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Comparative Study on Bactericidal Effect of Silver Nanoparticles, Synthesized Using Green Technology, in Combination with Antibiotics on *Salmonella typhi*

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ABSTRACT

In this work bactericidal study of silver nanoparticles was taken up in combination with two standard antibiotics, ampicillin and gentamycin, for *Salmonella typhi*. The antibacterial activities of antibiotics were increased in the presence of AgNPs against test strains. The higher enhancing effect was observed for ampicillin in comparison to gentamicin against test strains. Silver nanoparticles were synthesized elctrolytically using silver wire of 99% purity as anode and carbon rod wrapped with LDPE as cathode. Silver nitrate [of Merck] of 0.01N is used as an electrolyte. Here tea extract is added as capping and 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 coloured colloidal solution of silver nanoparticles is obtained. The as-synthesized silver nanoparticles were characterized using XRD, TEM and UV-Vis spectroscopy.

Keywords: Silver Nanoparticles; *Salmonella typhi*; Tea Extract; Ampicillin; Gentamicin

1. Introduction

Historically, silver has been extensively used for both hygienic and healing purposes (Cheng and Schluesener, 2008). 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. 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 had 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.

A variety of preparation routes have been reported for the synthesis of metallic nanoparticles [2,3] notable examples include, reverse micelles process [4,5], salt reduction [6], microwave dielectric heating reduction [7], ultrasonic irradiation [8], radiolysis [9], Solvothermal synthesis [10], electrochemical synthesis [11,12], etc. Chemical methods for metal nanoparticle fabrication usually involve toxic chemicals, which are usually expensive and potentially dangerous for the environment [13]. Green synthesis of nanoparticles is an easy, efficient and eco-friendly approach. 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. 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 (Sathishkumar *et al*., 2009), Neem leaves (Tripathi *et al*., 2009), Citrus limon (Prathna *et al*., 2011), Tannic acid (Sivaraman *et al*., 2009) and various plant leaves (Song and Kim, 2008).

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 gentamycin and ampicillin for *Salmonella typhi*. *Salmonella typhi* is an obligate parasite that has no known natural reservoir outside of humans. It is a multi-organ pathogen that inhabits the lymphatic tissues of the small intestine, liver, spleen, and bloodstream of infected humans. Infection of *S. typhi* leads to the development of typhoid, or enteric fever. Gentamicin is active against a wide range of human bacterial infections, mostly Gram-negative bacteria including Pseudomonas, Proteus, Serratia and the Gram-positive Staphylococcus. Ampicillin is a beta-lactam antibiotic that has been used extensively to treat bacterial infections since 1961. It is relatively non-toxic. The toxicity of silver nanoparticles synthesized using green technology is comparatively reduced.

2. Materials and Method

The experimental setup consists of a beaker filled with 40 ml of electrolyte silver nitrate (0.02 N) of MERK. The silver nitrate was further diluted with triple deionised distilled water to obtain a solution of 0.01 N (mol/l) strength. Two electrodes: silver wire (99% pure) as anode and carbon rod wrapped by LDPE (Low Density Poly Ethylene) material as cathode was used. LDPE material is used to collect silver nanoparticles produced in the synthesis process so that they can be easily extracted. The distance between the two electrodes is 1 cm. The diameter of the silver wire is 1.04 mm and the diameter of the carbon rod used is 4 mm. The length of the carbon rod as well as silver wire is 4.5 cm. 2 ml of tea extract is added to the electrolyte as a capping agent. The whole assembly is placed on magnetic stirrer which keeps the solution in the beaker stirring continuously. The process is carried at room temperature (30˚C) for 2 hours continuously. A Daniel cell of 1.1 volt and 2 ohm internal resistance is 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. **Figure 1** shows the set up. The beaker in the set-up is covered with silver foil with holes for the electrodes.

3. Results and Discussions

The as-synthesised silver nanoparticles were obtained in the form of colloidal solution of light brown colour as shown in **Figure 2**. The as-synthesized silver nanoparticles were characterized using XRD. **Figure 3** shows the graph obtained. The XRD results show Face-Centred-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. The intensive diffraction peak from the {111} lattice plane of face-centred cubic silver indicates that the particles are made of pure silver and that their basal plane, *i.e*., the top crystal plane

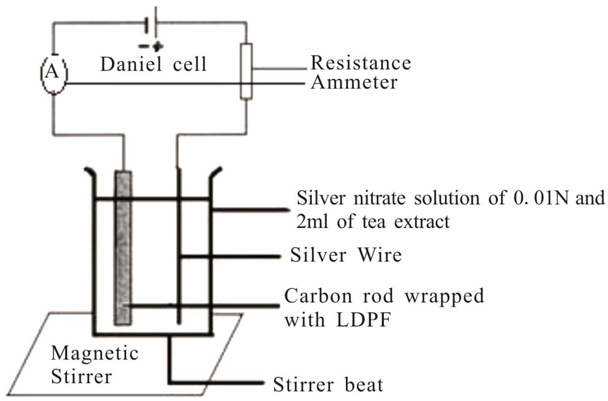

Figure 1. Experimental setup.

Figure 2. Synthesized colloidal solution of silver nanoparticles.

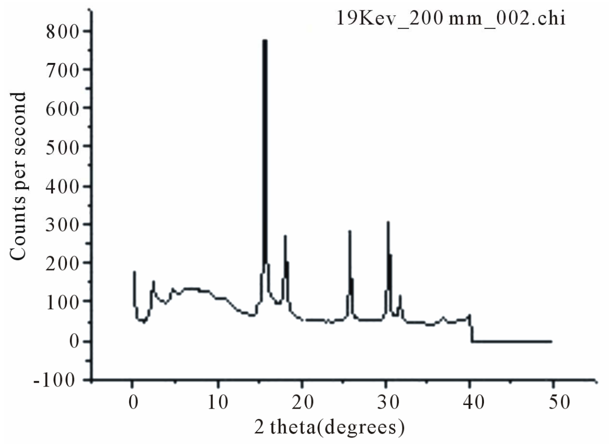

Figure 3. XRD graph of as-synthesized silver nanoparticles.

is the {111} plane. This 2θ value reflection angle confirms the presence of silver nanoparticles.

The morphology and the crystal structure of synthesized silver nanoparticles were examined using HR-TEM. HR-TEM unit used had a 120 kV class high-resolution transmission electron microscope JEM-1400, Jeol, Japan. The sample was placed on the carbon coated copper grid, making a thin film of sample on the grid and extra sample was removed using the cone of a blotting paper and kept in grid box sequentially. The TEM images show 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 [14]. The spherical and oval shape of the particle, as visible in **Figure 4**, is due to the fact that when a particle is formed, in its initial state, it tries to acquire a shape that corresponds to minimum potential energy. The spherical and oval shapes correspond to the state of minimum potential energy. Other factors that play role in determining the shape of silver nanoparticles are the fast rate of reaction and low concentration of silver nitrate solution as slower rate of reaction [15] and higher concentration of silver nitrate [16] leads to anisotropic silver nanoparticles.

The UV-vis spectroscopy was carried by Uv-vis spectrometer of Systronics. It is a dual beam spectrophotometer. The base solution was triple de-ionised distilled water to sample was added in small concentration. The UV-visible spectrum shows the formation of silver nanoparticles as the peak maxima 522 nm which is characteristic to silver nanoparticles. The specific characteristic peak, as shown in the **Figure 5**, for silver nanoparticles is 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.

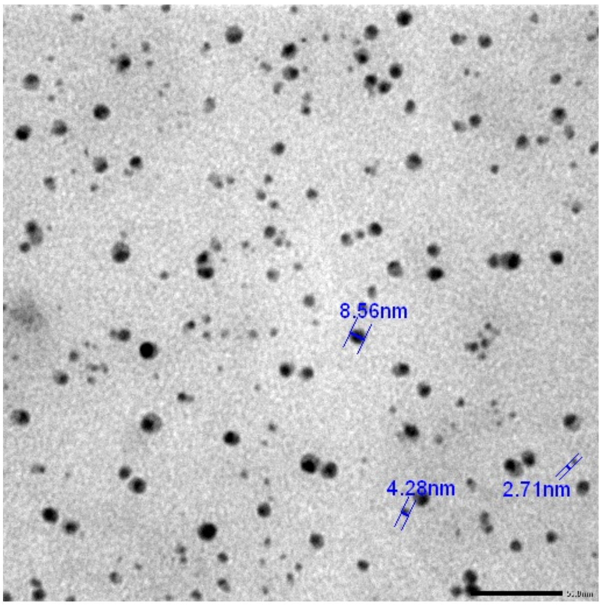

Figure 4. TEM image of silver nanoparticles.

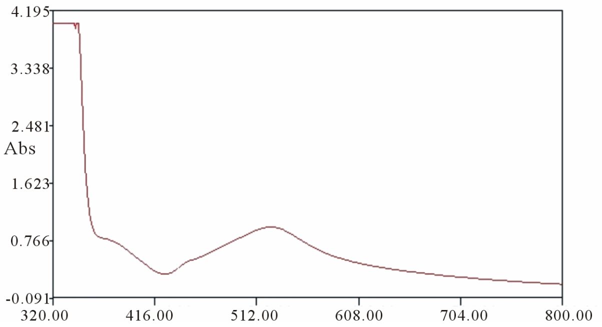

Figure 5. UV-Visible graph of silver nanoparticles.

Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications and even has shown to prevent HIV binding to host cells [17,18-21]. Additionally, silver has been used in water and air filtration to eliminate microorganisms [22-24].The antibacterial activity of nanoparticle along with antibiotics ampicillin and gentamicin was tested against *S. typhi*. **Figure 6** shows the zone of inhibition against *S. typhi*.The antibacterial activities 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 [25]. The mechanism of the bactericidal effect of AgNPs is that they may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell

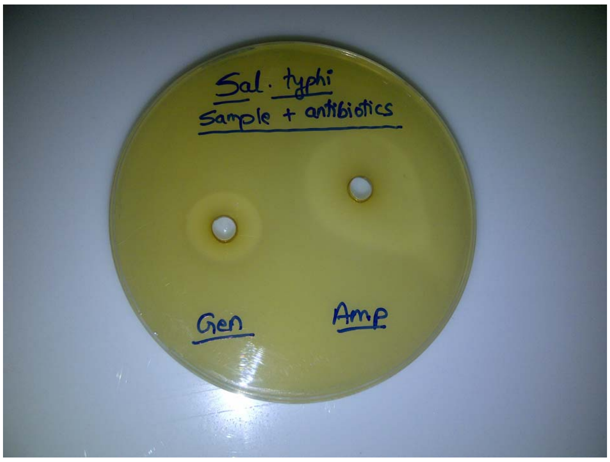

Figure 6. Zone of inhibition with silver nanoparticles.

Table 1. 1 ml 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.	*Salmonella typhi*	14	20	17	13	17	15

Plate 1 = P1; Plate 2 = P2.

[26]. Smaller AgNPs having the large surface area available for interaction would give more bactericidal effect than the larger AgNPs [26]. It is also possible that AgNPs not only interact with the surface of membrane, but can also penetrate inside the bacteria [27]. Zone of inhibition test was done for identification of degree of inhibition by silver nanoparticles in combination with gentamicin and ampicillin antibiotics. 1 ml of as-synthesized colloidal solution of silver nanoparticles was taken with 10 mcg of ampicillin and with 10 mcg of gentamicin for 15 minutes separately. Zone readings were taken for both. The comparative study given in **Table 1** show that the zone size for sample with ampicillin for *Salmonella typhi* is larger than the zone size for sample with gentamicin. Thus sample is more effective in combination with ampicillin for *S. typhi* in comparison to gentamicin.

4. 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 elctrolytically deposit highly pure silver nanoparticles with the plant extract like tea (containing antioxidant components) as capping agent in present synthesis me- thod 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 [28]. Silver nanoparticles have been reported to have antimicrobial activity against a wide range of microorganism. The use of silver nanoparticles antimicrobial effects are highly sought after because of its broad spectrum activity, high rate of effecttiveness, and low cost. Research is being done to find superior forms of silver-based antimicrobial agents.

The combination effect of nanosilver and ampicillin has more potential compared to the other antibiotics and may be caused by both, the cell wall lysis action of the ampicillin and the DNA binding action of the silver nanoparticles (Fayaz *et al*., 2009). The antibiotic molecules contain many active groups such as hydroxyl and amino groups, which reacts easily with silver nanoparticles by chelation, for this reason, the synergistic effect may be caused by the bonding reaction with antibiotic and silver nanoparticles.

Therefore a study for the combination of silver nanoparticles with most practised antibiotics *i.e*. ampicillin and gentamicin was undertaken with *S. typhi* which shows that the combination of silver nanoparticles and ampicillin is more effective than the combination of silver nanoparticles and gentamicin for *Salmonella typhi*.

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