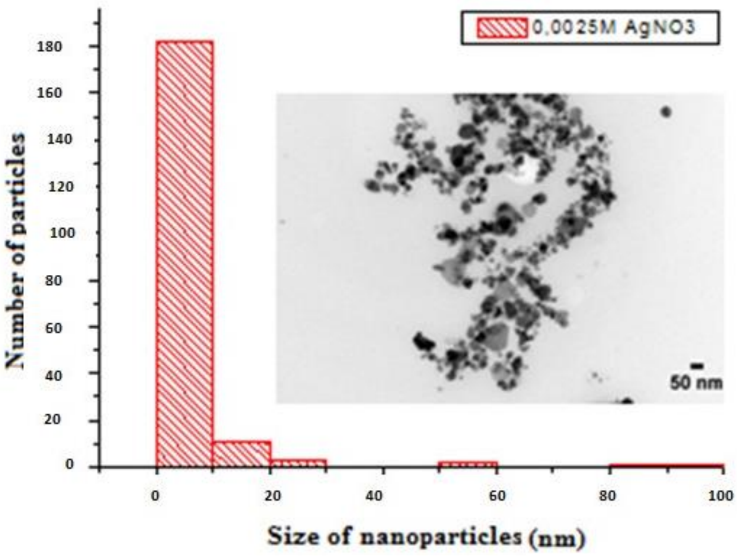

Figure 1: TEM image of synthesized Ag nanoparticles, with forms and sizes of AgNPsat 0.0025 mol

The size distribution of the silver nanoparticles was determined by DLS. The particle size distribution curve reveals tha tAgNPs obtained are rather monodispersed, with a mean diameter varying between 8 and 10 nm for different molar concentrations of the $AgNO_3$precursoras given in figure 2. As expected, the size of the nanoparticles measured by DLS is slightly greater than that measured by TEM. In fact, the hydrodynamic diameter obtained by DLS is always greater than the size estimated by TEM[14].

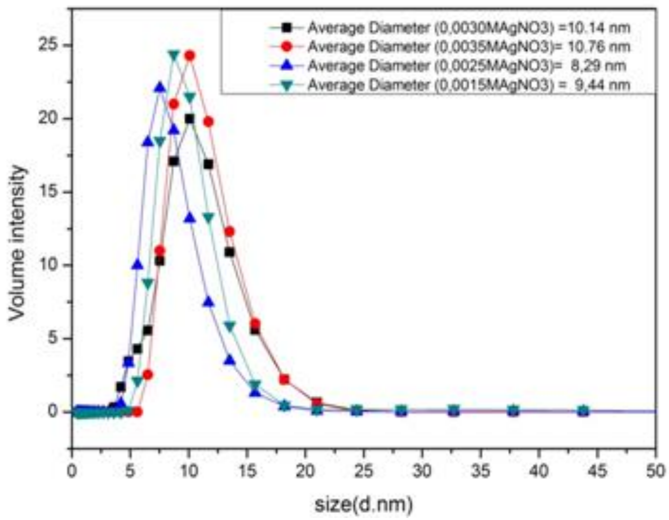

Figure 2: The particle size distribution of AgNPs measured by DLS

Remember that TEM and DLS results show that the colloidal solution of the silver nanoparticles is rather mono dispersed and the forms of the nanoparticles are spherical or oval (spheroidal). In addition, we noticed that the effect of the molar concentration is random. However, for the 0.0035 concentration, the precipitation phenomenon is important and nanoparticles with larger sizes are revealed at low quantities.

For the optical characterization of synthesized silver nanoparticles, we used UV-Visible spectroscopy. In Figure 3, we can observe the appearance of sharp peaks and surface plasmon resonance in the range of 438 to 441 nm. We note that a decrease in absorbance is observed for the concentration of 0.015 mol. On the other hand, the highest resonance peak with a decrease in the full width at half maximum (FWHM) value is obtained at a concentration of 0.0025 mol. This characterization confirms the TEM and DLS results with regard to the monodisperse aspect of AgNPs

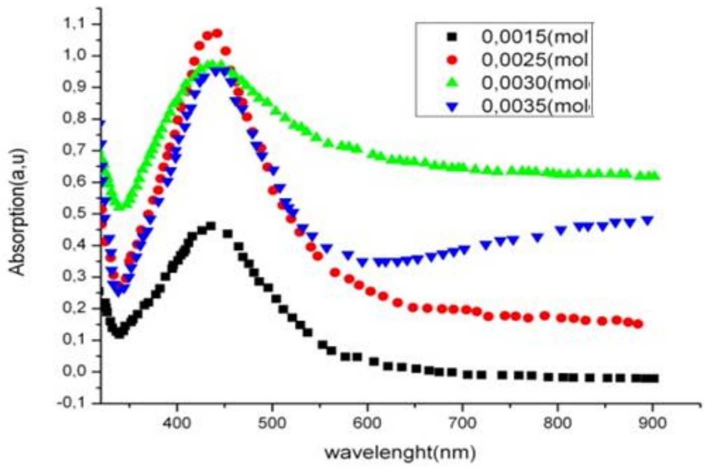

Figure 3: The ultraviolet-visible absorption spectrum of AgNPs, with a peak at different concentrations of $AgNO_3$

Theoretical approach

To generalize the previous experimental results obtained from different characterization techniques, we tried to evaluate the size and the shape of silver nanoparticles by a theoretical approach. This method is based on numerical analysis, depending on the refractive index of the colloidal solution obtained and a 3D simulation using the COMSOL Multiphysics software.

We considered three theoretical models: spherical, rod and spheroid for different diameters ranging from 8 nm to 12 nm and a shape ratio between 1.2 and 1.5; on the basis of the sizes measured by transmission electron microscopy that we obtained experimentally. The numerical calculations of the absorption spectra of the nanoparticles are made according to the size, the shape and the surrounding environment of the nanoparticles. Values of silver nanoparticles permittivity are extracted from experimental data of Palik[15]. Since the nanoparticles are supposed to be immersed in a spherical environment surrounded by perfectly matched layers (PML)[16], we have applied boundary conditions on the outer surfaces to consider an infinite domain. The nanoparticle is excited by an electromagnetic wave of 400 nm to 800 nm wavelength, polarized parallel to the major axis in the case of the spheroid and the rod.

As a general rule, we assume a diameter of 12 nm for the three types of nanoparticles, and then their length varies between 8 nm and 10 nm for the spheroid and the rod, which corresponds to the aspect ratio of 1.2 to 1.5 as given in Figure 4a.

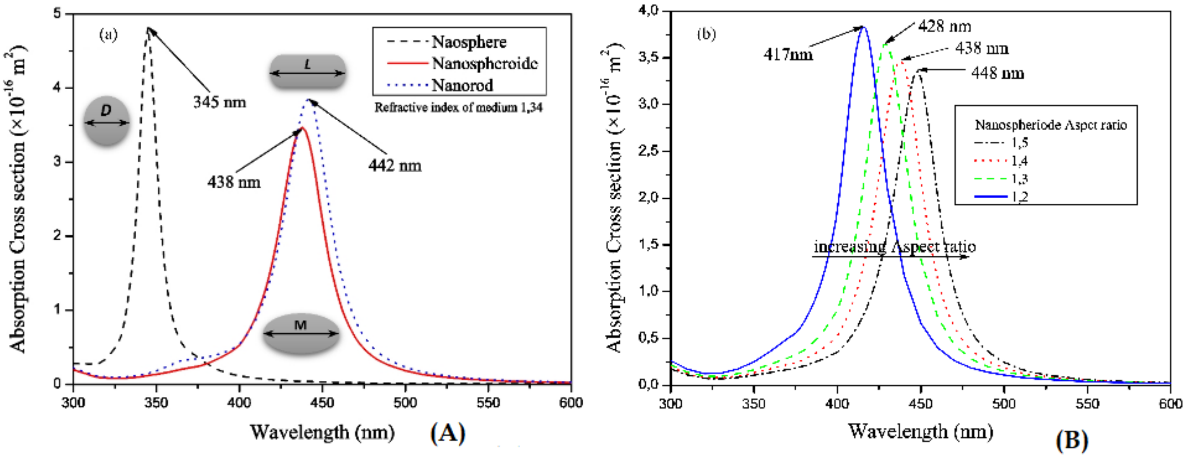

Figure 4: The study shows that the best results are obtained for nanospheroid; especially for geometrical parameter of (M =12 nm and minor axes of 8,5 nm)

The results of theoretical and numerical predictions show surface plasmon resonance peaks in the range of 417 to 448 nm for predominant spheroidal forms, based on experimental results by TEM. This difference in wavelengths with the experimental results, is certainly related to the synthesis process where the nanoparticles are not entirely homogeneous, residues of the other substances can infect the surface of the nanoparticles[17]. The best results are obtained for an aspect ratio equal to 1.4 and resonance peak at 438 nm (see figure 4b).The theoretical and experimental results are in agreement, especially for those forms and dimensions.

Antibacterial activity

We tested the silver nanoparticles synthesized with different concentrations of silver nitrate on the pathogenic resistant, *Coagulase-negative staphylococci* (CoNS), bacterium; at different molar concentrations of AgNP. We used the diffusion method of the antibiotic discs on a Muller Hinton agar medium (MH)[18]. The principle is as follows: a known quantity of silver nanoparticles (10 µl) is deposited on disks of antibiotics which proved to be ineffective on the bacterium tested during the antibiogram test. Control boxes are used to show the activity of antibiotics alone on the same bacteria. The antibiotics used alone or in combination with the naboparticles at different concentrations are Cefazolin KZ 30, Amoxicillin / clavulanic acid AMC30; TIC 75 Ticarcillin, Cefotaxim CTX-30, Amplicillin AMP 10, Ceftriaxone CRO 30. After 24 hours of incubation at 37°, the diameters of inhibition around the antibiotic discs loaded with silver nanoparticles are read in mm and then compared with the results obtained using the antibiotics alone to determine which active agent has the best activity. The results are summarized in Table 1 and Figure 5.

The different antibiotics used against the pathogenic strain of *Coagulase-negative staphylococci* (CoNS), showed their limits, and revealed a resistance of the bacterium. On the other hand, the synergistic effect of AgNPs with the different antibiotics was very important. In fact, all inhibition zones exceeded the sensitivity values according to Clinical and Laboratory Standards Institute - CLSI: S-sensitive (inhibition zones ≥ 13 mm)[19]. The highest inhibition zone was observed for AgNPs in combination with antibiotic CTX 30.

The increase of antibiotic inhibition zones with different solutions of nanoparticles, strongly depends on the penetration power of these small silver nanoparticles through the membranes of bacteria. In addition, the contact surface of these nanosilvers is important enough to overcome the bacteria. This contact surface with the bacteria is itself a function of physicochemical synthesis phenomena such as the shapes, the dimensions and the homogeneity of the solutions.

Bacteria tested. / ***Antibiotic***	***Staphylococcus Coagulase-Negative***				
	Antibiotic alone	*Antibiotic with 10µl of solution of AgNPs*			
		0.0015mol	*0.0025mol*	*0.0030mol*	*0.0035mol*
AMC30	*<6 R*	*15*	*15*	*14*	*16*
TIC75	*<6 R*	*15*	*23*	*15*	*15*
CTX30	*12R*	*16*	*17*	*25*	*13*
AMP10	*<6 R*	*20*	*13*	*15*	*15*
KZ30	*<6 R*	*13*	*17*	*15*	*9*
CRO30	*10R*	*20*	*15*	*16*	*12*

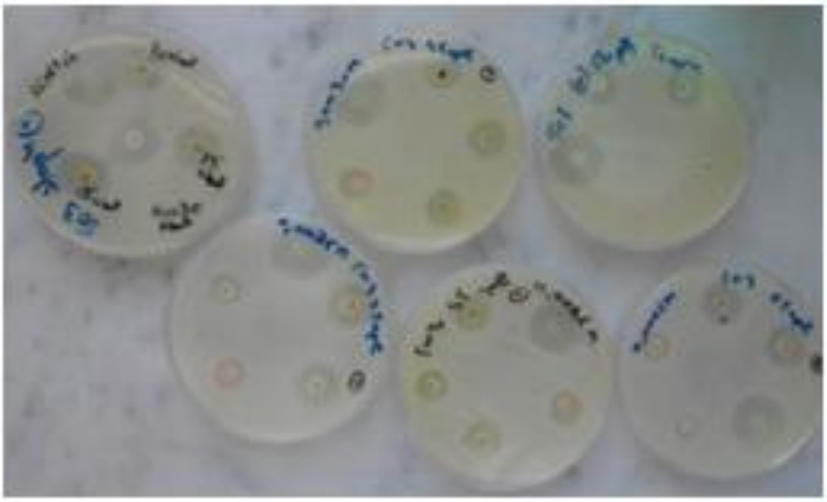

Figure 5: Test of susceptibility to antimicrobials. The antimicrobial activity of AgNPs is determined by measuring the inhibition zone around each disc

Conclusion

In this study, we synthesized silver nanoparticles by chemical reduction of the $AgNO_3$ precursor, for different molar masses (0.0015, 0.0025, 0.0030 and 0.0035). The colloidal solutions obtained are homogeneous and rather monodisperse. The characterization of these silver nanoparticles by TEM, DLS and spectroscopy revealed nanoparticles with a size ranging between 8 and 10 nm and a spheroidal shape with an aspect ratio between 1.2 and 1.5. Finally, we evaluated the antimicrobial activity of silver nanoparticles against the pathogenic bacterium *Coagulase-negative staphylococci* (CoNS) at different molar concentrations of AgNPs. The antibacterial effect of silver nanoparticles in combination with antibiotics is important. The zones of inhibition obtained with this combination are important, unlike the action of antibiotics alone.

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