



# Antibacterial activity of silver nanoparticles synthesized from serine



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## ABSTRACT

Silver nanoparticles (Ag NPs) were synthesized by a simple microwave irradiation method using polyvinyl pyrrolidone (PVP) as a capping agent and serine as a reducing agent. UV–Visible spectra were used to confirm the formation of Ag NPs by observing the surface plasmon resonance (SPR) band at 443 nm. The emission spectrum of Ag NPs showed an emission band at 484 nm. In the presence of microwave radiation, serine acts as a reducing agent, which was confirmed by Fourier transformed infrared (FT-IR) spectrum. High-resolution transmission electron microscopy (HR-TEM) and high-resolution scanning electron microscopy (HR-SEM) were used to investigate the morphology of the synthesized sample. These images showed the sphere-like morphology. The elemental composition of the sample was determined by the energy dispersive X-ray analysis (EDX). Selected area electron diffraction (SAED) was used to find the crystalline nature of the Ag NPs. The electrochemical behavior of the synthesized Ag NPs was analyzed by the cyclic voltammetry (CV). Antibacterial experiments showed that the prepared Ag NPs showed relatively similar antibacterial activities, when compared with AgNO<sub>3</sub> against Gram-positive and Gram-negative bacteria.

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## 1. Introduction

Metallic nanoparticles are used in different fields, such as catalysis, media recording, biosensing, optics, environmental remediation, biomedical, pharmaceutical, cosmetic, energy, electronic devices, imaging, drug delivery, and medicine [1–4]. Specific size, shape and surface morphology of the metallic nanoparticles play a vital role in controlling the properties of the nanoscopic materials [5,6]. Different synthetic methods are reported in the literature for the preparation of the metallic nanoparticles [7,8]. Though conventional (chemical and physical) methods are available to synthesize the metallic nanoparticles, they are expensive and required more time. Thus, there is an increasing need to develop the high-yield, low cost, non-toxic and eco-friendly procedures for the synthesis of metallic nanoparticles.

Among the various noble metal nanoparticles, Ag NPs have shown essential applications in various fields like catalysis, bio-sensing, imaging, drug delivery, nano-device fabrication and medicine [9–11]. Due to the strong anti-microbial activity, Ag NPs are also used in clothing, food industry, sunscreens and cosmetics [12–14]. Hence, Ag NPs have been projected as the future generation antimicrobial agents [15]. Oxidation of metallic silver is thermodynamically unfavorable, due to its higher positive reduction potential and thus, resulting in quite stable in aqueous and alcoholic suspensions without the aid of capping agents.

Chemical reduction methods are commonly used to synthesize the Ag NPs. Typical reducing agents, such as polyols [16,17], NaBH<sub>4</sub> [18–20], N<sub>2</sub>H<sub>4</sub> [21], sodium citrate [22], polymeric thiols [23], Triton X 100 [2], and amino acids [24–27] are used to synthesize the Ag NPs. Polyvinylpyrrolidone (PVP) and polyvinyl alcohol are eco-friendly polymers, since they are water soluble, and have extremely low cytotoxicity. These polymeric substances are frequently used as stabilizers, due to their optical clarity, which enables the investigation of the formation of nanoparticles [28,29]. The antibacterial activities of PVP modified Ag NPs are significant because the PVP is most effective in stabilizing the particles against aggregation [30]. Most of the chemical reduction reactions are carried out at high temperatures for a higher reaction rate, and it can be done by conventional heating [16,17], laser irradiation [31,32], ultrasonic [33], fixed frequency microwave radiation [34, 35], UV irradiation [36], and gamma-ray irradiation method [37].

Several methods for the synthesis of Ag NPs have been reported in the literature [38,39]. However, microwave irradiation method is known to have a faster heating rate than the conventional heating. The use of a fixed frequency microwave to synthesize platinum and silver nanoparticles is also reported [34,35,40]. Microwave irradiation method is more eco-friendly and requires less energy than the other conventional methods. This method offers rapid and uniform heating of solvents, reagents, and intermediates. Further, it provides uniform nucleation and growth conditions for the nanomaterials [27].

In the present work, we have described the synthesis of Ag NPs by a microwave irradiation method using serine as a reducing and PVP as a

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capping agent in aqueous medium. Thus, a very simple, low cost and green way to synthesize Ag NPs and the antibacterial activity studies are reported herein.

## 2. Experimental

### 2.1. Chemicals

Silver nitrate ( $\text{AgNO}_3$ ), serine and PVP (Avg. mol. wt 40,000) were purchased from Qualigens Fine Chemicals, Mumbai, India, and were used without further purification for the synthesis of Ag NPs. De-ionized water was used for the synthesis and characterization of Ag NPs.

### 2.2. Characterization techniques

The UV–Vis spectra were recorded by a Shimadzu 1800 spectrophotometer. Morphology was determined by using Jeol 200 CX high-resolution transmission electron microscopy (HR-TEM) and FE I Quata FEG 200 high-resolution scanning electron microscopy (HR-SEM). The sample for HR-TEM analysis was prepared by placing a drop of the solution on a carbon coated copper grid and dried in air. Elemental composition was obtained from energy dispersive X-ray analysis (EDX). Selected area electron diffraction (SAED) was used to find the crystalline nature of the Ag NPs. Emission spectrum of the Ag NPs was recorded by using a Varian Cary Eclipse fluorospectrometer. Fourier transform infrared (FT-IR) spectrum was obtained from a Fourier transform infrared spectrometer (BRUKER, Alpha T model). For FT-IR spectrum, Ag NPs were separated by centrifugation at 10,000 rpm for 15 min. The sample was dried, ground with KBr and made as a pellet which was analyzed by an FT-IR spectrometer. Electrochemical measurements were made with a CHI 600A electrochemical workstation controlled by a personnel computer. Three electrode systems were employed in this study. A platinum wire, glassy carbon electrode (GCE) and a saturated calomel electrode (SCE) were used as counter electrode, working electrode and reference electrode respectively. 0.1 M  $\text{KNO}_3$  was used as an electrolytic solution.

### 2.3. Synthesis of silver nanoparticles

A microwave oven (model: MS-2049 UW) was used in the typical synthesis of Ag NPs. 100 mL of 5 mM silver nitrate solution was prepared in 250 mL conical flask using the de-ionized water. 0.0525 g of serine and 0.75 g of PVP were added into the above-mentioned silver nitrate solution, and then 4 drops of 1 M NaOH were added by altering the pH for the favorable reduction. The above mixed solution was kept in the microwave oven (input power 1200 W, 50 Hz) for 90 s. The solution turned into yellowish brown color, which confirmed the formation of Ag NPs. Fig. 1 shows the digital photo of the synthesized Ag NP colloidal solutions at different time intervals of 15, 30, 45, 60, 75, and 90 s.

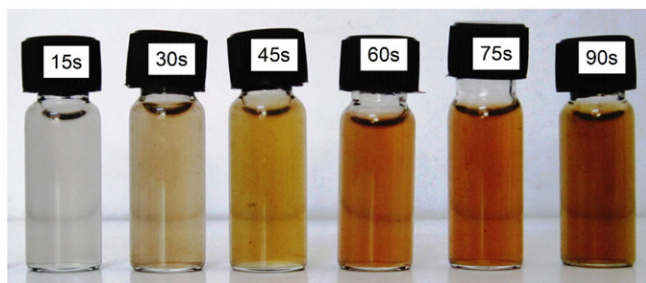


Fig. 1. Digital photo of the synthesized Ag NP colloidal solutions at different time intervals of 15, 30, 45, 60, 75, and 90 s.

### 2.4. Antibacterial activity

The Ag NPs in sterilized distilled water were tested for their antibacterial activity by the agar diffusion method or modified Kirby–Bauer method. Nine bacterial strains, *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis*, and *Enterococcus* as Gram-positive bacteria and *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, and *Klebsiella pneumonia* as Gram-negative bacteria were used for the antibacterial activity analysis. All human pathogens used in the antibacterial study were obtained from the Department of Medical Microbiology, Taramani Campus, University of Madras, India. These bacteria were grown on liquid nutrient agar media for 24 h prior to the experiment, and were seeded in agar plates by the pour plate technique. Different plates were prepared for every bacterial strain. In each petri plate, four cavities were made using a cork borer at an equal distance. In each cavity, 50  $\mu\text{L}$  of Ag NPs, serine,  $\text{AgNO}_3$  and PVP solutions was filled. All the plates were incubated at 35 °C for 24 h. All the experiments were repeated thrice to ensure the reproducibility.

## 3. Results and discussion

### 3.1. UV–Visible spectroscopic studies of Ag NPs

UV–Visible absorption spectra provide the information about the formation, particle size and surface properties of Ag NPs. Fig. 2 shows the UV–Visible absorption spectra of the solutions containing synthesized Ag NPs, serine, PVP, and  $\text{AgNO}_3$ . The formation of Ag NPs was confirmed by the presence of an intense peak at 443 nm. The obtained absorption spectrum of Ag NP colloidal solution is similar with that of our earlier report for the Ag nanospheres [2]. According to Mie's theory [41], spherical Ag NPs will give a single symmetric absorption peak, but anisotropic Ag NPs will exhibit two or more bands. Moreover, the UV–Visible spectrum of Ag NPs was symmetrical in nature, which suggested that the synthesized Ag NPs had sphere-like morphology.

Fig. 3 shows the UV–Visible absorption spectra of the solutions containing synthesized Ag NPs at different time intervals of 30, 45, 60, 75, 90, and 120 s. The UV–Visible absorption spectra confirmed the formation of Ag NPs at 90 s. There was no much difference in the spectra beyond 90 s. Fig. 4 displays the absorbance versus time plot at 5 mM of Ag NPs. It showed that the curve was sigmoid in nature, which suggested that the reaction had complicated kinetic features [25].

Fig. 5 shows the plot of  $\ln[a / (1 - a)]$  against time, where  $a = A_t / A_\infty$ , and  $A_t$  and  $A_\infty$  are the absorbance at time  $t$  and infinite time respectively. Fig. 5 indicates the involvement of the autocatalytic reaction paths in the formation of Ag NPs. The occurrence of autocatalytic reaction might be

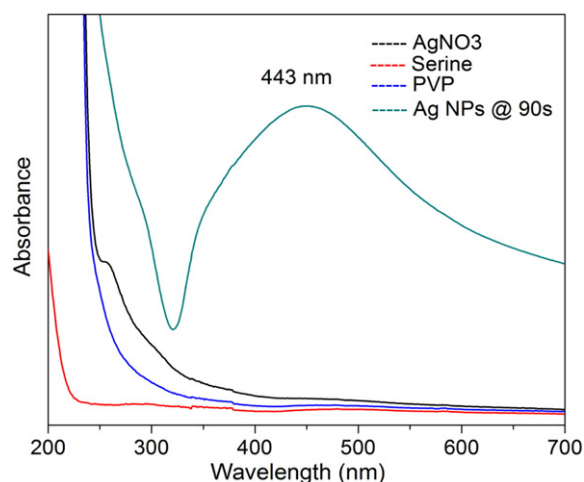


Fig. 2. UV–Visible absorption spectra of the solutions containing synthesized Ag NPs, serine, PVP, and  $\text{AgNO}_3$ .

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