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Optical properties and antimicrobial effects of silver nanoparticles synthesized by femtosecond laser photoreduction

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ABSTRACT

Silver nanoparticles exhibit a powerful antimicrobial action showing a pronounced potential to be widely used against drug resistance bacteria. The present work describes the optical properties and antimicrobial effect of silver nanoparticles produced by femtosecond laser photoreduction of $AgNO_3$ in the presence of tryptophan water solution. The advantages of this method are the absence of hazardous chemical reducing agents in the solution, and the versatile dimensional control achieved. The synthesized silver nanoparticles were characterized by absorption and fluorescence spectroscopy and their antibacterial activity were determined by monitoring the cell viability of *Escherichia coli*. The effects of the silver nanoparticles concentration and laser parameters (exposure time and pulse energy), on the formation of the nanoparticles, and its influence on the bacteria growth inhibition were studied. The prepared silver nanoparticles exhibited suitable antimicrobial properties. The results demonstrated that the nanoparticles concentration plays an important role in their bactericidal efficacy. The increase in the laser energy caused an increase in *E. coli* growth inhibition. Irradiations with energies around 300 μJ for 60 min presented high antimicrobial activity due to the presence of kynurenine, sub product of tryptophan photolysis. The first-time formation mechanism of tryptophan silver nanoparticles in high optical intensities was also discussed.

1. Introduction

Silver nanoparticles are an effective tool for killing microorganisms and have been used in catheters, bandages, water purification, cosmetics, toys, clothing, etc [1–3]. These nanoparticles have a broad spectrum of antibacterial properties against a wide range of gram-positive and gram-negative bacteria and do not contribute in the development of resistant strains [4], showing a great potential to be widely used against drug resistance bacteria.

Silver nanoparticles can be synthesized by a large variety of chemical, physical and biological methods [1,5,6], and among them, photoreduction by femtosecond laser pulses is used to produce nanoparticles with controlled sizes and concentrations [7,8]. The advantages of this method are the absence of hazardous chemical reducing agents in the solution, and the versatile dimensional control achieved. When synthesizing nanoparticle with focalized laser pulses, the temporal scale is the main parameter for the metallic nanoparticle fragmentation. On the femtosecond scale, the most common fragmentation process is the Coulomb explosion [9]. Several parameters such as the laser wavelength, intensity, pulse energy, pulse duration, repetition rate, influence in the growth and the aggregation mechanisms, and their control define the final size of the nanoparticles.

When silver nanoparticles interact with bacteria they produce reactive oxygen species and impairment of flagellar activity, which were observed for a broad range of silver species [10], and are responsible for bacteria inhibition [11]. Silver nanoparticles induce DNA degradation [11]. Inside the bacteria, spherical NPs interact and destroy the sulfur and phosphorus-containing complexes (soft bases) like DNA, and also disrupt the morphology of the membrane, finally leading to the cell death [12]. For a given quantity of silver, smaller nanoparticles show better inhibitory action due to a significantly larger surface area in contact with the bacterial effluent, resulting from the higher surface to volume ratio as compared to bigger nanoparticles. Therefore, smaller particles release more silver ions than larger particles to kill bacteria. Recently Raza et al. [12] observed that spherical silver nanoparticles with

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diameters in the range 15–50 nm exhibited maximum bactericidal efficacy against *E. coli* strains, followed by the antibacterial activity of triangular silver nanoparticles. Dror-Ehre et al. [13] showed that the bactericidal activity of silver nanoparticles against *E. coli* relies on the ratio NPs/cells, which determines the frequency of collisions of the NPs to the cells. Furthermore, the antibacterial performance of these particles was observed to be even better than that of Ciprofloxacin, suggesting that silver nanoparticles can be a good alternative for antibiotics that have promoted bacterial resistance.

The use of amino acid tryptophan (Trp) in nanoparticle synthesis as reducing/stabilizing agent has been reported in the literature [14–18]. The main advantages of using tryptophan in the synthesis of silver nanoparticles are low toxicity, biocompatibility, and ability to load various bioactive molecules potentializing drug delivery. Tryptophan nanoparticles can reduce potential hepato and nephrotoxicity [16]. Mukha et al. observed that the formation and stabilization of gold and silver nanoparticles in the presence of tryptophan is strongly influenced by acidity of initial components, and the tryptophan conversion in such systems goes through the kynurenine pathway [17].

The present study reports the synthesis of tryptophan silver nanoparticles by femtosecond laser pulses, investigating the effects of concentration and laser parameters (exposure time and pulse energy) on the formation of the nanoparticles and its influence on the bacteria growth inhibition. The formation mechanism of tryptophan silver nanoparticles in high optical intensities is also discussed.

2. Materials and methods

The green and environment friendly method to synthesize silver nanoparticles with ultrashort laser pulses for antimicrobial applications is described in the following paragraphs.

2.1. First Experiment: Concentration dependence

All the reagents used had analytical grade. Silver nitrate and commercial L-Tryptophan Vetec were purchased from Sigma–Aldrich. Initially, tryptophan silver nanoparticles (TrpAgNPs) solutions with different silver concentrations were prepared mixing $AgNO_3$ with tryptophan, according to Table 1, in 100 mL of distilled water at room temperature. The process was accompanied by vigorous stirring for 5 min.

The solutions 2, 3 and 4 were irradiated by 40 fs ultrashort pulses from an amplified Ti:Sapphire laser system (Odin, from Quantronix), centered at 800 nm, 400 μJ of maximum energy, at 1 kHz repetition rate. The laser beam was focused by a 50 mm lens, inside a 1 cm glass cuvette containing 1 mL of solutions, in such a way that no damage, or supercontinuum generation, occurred in the cuvette walls. Each solution was irradiated by 5 min in the first experiment.

2.2. Second Experiment: Irradiation time duration dependence

Solution 3 (1 mL) was irradiated with femtosecond pulses of 300 μJ, 40 fs pulses, 1 kHz, by 10, 20, 40 and 60 min.

2.3. Third Experiment: Pulse energy dependence

The influence of the pulses energy was studied (solution 3), fixing the irradiation time in 5 min, 1 kHz repetition rate and energies of 100, 200, 300, 350 and 400 μJ.

Table 1
Reagents used in TrpAgNps synthesis.

Sample	$AgNO_3$	Tryptophan
1	–	0.0621 g
2	0.0081 g	0.0621 g
3	0.0137 g	0.0617 g
4	0.0239 g	0.0618 g

2.3.1. Sample characterization

The UV–Vis absorption spectra of all samples were measured by a MultiSpec-1500 spectrophotometer (Shimadzu Scientific Instruments), using 1 cm quartz cells.

Fluorescence measurements were performed using a RF-5301 fluorimeter (Shimadzu Scientific Instruments). The samples fluorescence spectra under excitation at 280 nm were measured between 300 and 550 nm. All measurements were carried out at room temperature using a quartz cuvette with four polished faces and 1 mm of optical path.

Microscopic analyses were performed on a LEO 906E transmission electron microscope (Zeiss, Germany), with images captured using a Megaview III camera (Zeiss) and processed using the iTEM – Universal software HAS Imaging Platform (Olympus Soft Imaging Solutions GmbH, Germany). For analysis, 5 μl of each sample were deposited on a square copper mesh (37 μm/side), previously coated with parlodium and an amorphous carbon film. After allowing the sample to soak into the mesh (3 min), the excess sample was removed using absorbent paper and subjected to analysis. The ImageJ 1.46 software program was used to determine the average size of the nanoparticles by applying the Gaussian fitting in Origin 8.

Fourier Transforms Infra-Red spectroscopy (FTIR) of dried TrpAgNPs were grinded with KBr to make pellet and spectra was recorded using Shimadzu Spectrophotometer IRPrestige-21 in the region of 4000–700 cm^{-1}.

2.3.2. Growth inhibition assay

The *E. coli* ATCC 25,922 was transferred from glycerol (30 μl) to the culture medium TSB (trypticase soy broth), and left overnight in an incubator at 37 °C with 20% of CO_2. On the second day, the inoculum was transferred to the culture medium TSA (trypticase soy agar), and again incubated overnight at 37 °C with 20% of CO_2. On the third day, a solution of this inoculum was prepared at 0.5 McFarland scale, corresponding to 10^8 CFU/ml. This solution was prepared with saline 0.85% and a purview of the colony in TSA. After two dilutions in Mueller Hinton broth (MH) sterile, the solution final concentration was 10^6 CFU/ml.

The TrpAgNPs solutions obtained from the three experiments were diluted ten times in broth Mueller Hinton (20 μL of TrpAgNps + 180 μL MH). The viability tests were done in flat-bottom microplates (96 wells), in duplicate. 50 μl of TrpAgNps and 50 μL of inoculum were placed in each well. A control of TrpAgNps, was also made in duplicate, using 50 μL of TrpAgNps and 50 μl MH. Two wells were used for bacteria positive control (bacteria without nanoparticles), with 50 μL of inoculum and 50 μL MH. The plates were further incubated for 24 h at 37 °C. For cell viability study, after treatments for the microplate was placed in an incubator at 37 °C with 20% CO_2, and the reading was done by an ELISA reader at 595 nm. The percentage cell inhibition was calculated by the following formula:

$$\%\ \text{Cell inhibition} = 100 \times (\text{O.D. of control} - (\text{O.D. of treated} - \text{O.D. TrAgNPs}))/\text{O.D. of control}$$

were O.D is the Optical Density