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Detection of Routes of Interaction between Silver Nanoparticles and Bacterial Cell Membrane

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Abstract—*The synthesis, characterization and application of biologically synthesized nanoparticles are important aspects in nanotechnology. The present study deals with the synthesis of silver nanoparticles using plant extract Allium sativum (garlic). The formation of silver nanoparticles was confirmed by UV visible spectroscopy and FTIR (Fourier transform infrared spectroscopy). For understanding the detection of routes for killing bacteria we, further investigated the antibacterial properties of different concentrations of Ag nanoparticles against E.coli bacteria on agar plates. In this study we analysis of antibacterial activities of E.coli against different concentrations (10%, 20%, 50%, 70% and 100%) of Ag nanoparticles, the observed results indicates that the high concentration (100%) leads to more effective antibacterial activities against E. coli bacteria. Again we checked antibacterial activities of E. coli bacteria against four antibiotics (CF: Ciprofloxacin, G: Gentamycin, T: Tetracycline and CP: Cholramphenicol) the observed results indicates higher antibacterial activities were in CP then CF>G>T. In this comparative study on the bactericidal properties of AgNPs of different concentrations, and our results demonstrate that AgNPs undergo a concentration-dependent interaction with the bacterial species.*

1. INTRODUCTION

Nanotechnology is emerging as a rapidly growing field with its application in Science and Technology for the purpose of manufacturing new materials at the nanoscale level [1]. Nanotechnology is understood as "research and technology development at the atomic, molecular or macromolecular levels using a length scale of approximately 1-100 nm in any dimension", including the ability to "control or manipulate matter on an atomic scale". [2]. Ultrafine semiconductors particles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. They possess special properties such as a large surface to volume ratio, increased activity, special electronic properties and unexpected optical properties as they are small enough to confine their electrons and produce quantum effects. The development of biologically inspired experimental process for synthesis of nanoparticles is evolving into an important branch of nanotechnology [3,4]. Biologically synthesized silver nanoparticles could have many applications: they might be used as spectrally-selective coatings for solar energy absorption and intercalation material for electrical batteries; they also find use as optical receptors and as catalysts in chemical reactions [5]. The antibacterial activities of silver nanoparticles are related to their size, with the smaller particles having higher activities on the basis of equivalent silver mass content. In the present study plant extract *Allium sativum (garlic)* was used for the biological synthesis of silver nanoparticles which were then characterized using UV- Vis spectroscopy. The antimicrobial activity of biologically synthesized silver nanoparticles and antibiotics was checked against *E.coli.*

2. MATERIALS AND METHODS

2.1 Sample collection

All chemicals used in this experiment were of the highest purity and obtained from Sigma and Merck. Silver nitrate soluble of analytical grade was used for biological synthesis of silver nanoparticles. For antimicrobial activities against antibiotics we procure antibiotics disc. The cultures of *E. coli* were used to demonstrate the antimicrobial activity and toxic effects of silver nanoparticles collected from the microbiology laboratory of this Institute.

2.2 Collection of plant leaves and preparation of extracts

Allium sativum (garlic) were collected from the market. 10 gm of garlic was taken and thoroughly washed in distilled water, dried, and were crushed with motor piston then mixed into 75 ml sterile distilled water after that it was boiled for around 10 minutes and filtered through Whatman No.1 filter paper (pore size 45 μm) and centrifuged for 10 minutes at 4000 rpm then kept at 4°C for further experiments.

2.3 Synthesis of silver nanoparticles:

1mM aqueous solution of Silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. 10 ml of Garlic extract was added drop by drop into 90 ml of aqueous solution of 1 mM Silver nitrate for reduction into Ag+ ions. It was done on magnetic stirrer. The temperature was around 50 – 60º C and the rpm was around 30º. After addition of the extract a color change was seen and it was kept at room temperature for 48-72 hours.

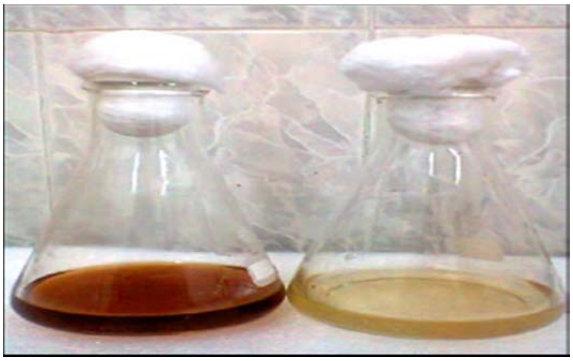

Fig. 1: Biological synthesis of silver nanoparticles

2.4 Characterization of CH-AgNPs

The Ag nanoparticle was characterized in a JASCO-V-530, UV-VIS spectrophotometer, to know the kinetic behavior of silver nanoparticles. The scanning range for the samples was 280-700 nm at a scan speed of 400 nm/min. Base line correction of the spectrophotometer was carried out by using a blank reference. The UV-Vis spectra analysis of AgNPs of all the samples was recorded. The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 2 h after diluting a small aliquot of the sample into distilled water. The color change in reaction mixture solution was recorded through visual observation which showed bioreduction of silver ions in aqueous solution.

2.5 Antimicrobial activity

Antibacterial activities of CH-AgNPs were observed against *E. coli* (gram negative) and combined with four antibiotics. Antimicrobial activities were determined, using the disc diffusion method. Approximately 20 mL of molten and cooled media (NA/SDA) was poured in sterilized petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The plates containing the test organism and CH-AgNPs were incubated at 37^0C for 24 - 48 h. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the disc. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in centimeter.

3. RESULT

In the present contribution, we describe the antimicrobial activities against gram negative bacterial species *E.coli* with understanding scientific study for does the antimicrobial activity of sliver nanoparticles depend on the concentration and detection of routes for killing bacteria after contact to highly dispersed biologically synthesized silver nanoparticles (AgNPs).

3.1 Characterization of BIO-AgNPs

The UV-Vis spectra analysis of CH-AgNPs samples was recorded at 280-700 nm. The reduction of pure Ag+ ions was monitored due to changes the color of solution turns transparent golden brownish which shows the presence of silver nanoparticles and on performing UV-Vis spectrophotometer the absorption peak is observed to be at 400 nm. [Fig 2]

Table 1: Antibacterial effect of *E.coli*.

Antibacterial effect of *E.coli*		Zone of inhibition (cm)
Sliver nanoparticle concentration (c) in (%)	10c	0.1
	20c	0.2
	50c	0.3
	70c	0.4
	100c	0.6
Antibiotics	Ciprofloaxacin(CF)	0.5
	Gentamycin (G)	0.4
	Tetracyclin (T)	0.3
	Chloramphenicol (CP)	0.6

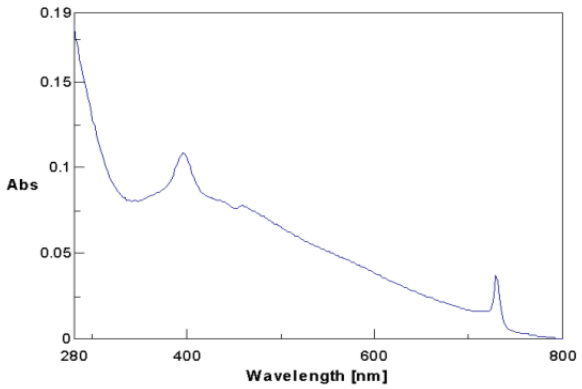

Fig. 2: UV-Vis spectrophotometer analysis of biologically synthesized silver nanoparticles

3.2 Antimicrobial activity of BIO-AgNPs

For understanding the detection of routes for killing bacteria we, further investigated the antibacterial properties of five differently concentrations of BIO-AgNPs (10%, 20%, 50%, 70% and 100%) against *E.coli* on agar plates. The observed results indicate that the high concentration (100%) of BIO-AgNPs leads to more effective antibacterial activities against all pathogenic bacteria as compare to rest concentrations (10%, 20%, 50% and 70%) of BIO-AgNPs

In the second way we checked antibacterial activities of eight pathogenic bacteria against four antibiotics e.g. Ciprofloxacin (CF), Gentamycin (G), Tetracycline (T) and Cholramphenicol (CP) on disc diffusion methods on NA agar plate. The observed results indicates higher antibacterial activities were in CP then CF>G>T. In this comparative study on the

bactericidal properties of AgNPs of different concentrations, and our results demonstrate that AgNPs undergo a concentration-dependent interaction with the bacterial species.

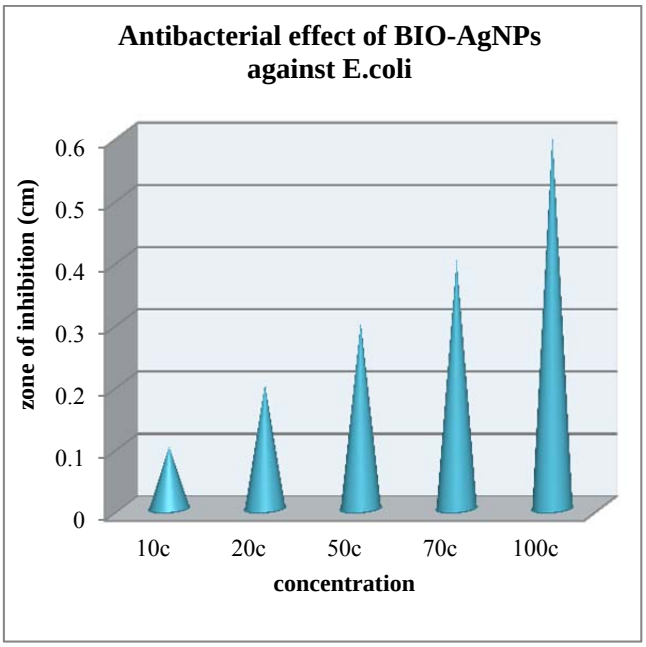

Fig. 3: Graph shows zone of inhibition of BIO-AgNPs against *E.coli*

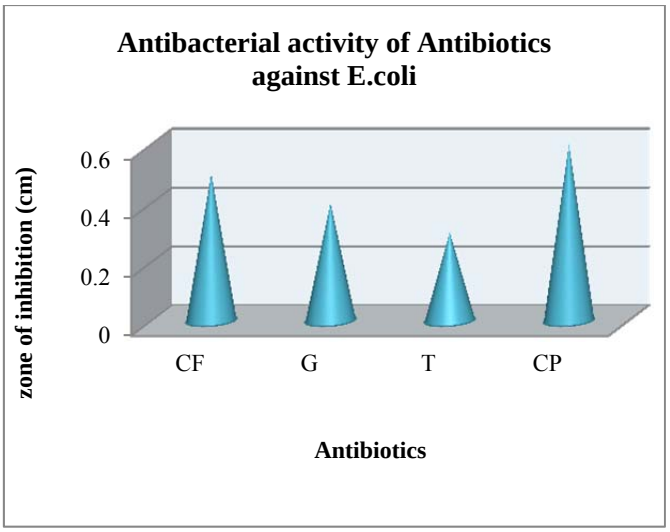

Fig. 4: Graph shows zone of inhibition of antibiotics against *E.coli*

Fig. 5: shows zone of inhibition of antibiotics and BIO-AgNPs against *E.coli*

4. CONCLUSION

Silver nanoparticles were biologically synthesized using *Allium sativum* which were characterized using UV-Vis spectrophotometer. Antimicrobial activity of BIO-AgNPs at different concentrations along with four antibiotics was checked against gram negative bacteria *E.coli.* The study revealed that the combination of antibiotics with AgNPs have better antimicrobial effects. Guzman et al. [6] had synthesized Ag nanoparticles via chemical route and studied their antimicrobial activities against *E.coli, P. aeruginosa,* and *S. aureus* bacteria as function of nanoparticle concentration and size. They observed a strong antimicrobial activity for a concentration of silver nanoparticles less than 7 ppm. In a recent work by Taglietti et al. [7], the mechanism of antimicrobial activities of silver nanoparticle coated with glutathione (GSH) were investigated on gram positive and gram negative bacterial strain in two ways (i) by dispersing the silver nanoparticles in solution (ii) grafted on thiol functionlized glass surfaces. Penetration of nanoparticles into bacterial cell wall can due to several reasons such as creating a pathole or generation free radicals for inhibiting the growth of bacteria. Bio-AgNPs extract also decreases the growth of pathogenic bacteria but lesser toxicity advantages [8]. Nanotechnology and nanofabrication has opened its doors to a world of metal nanoparticle synthesis with easy preparation protocols, less toxicity and a wide range of applications according to their size and shape. Metal nanoparticles of desired size and shape have been obtained successfully using living organisms (simple unicellular organisms) to highly complex eukaryotes [9]. The toxicity of metal nanoparticles toxicity was measured via toxtrak test and the percentage of inhabitation of CH-AgNPs was much greater than the Bio-AgNPs synthesized. Hence, Bio-AgNPs is the most suitable metallic coating material coat to drugs instead of CH-AgNPs in pharmaceutical industries [10].

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