Biomediated synthesis of silver nanoparticles using *Exiguobacterium mexicanum* PR 10.6

Aparna J. Padman, Janey Henderson, Simon Hodgson and Pattanathu K.S.M. Rahman*

School of Science and Engineering, Teesside University, Middlesbrough, TS13BA, UK.

*Author for Correspondence (Fax: 0044-1642-384669; Email: p.rahman@tees.ac.uk)

Abstract: The study reports the biomediated silver nanoparticle synthesis using the cell free extract of a soil bacterium, *Exiguobacterium mexicanum* PR 10.6. The silver nanoparticle samples were characterised using UV-Visible spectroscopy, Energy Dispersive Spectroscopy (EDS), Fourier Transform Infrared Spectroscopy (FTIR), and Transmission Electron Microscopy (TEM). The results show that silver nanoparticle of size range 5-40 nm could be synthesised using this method. The extracellular polymeric substance (EPS) plays the critical role in the silver ion reduction and nanoparticle stabilisation, when using the cell free extract. The results suggest that the biomediated synthesis using *Exiguobacterium mexicanum* PR 10.6 could be an effective eco-friendly rapid method for silver nanoparticle synthesis in an hour.

Key words: Biomediated synthesis, *Exiguobacterium mexicanum,* Silver nanoparticles

Introduction

Nanomaterials, which are defined as materials with at least one dimension roughly between 1 and 100 nm. The characteristic features of nanoparticles such as their high volume/surface ratio, surface tailorability, improved solubility and multifunctionality open many new possibilities for biomedicine (Gao and Xu 2009). The optical, electronic and electrical

properties of nanoparticles are size dependent and various novel methods for the size controlled synthesis of silver nanoparticles are being developed (Li et al. 2006). The high energy requirement in physical methods of nanoparticle synthesis and the waste disposal problems in the chemical synthesis due to the heavy use of organic solvents, toxic reducing agents and capping agents are major demerits of the conventional nanoparticle synthesis (Xie et al. 2005). These factors have led to a demand for the development of more environmental friendly methods for the nanoparticle synthesis for sustainability. Biological synthesis of metal nanoparticles has been considered as one of the eco- friendly approaches for the synthesis of the metal nanoparticles (Vigneshwaran et al. 2007). These process in which materials are synthesised using biological agents such as ,bacteria (Juibari et al. 2011), fungi (Castro-Longoria et al. 2011), yeast (Kowshik et al. 2003), live plants (Gardea-Torresday et al. 2003), plant extracts (Hebbalalu et al. 2013; Sivaraj et al. 2014), enzymes (Kumar et al. 2007) and peptides from phage library (Naik et al. 2002).

In this study, we report the biomediated synthesis of silver nanoparticles using a novel strain *Exiguobacterium mexicanum* PR 10.6 isolated from metal contaminated soil samples of North East of England. The silver nanoparticles are characterised using UV- Visible Spectroscopy, Energy Dispersive Spectroscopy (EDS), Fourier Transform Infrared Spectroscopy (FTIR) and High Resolution Transmission Electron Microscopy (HRTEM).

Materials and methods

Chemicals

Silver nitrate (Sigma) was used as the metal precursor solution for silver nanoparticle synthesis. Nutrient broth (Oxoid) and Nutrient agar (Oxoid) were used for the growth and subculturing of the bacterial strain.

Biomediated synthesis of silver nanoparticle

The bacterial culture, *Exiguobacterium mexicanum* PR 10.6 was subcultured in 100 ml nutrient broth and incubated at 30^{o}C for 48 h in a rotary shaker (New Brunswick-Innova) at 150 rpm and the culture was centrifuged using centrifuge (Thermo electron corporation - CR31) at 5,000 (*g*) for 10 min to separate the bacterial pellet from nutrient broth. The bacterial pellet was suspended in 100ml sterile distilled water and mixed thoroughly. The bacterial cell suspension was centrifuged at 14,000 (*g*) for 20 min. The supernatant was filtered through 0.2µm filter (Whatman filter) and the filtrate was used for the silver nanoparticle synthesis. In 90ml of the filtrate, 10ml of 10mM silver nitrate solution was added. The reaction mixture was incubated at room temperature (20±2^{o}C).

Instrumental characterisation of biomediated silver nanoparticle sample

An aliquot of sample was taken at 1 h from reaction mixture and analysed in UV-Visible Spectrophotometer (Jasco), using cuvette (Plastibrand). The wavelength scan measurement was performed between the wavelengths, 200 and 800 nm at resolution of 1 nm with a scanning speed of 0.1 nm/sec. The analysis in Energy Dispersive X-ray Spectroscopy (EDS; Inca Penta) equipped with Scanning Electron Microscopy (SEM; Hitachi S-3400 N) at an accelerating voltage of 20 KeV was carried out using dried samples at 40^{o}C and fixed on carbon tabs and mounted on sample holders. The liquid sample (100 µl) were added on the lacey grids (Agar) and air dried for 15 min and analysed in Transmission Electron Microscopy (TEM; Jeol 4000 EX HREM) at voltage 400 KV with vacuum of 4.5 *10^{5} Torr. The aliquot of sample was freeze dried (ThermoScientific-Heto PowerDry LL1500 and

Fourier Transform Infrared Spectroscopy (FTIR) analysis (Thermo electronic corporation-Nicolet 5700) was carried out using the between 400 and 4000 cm^{-1}. The result was analysed using OMNIC software.

Results

Biomediated synthesis of silver nanoparticle

The bacterial cell free filtrate when mixed with 1mM silver nitrate was initially colourless. Within 10 min, a gradual colour change was observed. In 30 min, the colourless solution had changed to a brown colour, which became intense after1 h (Fig 1-inset). This dark brown colour is an indication of the formation of the silver nanoparticles (Bhainsa and D'Souza 2006).

Note 1: (Insert Figure 1)

Instrumental characterisation of the biomediated silver nanoparticles

The UV-Visible spectroscopy spectrum results of the samples after 1 h exhibited a peak at 412 nm (Fig. 1) indicating the formation of silver nanoparticles. Silver nanoparticles characteristically produce a peak in the region 350-450 nm (Mulvaney 1996). EDS analysis of these particles confirmed that the sample contained predominantly silver (Fig 2). The sample has other elements such as silicon, oxygen, phosphorus, chlorine, and calcium. The transmission electron microscope (TEM) images (Fig. 3a) show that the silver particles are nanosize, typically less than 50 nm in diameter, being present in two size populations comprising smaller particles in the range 5-13 nm and larger particles in the range of 20-30. Under higher magnification (Fig. 3b), the crystal lattice was evident, confirming the

crystallinity of the nanoparticles. The FTIR spectrum obtained from the biomediated silver nanoparticle sample (Fig 4) exhibited major peaks at 3247 (cm^{-1}), 2916 (cm^{-1}), 1635 (cm^{-1}), 1547 (cm^{-1}) and 1051 (cm^{-1}) indicating the presence of amides.

Note 2: (Insert Figure 2, 3 and 4)

Discussion

Biomediated synthesis of nanoparticles is an environment benign silver nanoparticle synthesis process The process helps to obtain nano structures with less defects and better short and long range ordering, as the a process is mainly driven by reduction of Gibb's free energy (Leela and Vivekanandan 2008). The bacterial based nanoparticle synthesis also has advantages such as easiness in downstream processing, genetic manipulation, short doubling time etc (Sastry et al. 2003). In the biomediated silver synthesis, the colour change of the solution after adding silver nitrate is the indication of the formation of nanoparticles (Fig. 1-inset). The colour change of the solution can be attributed to the specific optical properties of the nanoparticles (Mulvaney 1996). The silver nanoparticles exhibit characteristic peaks between 350 - 450 nm due to Surface Plasmon Resonance (SPR) effects. This work demonstrated that the SPR at 412 nm (Fig. 1) was indicative of spherical nanoparticles without size variation (Mock et al. 2002).

The other elements (phosphorus, calcium, chlorine and silicon) identified in the EDS (Fig. 2) indicate the presence of biological matrix present in the sample. The silicon peak could have been attributed by the stub used for the analysis. The HRTEM images (Fig 3a) confirm that the particles are between 5 and 30 nm in diameter. The FTIR result of the sample (Fig.4) shows characteristic stretching vibrations of N-H bonds in the region of 3247 cm^{-1}. The

126 intense peak at 1635 cm^{-1} could be the stretching vibrations of the Carbonyl group (C=O).
127 The combination of N-H deformation and C-N stretching vibrations attributes the peak of
128 1547 cm^{-1}. The peak at 1051 cm^{-1} could be the stretching vibration N-H bond. The aliphatic
129 –N $(CH_3)_2$ groups in the sample are indicated by the absorption bands at the 2916 cm^{-1}. The
130 peak pattern in the FTIR correlates to the absorption bands of the secondary amides and the–
131 N $(CH_3)_2$ bond refers to tertiary amides (Simons 1978). The presence of amides is evidenced
132 as the indication of proteins in the sample (Sanghi and Verma 2009). The mechanism of
133 biomediated synthesis is not completely elucidated and there were several proposals for the
134 mechanism of nanoparticle synthesis. Gadd et al. (1989) had reported the accumulation of
135 silver using *Pseudomonas stutzeri* AG259, which was isolated from silver mine. The
136 mechanism of the intracellular synthesis of silver nanoparticles was related to the metal
137 resistance property of the organism against the toxicity of the metal. Schultze-Lam et al
138 (1996) had suggested that bacteria could precipitate an amount of metal equal to, or
139 exceeding their cellular weight. It could be an explanation for the extracellular synthesis of
140 metals.
141
142 In this study, the bacterial cell free extract is used for the silver nanoparticle synthesis
143 (Materials and Methods section) and it is suggested that the Extracellular Polymeric
144 Substance (EPS) play role in the silver nanoparticle formation. EPS are the microbially
145 produced organic compounds constitutes of polysaccharide, protein, nucleic acids, uronic
146 acids, lipids and functional groups such as carboxyl, phosphoric, amine and hydroxyl groups.,
147 Proteins have suggested playing key role in the biomediated synthesis of nanoparticles
148 (Sanghi and Verma 2009). Naik et al. (2002) had shown peptides from the phage library
149 could form silver nanoparticles and Kumar et al. (2007) demonstrated that the enzyme,

reductases could perform metal nanoparticle synthesis. The carbohydrates are also reported to play role in the silver reduction (Vigneshwaran et al. 2006).

EPS is loosely attached to the bacterial cell surface. Adav and Lee (2008) had suggested that high speed centrifugation can extract the soluble EPS to the solution. EPS contains charged moieties and have adsorptive and adhesive properties. It serves as a natural ligand and binding sites of metals (Bhaskar and Bhosle 2006; Comte et al. 2008). It is suggested that the EPS of bacteria acts as the electron donor (Fig. 5) in the biomediated silver synthesis using cell free extract of bacteria.

Note 3: (Insert Figure 5)

In the biomediated synthesis silver nanoparticle (Fig. 5), the silver nitrate ionises to silver (Ag^+) ions and nitrate (NO_3^-) ions in the solution, followed by the reduction of the Ag^+ ions, to metallic silver (Ag^0).

$$AgNO_3 \xrightarrow[H_2O]{\text{Ionisation}} Ag^+_{(aq)} + NO_{3(aq)} \qquad (1)$$

EPS are not active cells but have electrons (Laspidou and Rittmann 2002). The electrons from the EPS could donate electrons to the Ag^+ ions, reduce them to metallic silver and stabilise as nanoparticles.

$$Ag^+_{(aq)} + e^- \xrightarrow{\text{Reduction}} Ag^0 \qquad (2)$$

This study reports an environmental friendly method for the synthesis of nanoparticles. EPS mediated method helps for a fast, inexpensive and safe nanoparticle synthesis, by using bacterial cell filtrate.

Conclusion

This study focuses on the biomediated silver nanoparticle synthesis using the cell free extract of bacterium, *Exiguobacterium mexicanum* PR 10.6, isolated from the soil sample of the North East England. The instrumental characterisation results show that the cell free extract of *Exiguobacterium mexicanum* PR 10.6 could synthesise silver nanoparticle of size range 5-40 nm at room temperature in 1 h incubation time. The study establishes that the biomediated synthesis is a sustainable way of synthesising metallic nanoparticle without the use of any toxic chemicals or stringent conditions. It is assumed that the extracellular polymeric substance (EPS) present in the cell free extract plays the critical role in the silver nanoparticle reduction and stabilisation.

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References

Adav SS, Lee D-J (2008) Extraction of extracellular polymeric substances from aerobic granule with compact interior structure. J Hazard Mater 154:1120–1126

Bhainsa KC, D'Souza SF (2006) Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigates.* Colloid Surface B 47:160-164

Bhaskar PV, Bhosle NB (2006) Bacterial extracellular polymeric substance (EPS): A carrier of heavy metals in the marine food-chain. Environ Int 32:191-198

Castro-Longoria E, Vilchis-Nestor AR , Avalos-Borja M (2011) Biosynthesis of silver, gold and bimetallic nanoparticles using the filamentous fungus *Neurospora crassa.* Colloid Surface B 83:42-48

Comte S, Guibaud G, Baudu M (2008) Biosorption properties of extracellular polymeric substances (EPS) towards Cd, Cu and Pb for different pH values. J Hazard Mater 151:185-193

Gadd GM, Laurence OS, Briscoe PA, Trevors JT (1989) Silver accumulation in *Pseudomonas stutzeri* AG259. Biometals 2:168-173

Gao J, Xu B (2009) Applications of nanomaterials inside cells. Nano Today 4:37-51

Gardea-Torresdey JL, Gomez E, Peralta-Videa JR, Parsons JG, Troiani H, Jose-Yacaman M. (2003) Alfalfa sprouts: a natural source for the synthesis of silver nanoparticles. Langmuir 19:1357-1361

Hebbalalu D, Lalley J, Nadagouda M.N, Varma, R.S. (2013), Greener techniques for the synthesis of silver nanoparticles using plant extracts, enzymes, bacteria, biodegradable polymers, and microwaves. ACS Sustainable Chem. Eng 1*, 703–712*

Juibari MM, Abbasalizadeh A, Jouzani GhS, Noruzi M (2011) Intensified biosynthesis of silver nanoparticles using a native extremophilic *Ureibacillus thermosphaericus* strain. Mater Lett 65:1014-1017

Kowshik M, Ashtaputre S, Kharrazi S, Vogel W, Urban J, Kulkarni SK, Paknikar KM (2003) Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. Nanotechnol 14:95-100

Kumar SA, Abyaneh MK, Gosavi SW, Kulkarni SK, Pasricha R, Ahmad A, Khan MI (2007) Nitrate reductase- mediated synthesis of silver nanoparticles from $AgNO_3$. Biotechnol Lett 29:439-445

Laspidou CS, Rittmann BE (2002) A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass. Water Res 36:2711-2720

Leela A, Vivekanandan M (2008) Tapping the unexploited plant resources for the synthesis of silver nanoparticles. Afr J Biotechnol 7:3162-3165

Li Y, Kim NY, Lee EJ, Cai WP, Cho SO (2006) Synthesis of silver nanoparticles by electron irradiation of silver acetate. Nucl Instrum Meth B 251:425–428

Mock JJ, Barbic M, Smith DR, Schultz DA, Schultz S (2002) Shape effects in plasmon resonance of individual colloidal silver nanoparticles. J Chem Phys 116:6755-6759

Mulvaney P (1996) Surface Plasmon Spectroscopy of nanosized metal particles. Langmuir 12:788-800

Naik RR, Stringer SJ, Agarwal G, Jones SE, Stone MO (2002) Biomimetic synthesis and patterning of silver nanoparticles. Nat Mater 1:169-172

Sanghi R, Verma P (2009) Biomimetic synthesis and characterisation of protein capped silver nanoparticles. Bioresource Technol 100:501-504

Sastry M, Ahmad A, Khan MI, Kumar R (2003) Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr Sci India 85:162-170

Schultze-Lam S, Fortin D, Davis BS, Beveridge TJ (1996) Mineralization of bacterial surfaces. Chem Geol 132:171-181

Simons WW (1978) The Sadtler handbook of Infrared spectra. Sadlter Research Laboratories Inc. Philadelphia and Heyden & Son Ltd. London.

Sivaraj R, Rahman PKSM, Rajiv P, Narendhran S, Venckatesh R (2014) Biosynthesis and characterization of *Acalypha indica* mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity, Spectrochim Acta A 129: 255-258

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. P Natl Acad Sci USA 101:11030-11035

Vigneshwaran N, Nachane RP, Balasubramanya RH, Varadarajan PV (2006) A novel one pot 'green' synthesis of stable silver nanoparticles using soluble starch. Carbohyd Res 341:2012-2018

Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paralikar KM, Balasubramanya RH (2007) Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. Mater Lett 61:1413-1418

Xie J, Lee JY, Wang DIC, Ting YP (2007) Identification of active biomolecules in the high yield synthesis of single crystalline gold nanoplates in algal solutions. Small 3:672-682

List of Figures captions

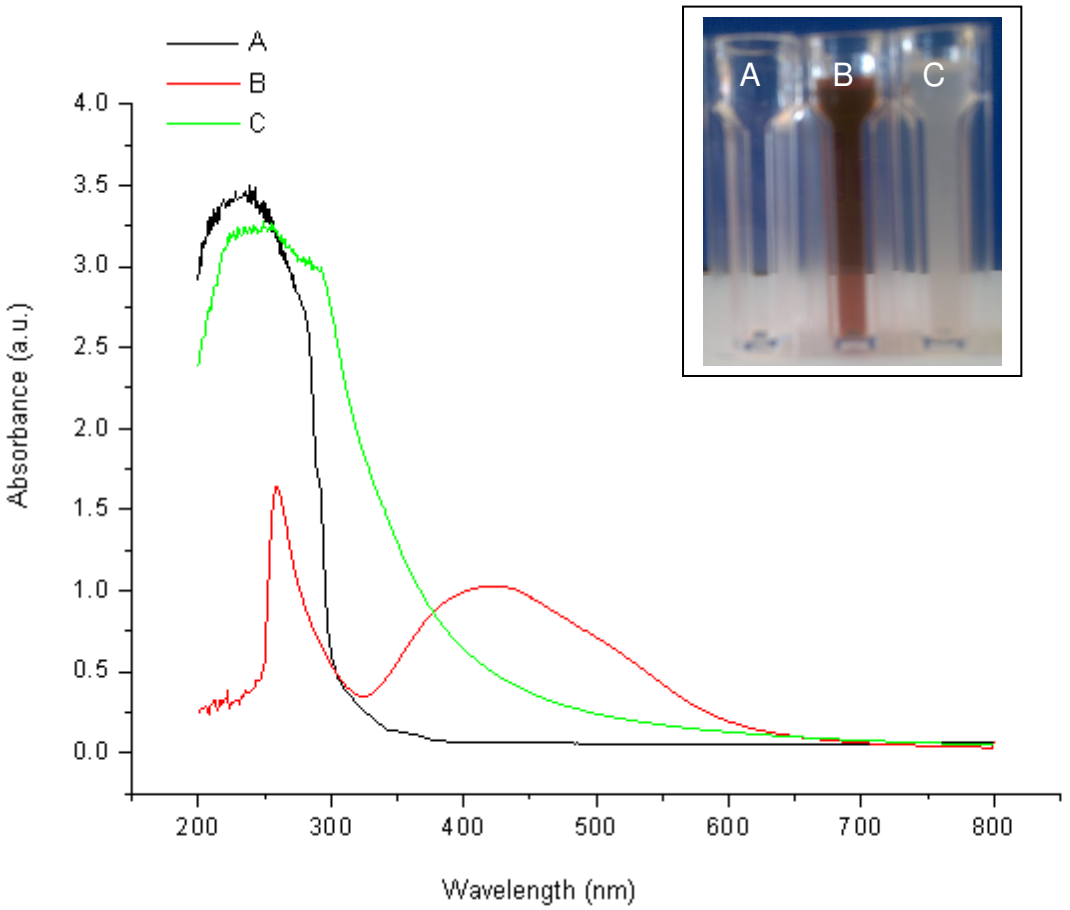
Figure 1. UV- Visible spectrum of the sample from biomediated silver nanoparticle synthesis using cell free extract of the *Exiguobacterium mexicanum* PR10.6. The inset represents the visual observations of the sample. In both figure and inset; (A) Silver nitrate, (B) Biomediated silver sample, (C) Cell free extract (blank). The spectrum scanning was between wavelength 250-800 nm. The biomediated sample (B) has turned to dark brown in colour (inset) and shows the characteristic SPR peak at 412 nm in the spectrum

Figure 2. Energy Dispersive Spectroscopy (EDS) spectrum of the biomediated silver nanoparticle sample. The EDS spectrum shows the peaks for: chlorine (Cl), calcium (Ca), oxygen (O), silicon (Si), phosphorus (P) and silver (Ag)

Figure 3. Transmission Electron Microscopy (TEM) image of the biomediated silver nanoparticle sample. The Fig 3A shows the nanoparticle distribution at the maginification 50000X. The Fig. 3b shows the magnified image of a single particle, at a magnification of 400000X. The scale bar in A is 50 nm and scale bar in Fig 3B is 5 nm

Figure 4. Fourier Transform Infrared Spectroscopy (FTIR) spectrum. The spectrum shows peaks at 3247 (cm^{-1}), 2916 (cm^{-1}), 1635 (cm^{-1}), 1547 (cm^{-1}) and 1051 (cm^{-1}). The peak locations correspond to the stretching and bending vibrations of the amides

Figure 5. The schematic representation of the biomediated synthesis of silver nanoparticle. Silver nitrate ($AgNO_3$) ionises to silver ion (Ag^+) and [$(NO_3)^{-1}$]. The bacterial cell wall has loosely extracellular polymeric substance (EPS; ∿∿∿). Some of EPS has charged moieties (-). The silver ion (Ag^+) is reduced to metallic particle using the electron provided by extracellular polymeric substance (∿∿∿). The extracellular polymeric substance (O) forms layer around the silver nanoparticles and stabilizes metallic silver as individual particles (AgNP)


A
B
C
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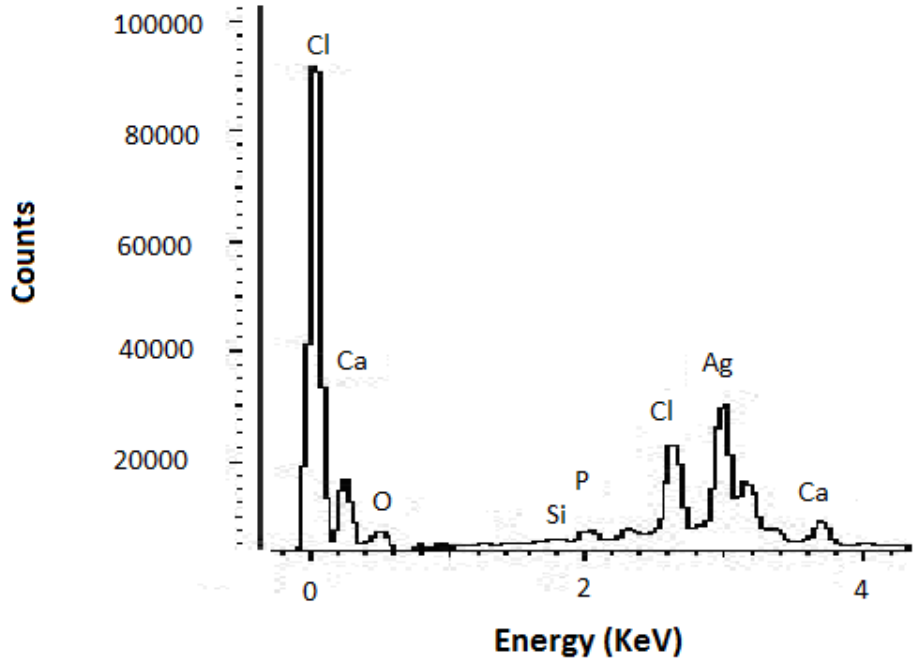
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Absorbance (a.u.)
200
300
400
500
600
700
800
Wavelength (nm)
A
B
C

Figure 1


Counts
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Si
P
Cl
Ag
Ca
0
2
4
Energy (KeV)

Figure 2

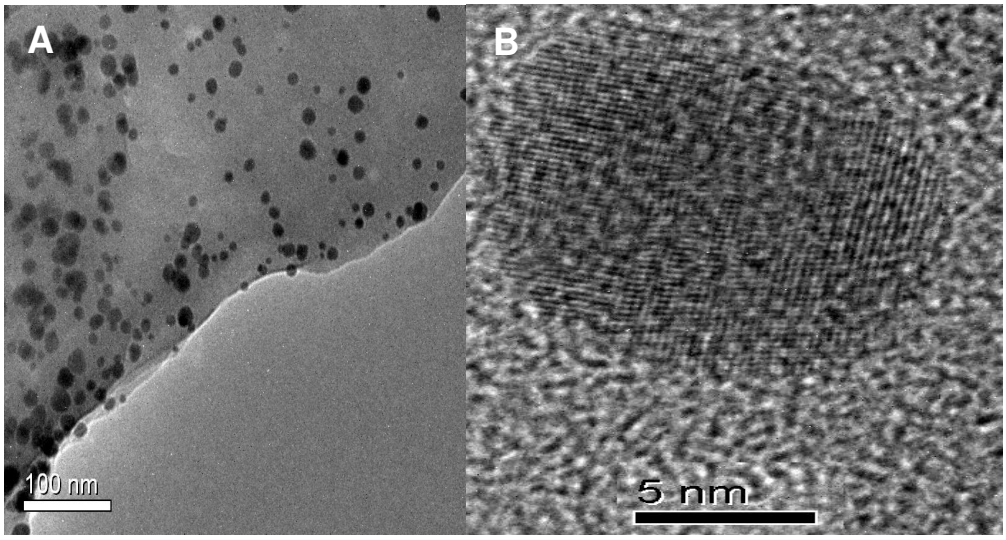
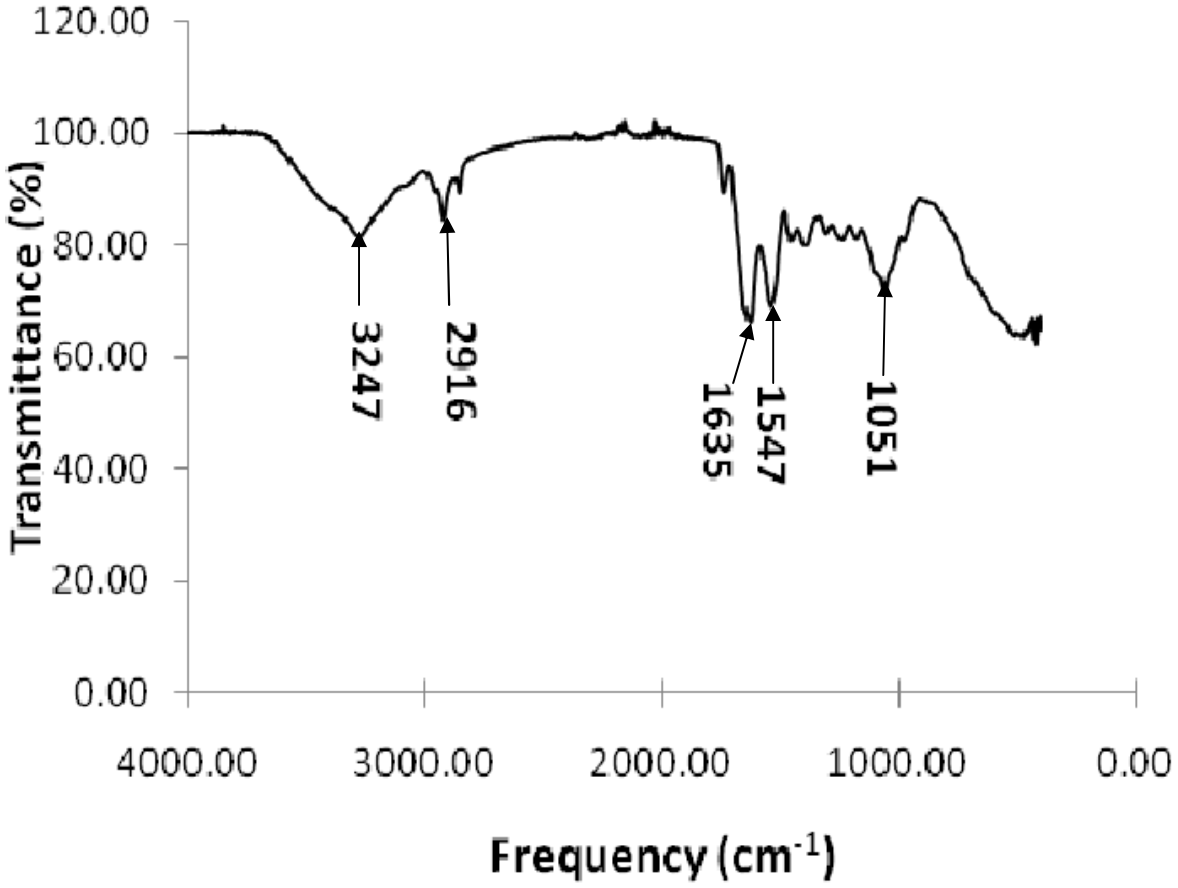


Figure 3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80.00
60.00
40.00
20.00
0.00
Transmittance (%)
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1635
1547
1051
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3000.00
2000.00
1000.00
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Frequency (cm-1)

Figure 4

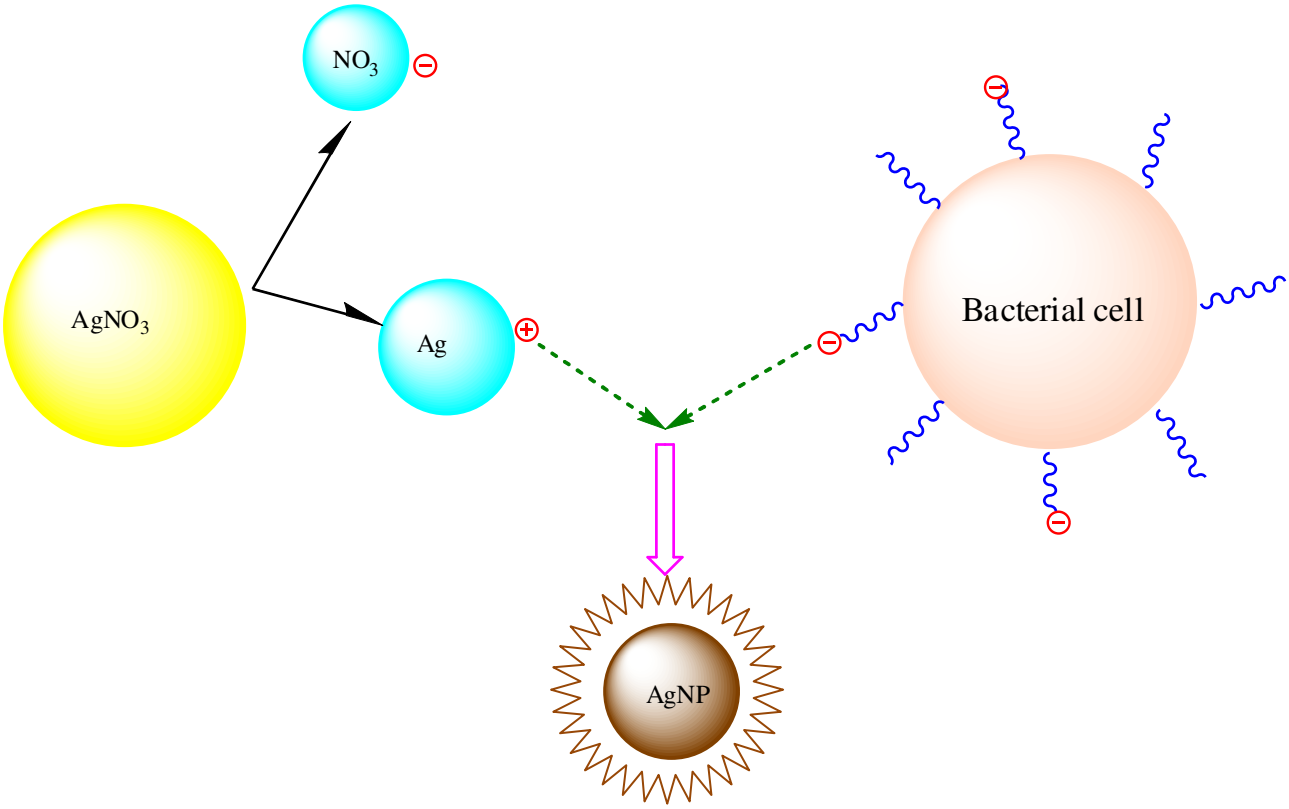

Figure 5