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# Eco-Friendly Microwave Assisted Green Synthesis, Characterization and Antibacterial Activity of Silver Nanoparticles

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#### ABSTRACT

This paper deals a novel method for the synthesis of silver nanoparticles (AgNPs) by microwave assisted approach using silver nitrate and trisodium citrate. The formation of nanoparticles was confirmed by UV-visible spectral studies. Particle size and morphology was determined by P-XRD and HR-TEM studies which revealed the average particle size of synthesized nanoparticles 21.49 and 20.08 nm respectively with spherical shape. The synthesized nanoparticles were screened for antibacterial activity in *vitro* against gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* by adopting disk diffusion method. The results of antibacterial studies exhibited that AgNPs were potential antibacterial agent.

#### 1. Introduction

In modern materials science the field of nanotechnology is one of the most important active areas of research. Nanoparticles showed entirely novel or better properties based on specific characteristics like size, distribution and morphology [1]. Metallic nanoparticles are unique and can significantly modify physical, chemical and biological properties compared to bulk material of same composition because of their surfaceto-volume ratio. So, these nanoparticles have been utilized for a variety of applications [2-5]. Silver nanoparticles (AgNPs) paying attention to the researchers due to their attractive optical [6], electronic [7] and excellent catalytic activities [8, 9]. The biological activity of silver nanoparticles depends on numerous factors including size, shape dependent distribution, composition of particles, particles reactivity in solution, morphology, effectiveness of ion liberates, type of cell and the nature of reducing agents used for the synthesis of AgNPs [10]. The advantage of AgNPs over bulk metal or salts is due to the slow and regular release of silver from nanoparticles, in that way causing permanent protection against bacteria [11-13]. Previous literature reported anticancer activity of chloroquine-based silver nanoparticles against neuroblastoma cells [14]. A number of methods are used for the preparation of nanoparticles such as reduction method [15], reversed micelles [16], chemical coprecipitation [17], spray pyrolysis [18], sol-gel [19], laser ablation [20], plasma polymerization [21]. However, these methods are non-ecofriendly since they frequently need large amounts of toxic chemicals and high temperature processing conditions. Recently, microwave assisted method has fascinated broad attention in material science which helps to enhance the nucleation rate, minimize the synthesis time and provide the small particles with fine particle size and high purity [22]. With the increasing awareness towards the environmental safety people are ready to begin the eco-friendly approach for the synthesis of nanoparticles. In continuation [23] of our previous work the purpose of the present work is to extend an easy, rapid and completely green method for synthesizing AgNPs using trisodium citrate as reducing and stabilizing agent in aqueous medium by microwave irradiation. The prepared silver nanoparticles have been screened for their antibacterial activity in vitro against gram +ve bacteria Staphylococcus aureus and Bacillus subtilis and gram -ve bacteria Escherichia coli and Klebsiella pneumoniae by adopting disk diffusion method.

## 2. Experimental Methods

#### 2.1 Synthesis of Silver Nanoparticles

0.8~g silver nitrate (AgNO3) was dissolved in 25 mL deionised water in a 100 mL round bottom flask. 25 mL aqueous solution of trisodium citrate (1.25 g) was added drop wise to the above solution under vigorous stirring at 110 W in 20 min by maintaining the temperature 70 °C in a microwave synthesizer. The obtained precipitate was separated by using centrifugation and washed with deionised water for several times. Finally, it was dried at 150 °C for 1 h to get the silver nanoparticles.

# 2.2 Characterization

Microwave synthesizer Discover Lab Mate with Intelli-Vent Pressure, 240 V/50 Hz was used to synthesize nanoparticles. UV-visible spectrum of silver nanoparticles was recorded by UV-visible spectrophotometer Shimadzu 1800+ in DMSO at room temperature. The P-XRD spectra of nanoparticles have been recorded on XPERT-PRO X-ray diffractometer manages at a voltage of 45 kV and a current of 40 mA with Cu  $K\alpha$  radiation in a  $\theta$ -20 configuration. The transmission electron microscopic measurements of synthesized nanoparticles have been performed by using JEOL model JEM 2100.

#### 2.3 Antibacterial Activity

The antibacterial activity of the synthesized nanoparticles has been screened on two gram-positive Staphylococcus aureus and Bacillus subtilis and two gram-negative Escherichia coli and Klebsiella pneumoniae microorganisms by using disk diffusion method [24]. The culture media was sterilized by moist heat sterilization method in an autoclave at 121°C temperature at 15 pounds pressure for about 30 minutes. Now well sterilized culture media was spread homogeneously on each petri plates. The culture media was then allowed to solidify. After solidification a sterilized swab of cotton was dipped in the bacterial culture broth and applied carefully on the entire surface of the petri plate. The petri plates were incubated at 37 °C for 24 h and then sterilised discs of 6 mm have been loaded at different places on the solidified culture plates. Five different concentrations (50, 25, 12.5, 6.25 and 3.12 µg/mL) of silver nanoparticles and 3.12 μg/mL for standard antibiotic amikacin, were prepared in ethanol. From the above solutions 20  $\mu L$  solution of each concentration was pipette out by using micropipette and loaded to different sterile discs separately. The petri plates have been subjected for incubation at 37 °C for 24 h. After 24 h the zone of inhibition values in diameter (in mm) was measured with the help of a ruler.

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#### 3. Results and Discussion

#### 3.1 UV-Visible Spectral Studies

The UV-visible absorption spectrum (Fig. 1) of synthesized silver nanoparticles [25] exhibited a strong absorption peak at 420 nm which confirmed the formation of silver nanoparticles.

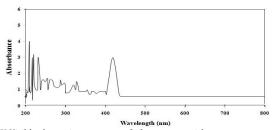


Fig. 1 UV-Visible absorption spectrum of silver nanoparticles

#### 3.2 P-XRD Studies

The structure and composition of synthesized silver nanoparicles was determined by X-ray diffraction technique. The P-XRD spectra of nanoparticles showed that particles were crystalline in nature. The crystalline size of nanoparticles has been calculated by using the width of X- ray peaks supposing they are free from non-uniform strains using Debye-Scherrer's formula,  $D = k\lambda/\beta\cos\theta$ , where D = Mean crystallite domain size, at right angles to the reflecting planes, k = Constant equals to unity,  $\lambda = x$ -ray wavelength,  $\beta = Full$  width at half of the maximum intensity (FWHM) and  $\theta$  =Diffraction angle. Full width at half maximum has been used to find the size distribution of nanoparticles. It has been observed that the broader is the peak, broader is the size distribution of nanoparticles. On applying Debye Scherrer formula on different peaks of P-XRD graph of silver nanoparticles (Fig. 2). It has been found that the synthesized nanoparticles have different size like 23.36, 7.35, 29.24, 38.84, 1.74, 21.24, 21.62, 15.08, 26.15 and 27.34 nm. The average particle size of synthesized silver nanoparticles has been found to be 21.49 nm.

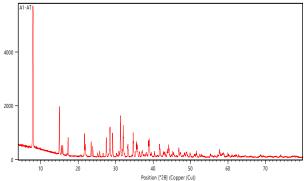
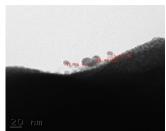


Fig. 2 P-XRD graph of silver nanoparticles

### 3.3 Transmission Electron Microscopic Studies

The transmission electron microscopic (TEM) images indicated that the particle size of synthesized silver nanoparticles lies in the range of 11.05 to 38.63 nm. The average size of silver nanoparticles was 20.08 nm. TEM images of silver nanoparticles indicated spherical shape of the nanoparticles. The TEM images of silver nanoparticles have been shown in Fig. 3.



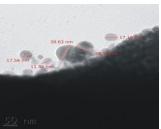


Fig. 3 TEM images of synthesized silver nanoparticles

# 3.4 Antibacterial Activity

The antibacterial activity of the synthesized silver nanoparticles was measured against two gram-positive *Staphylococcus aureus* and *Bacillus subtilis* and two gram-negative *Escherichia coli* and *Klebsiella pneumoniae* 

bacteria in diameter (in mm). The zone of inhibition (Fig. 4, Table 1) was found 24, 19, 16, 14 and 8 mm corresponding to the concentration 50, 25,12.5, 6.25 and 3.12  $\mu g/mL$  against the bacteria Staphylococcus aureus.The zone of inhibition (Fig. 4, Table 1) was found 19, 16, 14, 12 and 7 mm with respect to the concentration 50, 25, 12.5, 6.25 and 3.12  $\mu$ g/mL against the bacteria Bacillus subtilis. However, zone of inhibition for standard drug Amikacin was observed 13 mm and 11 mm at the concentration 3.12 µg/mL against Staphylococcus aureus and Bacillus subtilis respectively. Synthesized silver nanoparticles exhibited the zone of inhibition (Fig. 5, Table 2) at 22, 16, 13, 12 and 9 mm corresponding to the concentration 50, 25, 12.5, 6.25, 3.12 μg/mL against the bacteria Escherichia coli. The zone of inhibition (Fig. 5, Table 2) was found 20, 14, 12, 9 and 7 mm with respect to the concentration 50, 25, 12.5, 6.25 and 3.12  $\mu$ g/mL against the bacteria Klebsiella pneumoniae. However, zone of inhibition for standard drug Amikacin was found at 12 mm and 8 mm at 3.12 µg/mL concentration against Escherichia coli and Klebsiella pneumoniae respectively. From the antibacterial studies it has been found that the silver nanoparticles were potential active on the microorganisms exploring the further research on this noble metal.

**Table 1** Maximum zone of inhibition values (in mm) for silver nanoparticles against gram-positive micro-organisms

S. No.	Test conc. (µg/mL)	Staphylococcus aureus	Bacillus subtilis
1.	50	24	19
2	25	19	16
3	12.5	16	14
4	6.25	14	12
5	3.12	8	7
STD Amikacin	3.12	13	11





Fig. 4 Petri plates showing zone of inhibition against  $Staphylococcus\ aureus$  and  $Bacillus\ subtilis$ 

 $\textbf{Table 2} \ \ \text{Maximum zone of inhibition values (in mm) for silver nanoparticles against gram-negative micro-organisms$ 

S. No.	Test conc. (μg/mL)	Escherichia coli	Klebsiella pneumoniae
1.	50	22	20
2	25	16	14
3	12.5	13	12
4	6.25	12	9
5	3.12	9	7
STD Amikacin	3.12	12	8



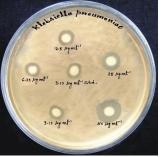


Fig. 5 Petri plates showing zone of inhibition against *Escherichia coli* and *Klebsiella pneumoniae* 

#### 4. Conclusion

In the present paper ecofriendly microwave assisted synthesis method has been used for the synthesis of silver nanoparticles. This method is a novel and cost effective and less energy consuming. The results of P-XRD and HR-TEM studies revealed that the average particle size of synthesized nanoparticles was 21.49 and 20.08 nm respectively with spherical shape. The synthesized nanoparticles were screened for antibacterial activity in

vitro against two gram +ve bacteria Staphylococcus aureus and Bacillus subtilis and two gram -ve bacteria Escherichia coli and Klebsiella pneumoniae bacteria by adopting disk diffusion method. The results of antibacterial studies exhibited that AgNPs were potential antibacterial agent. AgNPs may be used as bound dressing ointments, lotions, emulsions to control the bacterial growth.

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