# FACILE SYNTHESIS OF SILVER NANOPARTICLES AND ANTIBIOTIC CONJUGATED SILVER NANOPARTICLES: A PROFICIENT RECYCLABLE NANO-CATALYST AND ANTIMICROBIAL AGENT


**SUBMITTED**

**BY**

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This is to certify that Ms. Sayantani Das, student of Department of life Science & Biotechnology, Jadavpur University has successfully completed her project work entitled "**Facile synthesis of silver nanoparticles and antibiotic conjugated silver nanoparticles: A proficient recyclable nano-catalyst and antimicrobial agent**" under my guidance during the period of 21st June, 2018 to 31st August, 2018 in the Department of Biochemistry, University of Calcutta for the partial fulfilment of her M.Sc. Degree. I am satisfied with her performance and experimental aptitude.

I wish her all success ahead.

Date: 11/05/19

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**Date: 25/03/2019**

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# Content

# OVERVIEW

Microbial synthesis of silver nanoparticles (AgNPs) has attracted considerable attention in recent times due to their exceptional versatile properties. These nanoparticles provide a promising technology to enhance the drug efficacy, effective vehicles for drug delivery, agents for degrading pollutants and many more novel functions.A bacterialstrain *Staphylococcus warneri* SuMS_NO3, isolated from the soil sediment of Sundarbans estuaryis capable to produce highly stable AgNPs by reducing silver nitrate ($AgNO_3$) saltusing its intracellular protein extract. Then, Gentamicin, a common antibiotic was conjugated with as-synthesized AgNP. AgNPs werecharacterized utilizing ultraviolet-visible spectrophotometry,transmission electron microscopy, X-ray diffraction, dynamic light scattering and Fourier transformed infrared spectroscopic measurement. The particles are found to be spherical, 2–10 nm in size, highly dispersed and also highly crystalline with face-centered cubic structures.Recyclable catalytic activity of as-synthesized AgNPs was evidenced by complete degradation of nitro aromatic pollutants like 2-nitroaniline, 4-nitroaniline, 2-nitrophenoland 4-nitrophenol.This green technology for bioremediation of toxic nitro aromatic pollutants issafe and economically beneficial to challenge the development and sustainability issue.The antimicrobial activity assay of AgNP and AgNP-Gentamicin was carried out against different gram negative and gram-positive bacteria.

**Keywords:**Bacterial intracellular protein. Silvernanoparticles, Gentamicin, Nanoparticle-conjugate, Recyclable catalysis, Nitro aromaticpollutants, Antimicrobial effect

# INTRODUCTION

The concept of nanotechnology was first presented by Richard Feynman through his famous lecture, entitled “There’s aplenty of room at the bottom” at the American Institute of Technology. The word nanotechnology was introduced by Prof. Norio Taniguchi of Tokyo Science University. The nanotechnology is emerging as a cutting-edge technology involving many academic disciplines such as physics, material science, chemistry, biology and medicine. The prefix “nano” in the term nanotechnology is derived from a Greek word nanos, which means “dwarf”. Particles having one or more dimensions of the order of 100nm or less are referred to as nanoparticles have attracted great attention Nanoparticles are interesting nanoscale systems because of the ease with which they can be produced in different shapes. This infinitesimal particle is the answer to what science dreams to achieve in the near future. Activities involving the synthesis of nanoparticles is the present area of interest due to their unusual and fascinating properties, and applications that are advantageous over their bulk counterparts (Nasreen et. al 2014, Peto et al 2002) and unique catalytic, electronic, magnetic, chemical, photo electrochemical and opticalproperties (Kumar et al. 2003, Chandrasekharan et al. 2000 ).

Nanotechnology can be defined as a research for the design, synthesis and manipulation of structure of particles with dimension smaller than 100 nm. Nanotechnology, inextricably a multidisciplinary field, has an explosive growth in the past decade due to its extremely hefty applications in various fields of science and technology including physics, material science, chemistry, biology, and medicine etc. Nano-biotechnology or Green nanotechnology, a special branch of nanotechnology combines biological principles with chemical and physical procedures to generate nano-sized particles with specific function. Green nanotechnology have attracted tremendous interest because it reduce or eliminate toxic substances, require less energy, also has low manufacture costs of scalability, and better nanoparticle stabilization compared to chemically synthesized nanoparticles. Actually this process represents an economic alternative for chemical and physical methods of nanoparticles formation. In this case microorganisms have received maximum attention in the area of metal nanoparticles synthesis. This is probably due to the fact that microorganism cells have the ability to resist environmental stresses and have the capability of growing in presence of high metal concentrations (Correa-Llantén et al. 2013).They

are often exposed to extreme environmental, forcing them to resort to specific defense mechanisms to quell such stresses, including the toxicity of foreign metal ions or metals. These microorganisms play an important role in remediation or bio-mineralization of metals through reduction of metal ions. The toxicity of metal ions is reduced or eliminated by changing the redox state of the metal ions and/or precipitation of the metals intracellularly, thus, forming the basis of the synthesis of nanoparticles (Nangia et al. 2009). Biosynthesis of nanoparticles can be classified into intracellular and extracellular synthesis according to the location where nanoparticles are formed .Nanoparticles are biosynthesized when the microorganisms grab target ions from their environment and then turn the metal ions into the element metal through enzymes generated by the cell activities or the enzymes are extracted to carry out this process *in vitro*.

In recent years, nanoparticles are widely used in catalytic applications especially in case of degrading pollutants in wastewater. Among several water pollutants, nitro aromatic compounds are considered to be the most toxic. These are commonly used in the production of dyes, explosives and pesticides in industry. Being released into the environment, these compounds show serious carcinogenic and mutagenic threats to human health (Booth 2000). Although, several methods like ion exchange adsorption, filtration, chemical precipitation are available for eliminating pollutants, but these are not very effective. Therefore, there is always a strong need to eliminate these highly toxic pollutants easily and quickly using efficient catalytic agent. The nanoparticles can work as a better catalyst than bulk metal due to their greater accessibility to surface and low coordination number. Few previous report revealed that nanoparticles could catalyzed the degradation of 4-nitroaniline and 4-nitrophenol (Chirea et al. 2011; Srivastava et al. 2013; Reddy et al. 2013). Generally the biosynthesized nanoparticles show better catalytic efficiency than chemically synthesized. It should be noted that the catalytic behavior of nanoparticles significantly depends on the capping molecules (protein, nucleic acid, polysaccharides etc.) of biosynthesized nanomaterials (Das et al. 2012; Zhan et al. 2012).

In recent years noble metal nanoparticles such as gold, platinum and silver have received great attention in research field due to several applications for the sustenance of the societal development (Narayanan and Sakthivel 2010; Sperling et al. 2008). Among these noble metal nanoparticles (gold, silver, platinum) silver nanoparticles have attracted increasing attention for the wide range of applications. Additionally, owning to their broad spectrum antimicrobial ability, silver nanoparticles have also become the most widely used sterilizing nanomaterials in

consuming and medical products, for instance textiles, food storage bags, refrigerator surfaces and personal care products. Moreover Silver nanoparticles, generally smaller than 100 nm and contain 20 – 15,000 silver atoms, have distinct physical, chemical and biological properties compared to their bulk parent materials. The optical, thermal and catalytic properties of silver nanoparticles are strongly influenced by their size and shape.The optical properties may also change when particles aggregate and conduction electrons near each particle surface become delocalized.The absorption and scattering properties of silver nanoparticles can be changed by controlling the particle size, shape and refractive index near the particle surface. For e.g.: smaller nanoparticles mostly absorb light and have peaks near 400 nm, while larger nanoparticles exhibit increased scattering and have peaks that broaden and shift towards longer wavelengths.

In several studies, when nanomaterials like silver, gold, copper etc. were functionalized with different types of antibiotics, they became potent bactericidal agents with unique properties that subverted antibiotic resistance mechanisms of multiple-drug-resistant bacteria. Many pathogenic bacteria can form biofilm and become resistant towards antibiotics as normal antibacterial drugs cannot cross the barrier of biofilm. In this context antibiotic conjugated nanomaterials easily penetrate the barrier of biofilm. Labrenz et al. repoted that spherical aggregates of 2 to 5 nm diameter sphalerite (ZnS) particles were formed within the natural biofilms dominated by aerotolerantsulfate-reducing bacteria of the family Desulfobacteriaceae (Labrenz et al. 2000)

# BACKGROUND

Microbial synthesis of nanoparticles is a green chemistry approach that interconnects nanotechnology and microbial biotechnology. Biosynthesis of gold, silver, gold–silveralloy, selenium, tellurium, platinum, palladium, silica, titania, zirconia, quantum dots, magnetite and uraninite nanoparticles by bacteria, actinomycetes, fungi, yeasts and viruses have been reported. (Narayanan et. al 2010)The formation of extracellular and intracellular silver nanoparticles by bacteria (*Pseudomonasstulzeri*, *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Salmonells typlus*, and*Staphylococcus currens* has been investigated (Lengke et al. 2007).Biogenic Au and Ag NPs exhibit equivalent catalytic properties compared to commercially available (chemically grown) NPs. The dependence of the catalytic rate on parameters such as (i) the plant extract concentration used in NP synthesis, (ii) NP concentration, and(iii) the nature of metal (AuNPs vs AgNPs) in the catalytic conversion of 4-nitrophenol (4-

NP) to 4-aminophenol (4-AP) (Gangula et. al 2011). o and p-Nitroaniline are the nitro compounds which are readily soluble in waterand alcohol. They are mainly used for synthesis of phenylene diamine, which isused in the synthesis of many organic dyes. o and p-nitroaniline are quitetoxic to humans and aquatic organisms. Nitroaniline can harm the environment if released as a pollutant. Reduction of o and p-nitroaniline is, therefore, industrially and environmentally important. (Lee et. al 2015). Aminoglycosides are a group of broad spectrum antibiotics with bactericidal activity against the majority of grampositive and negative bacteria except obligate anaerobes.(Mazdeh et. al 2016)

Silver has been thought of as a promising agent for overcoming the resistance mechanism of antibacterial action on a range of targets as compared toa specific site of action in the case of antibiotics. Hence, nanoparticle-based antibacterial formulations could be effective bactericidal materials as they will exhibit combined effects of silver and antibacterial agents. The enhancedactivity of silver nanoparticles and antibiotics together has been reported. (Kora et. al 2013)

# SIGNIFICANCE

- Why biosynthesis?

There are a variety of chemical and physical preparation methods available for the fabrication of nanoparticles including radiation, chemical precipitation, photochemical methods, electrochemical and Langmuir- Blodgett techniques. But these methods are often extremely expensive and non-environment friendly due to the use of toxic, combustible and hazardous chemicals which may pose potential environmental and biological risk and high energy requirement. The drawbacks of low production rate, structural particle deformation and inhibition of particle growth are also encountered in this nanoparticle synthesis. Currently there is a growing need to develop sustainable preparation of nanoparticles that get rid of using harmful organic chemical substances since noble nanoparticles are widely applied to areas of human contact (Kumari et. al. 2015).

Through biological method extracts from biological agents such as microbes and plants can be employed either as reducing or protective agents for the fabrication of metal nanoparticles. In these extracts, various combination of biomolecules which have the reducing potential can be

found such as amino acids, vitamins, proteins, enzymes and polysaccharides that are environmentally benign yet chemically complex (Reddy et. al. 2013).

- Importance of catalysis

Among several water pollutants, nitro aromatic compounds are considered to be the most toxic. These are commonly used in the production of dyes, explosives, and pesticides in industry. Being released into the environment, these compounds show serious carcinogenic and mutagenic threats to human health (Booth 2000). Although, several methods like ion exchange adsorption, filtration, and chemical precipitation are available for eliminating pollutants, but these are not very effective. Therefore, there is always a strong need to eliminate these highly toxic pollutants easily and quickly using efficient catalytic agent. The nanoparticles can work as a better catalyst than bulk metal due to their greater accessibility to surface and low coordination number.

- Importance of AgNP and AgNP- antibiotic conjugate

The growing resistance of pathogenic bacterial strains to traditional antibacterial treatments has encouraged alternate strategies to control infections. During the past decade, a great potential in nanomedicine has been realized due to effectiveness of various nanoconjugates against pathogenic microbes. One approach to countering bacterial drug resistance is the application of metal composites, especially at nano scale, to control bacterial infections. Several enzymes and mutations in genetic sequences may impede multidrug resistance mechanisms by changing the medicine efflux from the cells, which thereby decreases the vulnerability of bacteria to antibacterial agents. Therefore, scientists are developing new ways to control resistant pathogens. Advancement in nanotechnology has prompted microbiologists to apply metal nanoparticles as an effective way to control certain pathogenic microbes involved in infectious diseases.Metal nanoparticles of silver, copper, and gold have been found to be active against certain pathogenic bacteria and fungi. Comparatively, AgNPs have been intensely studied owing to their distinct properties such as conductivity, chemical stability, catalytic activity, nonlinear optical behavior and bactericidal activity. These properties make them suitable for use as a microbial disinfectant in catheters and medical textiles. The production of AgNPs is relatively inexpensive, and the addition of these particles into goods such as plastics, clothing, creams, and soaps increase their

market value. New classes of compounds that include nanoparticle-antibiotic conjugates are undergoing clinical evaluation. The combination of antibiotics and metal nanoparticles could increase the antibiotics' efficacy against resistant pathogens (Brown et. al. 2013). Moreover, nanoparticle-antibiotic conjugates lower the amount of both agents in the dosage, which reduces noxiousness and increases antimicrobial properties. These conjugates were effective against resistant bacteria. Additionally, due to this conjugation, the concentrations of antibiotics were increased at the place of antibiotic-microbe contact and thus expedited the binding between microbes and antibiotics (Parandhaman et. al. 2018)

# OBJECTIVES

1. Synthesis of silver nanoparticles (AgNP) using intracellular protein extract of bacteria and conjugation of gentamicin, a common antibiotic with AgNP.
2. Optimization ofsynthesis parameters like, pH, concentration, time, temperature.
3. Characterization of AgNP and AgNP-Gentamicin conjugate using different techniques likeUV-Vis, TEM, XRD, DLS, and FTIR.
4. Investigation of catalysis efficiency of gold nanoparticles for bioremediation of nitro aromatic pollutants
5. Investigation of antimicrobial activity of AgNP and AgNP-Gentamicin conjugate against different bacterial strain.

# METHODS AND MATERIALS

## Preparation of Intracellular Protein Extract (IPE)

Overnight grown bacterial cell pellet was collected by centrifugation done at 9000 rpm for 10 minutes at 4°C and washed with 0.1(M) sodium phosphate buffer. Then cell pellet was resuspended in the same buffer (pH-7). The cells were then disrupted using ultrasonic processor (Hielscher-UP200S) for 10 minutes with 1 minute time intervals under 0.7 sonication cycle and 80% amplitude. Suspension was then again centrifuged and supernatant was collected to use for the synthesis of silver nanoparticles.

## Optimization of reaction parameters for synthesis of AgNPs

IPE was mixed with aqueous solution of $AgNO_3$ in different sets of pH ranging from 2.0 to 13.0 to optimize the pH condition. To estimate the optimum concentration of $AgNO_3$, IPE was mixed with the $AgNO_3$ solutions at different concentrations (1.0, 1.5, 2.0, 2.5, 3.0 mM) and maintained in the above mentioned conditions. To attain the optimized incubation time, IPE and $AgNO_3$ were mixed at their respective optimal pH and salt concentration for various time periods (18, 42, 62, 86, 112 hours). Now, finally, the temperature optimization was carried out by performing reaction between IPE and $AgNO_3$ aqueous solution in 25° Celsius and 37° Celsius, at their respective pH, salt concentration and incubation time. Absorbance spectra were recorded from each sample to find out the optimum reaction parameter.

## Synthesis of AgNPs

AgNPs were synthesized by mixing 1:1 (v/v) of IPE and aqueous solution of $AgNO_3$ in a conical flask, pH was adjusted to optimal condition,i.e. upto 12.24 by drop by drop addition of NaOH solution and measured with pH-meter. The solution was kept in shaking condition for overnight at 138rpm at 37°C.

## Synthesis of AgNP-Conjugate

AgNP-conjugate solutions were prepared based on AgNP interaction with antibiotics, specifically gentamicin. Gentamicin (Mol. Wt.: 694 - 723) stock solution of 5mg/ml(6.91mM) was prepared. From this a 1ml working solution of same strength was made. AgNPs were synthesized by mixing 5 ml. of IPE and 5 ml. aqueous solution of $AgNO_3$ in a 100 ml. conical flask, pH was adjusted upto 12.24 and the prepared gentamicin working solution was added dropwise. The solution was kept in shaking condition for overnight at 138 rpm at 37°C. Absorbance spectra was recorded later.

## Purification of AgNP and AgNP-Gentamicin

To remove excess salt and other byproducts along with maximal recovery of AgNPs, the solution was centrifuged at 13,900 rpm for 20 minutes at 28°C(Sigma-3AK30). The pellet of the AgNP and the AgNP-Conjugate was washed with double distilled water, then centrifuged again and

then washed once again. The pellets were re-suspended in double distilled water and they were stored respectively.

## Characterization of AgNPs

*UV-Vis Spectroscopy*

The formation of AgNPs and AgNP- Gentamicin was observed in the double beam UV-vis spectrophotometer (Hitachi_U2900) by recording the spectrum within the range of 200-700 nm with a resolution of 1 nm.

*Transmission electron microscopic (TEM), EDAX, and SAEDStudy*

AgNPs were pipetted onto carbon-coated copper grid and allowed to air dry for overnight in dark at room temperature to evaporate the excess solvent. Sample was analyzed in transmission electron microscope (JEOL-JEM 2100, Japan) operated at 200 kV. Selected area electron diffraction (SAED)patterns were taken using the same instrument. Elemental analyses of synthesized AgNPs were conducted by using energy dispersive X-ray analysis which was carried out using transmission electron microscope equipped with an energy dispersive X-ray spectrometer (Oxford-INCA).

*Dynamic light scattering (DLS) measurement*

Hydrodynamic diameter (dh) and zeta potential (ξ) of AgNPs was measured by dynamic light scattering using the Zetasizermodel NANO ZS90 (Malvern Instruments Ltd.,Worcestershire, UK) (5 mW HeNe laser $\lambda = 632$ nm).

*Fourier transformed infrared (FTIR) spectroscopic Measurement*

The FTIR measurement was carried out to identify possible functional group involved in intracellular protein extract (IPE)which may be responsible for the synthesis of AgNPs. The AgNPs solution was lyophilized to get solid powder and was mixed with calcium fluoride (CaF) pellet. The spectrums were recorded for the IPE in liquid state and AgNPs in solid state at FTIR

spectrophotometer (Spectrum 100, Perkin Elmer) in the range of 400–4000 cm−1 at a resolution of 4.0 cm−1.

## Antimicrobial activity of AgNPs and AgNP-Conjugates

The antimicrobial activity of the biogenic AgNPs was tested using Agar well-diffusion method. The experiment was tested against reference gram-negative (*Pseudomonas aeruginosa* ATCC 27853, *Vibrio cholarae*MTCC 3906) and gram-positive bacteria (*Bacillus subtilis*ATCC 6633) procured from Microbial Type Culture Collection (MTCC), Chandigarh, India. An inoculum of 100 µl was taken from stock and it was diluted in 1 ml luria broth, from which 100 µl was taken and again diluted in another 1 ml Luria broth. Mueller-Hinton Agar (MHA,HiMedia,India) was then poured into petri dishes and allowed to solidify. 100 µl of solution was taken from the second dilution and spread onto the MHA plates. 5 mm wells were cut. 120 µl of AgNP, Gentamicin and AgNP-Gentamicin each were poured into the wells and incubated at 37°C for 24h. Diameters of cleared zones of inhibition formed around the wells were measured.

## Catalytic degradation of nitro aromatic pollutants using AgNPs

To test the efficacy of as-synthesized AgNPs for their catalytic activity, the degradation of four different nitro aromatic compoundsviz. 2-NA, 4-NA, 2-NP, and 4-NP in the presence ofNaBH4 was studied as a model reaction. (Shi et al. 2015; Gangula et al. 2011; Guria et al.2016) with necessary modifications. The concentrations of the AgNPs were fixed by the intensity of absorbance to 0.3 a.u. In this reaction, ultrapure water(1.8ml), aqueous solution of 2-NA (0.119ml, 0.01 M), and freshly prepared ice-cold solution of NaBH4 (1 ml, 0.05 M)were mixed homogeneously. Then 0.35 ml ultrasonically dispersed AgNPs solution was added to the reaction mixtures. The catalytic process was investigated by monitoring the alteration of absorbance spectra in a time-dependent manner of 1-mininterval in the scanning range of 200 to 800 nm by using a UV-Vis spectrophotometer (Hitachi-U2900).

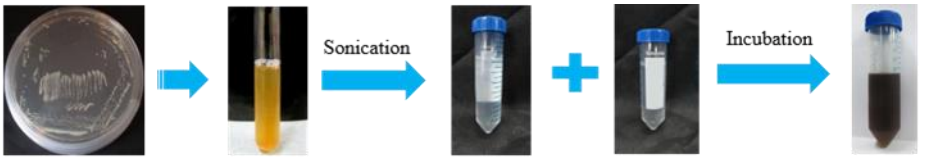
The same procedure was followed for the degradation of other nitro aromatic compounds with required changes in their concentration depending on their molar absorptivity. We also examined the recyclability of catalytic role of as-synthesized AgNPs up to the third cycle in case of all degradation reactions of four nitro aromatic compounds. After every cycle, AgNPs are recovered from reaction solution, centrifuged and the pellet was washed three times with

ultrapurewater and resuspended in 0.3 ml water to keep the concentration and volume of catalyst constant to be reused in the next cycle of reaction. The same process was repeated up to three cycles. The control reaction was also performed without adding AgNPs in the reaction solution. In all these catalysis reactions, the volume and concentration of catalyst as well as NaBH4 solution were also kept constant. To find out the actual concentration of nitro aromatic compound at each point of maximum absorbance, calibration curves of all the compounds were prepared using standard solutions.

# RESULT AND DISCUSSION

## Biosynthesis of AgNPs and optimization of regulating Parameters

The qualitative analysis AgNPs formation using IPE of bacterialstrain *Staphylococcus warneri* SuMS_N03 was carried out based on visual observation of color change from colorless to deep brown.




The quantitative analysis of AgNPs formation was performed using UV-Vis spectroscopy. The intense surface plasmon resonance (SPR) peak at 424 nm indicated the formation of AgNPs. The color change is due to excitation of surface plasmon vibration, i.e., collective oscillation of free electrons induced by an interacting electromagnetic field in metallic nanoparticles which is the characteristic feature of metal nanoparticles (Karthick et al. 2014).

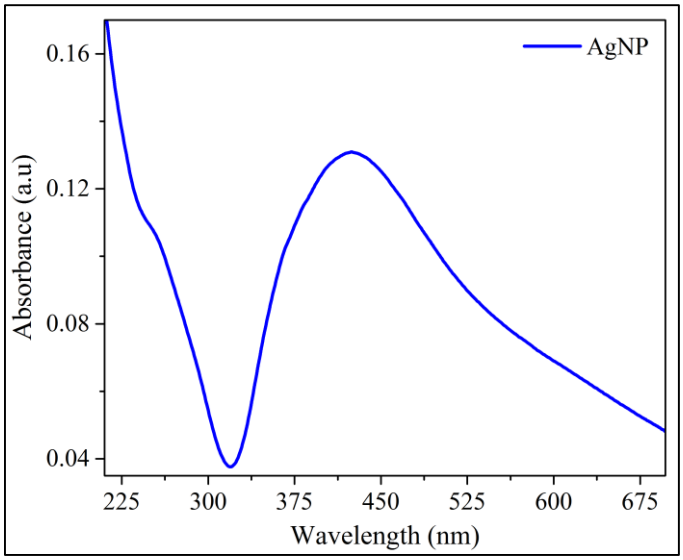



Fig.1:UV-Vis absorption spectra of AgNPs at 424.0 nm due to surface plasmon resonance

Among different salt concentrations (1.0–3.0 mM), 2.0 mM was found to be the most optimize one to get highest concentration of AgNPs with stable and monodisperse particles. During synthesis, significant colour change was observed at alkaline pH (12), out of various pH range, no change was observed in acidic pH.The AgNPs were formed within 18 h incubation time and intensity of SPR peak increased significantly with time up to 112 h with a noticeable peak shift and finally reached a saturation. Experiments were carried out at 25°C and 37°C, between which well-dispersed and most stable particles were formed at 37°C.

This feature indicates the bio-reduction of Ag+ ion to form silver nanoparticles as Ag0 which was increased with time to certain extent. Even after 1 month of synthesis, no significant change was observed in position or intensity of SPR peak confirming the high stability of as-synthesized AgNPs.

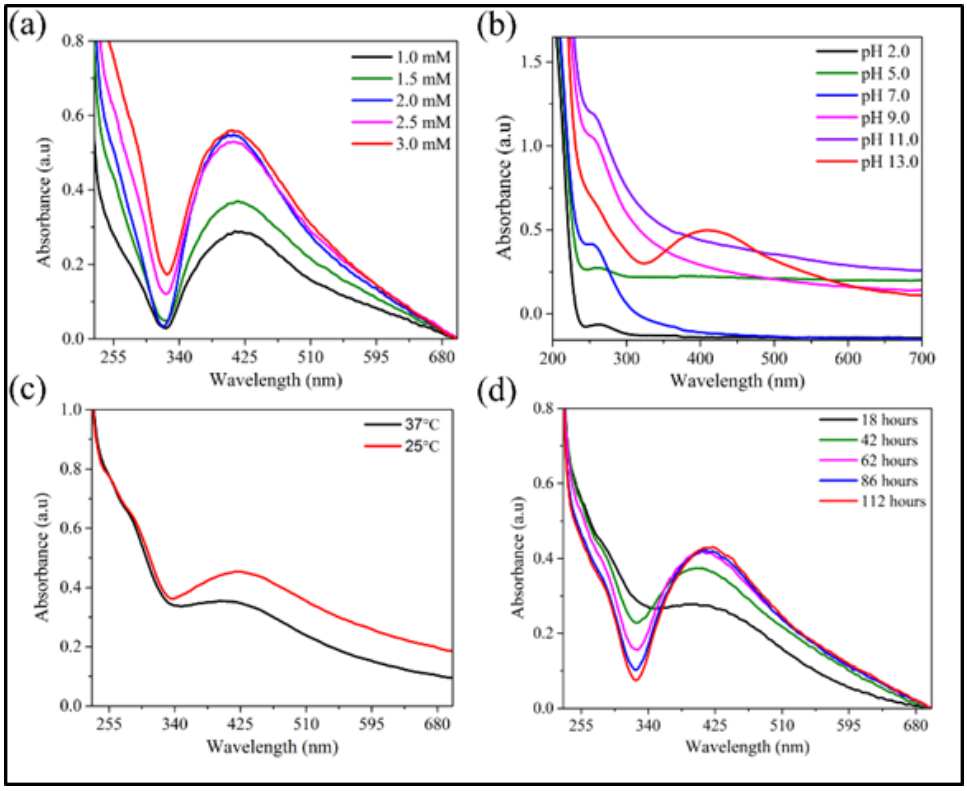



Fig.2: UV-Vis spectrum pf silver nanoparticles under different condition (a) optimization of salt concentration, (b) optimization of pH of reaction, (c) effect of temperature on synthesis and (d) optimization of incubation time

## TEM, SAED, and EDAX analysis

The size, shape, and morphology of the AgNPs were represented at different magnification in The TEM images which indicated that most of the nanoparticles formed at optimized conditions were well dispersed and mainly spherical shaped. These images also revealed that most of the particles were frequently distributed within 2 to 6 nm, although larger particles having about 8-10 nm diameter was also found. The selected area electron diffraction (SAED)pattern of as-synthesized AgNPs indicated that the nanoparticles were pure crystalline in nature and the lattice structure is face-centered cubic (FCC). In energy dispersive X-ray spectroscopic (EDAX) pattern, the strong peak at 3.0 KeV confirmed the presence of silver atom and the peaks for C and Cu were due to background signals from carbon-coated copper grid.

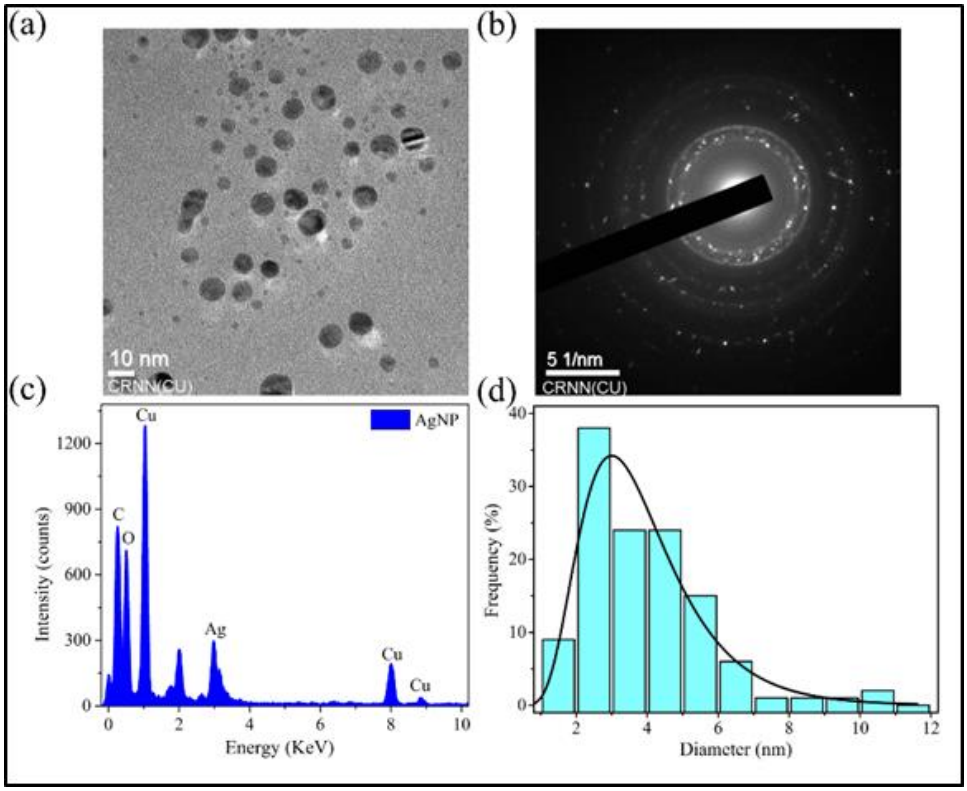



Fig.3: (a) TEM images of silver nanoparticles (Scale bar 10 nm), (b) Specific Area Electron Diffraction (SAED) pattern of silver nanoparticles. (c) Energy dispersive X-ray (EDX) spectrum of silver nanoparticles. (d) Particle size distribution histogram determined from the TEM image

## DLS analysis

DLS parameter represents the average particle size (Z average)in terms of hydrodynamic diameters. The average size of AgNPs was estimated to be 64.86 nm and AgNP-Gentamicin was 242.3 nm.DLS analysis also revealed that the polydispersity index of this AgNPs was 0.195.

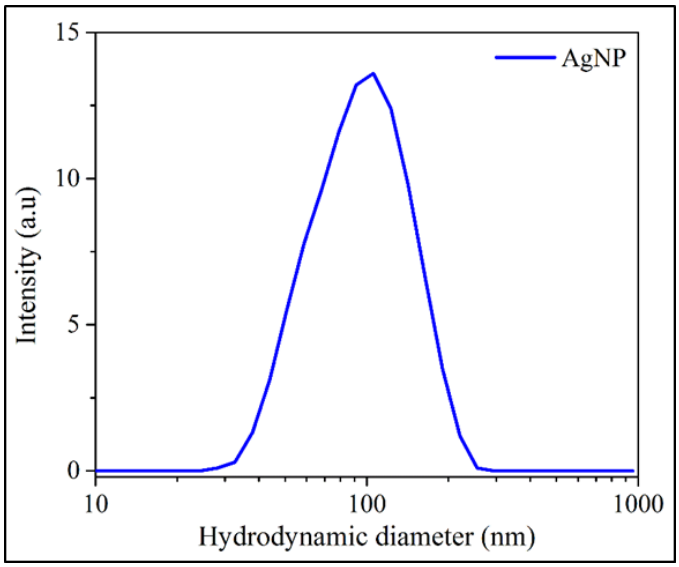



Fig.4: DLS profile of silver nanoparticles with average hydrodynamic diameter 64.86 nm

## FTIR spectroscopic analysis

The peak at 3246 $cm^{-1}$ indicates the role of alcoholic/ carboxylic –OH group for the formation of nanoparticles and also capped around the surface of AgNP. The single peak in dry sample at 1630 $cm^{-1}$ for >C=O stretching vibration of amide linkage. The peak at 1384 $cm^{-1}$ confirms the contribution of carboxylate ion (−COO−) in the synthesis and present towards outside of metal nanocore. The peak at 1015 $cm^{-1}$ originates for C-C/C-O vibration.

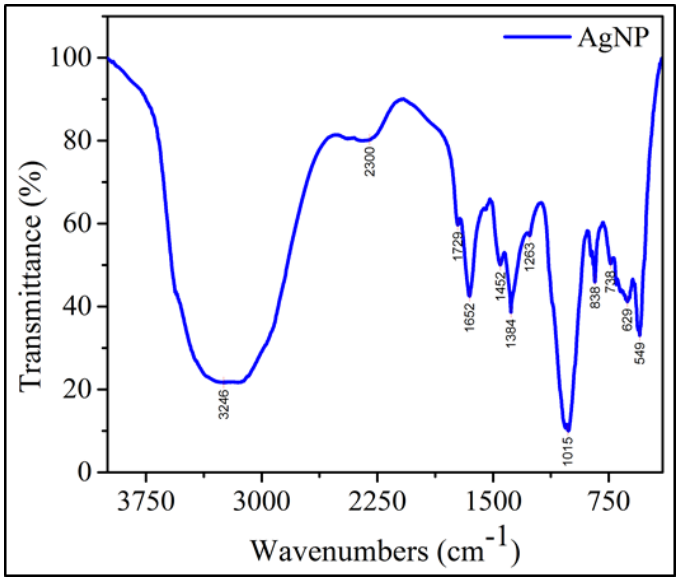



Fig.5: FT-IR spectra of AgNP

## Synthesis and characterization of AgNP-Gentamicin

Color change from brown to light greenish brown indicate AgNP-Gentamicin conjugate formation. The increase of hydrodynamic diameter from 64.86 to 242.3 nm confirm the attachment of Gentamicin with AgNP.

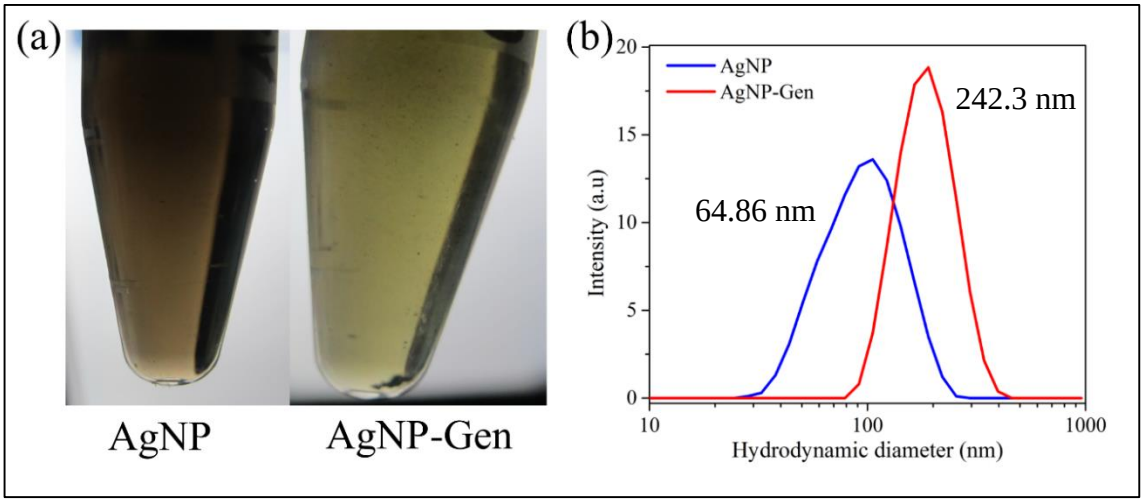



Fig.6: (a) Color images of silver nanoparticles and silver nanoparticles-gentamycin nano hybrid, (b) DLS profile of silver nanoparticles ($Z_{av}$ -64.86 nm) and silver nanoparticles-gentamycin nano hybrid ($Z_{av}$ -242.3 nm)

## Catalytic activity of silver nanoparticles: application for the removal of toxic compounds

The aqueous solution of 2-NA and 4-NA exhibited vivid yellow color with absorption maxima at about 380 and 412 nm, respectively. Addition of $NaBH_4$ into the aqueous solution of2-NA and 4-NA did not lead to the reduction reaction. But when AgNPs were added to the mixture of 2-NA/4-NA andNaBH4, reduction reaction started immediately. As a result, the solution containing 2-NA turned colorless and the intensity of the characteristic peak at 412 nm decreased progressively with simultaneous appearance of a new peak near225 nm which is associated with ortho-phenylenediamine(o-PDA) (Anantharaj et al. 2016). The reduction of 2-NA too-PDA was completed within 8 min in the first cycle, 11 min in the second cycle, and 18 min in the third cycle.

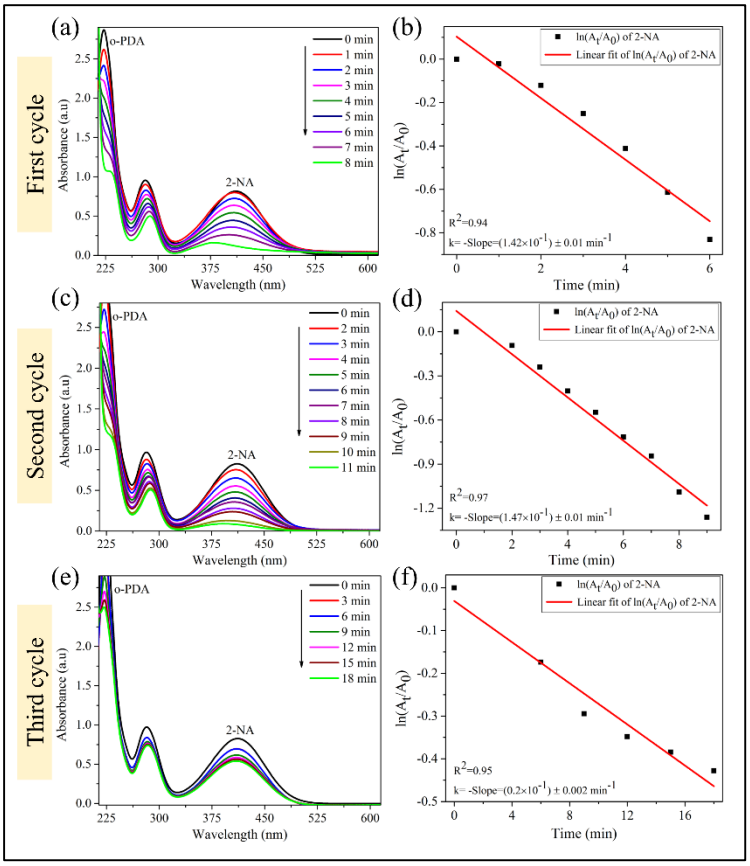



Fig.7: (a, c, e) time dependent UV-Vis absorption spectra and (b, d, f) first order kinetics plot for the catalytic reduction of 2-Nitroaniline by $NaBH_4$ in presence of silver nanoparticles for first, second and third cycle respectively.

On reduction in the presence of AgNPs, the solution of4-NA turned colorless and the intensity of absorption peak at 380 nm showed progressive decrease with appearance of new absorption peaks at 305 and 240 nm due to the formation of para-phenylenediamine (p-PDA) (Reddy et al. 2013). The reduction reaction was completed within5 min in the first cycle, 8 min in the second cycle, and 14 min in the third cycle.

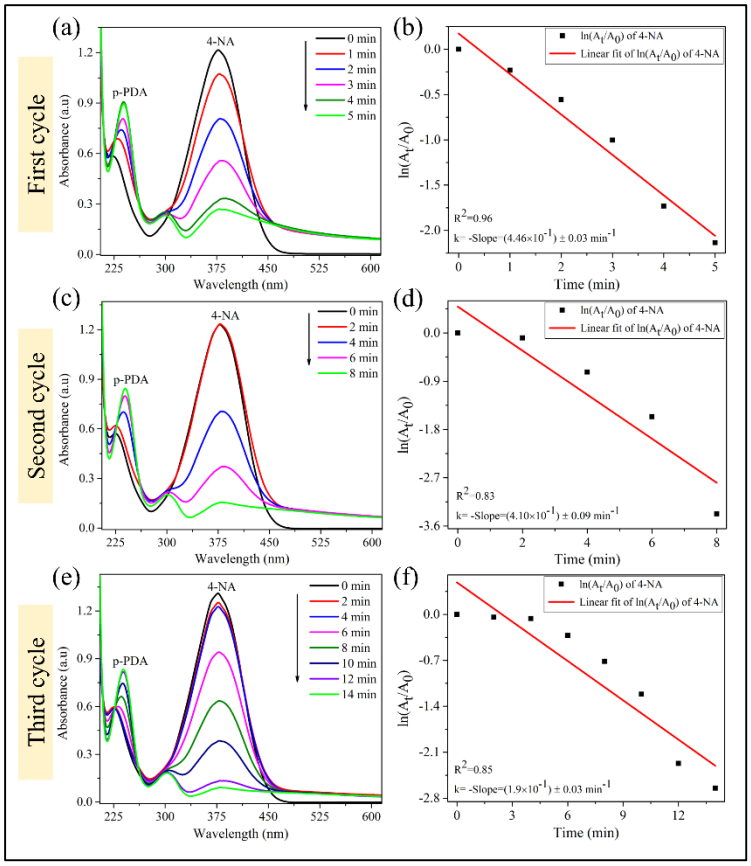



Fig.8: (a, c, e) time dependent UV-Vis absorption spectra and (b, d, f) first order kinetics plot for the catalytic reduction of 4-Nitroaniline by $NaBH_4$ in presence of silver nanoparticles for first, second and third cycle respectively.

Aqueous solution of 2-NP was pale yellow in color and showed two distinct absorption peaks at 278 and 351 nm.When NaBH4 (pH > 12) was added to the aqueous solution of 2-NP, an intense yellow color appeared due to formation of2-nitrophenolate ion in alkaline medium with a red-shift of the absorption peaks to 281 and 415 nm, respectively (Liu andYang 2011), but no reduction was observed. Thus, the progress of the reaction was monitored by tracking the changes in the intensity of absorption peak of 2-nitrophenolate ion at415 nm. Introduction of AgNPs into the reaction solution led to the disappearance of the yellow color of the solutionprogressively with decrease in the intensity of the absorption peak at 415 nm and appearance of a new peak at229 nm, indicating the formation of the reduction product2-aminophenol (2-AP). 2-NP was reduced completely within 8 min in the first cycle, 12 min in the second cycle,and 20 min in the third cycle.

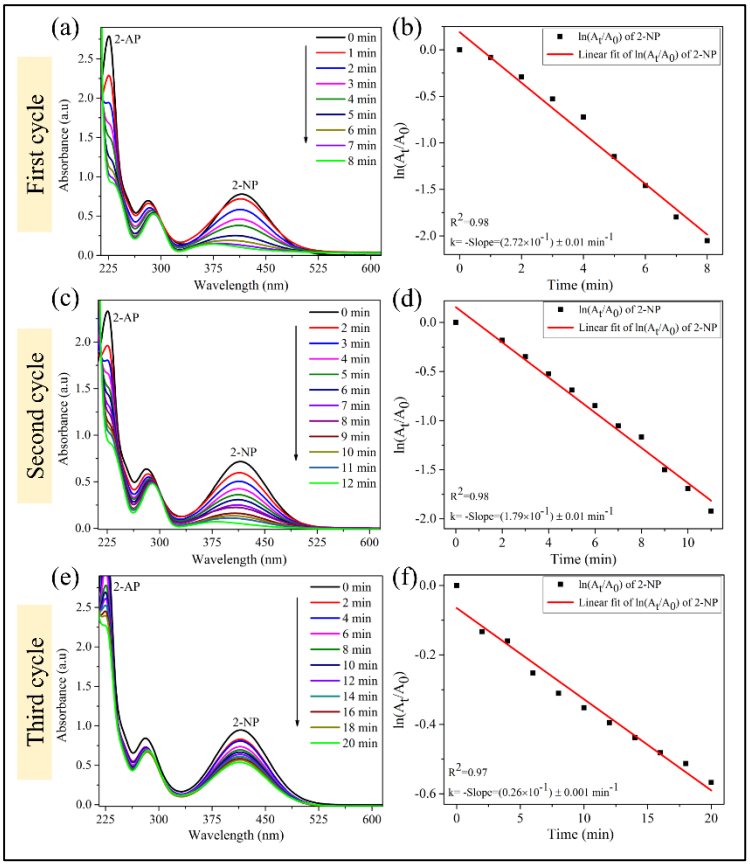



Fig.9: (a, c, e) time dependent UV-Vis absorption spectra and (b, d, f) first order kinetics plot for the catalytic reduction of 2-Nitrophenol by $NaBH_4$ in presence of silver nanoparticles for first, second and third cycle respectively.

On addition of NaBH4 to the aqueous solution of 4-NP, the solution turned into intense yellow color from pale yellow color and the absorption maximum peak at 317 nm was red shifted to 400 nm due to formation of 4-nitrophenolate ion in alkaline medium, although no reduction occurred. Here, the progress of the reduction reaction was monitored by tracking the changes in the intensity of absorption peak at 400 nm. The reduction process started immediately with addition of AgNPs into the solution. The fading of yellow color of the solution and progressive decrease of intensity of absorption peak at400 nm with simultaneous appearance of new peak at298 nm indicated the reduction of 4-NP with formation of 4-aminophenol (4-AP) (Gangula et al. 2011). This reaction was completed within 8 min in the first cycle, 19 min in the second cycle, and 25 min in the third cycle.

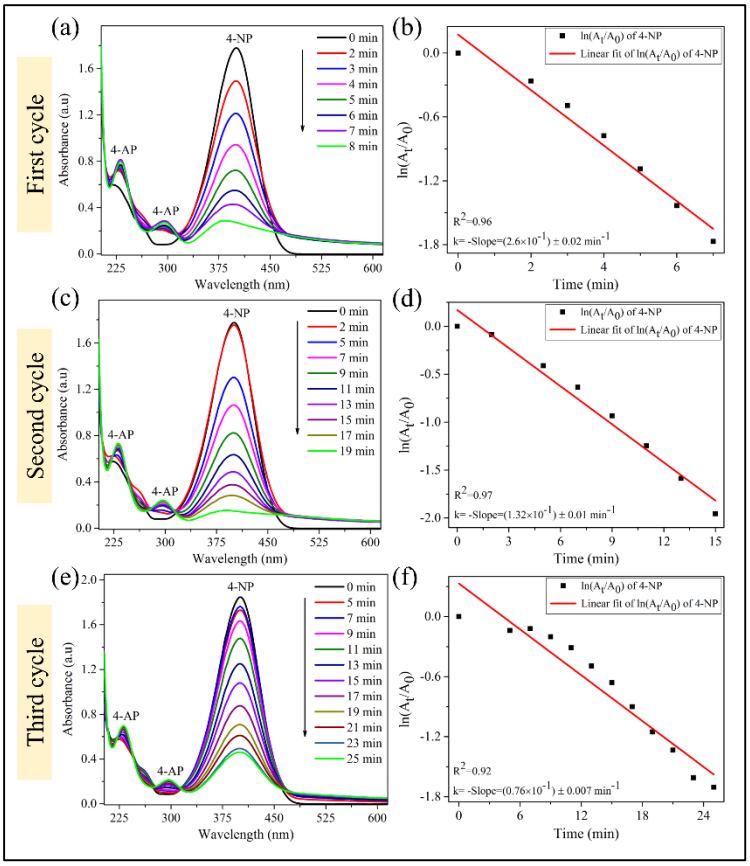



Fig.10: (a, c, e) time dependent UV-Vis absorption spectra and (b, d, f) first order kinetics plot for the catalytic reduction of 4-Nitrophenol by $NaBH_4$ in presence of silver nanoparticles for first, second and third cycle respectively.

The rate of reduction should depend on the concentrations of NaBH4 and AgNPs. As the concentration of NaBH4 used in reactions was much higher than that of nitro aromatic compounds and AgNPs, so it is expected that the concentration ofNaBH4 remains constant throughout the reaction. So the rates of these reactions may be considered to be independent of the concentration of NaBH4 and thus the reactions were considered as pseudo first-order reaction with respect to the concentration of corresponding nitro aromatic compound. However, it should be noted that here the rate of the reaction was not influenced by the concentration of nanoparticles due to use of relatively low and fixed concentration of nanoparticles throughout the reaction system. We found a good linear correlation of ln(At/A0) with time (t) where the logarithm of absorption decreased linearly with reaction time indicating pseudo first-order kinetics, where At is the absorption of nitro aromatic compound at any time t and A0 is the

absorption of nitro aromatic compound at time 0. The rate constant (k) of reaction was calculated from the slope of the linear sections of the plots and has been provided in details in Table 1. Apart from time, order and rate of the reaction, some important quantitative parameters like conversion, selectivity, yield, turnover number (TON), and turnover frequency (TOF)were also derived using calibration curve according to the previous literatures (Anantharaj et al. 2016; Kim et al. 2012; Fan and Huang 2014) and the results are shown in Table 1.

Table 1: Relevant parameters of catalytic degradation reaction of all nitro aromatic compounds by AgNPs

| Reactant | Number of cycles | Time (min) | Conversion (%) | Selectivity (%) | Yield (%) | TON ($\times 10^3$) | TOF ($\times 10^3$) ($h^{-1}$) | Rate constant (k) ($\times 10^{-1}$)($min^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 2-NA | 1 | 8 | 90.88 | 100 | 90.88 | 1.149 | 6.510 | 1.42 ± 0.01 |
| | 2 | 11 | 86.18 | 100 | 86.18 | 1.076 | 6.325 | 1.47 ± 0.01 |
| | 3 | 18 | 35.03 | 100 | 35.03 | 0.444 | 2.168 | 0.2 ± 0.002 |
| 4-NA | 1 | 5 | 97.16 | 100 | 97.16 | 0.925 | 10.00 | 4.46 ± 0.03 |
| | 2 | 8 | 91.55 | 100 | 91.55 | 0.825 | 6.043 | 4.10 ± 0.09 |
| | 3 | 14 | 81.58 | 100 | 81.58 | 0.722 | 3.822 | 1.9 ± 0.03 |
| 2-NP | 1 | 8 | 93.56 | 100 | 93.56 | 0.957 | 8.436 | 2.72 ± 0.01 |
| | 2 | 12 | 87.74 | 100 | 87.74 | 0.939 | 5.780 | 1.79 ± 0.01 |
| | 3 | 20 | 43.53 | 100 | 43.53 | 0.578 | 2.371 | 0.26 ± 0.001 |
| 4-NP | 1 | 8 | 92.07 | 100 | 92.07 | 0.485 | 4.265 | 2.6 ± 0.02 |
| | 2 | 19 | 91.49 | 100 | 91.49 | 0.481 | 2.136 | 1.32 ± 0.01 |
| | 3 | 25 | 82.13 | 100 | 82.13 | 0.449 | 0.986 | 0.76 ± 0.007 |

## Antimicrobial Assay of AgNPs and AgNP-Gentamicin

The antibacterial activity of the biogenic AgNPs conjugated with gentamicin was tested against *Pseudomonas aeruginosa*, *Vibrio cholarae* and *Bacillus subtilis* by agar well diffusion method. In this study, the highest antimicrobial activity of AgNPs observed against *Vibrio cholarae* was 10.70 mm, whereas biogenic AgNPs conjugated with gentamicin showed 12.51 mm. However, antimicrobial activity of AgNPs observed against *Pseudomonas aeruginosa* 7.97 mm, whereas biogenic AgNP-gentamicin showed 12.24 mm. Antimicrobial activity of AgNPs against *Bacillus subtilis* was 9.67 mm whereas biogenic AgNPs conjugated with gentamicin

12.97 mm. AgNP-conjugates showed overall better results against tested pathogens than AgNP and gentamicin alone.

Gentamicin is a broad spectrum aminoglycoside antibiotic produced by fermentation of *Micromonospora purpurea.* Gentamicin is an antibiotic complex consisting of four major (C1, C1a, C2 and C2a) and several minor components. This agent irreversibly binds to the bacterial 30s ribosomal subunit. Specifically, this antibiotic is lodged between 16s rRNA and S12 protein within the 30s subunit. This leads to interference with translational initiation complex, misreading of mRNA, thereby hampering protein synthesis and resulting in bactericidal effect.

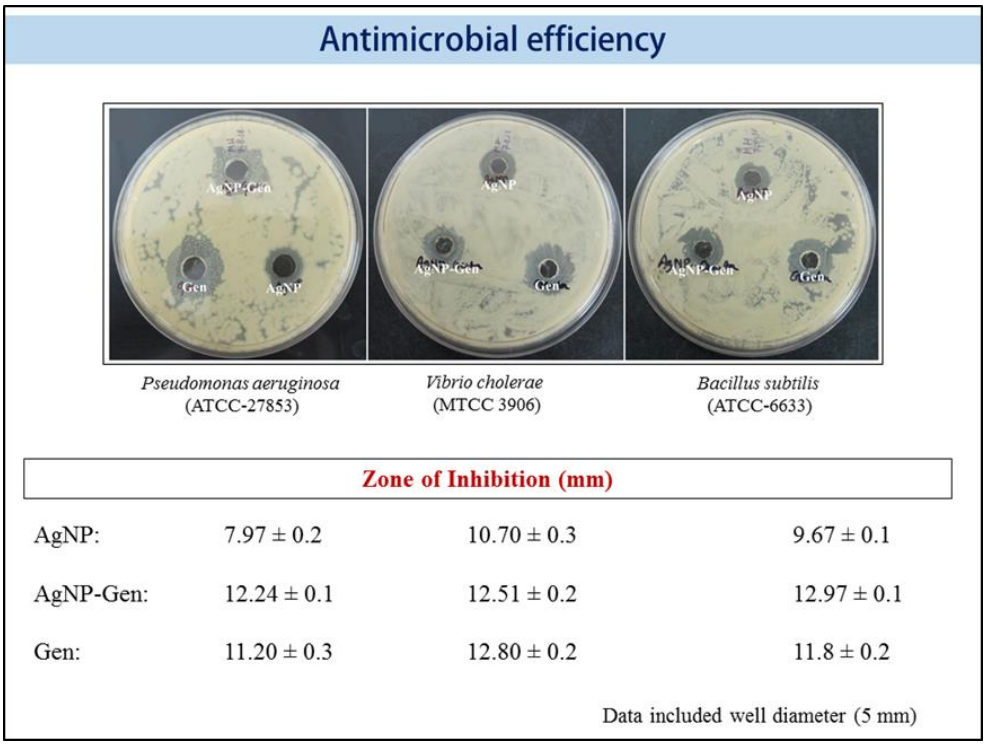



Fig.11: Antimicrobial activity of AgNP and AgNP-Gentamicin against different gram positive and gram negative bacteria pathogens

# CONCLUSION

A simple and effective eco-friendly process alternative to common physical and chemical method for the production of well-dispersed nanoparticles was developed using intracellular protein extract (IPE) of bacterial strain, *Staphylococcus warneri* SuMS_N03. AgNPs were

synthesized at room temperature and pH 13.Most of the nanoparticles were obtained in spherical shape with the size range of 2–6 nm. FTIR analysis inferred that intracellular protein moieties with functional groups including hydroxyl, carboxyl, and amine were probably responsible both for the reduction of silver ions and stabilization of the particles. Finally, we provided two effective approaches. First the utilization of as-synthesized AgNPs as recyclable nanocatalyst for the degradation of different toxic nitro aromatic pollutants. The high rate constant values (10−1 order), percentage of conversion (> 80%), and high turnover frequency ($10^3$ $h^{-1}$ order) ensured that as-synthesized AgNPs exhibited significant catalytic efficiency even up to the third cycle. Second is the utilization of AgNP and AgNP-Gentamicin conjugate as efficient antimicrobial agent against different gram positive and gram negative bacterial strains. This investigation is very useful against different biofilm forming microorganism and multi drug resistant pathogenic bacteria.

# REFERENCE

A. Krolikowska, A. Kudelski, A. Michota, J. Bukowska, Surf. Sci. 532 (2003) 227

A. Kumar, S. Mandal, P.R. Selvakannan, R. Parischa, A.B. Mandale, M. Sastry,Langmuir 19 (2003) 6277

Abilash Gangula,† Ramakrishna Podila,‡ Ramakrishna M,2013† Catalytic Reduction of 4-Nitrophenol using Biogenic Gold and Silver Nanoparticles Derived from Breynia rhamnoideshttps://doi.org/10.1021/la2034559

Amiya Kumar Prusty,Preparation Of Silver Nanoparticle By Microorganism And Its Application In Pharmacyassisted by Sapindus mukorossi Gaertn. Fruit pericarp and their catalytic application for the reduction of p-nitroanilinehttp://dx.doi.org/10.21474/IJAR01/2266

Awwad A, Salem N, Abdeen A (2013) Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity. Int J IndChem 4(1):1-6https://doi.org/10.1186/2228-5547-4-29

Binupriya AR, Sathishkumar M, Vijayaraghavan K, Yun S-I (2010)Bioreduction of trivalent aurum to nano-crystalline gold particlesby active and inactive cells and cell-free extract of Aspergillusby microbes.https://doi.org/10.1016/j.jhazmat.2009.12.066

Chakraborty A, Bera A, Mukherjee A et al (2015) Changing bacterial profile of Sundarbans, the world heritage mangrove: impact of anthropogenic interventionshttps://doi.org/10.1007/s11274-015-1814-5

Edison et. al. (2015) NaBH4 reduction of ortho and para-nitroaniline catalyzed by silver nanoparticles synthesized using Tamarindus indica seed coat extractfoams with high catalytic activity for 2-nitrophenol reduction and SERS propertyhttps://doi.org/10.1021/cs500379u

G. Peto, G.L. Molnar, Z. Paszti, O. Geszti, A. Beck, L. Guczi, Mater. Sci. Eng. 19(2002) 95

Gangula A, Podila R, Rao M et al (2011) Catalytic reduction of 4- nitrophenol using biogenic gold and silver nanoparticles derived from Breynia rhamnoides.

Guria MK, Majumdar M, Bhattacharyya M (2016) Green synthesis of protein capped nano-gold particle: an excellent recyclable nanocatalyst for the reduction of nitro-aromatic pollutants at higher concentration.https://doi.org/10.11113/jt.v81.11409

Iravani et. al.(2014)Bacteria in Nanoparticle Synthesis: Current Status and Future Prospectshttp://dx.doi.org/10.1155/2014/359316

J.H.P. Watson, D.C. Ellwood, A.K. Soper, J. Charnock, J. Magn. Magn. Mater. 203(1999) 69.

Jiang H, Dong H, Zhang G et al (2006) Microbial diversity in water andsediment of Lake Chaka, an Athalassohaline Lake in northwesternChinahttps://doi.org/10.1128/AEM.02869-05

Liu H, Yang Q (2011) Facile fabrication of nanoporous Au–Pd bimetallic
Meena Kumari M, Jacob J, Philip D (2015) Green synthesis and applications of Au–Ag bimetallic nanoparticles doi: 10.1016/j.saa.2014.08.079

Moghaddam KM (2010) An Introduction to microbial metal nanoparticle preparation method. J Young Invest 19(19):1-6

N. Chandrasekharan, P.V. Kamat, J. Phys. Chem. B 104 (2000) 10851.

Narayanan KB, Sakthivel N (2010) Biological synthesis of metal nanoparticles https://doi.org/10.1016/j.cis.2010.02.001

Nasreen I. Hulkoti, T.C. Taranath 475(2014)13 Biosynthesis of nanoparticles using microbes— a review https://doi.org/10.1016/j.colsurfb.2014.05.027

Synthesis of Gold Nanoparticles Using Leaf Extract of Ziziphus zizyphus and their Antimicrobial ActivityAlaa A. A. Aljabali, Yazan Akkamand David J. Evanshttps://dx.doi.org/10.3390%2Fnano8030174

Sharma S (2015) Metal dependent catalytic hydrogenation of nitroarenes over water-soluble glutathione cappedmetal nanoparticles.doi: 10.1016/j.jcis

Tokeer Ahmada,∗, Irshad A. Wania, Nikhat Manzoorb,1(2013) Biosynthesis, structural characterization and antimicrobial activity of gold and silver nanoparticles https://dx.doi.org/10.4155%2Ffsoa-2017-0062

Xiangqian Li,1, 2 Huizhong Xu,3 Zhe-Sheng Chen,2 (2011)Biosynthesis of Nanoparticles by Microorganisms and Their Applicationshttp://dx.doi.org/10.1155/2011/270974

Rastogi et.al. (2013) NaBH4 reduction of ortho and para-nitroaniline catalyzed by silver nanoparticles synthesized using Tamarindus indica seed coat extractDOI :10.1007/s11164-015-2051-0

Ashley N. Brown,a Kathryn Smith,a Tova A. Samuels,b Jiangrui Lu,b Sherine O. Obare,b and Maria E. Scotta (2012) ,Nanoparticles Functionalized with Ampicillin Destroy Multiple-Antibiotic-Resistant Isolates of *Pseudomonas aeruginosa* and*Enterobacter aerogenes* and Methicillin-Resistant *Staphylococcus aureus*
*DOI: 10.1128/AEM.06513-11*

Parandhaman et. al. (2018)Facile synthesis, biofilm disruption properties and biocompatibility study of a poly-cationic peptide functionalized graphene–silver nanocompositeDOI:10.1039/C8BM01003J