SYNTHESIS OF SILVER NANOPARTICLES BY CHEMICAL REDUCTION AND THEIR ANTIMICROBIAL ACTIVITY

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Abstract

In this article we have studied the antimicrobial activity of silver nanoparticles with five different types of bacteria. Silver nanoparticles were chemically synthesized using silver nitrate as a precursor material and tri sodium citrate as reducing agent. UV-Vis spectroscopy and Scanning Tunnelling Microscopy (STM) were used to study the absorption spectra and surface morphology of Silver nanoparticles. The antimicrobial activity of silver nanoparticles was investigated against five different types of microorganisms such as Bacillus subtilis, Bacillus megaterium, Escherichia coli, Salmonella typhimurium, and Staphylococcus aureus.

Keywords: silver nanoparticles, scanning tunnelling microscope, UV Spectroscopy,

1. Introduction

Nanotechnology provides the ability to design nanoscale materials which have unique properties and potentials applications, particles which are smaller than the characteristics length display new physics and chemistry leads to new behaviour which depends on the size [1]. while metal nanoparticles exhibit unusual optical, thermal, chemical, and physical properties which could be due to the combination of a large proportion of high energy surface atoms relative to the bulk solid and the nanometre scale, mean free path of an electron changing its conductivity and mobility [2], and can be used in numerous physical, biological, biomedical, and pharmaceutical applications [3, 4, 5]. The most important property of silver nanoparticles is its antimicrobial effect [6, 7], physical, chemical and biological methodologies are used to synthesize metal nanoparticles. Biological methods gained much more interest due to low cost and eco friendliness [8, 9]. Methods of biosynthesis of silver nanoparticles includes bacteria [10, 11], fungi [12, 13], plants extract [14] etc. It is known that Ag ions and Ag based compounds are highly toxic to microorganism [15]. Thus, in this present work we investigated the biological interaction of nanodimenstional silver nanoparticles against *Bacillus subtilis, Bacillus megaterium, Escherichia coli, Salmonella typhimurium,* and *Staphylococcus aureus.* Prior to that, we report the synthesis of stable nanometre-sized silver particles size ranging from 20-30nm using silver nitrate as a precursor with different preparation procedures. The surface morphology of the nanoparticles and optical properties are revealed by Scanning Tunnelling Microscope (STM) and UV-Vis spectroscopy, respectively.

Materials and Methods:

2.1. Materials:

Silver nitrate ($AgNO_3$), Trisodium citrate ($Na_3C_6H_5O_7$), were used, all analytical grades without further purification. Nutrient agar is prepared by standard protocol. All the bacteria like *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus* are collected from National Collection of Industrial Microorganisms (NCIM), double distilled water is used for this experiment.

2.2. Synthesis of Silver Nanoparticles

Most of the size controlled growth mechanism reaction takes place only in alcoholic media. Alcoholic media such as methanol, ethanol or propanol are desirable, because in alcoholic media growth of oxide particles is slow and controllable [16]. Solution of silver nitrate $AgNO_3$ was prepared by dissolving 0.0169g of silver nitrate in 100 ml of distilled water/ethanol and boiled in ambient atmosphere and 1% tri sodium citrate solution is prepared by dissolving 1g of trisodium citrate into 100 ml of distilled water. 20ml of silver nitrate solution was kept in hot plate at 90^{0}C for 5 minutes and then add 2.5 ml of trisodium citrate drop by drop once the reduction process begins colour change appears and the solution turn into pale yellow. After the changes in colour, solution was stirred in magnetic stirrer for 15mins.

2.3. Antimicrobial Test:

The antimicrobial activity of silver nanoparticles is tested on five different types of pathogenic bacteria such as *Bacillus subtilis, Bacillus megaterium, Escherichia coli, Salmonella typhimurium,* and *Staphylococcus aureus* which were cultured on agar plates supplemented with different concentration of silver nanoparticles by Bore well method. The plates were incubated for 24 hr at 37^{o}C.

2.4. Characterization:

The optical properties of synthesized silver nanoparticles by various methods were characterized by using UV-Vis spectrometry (Thermo Scientific UV-10) and Scanning Tunnelling Microscope (STM-Nano Surf Easy Scan 2). Figure-1 is the result of characterized silver nanoparticles by UV -Vis spectrometry. The surface topography of nanoparticles is concluded by Scanning Tunnelling Microscope. Figure-2 Represent, topography image of silver nanoparticles in the area range of 200 X 200 nm.

3. Results and Discussions

The formation of silver nanoparticles occurs after the reduction of aqueous silver salts with tri sodium citrate (1%) within duration of 10-20 minutes, colour change appear after the completion of reaction, it is well known that, the silver nanoparticles exhibit pale yellow colour. When the reducing agent is added into aqueous silver salt in drop wise manner, growth of silver "seed nucleation" is controlled in nano dimension. This control growth of silver atoms lead to another phenomenon called "localized surface plasmon oscillation". Due to this oscillation, optical property of (colour-pale yellow) synthesized silver nanoparticles differ from bulk silver salt.

UV-Vis spectroscopy is used to study the absorption spectra of silver nanoparticles, silver nanoparticles shown surface plasmon resonance in the range 420 nm which is similar in this case and peak boarding indicates that the particles are poly dispersed sharp peak indicates the uniformity of particles size (Figure 1). Recent study revealed that the size of metal nanoparticles can be predicated based on its colour observation [17].

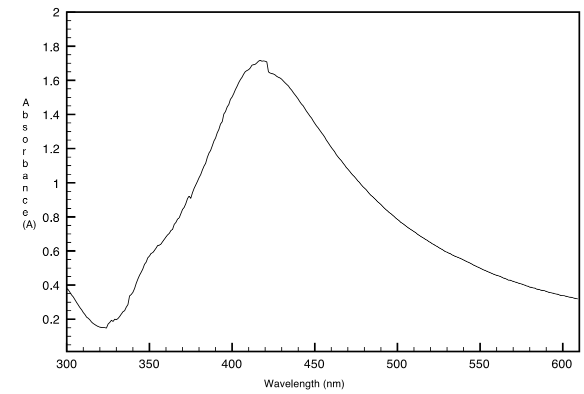

Figure 1: UV-Vis absorption spectra of silver nanoparticles

Figure 2 shows the topography image of Scanning Tunnelling Microscope (STM) high density surface morphology of silver nanoparticles. The line graph for silver nanoparticles is almost stable, this indicates the preparation of tip, sample and approach were done successfully and it also indicates good tunnelling contact with sample and tip. It is more difficult to obtain good image of silver nanoparticles, atomic structure are difficult to observe because the electrons are distributed homogeneously on the surface [18]. The particle size observed from the STM image is in the range of 5 - 10 nm.

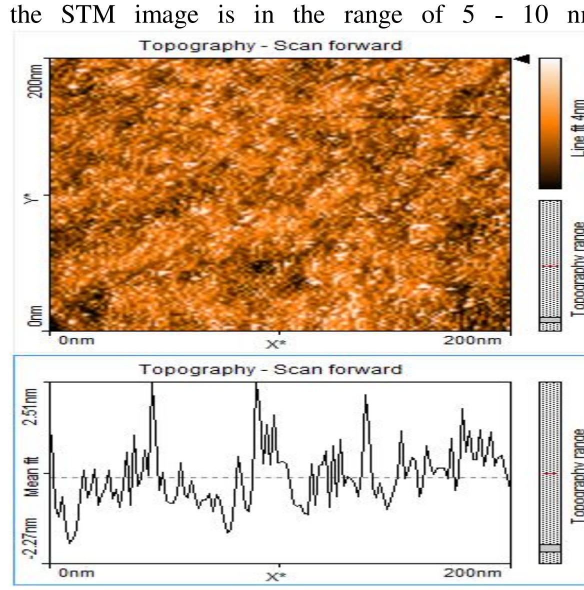

Figure 2: STM image of silver nanoparticles

Silver nanoparticles synthesized by chemical method have been found highly toxic against pathogenic bacteria. Herein, the synthesized silver nanoparticles exhibited antibacterial activity against *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* as it shown clear inhibition zone (Figure 3).

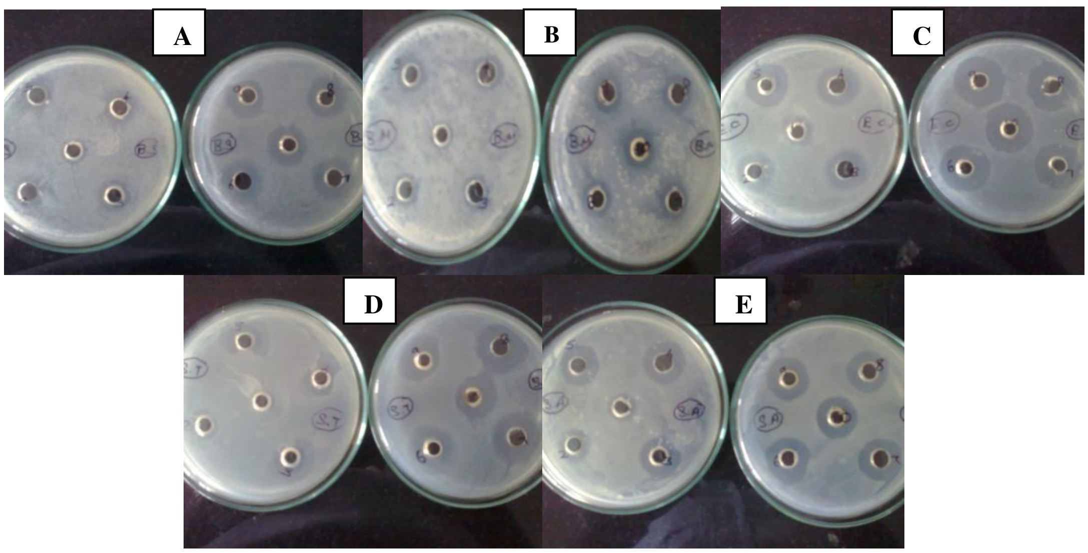

Figure 3: Result of Antimicrobial activity of silver nanoparticles on (A) *Bacillus subtilis* (B) *Bacillus megaterium* (C) *Escherichia coli* (D) *Salmonella typhimurium* (E) *Staphylococcus aureus*

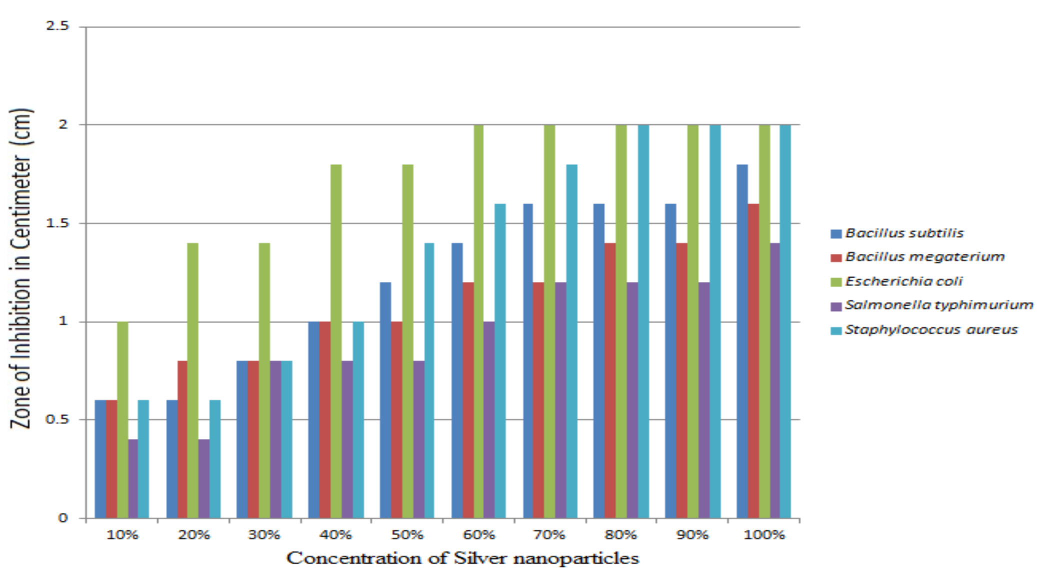

Figure 4: Zone of Inhibition Vs Concentration of silver nanoparticles on pathogenic micro-organisms.

Several studies reveal that silver nanoparticles interact into the bacterial cell membrane interrupting their metabolic activities like permeability and respiration functions and in the most of the studies it is found that smaller particles have large surface area and are more effective then bulk particles towards pathogenic bacteria [9]. Based on the analysis and result of figure 4 proves that, silver nanoparticles having antimicrobial activities towards bacterial cells.

Figure 4 represents effect of silver nanoparticles on all five bacteria. This graph shows that, Silver nanoparticles are more effective towards *Escherichia coli* and *Staphylococcus aureus,* than all other micro-organisms. Effect of concentration of silver nanoparticles on *Escherichia coli* is saturated from 100-60% where us *Staphylococcus aureus is* 100-80%. It is least effective towards *Salmonella typhimurium.*

Conclusion

Silver nanoparticles were successfully synthesized by two step chemical reduction process using thermal reduction technique, reduction of aqueous silver nitrate using tri sodium citrate. The STM analysis display that the surface topography of silver nanoparticles are spherical in nature. The antimicrobial assay showed that silver nanoparticles presented good antibacterial performance against clinical pathogens. The synthesis of nanoparticles using this technique is simple and low-cost approach. The process is useful in preparation of antimicrobial agents and could be exploited for further biomedical application.

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