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A simple and effective method to synthesize fluorescent nanoparticles using tryptophan and light and their lethal effect against bacteria

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ABSTRACT

A simple, environmentally friendly and cost-effective method was used to synthesize silver nanoparticles using tryptophan and light. To prepare the nanoparticles, the following components were used: deionized water, silver nitrate, light and tryptophan. The effects of the tryptophan concentration and light exposure time on the formation of tryptophan silver nanoparticles (Tnnps) were studied. The synthesized Tnnps were characterized using transmission electron microscopy (TEM), absorption and fluorescence spectroscopy and zeta potential measurements. The synthesized Tnnps were nearly spherical, with sizes of approximately 17 nm. In addition, the antibacterial activity of Tnnps was determined by monitoring the growth curves of strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Serratia marcescens*, and *Enterococcus faecalis* using the microdilution test. The Minimum Inhibitory Concentration (MIC) for 4 of 5 tested bacteria was determined to be between 20.0 and 17.5 μg/mL for 48 h and between 22.5 and 20.0 μg/mL for 72 h.

1. Introduction

Silver has a strong antimicrobial potential and has been used since the times of antiquity [1,2]. However, with the discovery of antibiotics, the use of silver as an antimicrobial agent has declined. Because of the recent observation of the increase of the antimicrobial effects of silver when in the form of silver nanoparticles, interest in the use of silver as a potential antimicrobial agent has made an extraordinary comeback [3,4]. The bactericidal efficacy of silver nanoparticles has been investigated by many researchers [3–12]. Microorganisms are unlikely to develop resistance against silver, in contrast to antibiotics, as silver attacks a broad range of targets within the microbes [9].

There are various theories modeling the antimicrobial effect of silver nanoparticles [1,4,6,13–16]. Due to the nanoparticles size, a large surface area comes into contact with the bacterial cells to provide a higher percentage of interaction when compared to bigger particles [4]. Yen et al. [17] reported that smaller silver nanoparticles (radius around 3 nm) are more cytotoxic than larger particles (25 nm) at a concentration of 10 μg/mL, highlighting the importance of the nanoparticle size. The bactericidal potential of nanoparticles is also influenced by their shapes. Pal et al. [9] reported the effect of spherical, rod and triangular nanoparticles, synthesized by citrate reduction, against *Escherichia coli*. Triangular nanoparticles were found to be more effective against this pathogen than spherical nanoparticles, which are more active than rod-shaped nanoparticles.

The synthesis of silver nanoparticles is carried out by several physical and chemical methods that include photochemical reduction [18,19], chemical reduction [20], laser ablation [3], sonochemical methods [21] and microwave radiation [22,23].

For the photochemical reduction of silver salts, very small colloidal particles can be produced, and the reduction of metal ions can be controlled through the control of the exposure time [24]. There are other factors that are also responsible for defining the particles sizes in photochemical reactions like the reactant concentration, presence of stabilizers [25], and the reaction medium [26]. The chemical and physical methods used to synthesize silver nanoparticles frequently raise questions regarding environmental risks due to the use of toxic, hazardous chemicals [27]. The synthetic methods also use organic solvents to counterbalance the hydrophobicity of the capping agents [28]. In addition, there are other various biochemical routes demonstrated in the literature [29].

Several studies have described the controlled synthesis of metal nanoparticles with different sizes and shapes mediated by biomolecules that are nontoxic and minimize environment damages. Tryptophan has already been used for silver nanoparticles

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synthesis in several reports [30,31], nevertheless, this is the first report that uses a photochemical reduction. This paper describes the synthesis of silver nanoparticles mediated by a tryptophan/light system and the antibacterial activity of the resultant nanoparticles. The characterization of the synthesized silver nanoparticles was done by optical/fluorescence spectroscopy, zeta potential measurements and transmission electron microscopy.

2. Materials and methods

2.1. Silver nanoparticles generation and evaluation

Silver nitrate ($AgNO_3$, 99%, CAS# 7761.88.8) and tryptophan (CAS 73-22-3) were purchased from Sigma–Aldrich. No other chemical reagents were used in the synthesis. The silver nanoparticles were obtained by mixing the reagents in water solution, followed by vigorous stirring for 5 min. The concentration rate study of silver nitrate and tryptophan aqueous solution was performed according to the conditions presented in Table 1. The transparent solutions were illuminated by a xenon lamp (Cermax 400 Watts, not focused and placed 10 cm from the sample reservoir ~3.6 W/cm^2). The Xe lamp power measurement was done condensing its output in a broadband power meter (Spectra Physics 407A), using a lens and a calibrated beam splitter.

The effects of the exposure time on the nanoparticle synthesis were studied, and the results are presented in Table 2.

UV–vis absorption spectra were recorded by a Shimadzu Multispec-1501 UV–vis spectrophotometer using 10-mm quartz cells.

The emission spectra were obtained by exciting the samples, inside a 1-mm optical path cuvette. The emissions of the samples were analyzed using a Fluorimeter Jobin Yvon exciting samples at 280 nm. Emission spectra were obtained in the range of 300–550 nm. The silver nanoparticles zeta potential dependence on the solution pH of silver nanoparticles was measured by the analytical instrument Zetasizer NanoZS. To construct a curve of zeta potential as a function of pH, the adjustment to different pH values was performed using standard solutions of HCl (5 mol/L) and NaOH (5 mol/L).

The morphology of the tryptophan nanoparticles (Tnnps) was determined by transmission electron microscopy using a LEO 906E instrument from Zeiss, Germany (6 μA and 80 kV). For the measurements, a drop of silver nanoparticles (5 μL) dispersed in bi-deionized water was placed onto a carbon-coated copper grid. The excess liquid was removed using a paper wick, and the deposit was dried in air for 5 min prior to imaging. The images were captured by a Megaview III camera and processed using the iTEM universal TEM imaging platform (Olympus Soft Imaging Solutions GmbH, Germany).

Table 1
Concentration rate study of silver nitrate and Tryptophan.

Tryptophan (mmol L^{-1})	$AgNO_3$ (mmol L^{-1})	[Tryptophan/$AgNO_3$]	Xe-lamp illumination times (min)
11.75	–	–	1
12.38	**0.83**	14.92	1
12.24	**3.41**	3.59	1
12.03	**5.41**	2.22	1
12.10	**11.77**	1.08	1
12.41	**23.72**	0.52	1
12.11	**47.34**	0.25	1

Table 2
Study of Xe exposure time.

Tryptophan (mmol L^{-1})	$AgNO_3$ (mmol L^{-1})	[Tryptophan/$AgNO_3$]	Xe-lamp illumination times (min)
12.03	**5.41**	2.22	30″
12.03	**5.41**	2.22	1′
12.03	**5.41**	2.22	3′
12.03	**5.41**	2.22	5′
12.03	**5.41**	2.22	10′
12.03	**5.41**	2.22	15′
12.03	**5.41**	2.22	

2.2. Broth microdilution assay for the determination of the Minimum Inhibitory Concentration (MIC)

The preparation of *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Serratia marcescens*, and *Enterococcus faecalis* strains were performed by a 1:20 dilution in a LB (Luria–Bertani) broth. Microtiter plates (96 wells) were used for the broth microdilution assays to ascertain the MIC for each tested strain. Two independent assays were conducted according to the guidelines of the National Committee for Clinical Laboratory Standards (CLSI, M100-S9). The following minor modifications were implemented: the target microorganisms were grown in test tubes overnight in 3 mL of the respective medium at 30 °C and agitated in a rotary shaker (150 rpm). After a 1:20 dilution in a LB (Luria–Bertani) broth, the expected density for the broth microdilution method was 5×10^5 CFU/mL, which is equivalent to approximately 100 colonies isolated on the plates containing a medium solid culture. Finally, a 90-μL aliquot of this suspension was used in each well of a 96-well plate acrylic (ELISA), resulting in a concentration of 5×10^4 CFU/mL on a well acrylic plate. The concentration was confirmed by viability counts on the LB plates. The microtiter plates were subsequently incubated at 30 °C for 24 h, 48 h and 72 h. Finally, the absorbance at 530 nm was measured in a plate reader (Epoch, Bio-Tek, Winooski, VT, USA). The threshold for the MIC was set at a minimum of an 80% growth inhibition. All of the tests were performed in triplicate in 100 μL of reaction volume.

2.3. Evaluation of the antibacterial activity of silver nanoparticles

The concentration of the silver nanoparticle samples ranged from 15 to 30 μg/mL (10.0 μL stated on each well). A positive growth control containing only culture medium and the strains under study was also prepared. The LB liquid culture medium was used as negative control in the study.

After preparation of the experiment, the plates were incubated at 37 °C for 24 h, 48 h and 72 h and analyzed using a microplate reader (Biotek – Epoch Microplate Spectrophotometer).

All of the tests were performed in triplicate.

3. Results and discussions

3.1. Synthesis and characterization of tryptophan silver nanoparticles (Tnnps)

During light exposure, a modification of the solution color was noted, which was observed to change from colorless to a brownish/yellow due to the presence of silver nanoparticles in suspension (Fig. 1).

3.1.1. Variations of the silver nitrate concentrations

The absorbance of the silver colloidal solutions was measured for the solutions prepared with different $AgNO_3$ concentrations. The results are shown in Fig. 2. With low silver nitrate concentration (0.83 mM), a small absorption band at approximately 440 nm was observed. The peak was low in intensity and very broad. According to the literature, broad peaks in the beginning of the formation of nanoparticles are attributed to very small particles