Synthesis of colloidal silver nanoparticles and their bactericidal effects on *E. coli*, *S. epidermidis* and oral plaque

Juan Fernando Ramirez¹, Juliana Ortiz¹, Jorge Andrés Cuellar², Carlos Andrés Naranjo², Francy Nelly Jiménez¹,³ and Oscar Moscoso Londoño¹

¹Departamento de Física y Matemáticas, Universidad Autónoma de Manizales, Colombia.
²Departamento de Ciencias Básicas Biológicas, Universidad Autónoma de Manizales, Colombia.
³Departamento de Física y Química, Universidad Nacional de Colombia – Sede Manizales, Colombia.

*corresponding author: juan.ramirezhe@autonoma.edu.co

Abstract. Silver nanoparticles have been obtained by colloidal synthesis using two different reducing agents: ascorbic acid and ethylene glycol. The colloids have been characterized by UV-Vis Spectroscopy, atomic absorption and transmission electron microscopy (TEM). The UV-visible spectra show the typical peak with a maximum ranging between 390-420 nm, due to the plasmon resonance of spherical silver nanoparticles. TEM micrographs show non-aggregated spherical silver nanoparticles with diameters between 30 and 50 nm. The antibacterial effect was tested in three different bacteria cultures: *Escherichia coli* (gram negative), *Staphylococcus epidermidis* (gram positive) and dental plaque, which were grown in Mueller-Hinton agar. For comparative purposes the bactericidal effect of silver nitrate was also evaluated. Inhibition index (IIC) was calculated, obtaining satisfactory results for the three kinds of microorganism when silver nanoparticles are used.

1. Introduction

The capacity to produce nanoscale materials with controlled physicochemical properties has given rise to new applications in several fields, including technological and biomedical [1,2]. In particular, metal nanoparticles have been a focus of interest for numerous applications in engineering as in biomedical sciences [3]. Due to the surface activity of the nanoparticles, emerged from the surface / volume ratio, these systems can be functionalized or coated with drugs, antibodies, organic and inorganic molecules, as well as a wide variety of chemical agents. Specially, silver compounds are used as disinfectant for thousands of years. For instance, Hippocrates describes the use of silver powder for the healing of wounds. In the XVII and XVIII centuries, silver salts were used in the treatment of ulcer and in the XIX century, the antimicrobial effect of silver nitrate was discovered [4]. Currently, silver nanoparticles are used in many fields including medical, food, health care, consumer, even in industrial purposes [5]. Moreover, recent studies report a promising effect of silver nanoparticles (AgNPs) in cancerous cells [6] and HIV virus [7, 8]. AgNPs are also used in recent investigation as a colorimetric sensor for detection of sialic acid and melamine [9].
The high surface area to volume ratio is a fundamental factor for antimicrobial activity of silver nanoparticles, therefore this is an important factor to control in nanoparticle production [10]. The exact mechanism by which AgNPs exert toxicity over the bacteria and other organisms is still under discussion. For instance, Z. Xiu and coworkers argue that silver ions (Ag⁺) located at the AgNPs surface interact with oxygen, which produce cell lysis in the bacteria [11]. Other work, published by E. Fauss et al., describes how Ag⁺ ions generate reactive oxygen species (ROS), which are responsible for the disinfection mechanism [12]. Figure 1 (a and b) shows an illustration of these two approaches.

![Figure 1](image.png)

Figure 1. (a) Silver ions directly produce cell lysis (adapted from Xiu, 2012). (b) Silver ions produce reactive oxygen species (ROS) (adapted from Fauss, 2014)

2. Experimental

2.1. Synthesis of silver nanoparticle colloids

Silver nanoparticles were synthesized by a bottom-up approach using the Turkevich method [13]. Briefly, 0.1124 g of silver nitrate (AgNO₃) were dissolve in 6.5 mL of distilled water. Then, 0.0196 g of potassium citrate were dissolved and 0.0024 ascorbic acid were dissolved in 21 mL and 23 mL of distilled water, respectively. Afterwards, 4 mL of the solution with potassium citrate and 4 mL of the solution with ascorbic acid were mixed with 20 mL of distilled water to then drip the AgNO₃ solution until reaches a pH of 9. This solution was heated to 65 °C and kept at this temperature for 10 min. Ascorbic acid was used to give an electron to the cation Ag⁺ to then it becomes into Ag⁰. Potassium citrate was used as surfactant to cover and prevent aggregation of silver nanoparticles. The sample obtained under this protocol is labeled as Ag₁. Other sample was synthesized, this time were used 10 mL ethylene glycol as reducing agent and 4 mL polyvinylpyrrolidone as surfactant. The sample obtained under this protocol is labeled as Ag₂.

In both cases the solution turned yellow, which is an indication of the formation of spherical silver nanoparticles. All chemicals were used as received without further purification. The figure 2 display the production sequence of silver nanoparticles using this way.
2.2. Characterization techniques

Transmission electron microscopies of silver nanoparticles were obtained by placing a drop of silver colloid (sample \(Ag1\)) onto a copper grid (Ted Pella, Inc), followed by water evaporation in atmospheric conditions. Samples were studied using a JEOL–TEM transmission electron microscope. Absorption spectra were collected using a PerkinElmer UV–vis spectrophotometer. Atomic absorption spectroscopy was used to determine the concentration of silver nanoparticles in the colloidal solutions.

3. Results and discussion

3.1. Characterization

Figure 3 (a) shows the \(Uv–vis\) absorption spectra of silver nanoparticle colloids synthesized at different \(\text{AgNO}_3/\text{ascorbic acid}\) ratio. As can be noted, the maximums, associated with the plasmon surface resonance of the silver nanoparticles, are located at different wavelengths (ranging between 406 and 422 nm). This fact is a consequence of the obtention of colloids with Ag nanoparticles with different morphological properties [15], which can be promote by changes in the \(\text{AgNO}_3/\text{ascorbic acid}\) ratio. The localization of the absorbance peak mainly depends of the size and shape of the AgNPs. According to scientific literature, for spherical silver nanoparticles with a diameter smaller than 100 nm, this peak ranging between 390 to 430 nm [16, 17, 18]. Figure 3 (b) shows the \(Uv–vis\) absorption spectra of sample \(Ag1\). The plasmon surface resonance peak was found at 416 nm, which is a typical value of silver nanoparticle colloids.

\[ \text{AgNO}_3 \rightarrow \text{Reducing agent} \rightarrow \text{H}_2\text{O} \]
\[ \text{Free silver atoms} \rightarrow \text{Colloidal solution of silver nanoparticles} \]
Figure 3. (a) Uv–vis absorption spectra of silver nanoparticle colloids synthesized at different AgNO$_3$/ascorbic acid ratio. (b) Uv–vis absorption spectra of sample Ag1.

The nanoparticle concentration, determined by atomic absorption spectroscopy, were 74.79 mg/L and 8206 mg/L for samples Ag1 and Ag2, respectively. Despite the same amount of silver nitrate was used, it is possible to note that the silver concentration for both samples is strongly different. These results expose a larger efficiency when the ethylene glycol is used as reducing agent.

Transmission electron microscopies of silver nanoparticles of sample Ag1 and Ag2 sample are displayed in figure 4. Due to the high dilution of the silver nanoparticle colloids at the time of preparing the samples, it was not possible to obtain a representative number of nanoparticles to build a size distribution. However, the results show non-aggregates of Ag NPs (fundamental factor for biomedical applications). According to the results, Ag NPs nanoparticles have a quasi-spherical shape. Moreover, it is possible to note facets on each nanoparticle (dark and light regions), which suggest that the growth of the nanoparticles does not occur in a single step, it means that after NP nucleation, a process of multi-stage growth occurs. In both cases a broad size distribution is presumed, since, for example, in sample Ag1, it is possible to observe a diameter variation between 20 nm and 50 nm. Similar trend is observed in sample Ag2.
3.2 Bactericidal test on three different bacteria cultures.

The antibacterial effect was tested on *Escherichia coli*, *Staphylococcus epidermidis*, as well as in oral plaque. The *E. coli* is a gram-negative bacillus present in a great proportion of human and animal infections [19]. The *S. epidermidis* is a gram-positive coccus that has become the most important cause of nosocomial infections [20]. The mouth harbors one of the most diverse microbiomes in the body which includes over 700 different microbial species including viruses, fungi, protozoa and archaea as well as bacteria [21].

The antibacterial effect was tested using Kirby-Bauer method consisting in incubating the bacteria in a Petri dish with agar Müller-Hinton for 24 hours at 37 °C. Silver nanoparticles were impregnated on discs (*Ag1* and *Ag2* colloids) to then placed in the agar before incubation. In order to compare the antibacterial effect, other discs were impregnated with 0.25 M of silver nitrate (AgNO₃) and ethylene glycol. Sodium citrate and ascorbic acid are not used as blanks because they do not have any bactericidal effect.

Index of bacteria inhibition is calculated by the equation 1 [22].

\[
IIC = \frac{DIZ - DD}{DD} \quad \text{(Eq. 1)}
\]

Where *IIC* is inhibition index, *DIZ* is the diameter of inhibition zone and *DD* is the disc diameter (9 mm).

The results of the bactericidal tests on *E. coli*, *S. epidermidis* and oral plaque are presented in figure 5 (two tests for each bacteria). Results indicate that sample *Ag2* has an *IIC* slightly larger than sample *Ag1*. The larger concentration in *Ag2* is the most probable explanation. Silver nitrate and ethylene glycol

![Figure 4. Transmission electron microscopies (TEM) for samples Ag1 (a, b and c) and Ag2 (d, e and f)](image-url)
have insignificant diameter of inhibition corresponding to an IIC of 0. For *E. coli* bacteria, samples Ag1 and Ag2 have similar diameter of inhibition around 18 mm corresponding to an IIC of 1. The bacteria inhibition on *S. epidermidis* was larger with sample Ag2 (23 - 30 mm) in comparison to the obtained with sample Ag1 (10 – 25 mm). Contrary to the previous test, silver nitrate and ethylene glycol showed bactericidal effect with an inhibition diameter around 18 mm. Ag1 presents an inhibition diameter between 14 and 20 mm (corresponding to IIC of 0.55 and 1.22), while for sample Ag2 diameters between 17 and 25 mm were obtained (IIC in the range 0.89 – 1.78).

![Figure 5. Results of bactericidal test on E. coli (a and b), S. epidermidis (c and d) and oral plaque (e and f).](image-url)
3.3 Concentration dependence of inhibition

In general terms, with sample $Ag_2$ a larger diameter of inhibition was obtained in comparison with sample $Ag_1$. Moreover, ethylene glycol does not show a significant bactericidal effect. Fact related with one of the most important differences between $Ag_1$ and $Ag_2$ is the AgNPs, it means Ag NPs concentration. Figure 6 shows the $IIC$ versus AgNPs concentration for the three different microorganisms studied in this work. It can be observed that to a larger AgNPs concentration results in larger $IIC$.

![Figure 6. Inhibition index (IIC) as function of the AgNPs concentrations.](image)

4. Conclusions

While most of commercial antibiotics only works in similar bacteria, for example grams positives cocci such as $S. epidermidis$ or gram negatives bacilli such as $E. coli$, AgNPs showed inhibition in these two types of bacteria and additionally on oral plaque, so it could be used in a wide variety of application including: infection treatment, food preservation, water purification etc. The inhibition is dependent of the AgNPs concentration, but evidently there are other variables that affect $IIC$, some of these are particles diameter and surface coating.
Acknowledgements

We acknowledge support of Unidad de Investigaciones and Vicerrectoría Académica of Universidad Autónoma de Manizales - UAM (project # 580-085). We thank M. Delgado Loaiza for the support during antibacterial effect tests. We acknowledge the Chemistry Lab of Universidad Nacional de Colombia for the use of UV/VIS spectrophotometer. We also acknowledge Dr Sebastian Calderón Velasco of the International Iberian Nanotechnology Laboratory (INL) for TEM images.

References

[1] Wang C, Yin H, Dai S and Sun S, 2010 Chem. Mater. 22 3277–82
[2] Amarjargal A, Tijing L D, Im I T and Kim C S 2013, Chem. Eng. J. 226 243–54
[3] Mody V, Siwale R, Singh A and Mody H, 2010, J. Pharm. Bioallied Sci. 2 (4) 282–289
[4] Monge M, 2009 An. Quim 105 33-41
[5] Panacek A, Kvıtek L, Prucek R, Kolar M, Vecerova R, Pizurova N, Sharma V, Nevecna T and Zboril R, 2006 J. Phys. Chem. 110 16248-16253
[6] Zhang X, Liu Z, Shen W and Gurunathan S, 2016 Int. J. Mol. Sci. 17 1534
[7] Ayala N, 2010 Nanopartículas de plata como microbicidas: actividad y mecanismos de acción contra la infección por el virus de inmunodeficiencia humana (VIH) y diferentes bacterias resistentes a antibióticos
[8] Ávalos A, Haza A, Mateo D and Morales P, 2013 Revista Complutense de Ciencias Veterinarias 7 (2) 1-23
[9] H I Badi’ah et al 2019 IOP Conf. Ser.: Earth Environ. Sci. 217 012005
[10] Gurunathan S, Kalishwaralal K, Vaidyanathan R, Deepak, Sureshbabu R, Muniyandi J, Hariharan N and Eom S, 2009 Colloids and Surfaces B: Biointerfaces 74 328-335
[11] Xi Z, Zhang Q, Puppala H and Colvin V. Alvarez P, 2012, Nanoletters 12 4271-4275.
[12] Fauss E, MacCuspie R, Oyanedel-Craver V, Smith J and Swami N, 2014 Colloids and Surfaces B: Biointerfaces 113 77-84
[13] Turkevich J, Stevenson P and Hillier J, 1951 Discuss. Faraday Soc. 11 55–75.
[14] Torrecilla A, 2014 Determinación en el contenido de plata en nanomateriales de plata inorgánicos y polímericos mediante la digestion asistida por microondas y espectroscopía de de absorción de llama 8
[15] Martinez F, Zuniga E and Sanchez A, 2013 Mundo Nano 10 101-108
[16] Kelly K, Coronado E, Zhao L and Schatz G, 2003. J. Phys Chem B 107 (3), 668-677
[17] Serra A, Filippo E, Re M, Palmisano M, Vittori-Antisari M, Buccolieri M and Manno D, 2009 Nanomaterials 20 681
[18] Pradhan N, Pal A and Pal T, 2001 Colloids and Surfaces A: Physicochemical and Engineering Aspects 196 257-257
[19] Bhainsa K and D’Souza S, 2006 Colloids and Surfaces B: Biointerfaces 47 160-164
[20] Desai R, Mankad V, Gupta S and Prafulla J, 2012 Nanoscience and nanotechnology letters 4 30-34
[21] Young C and Otto M, 2002 Microbes and Infection 4 481-489
[22] Adams S, Arnold D, Murphy B, Green A, Smith A, Marsh P, Chen T, Marriott R and Brading M, 2017 Scientific Reports 7
[23] Charlena et al 2017 IOP Conf. Ser.: Earth Environ. Sci. 58 012064