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## *Original Article*

# A Study on Synthesis, Characterization and Toxicity of Silver Nanoparticles

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## *Abstract*

***Introduction:*** *Nanoparticles are increasing attention for the wide range of new applications in various fields of technology. Silver nanoparticles possess unique properties which find tremendous applications such as antimicrobial, anticancer, larvicidal, catalytic, and wound healing activities.*

***Methods:*** *This quasi experimental study was designed to synthesize silver nanoparticles (AgNPs) from silver nitrate ($AgNO_3$) solution using glucose as reducing agent and starch as capping agent during the period of July 2014 to June 2015. The characterization of nanoparticles was carried out using UV-Vis spectroscopy. The harmful effect of AgNPs was investigated using Brine shrimp (Artemia salina) in various micro molar concentrations (0.01µmol, 0.1µmol, 10 µmol, 25µmol, 50 µmol). The $LD_{50}$ and mortality rate was also evaluated.*

***Results:*** *The AgNPs containing colloidal solution showed distinctive color change and a sharp peaked surface plasmon resonance at 420 nm. Maximum mortality rate was observed at 50 µmol (61%). The $LD_{50}$ value was obtained at 1.62 µmol which signifies high toxicity of silver nanoparticles to Artemia salina.*

***Conclusion:*** *This study established that, the chemical synthesis guided AgNPs are small, spherical and have toxic effect on Artemia salina.*

***Key words:*** *Silver nanoparticles, Brine shrimp, Cytotoxicity*

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## Inroduction

Nanoparticle may be defined as particles of controlled size with at least one dimension less than 100 nm.[1] That is nanoparticles are the particles between 1 to 100 nm that behave as a whole unit in respect of its transport and properties. Nanoparticles are now being developed for a variety of biological applications such as medicines, antimicrobial agents, wound dressings, drug targeting and deliveries. Development of newer drug delivery system based on nanotechnology methods is being tried for conditions like cancer, diabetes, fungal infections, viral infections and gene therapy.[2] Nanotechnology has also found its use in diagnostic medicine as contrast agents like fluorescent dyes and magnetic nanoparticles.[3] Among metal nanoparticles silver have received considerable attention due to their attractive properties like size and shape depending optical, electrical and magnetic properties which can be incorporated into antimicrobial application, biosensor materials, cosmetic products and electronic components.[4] β-d-Glucose as reducing agent is renewable, inexpensive and nontoxic.[5] Starch capped nanoparticles can be radialy integrated into systems relevant for pharmaceuticals and biomedical applications.[6] The increase of manufacture and consumption of products containing silver nanoparticles can lead to metallic nanoparticles release in the environment if waste is not properly disposed. On the other hand, concentration of these nanoparticles is increasing in aquatic environment and can strongly affect and damage the biota.[7] Therefore, it is thought worthwhile to study the synthesis of AgNPs in a simple method using glucose as reducing substance and starch as stabilizing agent with gentle heating. Brine shrimp lethality study was also determined in order to observe cytotoxic effect of AgNPs.

## Materials and Methods

This study was carried out in the department of Pharmacology, Rajshahi Medical College, Rajshahi during the period July 2014 to June 2015. Silver nitrate ($AgNO_3$), β-d glucose, starch were purchased from local market. All glass wares and instruments (conical flasks, measuring cylinders, beakers, petri plates and test tubes, micropipettes etc.) were purchased from local market. Deionized water was used throughout the experiment.

**Preparation of Silver Nanoparticles**

For synthesis of silver nanoparticles 200 µl of 0.1 M silver nitrate and 500 µl of 0.1M glucose were placed into the conical flask using 1000 µl micropipette. Then 10 ml of 0.2% starch was added into the flask. The mixtures of solutions were gently heated in a hot plate for 10 minutes. A change in color of the solution was obtained from colorless to yellow indicating the formation of silver nanoparticles.

### UV-Vis Spectral Analysis

UV-Vis spectroscopy was used to analyze the presence of silver nanoparticles using MODEL 340 Spectrophotometer (SEQUOIA TURNER CORP). The scanning range for the samples was 200-800 nm. Baseline correction of the spectrophotometer was carried out by using a blank reference. Ultraviolet–visible spectroscopy (UV-Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. It is the measurement of light passing through a sample (I), and compares it to the intensity of light before it passes through the sample ($I_0$). The ratio $I / I_0$ is called the transmittance and usually expressed as a percentage (% T). The absorbance A is based on the transmittance: $A = - \log (\%T)$.

### Brine Shrimp Cytotoxicity Assay

Brine shrimp *Artemia salina* cysts were purchased and maintained in the laboratory conditions and were used for cytotoxicity assay. Briefly, *Artemia salina* cysts of 1 gm were aerated in 1 L capacity of glass jar containing 3.8 % of saline water (3.8 gm commercial salt in 100 ml of distilled water). The jar was aerated constantly for 48 hrs at room temperature (25-29 °C). After hatching, active free-floating young nauplii were collected from bright illumination and were used for the bioassay. Parallel negative control (without AgNPs) was also included for the experimental set up. Five test samples containing 0.01 µmol, 0.1 µmol, 10 µmol, 25 µmol, 50 µmol were prepared. 10 nauplii transferred to each test petridis (10 petridis for each test sample) using pipette. Then 1ml of nano solution and artificial sea water was added to make the final volume 5 ml. The experimental set up was allowed to remain 48 hrs in darkness. Survivors were counted with a magnifying glass after 48 hours. Percentages of mortality at each dose and control were determined. The results were calculated as means ± standard deviation (SD). $LD_{50}$ for tested concentration of Brine Shrimp cytotoxicity assay along with 95% confidence limit were determined using probit analysis. Percentage of mortality was calculated by the following formulae: % mortality (number of dead *Artemianauplii* / initial number of lice *Artemianauplii*) × 100.

## Results

When solution of glucose and starch was gently heated with aqueous solution of the $AgNO_3$, the color from colorless solution mixture started turning yellow just after 2-3 minutes of heating and after 10 minutes it became yellowish brown. On the other hand, control solution (deionized water, glucose and starch) did not develop color after heating.

The UV-Vis absorption spectra of the Ag NP were shown in Figure 1. UV-Vis spectroscopy allows high quality characterization of the AgNPs. Absorption spectra of Ag nanoparticles formed in the reaction media had a sharp peak and absorbance maxima at 420 nm which indicates that size of synthesized AgNPs were below 30nm. The absorption range of prepared silver nanoparticle solution was checked on 1st, 3rd and 45th day. There was no significant change observed in peak position for 3rd and 45th day. The control solution did not develop SPR peak observed in UV-Vis spectra confirming absence of nanoparticles.

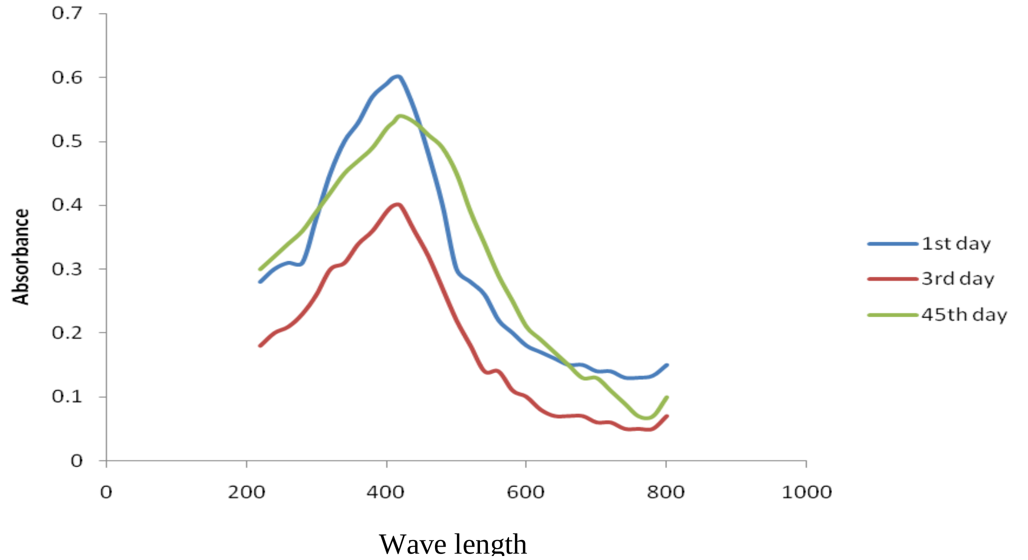


**Figure 1 : UV-Vis absorption spectra of silver nanoparticles**

The brine shrimp lethality assay was also performed to determine the cytotoxicity of AgNps, which could also provide an indication of possible toxicity of the test materials. It was demonstrated that early developmental stages of *Artemia* was highly vulnerable to toxins. The lethality was found to be directly proportional to the concentration of NPs. The results were found to be in such a way that in the control the mortality was about 0%. In minimum concentration of 0.01µmol the mortality was 37% and as the concentration increased to 0.1µmol, 10 µmol, 25 µmol and 50 µmol the mortality was increased to 39%, 50%, 56% and 61%. Maximum mortality rate was observed at 50µmol. While $LD_{50}$-48 hours was obtained at 1.62 µmol (.275 mcg/ml) as shown in Table I.

**Table I: $LD_{50}$ value of AgNPs**

| Test sample | $LD_{50}$-48 hours (µmol) | 95% confidence limit | | Regression equation | $\lambda^2$ (df) |
|---|---|---|---|---|---|
| | | lower | upper | | |
| AgNPs | 1.62 | 6.826 | 386.17 | Y= 4.673±.147 | .20 (3) |

(AgNPs= Silver nanoparticles, µmol= Micromol, $LD_{50}$= Median lethal dose ( dose at which 50% of shrimp nauplii died), $\lambda^2$= Chi-square, df = degree of freedom).

## Discussion

At the macroscale, silver always looks like silver. But it is well known that silver nanoparticles exhibit yellowish color in aqueous solution due to surface plasmon vibration.[8] In the silver nanoparticles, electrons oscillate collectively. These oscillations affect how light interacts with the nanoparticles. The specific oscillations depend on the particles size and shape. So particles of different sizes have different colors. The appearance of yellowish color in the solution suggests the formation of silver nanoparticles. Our observation is in agreement with the other studies reported earlier.[9-10]

UV-vis spectroscopy is one of the most widely used techniques for characterization of silver nanoparticles. The absorption phenomenon shown by the nanoparticles is due to surface plasmon resonance. In the present study, silver nanoparticles exhibited a single and well defined peak in the absorbance spectrum with maximum absorbance at 420 nm which corresponds to characteristic surface plasmon resonance of AgNPs. Only a single SPR band is expected in the absorption spectra of spherical nanoparticles, whereas anisotropic particles could give rise to two or more SPR bands depending on the shape of the particles. On the other hand, relatively narrow peak indicates that AgNPs were within a narrow size distribution.[11] Therefore; the overall findings concluded that synthesis of silver nanoparticles using glucose as reducing agent and starch as capping agent were spherical and roughly spherical in shape and having narrow size. The sharp narrow plasmon peak at 420 nm observed in this study was similar to an previous report[5] who found the surface plasmon absorbance spectrum of SNPs formed in aqueous starch dispersion at 419 nm and 90% of those particles were in the size range from 1 to 8 nm, which was further confirmed by TEM study.

In the present study, toxicity of silver nanoparticles (SNPs) was observed using brine shrimp lethality bioassay and the mortality rate of brine shrimp was found to be increased with increased concentration of nanoparticles. The highest mortality was found at 50 µmol and $LD_{50}$-48 hours was observed at 1.62 µmol (0.275 mcg/ml) indicates high toxicity to the *A. salina*. In another similar study,[7] evaluating toxicity of PVA (polyvinyl alcohol) stabilized silver nanoparticles to algae and micro crustaceans showed the $LD_{50}$ 48 hours was 0.55 mcg/ml which was also much higher than our study. The possible mechanism of toxicity effect of silver nanoparticles on brine shrimp was the nanoparticle aggregates at elevated levels in guts of *Artemia salina* resulting lack of food uptake since the guts were completely filled with the aggregates of silver nanoparticles.[12]

Therefore, the overall findings concluded that synthesis of silver nanoparticles using glucose as reducing agent and starch as capping agent were spherical and roughly spherical in shape and having narrow size range and highly stable. The synthesized silver nanoparticles using glucose and starch have potential harmful effects on aquatic invertebrates. Taking into account the mobility of SNPs into the cell and their fate in a bioprocess or even in the environment, other cytotoxicity tests and specific bioassays might be performed on cell lines for further studies.

## Conclusion

Nanotechnology stands for an important scientific advancement and can contribute with several benefits for human. In the present study, silver nanoparticles were successfully obtained from reduction of silver nitrate solution using glucose as reducing agent and starch as capping agent. UV-vis spectroscopy suggested the formation of uniform and spherical silver nanoparticles. The uncontrolled and unobserved release of these nanoparticles, either as by products or medical wastes, could have a large negative consequence on the aquatic, terrestrial organisms and also on humans. So, brine shrimp lethality assay was performed to investigate the toxicity of silver nanoparticles. AgNPs produced by using glucose and starch showed significant toxicity against *A. salina*. Silver nanoparticles produced by the method reported in this study using glucose as reducing agent and starch as capping agent have promising applications in biomedical, dental and pharmaceutical fields. Hence, effects and fates of this promising technology need further studies to evaluate the potential risks of nanoparticles on environment.

## Contribution of the Authors

First author was the principal researcher. Second and third authors did the statistical analysis and computer composing. Fourth author was the supervisor of the research work.

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