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Study on Bactericidal Effect of Biosynthesized Silver Nanoparticles in Combination with Gentamicin and Ampicillin on PSEUDOMONAS AERUGINOSA

Shweta Rajawat, M.S.Qureshi

Department of Physics, MANIT Bhopal (M.P) India

Email: srajawat73@gmail.com, shumsqureshi@rediffmail.com

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Abstract. Silver nanoparticles are the most promising nanomaterial with antibacterial properties. Recent study of resistance to most potential antibiotics promotes research in the bactericidal activity of the silver nanoparticles. In this work, the effect of biosynthesized silver nanoparticles, in combination with gentamicin and ampicillin, on Pseudomonas Aeruginosa bacteria has been studied. Pseudomonas Aeruginosa is a common bacterium that can cause infections which are generalized as inflammation and sepsis. The results show that the bactericidal properties of the nanoparticles depends on the size of the as-synthesized silver nanoparticles as nanoparticles of diameter ~1–20 nm only have a direct interaction with the bacteria. It is observed that the antibacterial activities of antibiotics increase in the presence of AgNPs against test strains. Silver nanoparticles were synthesized elctrolytically using silver wire of 99% purity as anode and carbon rod wrapped with LDPE as cathode. 0.01 N Silver nitrate was used as an electrolyte. The process is termed as biosynthesis, because tea extract was used used as the capping agent which is also a very mild reducing agent. The polyphenols theaflavins and thearubigins, present in tea perform the role of stabilizing or capping agents due to their bulky and steric nature. A brown colored colloidal solution of silver nanoparticles is obtained. The as-synthesized silver nanoparticles were characterized using XRD, TEM and UV-Vis spectroscopy.

Introduction

Silver has been extensively used for both hygienic and healing purposes. With time, the use of silver has reduced as an anti-infection agent due to the advent of antibiotics and other disinfectants and the poorly understood mechanisms of their toxic effects. However, resistance of bacteria to bactericides and antibiotics has increased in recent years. Some antimicrobial agents are extremely irritant and toxic. Hence there is a need to find ways to formulate safe and cost-effective biocidal materials. Previous studies show that antimicrobial formulations of silver in the form of nanoparticles could be used as effective bactericidal materials. Silver nanoparticles exhibit increased activity due to the large surface to volume ratios and crystallographic surface structure. Highly reactive metal oxide nanoparticles exhibit excellent biocidal action against Gram-positive and Gram- negative bacteria [1]. Thus, the syntheses, characterization, functionalization of nanosized particles open the possibility of formulation of a new generation of bactericidal materials.

Researchers have found that nanosilver and antibiotics used together have an extremely strong effect against gram positive and gram negative bacteria. Recently, due to the limitations of antibiotics, the synergetic effect of silver nanoparticles with antibiotics has been studied combining silver nanoparticles with different antibiotics against gram positive and gram negative bacteria. The importance of bactericidal nanomaterial's study is because of the increase in new resistant strains of bacteria against most potent antibiotics. This has promoted research in the activity of silver ions and silver-based compounds, including silver nanoparticles [2]. A variety of preparation routes have been reported for the preparation of metallic nanoparticles [3, 4], notable examples include, reverse micelles process [5], radiolysis [6], electrochemical synthesis [7] etc. Presently, the investigation of this phenomenon has regained importance due to the increase of bacterial resistance to antibiotics, caused by their overuse. Recently, silver nanoparticles exhibiting antimicrobial activity have been synthesized [8, 9]. Antibacterial activity of the silver-containing materials can be used, for example, in medicine to reduce infections as well as to prevent bacteria colonization on prostheses [10], catheters [11, 12], vascular grafts [13], and human skin [14]. Green synthesis of nanoparticles is an easy, efficient and eco-friendly approach. "Green" chemistry and chemical processes are progressively being integrated in science and industry for sustainable development [15]. Among several synthesizing methods, biosynthetic methods employing either biological microorganisms or plant extracts have emerged as a simple and viable alternative to chemical synthetic procedures and physical methods. Using dissimilatory properties of fungi, the biosynthesis of inorganic nanomaterial using eukaryotic organisms, such as, fungi is used to grow nanoparticles of silver [16] intracellularly in *Verticillium* fungal cells [17]. Recently, it was found that aqueous chloroaurate ions may be reduced extracellularly using the fungus *F. Oxysporum*, to generate extremely stable silver nanoparticles in water [18]. However synthesizing silver nanoparticles using microorganism is a very slow process therefore the synthesis of metal nanoparticles using biological systems is an expanding research area due to the potential applications in nano medicines. Plant extracts play an important role in remediation of toxic metals through reduction of the metal ions. Silver nanoparticle are synthesized from various parts of the herbal plants like bark of Cinnamon [19], Neem leaves [20], Citrus Limon [21], Tannic acid [22] and various plant leaves [23]. We describe a synthesis approach which is simple and "green" for the synthesis of metallic nanostructures of noble metal i.e. silver (Ag). The bactericidal effect of as- synthesized silver nanoparticles is then studied for combination with gentamicin and ampicillin on Pseudomonas Aeruginosa. P. Aeruginosa is a gram-negative, aerobic, rod- shaped bacterium. It is found in soil, water, skin flora and most man-made environments. It is a frequent cause of nosocomial infections such as pnenumonia, urinary tract infections (UTIs), and bacteremia. Pseudomonal infections are complicated and can be life threatening. Gentamicin is an aminoglycoside antibiotic, used to treat many types of bacterial infections, particularly those caused by Gram-negative organisms. It remains a mainstay for use in sepsis. Gentamicin is also used in molecular biology research as an antibacterial agent in tissue and cell culture, to prevent contamination of sterile cultures. Ampicillin is a beta- lactam antibiotic that has been used extensively to treat bacterial infections since 1961. It is relatively non-toxic and the toxicity of the synthesized (using the green route) silver nanoparticles is comparatively less [24].

Methodology

0.2 N solver nitrate solution was obtained from Merck, and it was further diluted to 0.1 N (mol/L). This silver nitrate solution was used an elctrolyte. The experimental setup consists of a beaker filled with 40 ml of electrolyte (0.1 N silver nitrate solution). Two electrodes: (1)silver wire (99% pure, 1.04 mm diamter)was used an an anode and (2) carbon rod(4 mm diameter) wrapped by LDPE (Low Density Poly Ethylene) was used as a cathode. The distance between the two electrodes was 1 cm and both the electrode were of 4.5 cm in length. 1 ml of tea extract was added to the electrolyte as a capping agent. A Daniel cell of 1.1 volt and 2 ohm internal resistance was used as current source. A potentiometer pot along with a rheostat is used in the circuit to increase the resistance and obtain current of different values in mamperes. Copper wires are used to connect the components of the circuit. All the parameters were same for the samples synthesized, except the current through the circuit. Fig. 1 shows the set up. The beaker in the set-up was covered with silver foil with holes for the electrodes. The whole assembly was placed on a magnetic stirrer and the sysntheis was carried at room temperature for 2 hours.

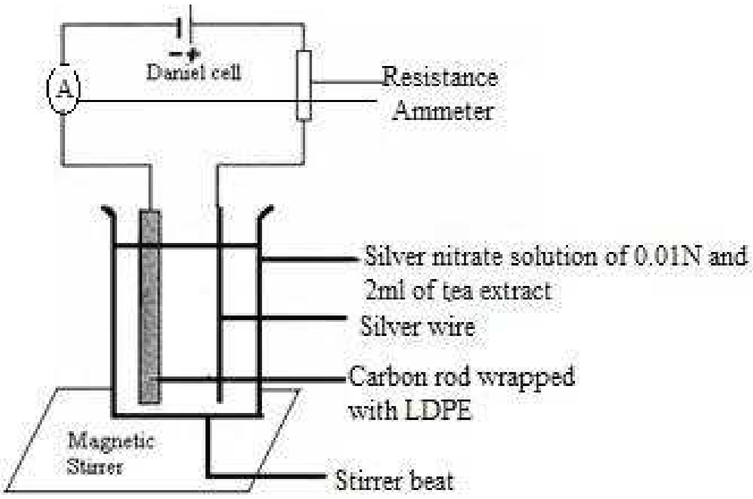

Fig. 1. Experimental Setup.

Results and Discussions

The as-synthesized silver nanoparticles were characterized using XRD. The XRD diffractogram is shown in Fig. 2. The XRD results show Face-Centered-Structure of pure silver nanoparticles oriented in planes (111), (200), (220), and (311). The peaks obtained for as-synthesized silver nanoparticles are well in accordance with JCPDS file No. 04-0783.

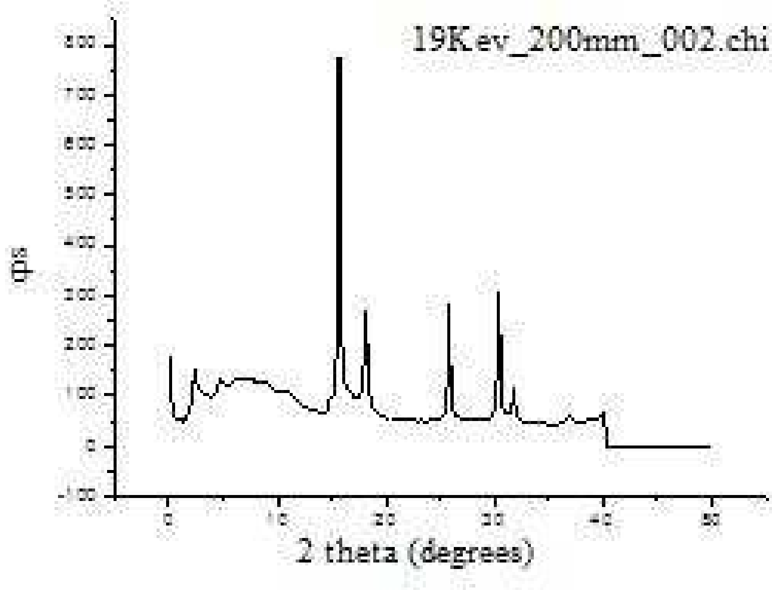

Fig. 2. XRD pattern of as-synthesized silver nanoparticles.

The size and shape of the silver nanoparticles were examined clearly under Transmission Electron Microscope (TEM). The image, in Fig. 3, shows that the particles are well dispersed and spherical in shape. The particle size is between 2 nm to 23 nm. The average size of the particle is 10 nm. The as-synthesized silver nanoparticles are polydispersed nanoparticles. Such variation in shape and size of nanoparticles synthesized by biological systems is common [25].

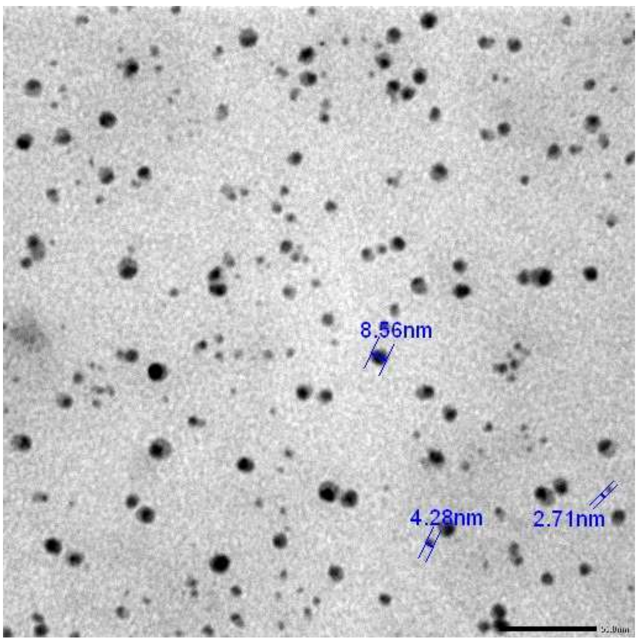

Fig. 3. TEM image of silver nanoparticles.

The UV-visible spectra, in Fig. 4, shows the formation of silver nanoparticles as the peak maxima 522 nm which is characteristic to silver nanoparticles. The specific characteristic peak for silver nanoparticles was due to the surface Plasmon resonance. The nanoparticles which are smaller than the wavelength of light can produce a coherent resonance waves at a particular absorbance wavelength which is in the visible range for silver nanoparticles.

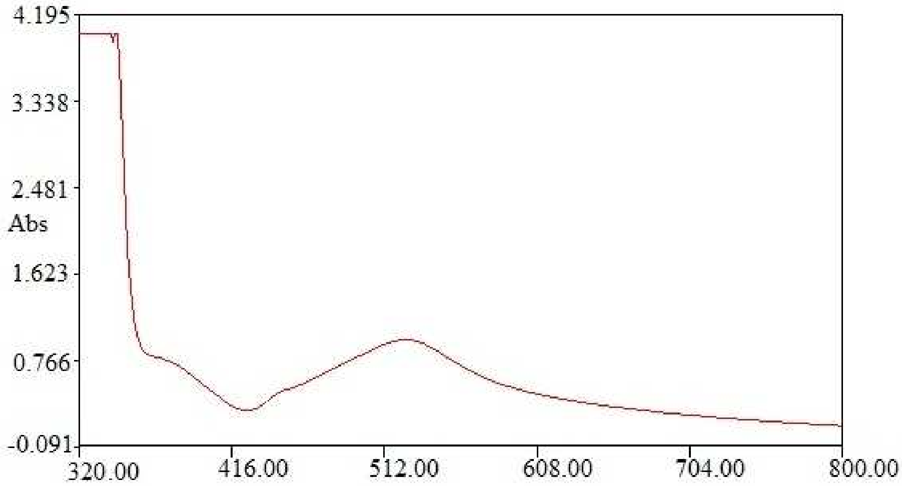

Fig. 4. UV-Visible graph of silver nanoparticles.

The antibacterial activity of nanoparticle along with antibiotics gentamicin and Ampicillin was tested against Pseudomonas Aeruginosa. The antibacterial activity of antibiotics increase in the presence of silver nanoparticles against gram positive and gram negative bacteria. The size of metallic nanoparticles ensures that a significantly large surface area of the particles is in contact with the bacterial cells. Such a large contact surface is expected to enhance the extent of bacterial elimination [26]. Zone of inhibition test was done for identification of degree of inhibition by silver nanoparticles in combination with gentamicin and ampicillin. 1ml of as-synthesized colloidal solution of silver nanoparticles was taken with 10 mcg of gentamicin and ampicillin for 15 minutes separately. The study shows an inhibition zone in Fig. 5, for sample with gentamicin for P. Aeruginosa whereas in case of combination of silver nanoparticles with ampicillin, P. Aeruginosa showed resistance i.e. no inhibition zone could be observed.

Fig. 5. Zone of inhibition with silver nanoparticles.

The table-1, shows the size of the inhibition zones obtained for the sample in combination with both, ampicillin and gentamicin. From the table it is obvious that the inhibition zone obtained are large enough to conclude that the sample in combination with gentamicin is very effective for Pseudomonas Aeruginosa.

Table-1 Comparative study (1ml sample + ampicillin and gentamycin [10 mcg] [kept for 15 mins] and zone readings taken)

SR. NO.	ORGANISM	ZONE FOR SAMPLE + AMPICILLIN			ZONE FOR SAMPLE + GENTAMICIN		
		P1	P2	MEAN	P1	P2	MEAN
1.	PSEUDOMONAS AERUGINOSA	RE	RE	RE	15	19	17

Plate 1=P1

Plate 2=P2

Conclusion

Production of silver nanoparticles can be achieved through different methods. Chemical approaches are the most popular methods for the production. However chemical methods cannot avoid use of toxic chemicals in synthesis methods. There is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals. So the approach to electrolytically deposit highly pure silver nanoparticles with the tea extract (containing antioxidant components) as capping agent in present synthesis method is well justified. Due to the antibiotic resistance developed by the bacteria it is very hard to manage different types of antibacterial drugs. There is great need of agents to kill bacteria and other microorganisms. Silver nanoparticles have been reported to have antimicrobial activity against a wide range of microorganism. The use of silver nanoparticles for antimicrobial effects are highly sought because of its broad spectrum activity, high rate of effectiveness, and low cost. Research is being done to find superior forms of silver based antimicrobial agents. The combined effect of nanosilver with gentamicin and ampicillin has more potential as compared to the other antibiotics. The antibiotic molecules contain many active groups such as hydroxyl and amino groups, which reacts easily with silver nanoparticles by chelation. Due to this reason, the synergistic effects will probably be caused by the bonding reaction with antibiotic and silver nanoparticles. Therefore a study on the combination of silver nanoparticles with most practiced antibiotics i.e. ampicillin and gentamicin was undertaken with P. Aeruginosa. The study shows that the combination of silver nanoparticles and gentamicin on P. Aeruginosa is more effective than ampicillin. It is found to be resistant towards combination of silver nanoparticles and ampicillin.

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