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### Synthesis, Characterization and Evaluation of its Antibacterial activity of Silver Nanoparticles

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#### ABSTRACT

In the present study aimed to Synthesis, characterization and evaluation of its antibacterial activity of silver Nanoparticles. Silver nanoparticles exhibit brown colour in aqueous solution due to excitation of surface plasmon vibrations in Silver nanoparticles. The appearances of creamy colour in the reaction vessels suggest the formation of Silver nanoparticles. UV-Visible and FTIR spectroscopy are further confirmed the structural characterization and functional group identification of Silver nanoparticles. The SEM analysis showed the particle size 84.90nm as well the spherical structure of the nanoparticles. Silver nanoparticles might be useful for the development of newer and more potent antibacterial agents. All the above data's represented in our study contribute to a novel and unexplored area of nanomaterials as medicine.

**Keywords:** Nanoparticles, Silver nanoparticles, Antibacterial activity, Antioxidant,

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#### INTRODUCTION

In the past decade, considerable attention has been paid for the development of novel strategies for the synthesis of different kind of nano-objects. The world of nanochemistry is a vast world from individual molecules to continual systems that constitute a phase. Nanoparticles (size up to 100 nm) are characterized by intermolecular interactions that deprive molecules of their personality; the properties and behavior of molecules in ensembles differ from those of single molecules. Nanoparticles exhibit completely new properties based on specific characteristics such as size, distribution and morphology. Many routes are available for the synthesis of nanoparticles, there is an increasing need to develop high-yield, low cost, non-toxic and environmentally friendly procedures. Therefore, the biological approach for the synthesis of nanoparticles becomes essential. In recent years, the biosynthesis of nanoparticles using chemicals has gained more importance. Chemistry celebrated the end of the 20th century with a remarkable result of strategic importance it has approached the threshold of being able to detect a single molecule and identify its physical and chemical behaviour. As a result, a new field of chemistry has appeared, viz., the chemistry of single molecules. A molecule acquires identity and becomes a person.

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This new field defines precisely the upper boundary of yet another field of chemistry, called nanochemistry, which studies small ensembles of molecules (or atoms) comprising more than one species. However, there is no strict quantitative criterion for defining the lower boundary of this region; furthermore, setting any artificial limits (e.g., size in nanometres or number of molecules or atoms in hundreds or thousands) is harmful. The world of nanochemistry is a vast world from individual molecules to continual systems that constitute a phase. Nanoparticles are characterised by intermolecular interactions that deprive molecules of their personality; the properties and behaviour of molecules in ensembles differ from those of single molecules (social communities of people and animals show some similar features) (Buchachenko, 2003).

#### **Different methods for metallic nanoparticle synthesis**

Several methods are used for synthesis of nanoparticles (NPs) such as physical, chemical, enzymatic and biological

#### **Types of nanoparticles:**

Nanoparticles can be broadly grouped into two, namely, organic nanoparticles which include carbon nanoparticles (fullerenes) while, some of the inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semi-conductor nanoparticles (like titanium oxide and zinc oxide). There is a growing interest in inorganic nanoparticles i.e. of noble metal nanoparticles (Gold and silver) as they provide superior material properties with functional versatility. Due to their size features and advantages over available chemical imaging drug agents and drugs, inorganic particles have been examined as potential tools for medical imaging as well as for treating diseases. Inorganic nonmaterial have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good compatibility, and capability of targeted drug delivery and controlled release of drugs (Xu *et al.*, 2005).

#### **Methods for nanoparticle synthesis:**

##### **Silver nanoparticle**

Most important physical approaches include evaporation-condensation and laser ablation. Various metal nanoparticles such as silver, gold, lead sulfide, cadmium sulfide, and fullerene have previously been synthesized using the evaporation-condensation method. The absence of solvent contamination in the prepared thin films and the uniformity of nanoparticles distribution are the advantages of physical approaches in comparison with chemical processes Kruis, *et al.*, (2000). It was demonstrated that silver nanoparticles could be synthesized via a small ceramic heater with a local heating source (Jung, *et al.*, 2011). The evaporated vapor can cool at a suitable rapid rate, because the temperature gradient in the vicinity of the heater surface is very steep in comparison with that of a tube furnace. This makes possible the formation of small nanoparticles in high concentration. This physical method can be useful as a nanoparticle generator for long-term experiments for inhalation toxicity studies, and as a calibration device for

nanoparticle measurement equipment Kruis, *et al.*, (2000). Titanium nanoparticles could be synthesized by laser ablation of metallic bulk materials in solution. The ablation efficiency and the characteristics of produced nanosilver depend upon many factors such as the wavelength of the laser impinging the metallic target, the duration of the laser pulses (in the femto-, pico- and nanosecond regime), the laser fluence, the ablation time duration and the effective liquid medium, with or without the presence of surfactants. One important advantage of laser ablation technique compared to other methods for production of metal colloids is the absence of chemical reagents in solutions. Therefore, pure and uncontaminated metal colloids for further applications can be prepared by this technique Tsuji *et al.*, (2002).

#### **Microbes:**

The world we live in is one full of microbes. Microbes, whether they are good, bad or benign are certainly everywhere. This includes on our body, in our homes, far below the earth's surface and up to the atmosphere, in cold, cool, warm and hot and very hot places, and even in places without oxygen. Our body temperature and wealth of nutrients provide an ideal home for these micro-organisms to thrive. Microorganisms always live in water (directly in aquatic environments, in water inside animals or plants, or in water around soil particles). They can eat all sorts of things, including oil, rocks, dead and living plants and animals (Needham, 2000).

There are 4 major types of Microbes: bacteria, fungi, protists and viruses. Prokaryotes (bacteria) are simple celled organisms, meaning that they have no membrane-bound organelles. In other words, the small organs and DNA are free-floating within the cell. Bacteria can occur as little round balls, tiny short sticks, or spirals that look like springs. These three basic forms are often stuck together in long strings or clusters that look like little squares, cubes, or random grape-like clusters (Lynch and Hobbie, 1988).

## **MATERIALS AND METHODS**

#### **Synthesis of Silver nanoparticles.**

To synthesize different-sized AgNPs, the spherical AgNPs were prepared according to the literature procedure by Fang *et al.* (2005), by reducing aqueous AgNO<sub>3</sub> with sodium citrate at boiling temperature. In typical procedure, 50 ml of 0.001 M AgNO<sub>3</sub> was heated to boiling. To this solution, 5 ml of 1% trisodium citrate was added drop by drop. The solution was heated at boiling point under continuous stirring. The reaction was allowed to take place until the color changed to a yellow solution. The solution was then cooled to room temperature. The AgNPs in this solution were called citrate-AgNPs.

#### **Characterization of silver nitrate nanoparticles**

##### **UV-Visible spectra analysis**

The reduction of pure Ag<sup>+</sup> ions was monitored by measuring the UV-Vis spectrum of the reaction medium and the absorption spectra were recorded over the range of 300-

700 nm using UV-Vis spectrophotometer (VARIAN CARY EL06023680).

#### Fourier transform infrared Spectroscopy

To determine Fourier transform infra-red (FTIR) pattern of the AgNO<sub>3</sub> nanoparticles was freeze-dried and the dried powder was diluted with potassium bromide in the ratio of 1:100 and recorded the spectrum in Perkin Elmer FTIR Spectrum BX (Wellesley, MA, USA).

#### SEM analysis of silver nanoparticles

The scanning electron microscopy (SEM) analysis of freeze dried sample was performed by mounting nanoparticles on specimen stubs with double-sided adhesive tape and coated with platinum in a sputter coater and examined under JEOL 63861 SEM (Japan) at 10 kV.

#### In vitro antioxidant activity

DPPH radical-scavenging activity (2,2-diphenylpicrylhydrazyl) DPPH radical-scavenging activity was determined by the method of Shimada, *et al.*, (1992).

#### Determination of antimicrobial activity

The antibacterial activity was performed by nutrient agar plate method (Cormican *et al.*, 1996).

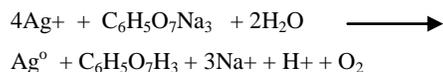
#### Antibacterial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007). Petri plates were prepared by pouring 30 ml of nutrient agar medium for bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mins. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar. Briefly, inoculums containing bacteria were spread on Nutrient agar plates. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the sample (30µl and 30µl for standard) was laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria. Each sample was tested in triplicate.

## RESULTS AND DISCUSSION

#### Synthesis of Silver nanoparticles

The synthesis of silver nitrate nanoparticles through trisodium citrate were carried out. Silver is used as reducing agent as silver nitrate has distinctive properties such as good conductivity, catalytic and chemical stability. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). Silver nitrate and trisodium citrate were used as starting materials for the preparation of silver nanoparticles. The silver colloid was prepared by using chemical reduction method (Li *et al.*, 2010). The mechanism of reaction could be expressed as follows ((Silva *et al.*, 2007: Hangxun, Xu and Kenneth, 2010).



The aqueous silver when exposed to trisodium citrate was reduced in solution, there by leading to the formation of silver nitrate hydrosol. The time duration of change in colour varies from chemical to chemical. It is well

known that silver nanoparticles exhibit brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Fig 1). The appearances of brown colour in the conical flask suggest the formation of silver nanoparticles (Mamun *et al.*, 2012).



Fig 1: Photographs of silver nitrate and silver nanoparticles colour

#### Ultraviolet/visible (UV/VIS) spectroscopy

UV-Visible spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles. It is quite sensitive to the presence of silver colloids because these nanoparticles exhibit an intense absorption peak due to the surface plasmon excitation. The absorption band in the 350 nm to 450 nm region is typical for the silver nanoparticles (Kadir *et al.*, 2005). With increasing particles size, the plasmon absorption shifts toward red. The adsorption spectra of the yellow silver solution (Figure 3) prepared by trisodium citrate reduction shows the surface plasmon resonance at about 430 nm, indicating the presence of spherical and roughly spherical Ag nanoparticles with an average size of 84.90 nm as confirmed by SEM photographs.

UV/Visible spectroscopy can be used as a characterization technique that provides information on whether the nanoparticle solution has destabilized over time. The optical properties of silver nanoparticles change when particles aggregate and the conduction electrons near each particle surface become delocalized and are shared amongst neighbouring particles. When this occurs, the surface plasmon resonance shifts to lower energies, causing the absorption and scattering peaks to red-shift to longer wavelengths. UV-Visible spectroscopy can be used as a simple and reliable method for monitoring the stability of nanoparticle solutions. It is observed that the maximum absorbance of silver nanoparticles occurs at 430 nm. As the particles destabilize, the original extinction peak will decrease in intensity (due to the depletion of stable nanoparticles), and often the peak will broaden or a secondary peak will form at longer wavelengths (due to the formation of aggregates). The rapid and irreversible change in the extinction spectrum clearly demonstrates that the nanoparticles are agglomerating. In the present investigation the peak was decreased due to destabilization of nanoparticles (Fig 2).



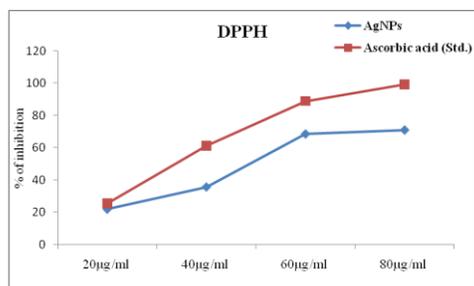


Fig 6: DPPH radical scavenging activity of AgNPs

### Antibacterial activity

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of AgNPs has a new awareness for the control of disease, besides being safe and no phytotoxic effects (Torresdey *et al.*, 2003). The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria of selected species. The AgNPs shows highest antibacterial activity was observed against *Escherichia coli* and *Staphylococcus aureus*. The inhibitory activities in culture media of the Ag nanoparticles reported in table were comparable with standard antimicrobial viz. chloramphenicol.

In this study, to evaluate the antibacterial effects Ag nanoparticles against various bacterial strain such as *Escherichia coli* and *Staphylococcus aureus*. There were distinct differences among them. When Ag nanoparticles were tested they effectively inhibited bacterial growth. The result shows that Ag nanoparticles having antibacterial activity against *E. coli* that was similar to that found by Sondi and Salopek-Sondi (2004).

In contrast, the inhibitory effect of Ag nanoparticles was mild in *S. aureus* as compared with other microorganisms; these results suggest that the antibacterial effects of Ag nanoparticles may be associated with characteristics of certain bacterial species. The growth of microorganisms was inhibited by the NPs showed variation in the inhibition of growth of microorganisms may be due to the presence of peptidoglycan, which is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less silver to reach the cytoplasmic membrane than the gram negative bacteria (Ahmad *et al.*, 2011). We think that the efficacy of the Ag nanoparticles against *Escherichia coli* and *Staphylococcus aureus* may derive from the difference as a point of membrane structure. The peptidoglycan layer is a specific membrane feature of bacterial species and not mammalian cells. Therefore, if the antibacterial effect of Ag nanoparticles is associated with the peptidoglycan layer, it will be easier and more specific to use Ag nanoparticles as an antibacterial agent. The SNPs synthesized from plant species are toxic to multidrug resistant microorganisms. It shows that they have great potential in biomedical applications.

Table 2: Antibacterial activity of AgNPs, AgNO<sub>3</sub> and control

| Microorganism                     | Silver nitrate (30µl) | Silver nanoparticles (30µl) | Standard (Chloramphenicol) (30µl) | Control (30µl) |
|-----------------------------------|-----------------------|-----------------------------|-----------------------------------|----------------|
| <i>Escherichia coli</i> (mm)      | 0.80 ±0.05            | 3.04 ±0.21                  | 6.65 ±0.46                        | 0              |
| <i>Staphylococcus aureus</i> (mm) | 0.53 ±0.03            | 2.64 ±0.18                  | 6.24 ±0.43                        | 0              |

Values were expressed as Mean ± SD.

### *Escherichia coli*      *Staphylococcus aureus*

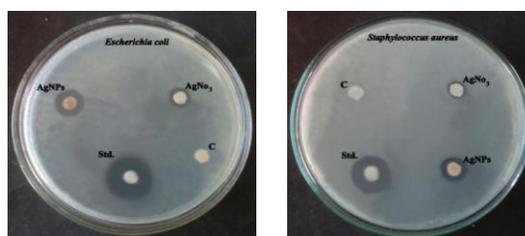


Fig 7: Antibacterial activity

Nanoscience has been established recently as a new interdisciplinary science. It is widely accepted in the context of nanoscience and nanotechnologies, the units should only be those of dimensions, rather than of any other unit of scientific measurement. It is widely agreed that nanoparticles are clusters of atoms in the size range of 1–100 nm. Metal nanoparticles can be prepared by two routes, the first one is a physical approach that utilizes several methods such as evaporation/condensation and laser ablation. The second one is a chemical approach in which the metal ions in solution are reduced in conditions favoring the subsequent formation of small metal clusters or aggregates.

It is confirmed that silver nanoparticles are capable of rendering high antibacterial efficacy and hence has a great potential in the preparation of drugs used against bacterial diseases. Applications of Ag nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antibacterial systems.

### CONCLUSION

The present study exhibit a simple method of synthesis of silver nanoparticles from a novel primitive chemical source. This method can be further used for industrial production of nanoparticles at room temperature and with a single step. Since the nanoparticles thus synthesized shows antibacterial activity, they can be used in the field of pharmaceutical industry. Silver nanoparticles might be useful for the development of newer and more potent antibacterial agents. All the above data's represented in our study contribute to a novel and unexplored area of nanomaterials as medicine.

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