

Synthesis of Silver nanoparticles (AgNPs) with Antibacterial Activity.

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Abstract. The synthesis of nanomaterials is currently one of the most active in nanoscience branches; especially those help improve the human quality life. Silver nanoparticles (AgNPs) are an example of this as it is known to have inhibitory and bactericidal effects. In this work, we report the synthesis of silver nanoparticles by chemical reduction method of silver nitrate (AgNO_3) from aqueous solution, using a mix of polyvinyl pyrrolidone (PVP) – *Aloe Vera* as reducing agent and for stabilization and control of particle size. Silver nanoparticles obtained were characterized by Scanning Electron Microscopy (SEM), UV–visible spectroscopy and measurements using Zetasizer Nano ZS were applied to size estimation. The existence of surface plasmon resonance peak at $\lambda_{\text{max}} \sim 420$ nm is evidence of silver nanoparticles formation. It was possible to standardize an appropriate protocol for the evaluation of bactericidal activity of the nanoparticles, for mesophilic microorganisms. Bactericidal activity above 90% against these kinds of bacteria was demonstrated.

Key words: Silver Nanoparticules, Antibacterial Activity, nanomaterial synthesis, mesophilic bacteria.

INTRODUCTION

Since ancient times it is known that silver has antibacterial properties, it has been used in biomedical applications, water and air purification, food production, cosmetics, clothing, and numerous household products [1]. Silver nanoparticles might exhibit additional antimicrobial capabilities not exerted by ionic silver, because its small size and large surface to volume ratios, which lead to both chemical and physical differences in their properties compared with their bulk counterparts [2]. AgNPs can be produced with various sizes and shapes depending on the fabrication method, among which the most widely used is the method of chemical reduction [3]. The aim of this study was to evaluate the antibacterial activity of AgNPs which were synthesized by chemical reduction method of silver nitrate (AgNO_3) in water, using polyvinylpyrrolidone (PVP) as reducing agent and *Aloe Vera* for stabilization and size control. The characterization of AgNPs was carried out by different techniques and antibacterial activity against mesophilic microorganisms was measured by number of bacteria colonies in Agar Plate Count.



EXPERIMENTAL

- **Preparation of Silver nanoparticles (AgNPs).**

The AgNPs were prepared by chemical reduction of an aqueous solution 12mM of AgNO_3 . At 70 mL of this solution is added PVP (keeping the molar ratio of the repeating unit of PVP and Ag equal to 34) and 21 mL of *Aloe Vera*, the whole reaction was carried out in argon atmosphere. The mixture was stirred in ultrasound at room temperature for 45 min, and then heated $2^\circ\text{C}/\text{min}$ to reach 80°C and left for 2 hours to obtaining a translucent solution with small suspended particles must be removed by simple filtration.

For purposes of comparison was done a synthesis with *Aloe Vera* only, to identify whether this natural extract may be used with dual function as a reducing agent and stabilizer. However, although using only *Aloe Vera*, a monodisperse suspension with an average particle size of 82.61 nm was obtained, this solution had no stability over time. Therefore, it was possible establish the size modulator and stabilization capacity of PVP, indicating that is necessary to use a PVP-*Aloe Vera* mixture to favor the formation of small nanoparticles and stabilized over time.

- **Characterization.**

The particle size analysis was performed 24 hours after synthesis; the samples were studied by use dynamic light scattering measurements a Nanozeta S (Malvern), UV-vis absorption spectroscopy (UV-Visible Cary-100 VARIAN) in the wavelength range from 200 to 900 nm. The size and morphology of silver nanoparticles were observed by Scanning Electron Microscopy (SEM) using a JEOL JSM-6490LV. Samples for SEM measurements were suspended in ethanol and ultrasonically dispersed and by Transmission Electron Microscopy (TEM) using a Tecnai F20 Super Twin TMP with Field emission source, resolution of 0.1 nm at 200 kV, 1.0 maximum magnification TEM MX camera GATAN US 1000XP-P.

- **Antibacterial activity.**

First we proceeded to prepare the inoculum for which was available fresh Rumen, the nutrient solution was prepared according to Siegert and Banks [4], 1 g of Rumen per liter of solution. It was cultured with 10 mL of the inoculated solution and stirred for 24 hours. Nutrient broth solution according to the manufacturer's recommendation (8g/L) was prepared and spread onto Agar Plate Count and incubated at $36-37^\circ\text{C}$ for 6 h. After incubation, the content of microorganisms was evaluated according to the scale McFarland (1.7×10^8 CFU/mL). To examine the bactericidal activity of AgNPs, different concentrations of AgNPs solution (0.1, 1, 10, 20, 40, 60, 80 and 100%) was used. An Agar Plate Count solution was used as nutrient medium, which was sterilized at 20 pounds of pressure for 30 minutes in autoclave GEMMYCO SA 232 mark.

In a series of Petri dishes previously sterilized it was added a volume of silver solution such that 10% remained in the volume concentration of agar to each system. When solidify the agar, it was cultured with 0.1mL of inoculum and incubated at 37°C (corresponding mesophilic microorganisms). After incubation, the number of colonies grown on the agar was counted [5]. The agar without nanoparticles and inoculated was considered as positive control, while a negative control, consisted of agar with nanoparticles but uninoculated.

RESULTS AND DISCUSSION

- **Synthesis and Characterization**

Due to the surface Plasmon resonance effect, is possible indicating of metal nanoparticles presence because the small metal nanoparticles exhibit the absorption of visible electromagnetic waves by the collective oscillation of conduction electrons at the surface [6]. UV-Vis spectroscopy was used to identify the presence of AgNPs, Figure

1a) shows the UV-Vis absorbance spectrum of AgNPs obtained, showing a peak associated with surface plasmon resonance at $\lambda_{max} \sim 420$ nm, although it can be turned by the fabrication technique [7]. Others works have reported a maximum peak between 405-418 nm [8,9]. The size distribution by intensity of synthesized AgNPs, is showed in Figure 1b, and confirms the presence of nanoparticles with size less than 20nm, as well as the presence of nanoparticles of greater size but, in minimum quantities. Therefore, it is clear that in this synthesis of AgNPs is obtained a polydisperse suspension, possibly due to the effect of the mixture of both stabilizers and a possible antioxidant activity of PVP that prevents aggregation of the nanoparticles in the system.

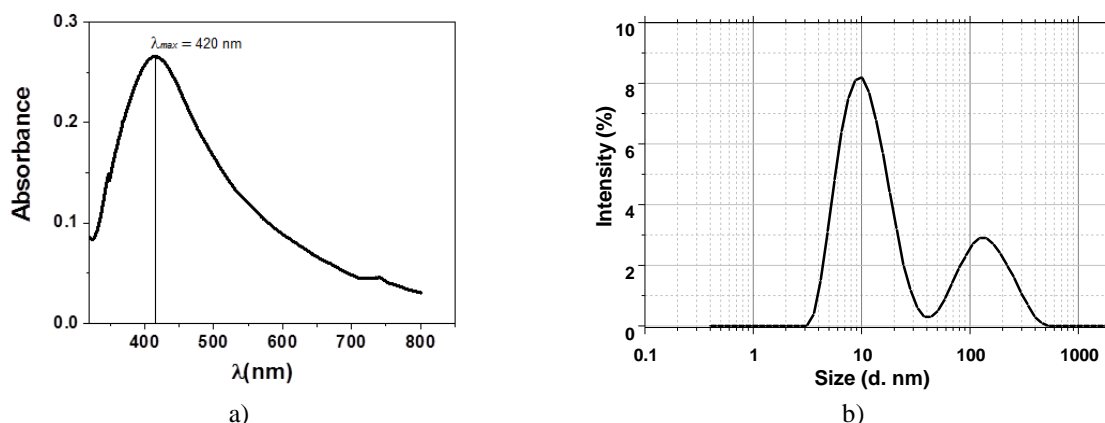


Figure 1: a) UV-vis spectrum and b) particle size distribution analysis of AgNPs

The shape and size distribution of AgNPs were characterized by SEM and TEM micrographies, which are shown in Figure 2. It confirms the presence of nanoparticles with a size between 10 nm at 130 nm, we used *Aloe Vera* as surfactant to avoid nuclei aggregation in order to decrease the total surface energy. The use of substances that lead to steric repulsion between individuals is one way to prevent nanoparticles from aggregation [10].

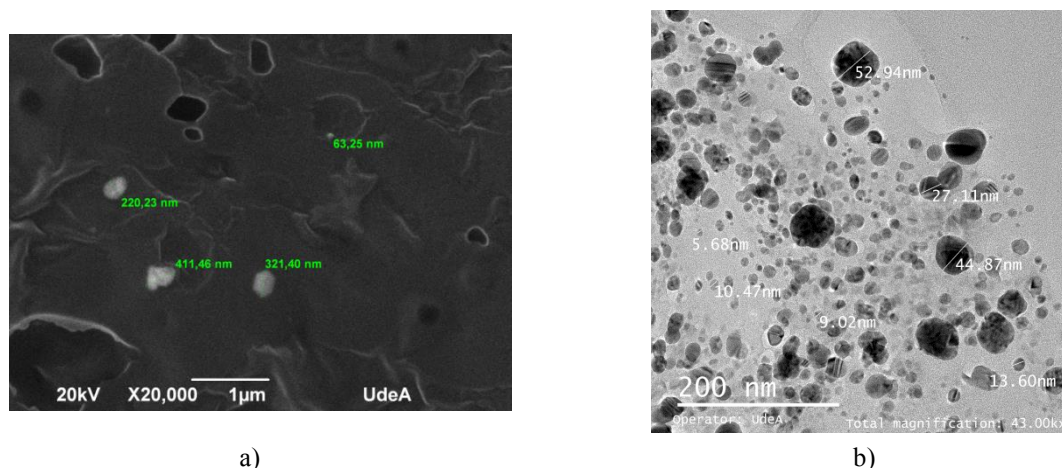


Figure 2: a) Micrographs SEM and b) TEM of AgNPs with *Aloe Vera*-PVP

• Antibacterial activity

The antibacterial activity of AgNPs against a mesophilic microorganism, the *Kocuria varians* a gram-positive cocci, was evaluated by triplicate in agar plates. Bactericidal activity was shown at higher concentration (20, 40, 60, 80 and 100%) other dilutions (0.1, 1 and 10%) didn't have antibacterial activity. Figure 3 shows photographs of the antibacterial test results, the lowest concentration that completely inhibited bacterial growth was 20%.



Figure 3: Photographs of the antibacterial test results of AgNPs at 100, 80, 60, 40 and 20% , made by triplicate.

CONCLUSIONS

Chemical reduction method was used to synthesize the AgNPs with an average size of 20 nm, from silver nitrate (AgNO_3) in water and using polyvinylpyrrolidone (PVP) as reducing agent and *Aloe Vera* for stabilization and size control with an average size of ~20nm. These AgNPs allowed standardizing an appropriate protocol for the evaluation of their bactericidal activity on mesophilic microorganisms. It was found that for all dilutions evaluated the nanoparticles exhibit bactericidal activity after 24 hours and the lowest concentration that completely inhibited bacterial growth was 20%.

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