# PERIODICAL REDUCTION OF SILVER NITRATE BY BACTERIAL METABOLITES USING PARTICLE SIZE ANALYSER

**Jayavarthanan R, Anima Nanda***
Department of Biomedical Engineering, Sathyabama University,
Rajiv Gandhi Salai, Chennai - 600119, India
***Corresponding author:** Email: animananda72@gmail.com; Ph.no: +91-9443786840

**ABSTRACT**

The present study reports the reduction pattern and time duration needed for bacterial metabolites to reduce silver nitrate. The nitrogen fixing bacterium *Rhizobium* species was isolated from rhizosphere soil and their extracellular components were used to reduce 1mM silver nitrate into silver nanoparticles. The reaction was initially confirmed by UV-Spectrophotometer. Samples were drawn at constant intervals and analyzed through particle size analyzer. The results show that the bacterial metabolites need 96 hrs to reduce the silver nitrate into silver nanoparticles and also their zeta potential was found as 17.54 mV, it shows that the particles have high positive charge.

**KEY WORDS**: Silver nanoparticles, UV-Spec, particle size analyzer, Zeta potential.

## 1. INTRODUCTION

Nanotechnology is the branch of science controls the molecules at nano level, they are known as nanoparticles. It's the most emerging field for its vast and new applications in both applied and medical field (Shirley, 2010). Because of their increased surface area to volume ratio, the nanoparticles can interact with more molecules and exhibits more activities. Mostly they are synthesized from noble metals like Ag, Au, Pt & Pd. Often used in various biomedical applications. Among those the silver was focused more for its chemical stability, good conductivity and potent antimicrobial activity (Vijaya Raj, 2012). The synthesis of nanoparticles includes Biological, Chemical and Physical methods. Out of these the biological mode of reducing silver nitrate was considered to be the most successful one because of its simple principle and Eco-friendly in nature (Habeeb, 2013). Most of the research stated that the bacterial metabolites have the ability to convert silver nitrate into silver nanoparticles. But their exact mechanisms were not yet explained clearly. Thus the present study focused on the periodical reduction of silver nitrate by bacterial metabolites and examined through particle size analyzer.

## 2. MATERIALS AND METHODS

**2.1. Bio Reduction of Silver Nitrate:** The *Rhizobium* colonies were inoculated in Yeast Mannitol Broth and incubated in orbital shaker at 180 rpm for 96 hrs. After the incubation the cells were separated by centrifugation at 10,000rpm for 10 min. The cells were suspended in 100ml of distilled water along with sodium nitrate and potassium nitrate at 5mM concentrations. The contents were again incubated for 72 hrs at 180 rpm in orbital shaker. This procedure will increase the production of nitrate reductase enzyme. After the incubation the cells were removed by centrifugation. The supernatant with metabolite was mixed with silver nitrate at the concentration of 1mM and incubated in dark condition at 180 rpm. [Sundaramoorthi et al., 2010 & Muthukkumarasamy et al., 2012].

**2.2. CONFIRMATION BY UV-SPECTROPHOTOMETER:** 10 µl of silver nanoparticles solution was mixed with 990 µl of distilled water. The solution was scanned by UV-visible spectrophotometer (SHIMADZU) at the wavelength of 200-800ηm to find out its absorption maxima [Natarajan, 2010 & Saravanan, 2010]

**2.3. PERIODICAL REDUCTION ANALYSIS:** After 4 hrs of incubation 100 µl of sample was drawn from the reaction mixture and placed on the processing area of particle size analyzer (Microtrac inc. nanotrac particle analyzer, MW12031907-U2839Z, USA) [Kohila et al., 2013]. The equipment was allowed analyze the sample and their readings noted in the form of graphs. The particle size and their charge potential were calculated from the graph. Same procedure was repeated for the following samples drawn from the reaction mixture at 4 hrs intervals till 96 hrs. [Nanda, 2014 and Nayak, 2013].

## 3. RESULTS AND DISCUSSION

**3.1. BIO REDUCTION OF SILVER NITRATE:** The flasks containing cells with silver nitrate were continuously examined during incubation. The color change of solution from white to pale yellow indicates the formation silver nanoparticles **Fig-1**. [Sundaramoorthi et al., 2010 & Muthukkumarasamy et al., 2012].

**3.2. CONFIRMATION BY UV SPECTROPHOTOMETER**

During the scanning in UV spectrophotometer the absorption maxima showing between 400-450 ηm indicates the reduction of silver nitrate into silver nanoparticles. For this solution the peak was obtained at 420 ηm confirms the presence of silver nanoparticles Fig.2 **[**Saravanan et al., 2010**]**

**Fig -1 shows the formation of silver nanopaticles**

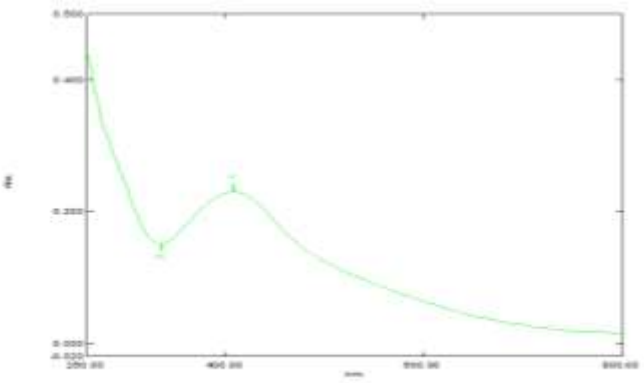

**Fig-2 shows the UV-spectrum for silver nanoparticles**

### 3.3. PERIODICAL REDUCTION ANALYSIS

Results of particle size analyzer shows that the silver nitrate molecule size was initially in the range of around 6 µm. The reduction reaction starts from the 2nd hour onwards. After 24 hrs of incubation the molecules were miniaturized up to 2 µm. Most of the molecules were reduced below 1 µm after 36 hrs of incubation but their size are mostly concentrated between 800-1000 ηm. After 72 hrs of incubation the particles were reduced up to 250-300 ηm. Further their sizes were reduced below 100 ηm after 96 hrs of incubation. [Nanda, 2014 & Nayak, 20.13] **Fig-3**

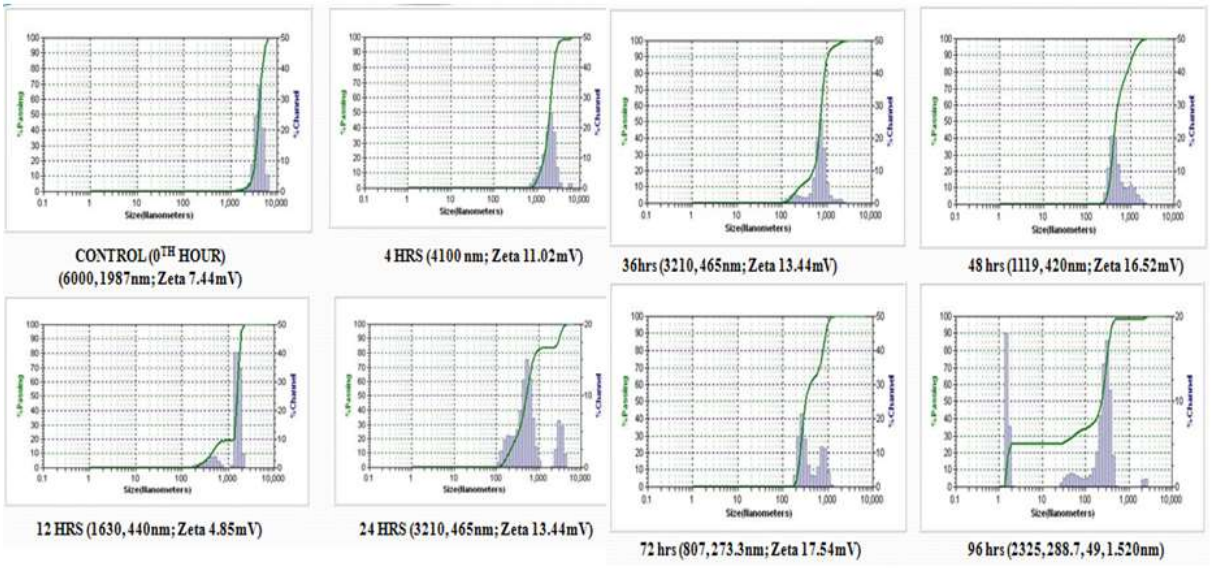


**Fig-3 Shows the periodical reduction results from particle size analyzer**

### 4. CONCLUSION

From this study it was confirmed that the metabolites has the abilities to reduce silver nitrate. The metabolites can reduce the compound below 100 ηm in size. From the above results it was concluded that the reduction reaction requires minimum of 72 hrs to reduce molecules at nano level. Further the report shows the synthesized particles are highly positive in charge.

### ACKNOWLEDGMENT

We would like thank the **DBT, New Delhi, India** for providing the funds for executing our research work. We give thanks to the ***Department Of Biomedical Engineering,*** **Sathyabama University, Chennai, India** for providing the facility and chemicals to carry out our research work.

### REFERENCES

Habeeb. M. K: Biosynthesis of Nanoparticles by Microorganisms and their Appications. International Journal of Advanced Scientific and Technical Research, 1(3), 2013, 44-51.

Muthukkumarasamy. S, Sharma. A, Vignesh. S, Dhanabalan. K, Grunathan. K: Extracellular Synthesis of Polygonal Silver Nanoparticles Using *Escherichia coli* ATCC 25922 and its Antibacterial Activities. Digest Journal of Nanomaterials and Biostructures, 7(4), 2012, 1419-1426.

Nanda. A, Lakshmipathy M: Biocatalysts of silver nanoparticles synthesized using an environmental isolate, *Serratia marcescens* S01. Int J ChemTech Res, 5(3), 2013, 1162-1168.

Nanda. A, Shahnaz Majeed: Enhanced antibacterial efficacy of biosynthesized AgNPs from Penicillium glabrum (MTCC1985) pooled with different drugs. International Journal of PharmTech Research, 6(1), 2014, 217-223.

Natarajan. K, Subbalaxmi Selvaraj, Ramachandra murty. V: Microbial Production of Silver Nanoparticles. Digest Journal of Nanomaterials and Biostructures, 5(1), 2010, 135-140.

Nayak. B.K., Bhat M. A., Nanda A**:** Amplification of antibacterial property of drugs in combination with silver nanoparticles synthesized from an airborne fungus International Journal of chemTech. Research, 6(4), 2014, 2368-2373.

Saravanan. M, Nanda. A**.** Biosynthesis of Silver nanoparticles from *Aspergillus clavatus* and its Antimicrobial Activity against MRSA and MRSE. Colloids and Surfaces B: Biointerfaces**,** 77(2), 2010, 214-218.

Shazhad. F, Shafee. M, Abbas. F, Tariq. M.M, Ahmad. Z: Isolation and Biochemical Characterization of Rhizobium melliloti from Root Nodules of Alfalfa (*Medico sativa*). The Journal of Animal and Plant Sciences, 22(2), 2012, 522-524.

Shirley, Dayanand. A, Sreedhar. B, Syed G Dastager: Antimicrobial Activity of Silver Nanoparticles Synthesized from Novel *Streptomyces Species*. Digest Journal of Nanomaterials and Biostructure , 5(2), 2010, 447-451.

Sundaramoorthi. C, Devarasu. S, Vegadesh Prabu. K: Antimicrobial and Wound Healing Activity of Silver Nanoparticles Synthesized from *Streptomyces aureofacians.* International Journal of Pharma. Research & Development, 2(12), 2010, 69-75.

Vani K, Thomas S, Prabhawathi V, Boobalana T, Sawant SN and Doble M: In vitro biocompatiblity of modified polycarbonate as a biomaterial. Colloid Surf. B: Biointerfaces, 108, 2013, 191-198.

Vijaya Raj. D, Anarkali. J, Rajathi. K, Sridher. S: Green Synthesis and Characterization of Silver Nanoparticles from the Leaf Extract of *Aristolochia bracteata* and its Antimicrobial Efficacy, 2(2), 2012, 11-15.