**"GREEN SYNTHESIS OF SILVER NANOPARTICLES USING DEXTROSE AND ITS ANTIMICROBIAL ACTIVITY"**

A thesis submitted to the

Faculty of engineering and biotechnology of Jadavpur University

for the partial fulfilment of the requirement for degree of

**Master of technology**

In

**Department of Food Technology and Biochemical Engineering**

**2015-2016**

By

**BANASHREE SAMANTA**

**ROLL NO- M4FTB1602**

Under the guidance of

**Dr. DIPANKAR HALDER**

Assistant Professor

Department of Food Technology and Biochemical Engineering

Jadavpur University, Kolkata

&

**Dr. ATANU MITRA**

Assistant Professor

Department of Chemistry

Sree Chaitanya College, Habra

***FACULTY OF ENGINEERING AND TECHNOLOGY***
***DEPARTMENT OF FOOD TECHNOLOGY AND BIOCHEMICAL ENGINEERING***
***JADAVPUR UNIVERSITY***
***KOLKATA-700032***

## DECLARATION OF ORIGINALITY & COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that thesis contains literature survey and original research work by the undersigned candidate, as part of her Master of Technology in Food technology and Biochemical engineering studies. All information in this document have been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

Name: Banashree Samanta

Examination Roll Number: M4FTB1602

Thesis Title: **Green synthesis of silver nanoparticles using dextrose and its antimicrobial activity**

Signature:

Date:

## SELF ATTESTATION

This is to certify that I have personally worked on the research project entitled "**Green synthesis of silver nanoparticles using dextrose and its antimicrobial activity**". The data mentioned in this report were obtained during genuine work done and collected by me. Data obtained from other agencies have been fully acknowledged. None of the finding pertaining to the work has been concealed. The results embodied in this project have not been submitted to any other university or institute for the award of any degree or diploma.

PLACE:

DATE:

BANASHREE SAMANTA

# ***FACULTY OF ENGINEERING AND TECHNOLOGY***
# ***DEPARTMENT OF FOOD TECHNOLOGY AND BIOCHEMICAL ENGINEERING***
# ***JADAVPUR UNIVERSITY***
# ***KOLKATA-700032***

## CERTIFICATE OF RECOMMENDATION

I hereby recommended the thesis entitled "**Green synthesis of silver nanoparticles using dextrose and its antimicrobial activity**". Prepared by Miss Banashree Samanta (Roll no: M4FTB1602), a student of M. tech second year, under supervision of Dr. Dipankar Halder and Dr. Atanu Mitra has been evaluated and been found satisfactory. It is therefore accepted and submitted as a dissertation for partial fulfilment for the requirement for awarding of degree of Master of Technology in Food Technology and Biochemical Engineering.


Dr. DIPANKAR HALDER

Assistant professor

Food Technology
& Biochemical Engineering Department

Jadavpur University

Kolkata-700032

Dr. ATANU MITRA

Assistant professor

Department of Chemistry
Sree Chaitanya College,

Habra

Dr. Runu Chakraborty

Head of the Department

Food Technology & Biochemical Engineering

Jadavpur University

Kolkata-700032

Prof. Sivaji Bandyopadhyay

Dean

Department of Food Technology
& Biochemical Engineering

Jadavpur University

Kolkata-700032

***FACULTY OF ENGINEERING AND TECHNOLOGY***
***DEPARTMENT OF FOOD TECHNOLOGY AND BIOCHEMICAL ENGINEERING***
***JADAVPUR UNIVERSITY***
***KOLKATA-700032***

## CERTIFICATE OF APPROVAL

This is to certify that Miss Banashree Samanta has carried out the research work entitled "**Green synthesis of silver nanoparticles using dextrose and its antimicrobial activity**" under our direct supervision, at department of Food Technology and Biochemical Engineering Jadavpur University, Kolkata. We are satisfied that she has carried out this work independently and with proper care and confidence. We hereby recommend that this dissertation be accepted in partial fulfilment of the requirements for the degree of Master of Food Technology and Biochemical Engineering.

We are very much pleased to forward this thesis for evaluation.


Dr. DIPANKAR HALDER
Assistant Professor,
Dept of Food Technology & Biochemical Engineering
Jadavpur University
Kolkata-700032

Dr. ATANU MITRA
Assistant Professor
Sree Chaitanya College
Habra

## ACKNOWLEDGEMENT

I would like to take this opportunity to express my sincere thanks to each and every individual whose contribution has been invaluable for successful completion of this thesis work entitled "**Green synthesis of silver nanoparticles using dextrose and its antimicrobial activity**".

I am very grateful to **Dr. Runu Chakraborty**, Head of the Department of Food Technology and Biochemical Engineering, Jadavpur University for giving me the opportunity to do this work and also for her precious suggestion and encouragement during the course of my work.

I would also like to extend my heartfelt gratitude to my project mentors; **Dr. Dipankar Halder** and **Dr. Atanu Mitra** for their constant inspiration, suggestions and active supports. I take this opportunity to express my sincere gratitude & thankfulness to esteemed professors of this department, **Prof. Amit Kumar Ghosh, Late Dr. Lalita gouri Roy, Prof. Utpal Ray Chowdhuri, Dr. Uma Ghosh and Dr. Paromita Bhattacharjee** for their valuable guidance. I would also like to mention my sincere thanks to all the Research Scholars, non-teaching staffs and sub-staffs of this department.

It is the perfect opportunity to convey my gratitude and appreciation to **Miss Gargi Dinda** and **Miss Koushiki Goswami** for enlightening me and guiding me throughout my project tenure. I would also like to communicate my regards to my batch-mates for their valuable cooperation and encouragement.

The authors of the various journals that I referred to, also deserve a sincere thanks on my behalf for being a constant source of encouragement & specially for providing valuable information.

Last but not the least, my heartfelt gratitude and love to my parents for their endless support throughout the years.

BANASHREE SAMANTA

## Abstract

Here the green synthesis of polydispersed, small sized silver nanoparticles (Ag-NPs) in aqueous medium is reported. The synthesis was achieved by reduction of silver ion using dextrose. Dextrose also acts as a stabilizing agent. Silver nitrate was used as a precursor of silver ion. Influence of two parameters, temperature and pH were examined on the synthesis of nanoparticles. The nanoparticles were characterized using UV–Vis absorption spectroscopy and fluorescence spectroscopy. The absorption maxima of the as-synthesized Ag-NPs showed characteristic surface plasmon resonance (SPR) peak. The antimicrobial properties of the as-synthesised Ag-NPs were investigated against the gram-negative strains, *Escherichia coli* and gram-positive strains, *Bacillus subtilis.* Better antimicrobial activity has been seen against gram-negative *E. coli.*

## Content

# Chapter1-Introduction

## 1.1 Introduction

It has been suggested for some time that materials and substances may be manipulated at the very small size scale through atom-by-atom assembly. Nanotechnology is the broad term used to represent an assemblage of processes, materials and applications that span physical, chemical, biological and electronic science and engineering fields. Manipulation of materials is done for the all fields at a size range in the nanometer scale. One nanometer (nm) is one-billionth of a meter. A nanomaterial has been defined as a 'material having one or more external dimensions in the nanoscale or which is nanostructured', where the nanoscale size ranges is approximately 1-100 nm. Nanomaterials also exist in other forms, such as nanorods or nanotubes with two dimensions in the nanoscale, or nanolayers, coatings or sheets with just one dimension in the nanoscale.

The nation 'a little goes a long way' is probably the single most powerful reasoning behind many of the nanotechnology applications in different sectors. As food packaging plastics ENPs may be used. The very small size of engineered nanoparticles ENPs can also offer other benefits. For example, nanosizing of water-insoluble substances can enable their uniform dispersion in aqueous formulations. This makes it possible to reduce the use of solvents in certain applications such as cosmetics, paints and coatings, and allows the dispersion of food additives such as water-insoluble colours, flavours and preservatives in low-fat systems. [1]

## 1.2 Importance of silver nanoparticles

The synthesis of noble metal nanoparticles has been gaining significant importance during the past few years owing to its increasing application in the field of medicine, biology, material science. Among the noble metals silver has been used for thousands of years for ornaments, medicine, food, textile, photography, electronics, dental fillings and utensils. Silver and its nanoparticles have been used as an antimicrobial in a variety of industrial, healthcare and domestic applications [2]. Silver is used to inhibit the growth of bacteria and fungi on socks. It reduces odours and the risk of bacterial and fungal infections [3]. The silver ion, $Ag^{+}$, is bioactive and in sufficient concentration readily kills bacteria in vitro. Exposure to silver nanoparticles has been associated with inflammatory, oxidative, genotoxic and cytotoxic consequences. The silver particulates primarily accumulate in the liver, but have also been shown to be toxic to brain. Nano silver applied to tissue-cultured human cells leads to the formation of free radicals, raising concerns of potential health risks [4, 5].

## 1.3 Methods of synthesis of silver nanoparticles

Nano-biotechnology is a rapidly growing scientific field of producing and constructing devices. An important area of research in nano-biotechnology is the synthesis of NPs with different chemical compositions, sizes and morphologies, and controlled disparities. Nano-biotechnology has turned up as an elementary division of contemporary nanotechnology and untied novel epoch in the fields of material science receiving global attention due to its ample

applications. It is a multidisciplinary approach resulting from the investigational use of NPs in biological systems including the disciplines of biology, biochemistry, chemistry, engineering, physics and medicine. Moreover, the nano-biotechnology also serves as an imperative technique in the development of clean, nontoxic, and eco-friendly procedures for the synthesis and congregation of metal NPs having the intrinsic ability to reduce metals by specific metabolic pathways [6-11].

Nowadays, development of eco-friendly processesis needed, where toxic chemicals are not used in the synthesisprotocols. Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, tollens, biological, and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity. Selection of solvent medium and selection of eco-friendly nontoxic reducing and stabilizing agents are the most important issues which must be considered in green synthesis of NPs.

Silver NPs have unique properties which can be incorporated into antimicrobial applications, composite fibres, biosensor materials, cosmetic products, cryogenic super-conducting materials, and electronic components.Several physical and chemical methods have been used for synthesizing and stabilizing silver NPs [12, 13].

Most of these methods are still in development stage and the experienced problems are the stability and aggregation of NPs, control of crystal growth, morphology, size and size distribution.The most popular chemical approaches, including chemical reduction using a variety of organic and inorganic reducing agents, physicochemical reduction, electrochemical techniques and radiolysis are widely used for the synthesis of silver NPs. Furthermore, extraction and purification of produced NPs for further applications are still important issues [14-16].

### 1.3.1 Physical methods: [17-23]

**Table-1: Physical method**

| Synthesis method | Advantages | Disadvantages |
|---|---|---|
| Laser ablation | Absence of solvent contamination, absence of chemical reagent in solution | Low yield, high power requirement |
| Evaporation-condensation | Uniform particle distribution, absence of solvent contamination | High energy requirement, large space requirement, long time period requirement |
| Arc discharge method | High production rate, high energy efficiency | Expensive |

## 1.3.2 Chemical methods: [24-41]

**Table-2: Chemical method**

| Synthesis method | Advantages | Disadvantages |
|---|---|---|
| Chemical reduction | Easy operation, low energy requirement, easy availability of equipment | Presence of toxic chemicals, low productivity, inconsistency of particle size |
| Electrochemical synthetic method | Easy operation, low cost, high flexibility, easy availability of equipments, control particle size, improve homogeneity of nanoparticles | Presence of toxic chemicals, low productivity, inconsistency of particle size |
| Photo induced reduction | high spatial resolution, convenience of use, and great versatility | Presence of toxic chemicals, low productivity, inconsistency of particle size |
| Sonoelectrochemical reduction | Simple, high flexibility, carried out in ambient temperatures | Presence of toxic chemicals, low productivity, inconsistency of particle size |
| Microwave associated synthesis | consistently yielding nanostructures with smaller sizes, narrower size distributions, and a higher degree of crystallization, shorter reaction times, reduced energy consumption, and better product yield, drastically reduce chemical wastes, rapid heat transfer , volumetric and selective heating. | Presence of toxic chemicals, low productivity, inconsistency of particle size |
| Microemulsion | Uniform and size controllable nanoparticles | use of highly deleterious organic solvents |
| UV-initiated photoreduction | Simple and effective method | Presence of toxic chemicals, inconsistency of particle size |
| Irradiation method | Well defined shape and size distribution, short time period requirement | Presence of toxic chemicals, low productivity, |
| Polymers and polysaccharides | Highly stable, protected against nanoparticle aggregation | Presence of toxic chemicals, low productivity, inconsistency of particle size |
| Tollens method | Simple one step method, synthesis nanoparticle with a controlled size | Presence of toxic chemicals, low productivity, |

### 1.3.3 Bio-based methods:

A number of reports prevailed in the literatures indicate that synthesis of nanoparticles by chemical approaches are not eco-unfriendly and expensive. Thus, there is a growing need to develop environmentally and economically friendly processes, which do not use toxic chemicals in the synthesis protocols. This has conducted researchers to look at the organisms. The potential of organisms in nanoparticle synthesis ranges from simple prokaryotic bacterial cells to eukaryotic fungi and plants [42]. Some examples of nanoparticle production include using bacteria forgold, silver, cadmium, zinc, magnetite, and iron NPs; yeasts for silver, lead and cadmium NPs; fungi for gold, silver and cadmium NPs; algae for silver and gold NPs; plants for silver, gold, palladium, zinc oxide, platinum, and magnetite NPs [43,44] .

Bio-based protocols could be used for synthesis of highly stable and well-characterized NPs when critical aspects, such as types of organisms, inheritable and genetic properties of organisms, optimal conditions for cell growth and enzyme activity, optimal reaction conditions, and selection of the biocatalyst state have been considered [45].

**Table-3: Synthesis of nanostructured silver from various biological sources**

| Biological source | Intracellular/Extracellular | Size range | Ref. |
|---|---|---|---|
| **Bacteria** | | | |
| *Klebsiella pneumonia* | Extracellular | 5-32 nm | 46 |
| *Bacillus thuringiensis* | | 10-20 nm | 47 |
| *Bacillus licheniformis* | Extracellular | 50 nm | 48 |
| *P. stutzeri* AG259 | Intracellular | <200 nm | 49 |
| *Cornylococcusaureus* | | 5-15 nm | 50 |
| *Staphylococcus aureus* | Extracellular | 120-180 nm | 51 |
| **Plants** | | | |
| *Pelargonium graveolens* | Extracellular | 16-40 nm | 52 |
| *Hibiscus rosasinensis* | Extracellular | 13-20 nm | 53 |
| *Cinnamomumcamplora* | Extracellular | 55-80 nm | 54 |
| *Geranium leaf extract* | Extracellular | 16-40 nm | 55 |
| *Emblicaofficinalis* | Extracellular | 10-25 nm | 56 |
| *Jatrophacurcas* (latex) | Extracellular | 20-40 nm | 57 |
| *Aloe vera* | Extracellular | 15.2±4.2 nm | 58 |
| *Azadirachtaindica* (Neem) | Extracellular | 5-35 nm | 59 |
| **Yeast** | | | |
| Extremophilic yeast | | | 60 |
| MKY3 | Extracellular | 2-20 nm | 61 |
| **Fungi** | | | |
| *Aspergillusniger* | | 20 nm | 62 |
| *Fusariumoxysporum* | Extracellular | 2-50 nm | 63 |
| *Aspergillusfumigatus* | Extracellular | 5-25 nm | 64 |
| *Fusariumsolani* | | 5-35 nm | 65 |
| *Verticillum* | Intracellular | 25±12 nm | 66 |
| *Aspergillusflavus* | | 8.92±1.61 nm | 67 |
| *Trichodermaviride* | | 10-40 nm | 68 |

### 1.3.3.1 Synthesis of nanoparticles using plant-based extracts

Recently G. A. Kahrilas et al have studied Microwave-assisted synthesis of silver nanoparticles using orange peel extract. Besides orange peel extract, they also used extracts from other peels of citrus fruits like grapefruit, tangelo, lemon and lime. But only orange peel extract went successful. Orange peel is served as both reducing agent as well as capping agent. Nanospheres of TEM mean diameter of 7.36±8.06 nm were successfully synthesized in 15 min by reducing $Ag^+$ ions with orange peel extract. One of the main advantages of microwave heating over traditional heating is that it reduces reaction time and thus improves experimental efficiency. Main reductive compound present in orange peel extract is citronellal. Other compounds responsible for capping reducing are citrate and limonene. This microwave assisted green synthesis allows for preparation of nanoparticles without risking intervention from toxic reducing agents and capping agents. [69]

A. A. Kajani et al have reported synthesis of anisotropic silver nanoparticles using Taxus baccata extract in the year 2014. Here taxus baccata extracts are used as reducing, capping and stabilizing agents. Monodispersed nanoparticles were obtained with an average of 75.1 nm. The main parameter for this synthesis was the type of plant extract used. Type of plant extract used affected the physical, chemical and cytotoxic properties of the nanoparticles. Other factors that were investigated to synthesize silver nanoparticles of desired shape and size were concentration of silver nitrate, pH and temperature. Plants and plant extracts are well suitable for biosynthesis of nanoparticles of various shape and sizes. Biomolecules present in the plants like alkaloids, phenolic compounds, terpenoids and coenzymes can be used to reduce metal ions to the nanoparticles. Organic compounds of plants act both as reducing and stabilizing agents. Taxus species are known to produce diterpenoids named as toxoids. One of the most known taxoids, taxol is well known for its anticancer property. Thus the nanoparticles synthesized from this taxus baccata extract are known for its anticancer effects. The MTT assay revealed the anti-cancer effects of those synthesised nanoparticles. [70]

P. K. Singh et al have studied about Lantana camara leaf extract synthesized silver nanoparticles and about their UV-assisted sampling and antibacterial property in the year 2015. In this study silver nanoparticles are synthesised using weed plant Lantana camara's leaf extract. This green route synthesis is very quick, easy and cost effective route. Synthesised silver nanoparticle by this method was studied and discussed based on photoluminescence, optical absorption, dynamic light scattering, zeta potential, X-ray diffraction measurements, infrared spectroscopy, FE-SEM and TEM analysis. Lantana camara has recently been recognized for its natural healthcare potential. It is known for its antifungal and antibacterial activity. It is also used in HIV patients for boosting of immune system. Due to the presence of flavonoids and phenylpropanoid, lantana camara acts as potential anti-tumour agents. Here lantana camara leaf extract act as both reducing and stabilizing agent for synthesizing silver nanoparticles. Synthesised silver nanoparticles showed very high antibacterial activity against *E. coli* and *S. aureus* bacteria at very low concentration of 50 ppm. [71]

E. Roy et al have studied on shape-specific silver nanoparticles prepared by Microwave-assisted green synthesis using pomegranate juice in the year 2015. Here four different shaped silver nanoparticles were synthesized. Four different shapes include spherical, oval, rod and flower. Pomegranate juice was used as reducing agent here. Microwave irradiation is used here due to its advantage of reducing reaction time and its effectiveness in synthesis. Pomegranate juice contains mainly gallic acid, ellagic acid and flavonol glycosides that are able to reduce silver nitrate to silver nanoparticles very effectively. Four different shaped silver nanoparticles were synthesized. Synthesized silver nanoparticles have been characterized by UV-visible spectroscopy, transmission electron microscopy, field emission scanning electron microscopy and powder X-ray diffraction techniques. Bactericidal activity was studied for all prepared nanoparticles of varied shapes. Among the four different shaped silver nanoparticles, the flower shaped silver nanoparticles gave the best results and mediated the fastest bactericidal activity against all the tested strains at similar bacterial concentrations. [72]

### 1.3.3.2 Microbial synthesis of nanoparticles

P. Mukherjee et al have studied on Fungus-mediated synthesis of silver nanoparticles and their immobilization in the Mycelial Matrix in the year 2001. In this study silver nanoparticles were synthesised using the fungus Verticillium. Here this fungus Verticillium, when exposed to aqueous silver nitrate solution, it reduces the metal ions and thus leads to the formation of silver nanoparticles of 25nm diameter. Silver nanoparticles were formed below the fungal cells. Fungal cells continued to multiply after biosynthesis of silver nanoparticles. [73]

B. Ramalingam et al have studied on Antibacterial effects of biosynthesized silver nanoparticles on surface ultra-structure and nano mechanical properties of gram-negative bacteria in the year 2016. Here biosynthesis was done by in situ reduction of silver nitrate using cell free protein of *Rhizopus oryzae*. Synthesized nanoparticles were characterized UV-Visible spectroscopy, dynamic light scattering, transmission electron microscopy and FTIR spectroscopy. High resolution transmission microscopy was also done for the synthesized nanoparticles and confirmed the formation of 7.1±1.2nm silver nanoparticles. The antibacterial activity of silver nanoparticles was tested against *E. coli* and *Pseudomonas aeruginosa*. Silver nanoparticles showed concentration dependent antibacterial activity. 100% killing of *E. coli* and *Pseudomonas aeruginosa* achieved when the cells were treated with 4.5 and 2.7 μg/ml silver nanoparticles for 4h. Using fluorescence microscopy, AFM and FTIR, the alteration and morphological and physicochemical properties of bacterial surface has been monitored. [74]

H. Sowani et al have studied on green synthesis of gold and silver nanoparticles by an actinomycete Gordonia amicalis HS-11 and their biological application in the year 2015. Silver nanoparticles were prepared by incubating cell free supernatant of bacterium with 1mM silver nitrate at pH 9 in a boiling water bath. Similarly gold nanoparticles were also synthesized incubating with 1mM chloroauric acid. It was assumed that the cell free supernatant contained some thermo stable biomolecule that mediated metal reduction

reactions. The synthesized nanoparticles were characterized by using UV-Vis spectrophotometer, transmission electron microscope and X-ray difractometer. In this paper nanoparticle synthesis by actinomycete culture is highlighted, also identifies the biomolecule involved in the process and describes the associated antioxidant activity. [75]

P. Manivasagan et al have studied on Production of polysaccharide-based bioflocculant for the synthesis of silver nanoparticles by Streptomyces sp. in the year 2015. In this study production and optimization of polysaccharide based bioflocculants by Streptomyces sp. for the green synthesis of silver nanoparticles were reported. Bioflocculants are organic macromolecular substances secreted by microorganisms. Bioflocculants are well known for their wide use in waste water treatment, food and fermentation process and downstream processing. Using response surface methodology medium composition and culture conditions for polysaccharide-based bioflocculants were optimized. Synthesized silver nanoparticles were characterized using UV-Vis spectroscopy, FTIR, XRD, FESEM, EDXA and HRTEM. Strong antibacterial activity was shown by synthesized silver nanoparticles in sewage water. [76]

### 1.3.3.3 Carbohydrate based green synthesis

S. Mohan et al have studied on Green synthesis of dextrose reduced silver nanoparticles and its antimicrobial and sensing properties in the year 2014. In this study silver nanoparticles were synthesized using dextrose. Here water is used as the solvent. Dextrose acts as both reducing agent as well as stabilizing agent. In this work, they have reported, synthesis of highly mono-dispersed, water soluble, stable and small sized gelatine-capped silver nanoparticles. Synthesized nanoparticles were characterized using UV-Vis spectroscopy, transmission electron microscopy; X-ray diffraction, high resolution transmission electron microscopy and Fourier transform infra-red spectroscopy. These synthesized silver nanoparticles showed better antibacterial efficacy than the antibiotics; ciproflaxin and impinem against *Pseudomonas aeuruginosa* with minimum inhibition concentration of 6µg/ml and better efficacy than impinem against *E. coli* with minimum inhibition concentration of 10µg/ml. [77]

W. Haggren et al have studied about Microwave-assisted green synthesis of silver nanoparticles with soluble starch, dextrose and arabinose and its antibacterial activity in the year 2014. In this study silver nanoparticles were synthesized in three different schemes by reducing $Ag^{+}$ ions with reducing agents arabinose, dextrose and soluble starch. Nanoparticles formed in less than 15 min. Microwave heating was used here. The advantage of microwave heating over traditional heating like les reaction time and all made to select this microwave mode of heating. Here starch served as both capping agent as well as reducing agent. In this study protocols were developed using starch, starch + dextrose and starch + arabinose. The nanoparticles prepared by these methods were characterized using UV-Visible spectroscopy, powder X-ray diffraction, fluorescence spectroscopy, transmission electron microscopy and differential light scattering. Antibacterial activity of synthesized nanoparticles was examined using different test microorganisms. All silver nanoparticles prepared in this study showed

antibacterial effects on variety of microorganisms. This antibacterial activity was determined by a well diffusion assay. [78]

B. Xia et al have studied about Preparation of bimetallic nanoparticles using green synthesis method in the year 2013. In this study, green synthesis of well stabilized Au/Ag bimetallic nanoparticles was reported. Degraded pueraria starch (DPS) used for synthesis of bimetallic nanoparticles. DPS acted as both a reducing agent as well as a capping agent. Au/Ag bimetallic nanoparticles were characterized using energy-dispersive X-ray, X-ray diffraction and high-resolution transmission electron microscopy. Prepared bimetallic nanoparticles showed god catalytic activity in the reduction of 4-nitrophenol. [79]

O. S. Oluwafemi et al have studied on Maltose-reduced silver nanoparticles in the year 2012. In this study highly stable and small sized silver nanoparticle with narrow size distribution without the use of an accelerator was reported. Here maltose was used as the reducing agent and gelatine as the stabilizer. Synthesized silver nanoparticles were characterized using UV-Visible spectroscopy, transmission electron microscopy; X-ray diffraction, high resolution transmission electron microscopy, and Fourier transform infrared spectroscopy. Through transmission electron microscopy it was found that the particles formed are small, and spherical in shape. Fourier transform infrared spectroscopy confirmed the stabilisation of silver nanoparticles by the gelatine. Main advantages of this synthesis method are moderate temperature for preparation, avoidance of toxic starting materials, lack of need for an additional flow of inert gas, and the use of atmospheric pressure. [80]

## 1.4 Antibacterial activity of Silver nanoparticles:

Application of silver nanoparticles into bacterial cells induces a high degree of structural and morphological changes; as a result cell death is happened. As the silver nanoparticles come in contact with the bacteria, they adhere to the cell wall and cell membrane. Once bound, some of the silver passes through to the inside, and interacts with phosphate-containing compounds like DNA and RNA, while another portion adheres to the sulphur-containing proteins on the membrane. [81] The silver-sulphur interactions at the membrane cause the cell wall to undergo structural changes, like the formation of pits and pores. Simply due to the osmotic difference, through these pores, cellular components are released into the extracellular fluid. Within the cell, the integration of silver creates a low molecular weight region where the DNA then condenses. Having a condensed state DNA inhibits the cell's replication proteins contact with the DNA. Thus the application of silver nanoparticles inhibits replication and becomes a cause of cell death. Further increasing their effect, when silver comes in contact with fluids and tends to ionize which increases the nanoparticles bactericidal activity. [82] This has been correlated to the suppression of enzymes and inhibited expression of proteins that relate to the cell's ability to produce ATP. [83] This could also be partly due to the fact that as particle size decreases, reactivity increases due to the surface area to volume ratio increasing. It has been noted that the introduction of silver nanoparticles has shown to have synergistic activity with common antibiotics already used today, such as; penicillin G, ampicillin, erythromycin, clindamycin, and vancomycin against *E. coli* and *S. aureus*. [84] In medical equipment, it has been shown that silver nanoparticles drastically lower the bacterial count on devices used. However, the problem arises when the procedure is over and a new one must be done. In the process of washing the instruments a large portion of the silver nanoparticles become less effective due to the loss of silver ions. They are more commonly used in skin grafts for burn victims as the silver nanoparticles embedded with the graft provide better antimicrobial activity and result in significantly less scarring of the victim. [85]

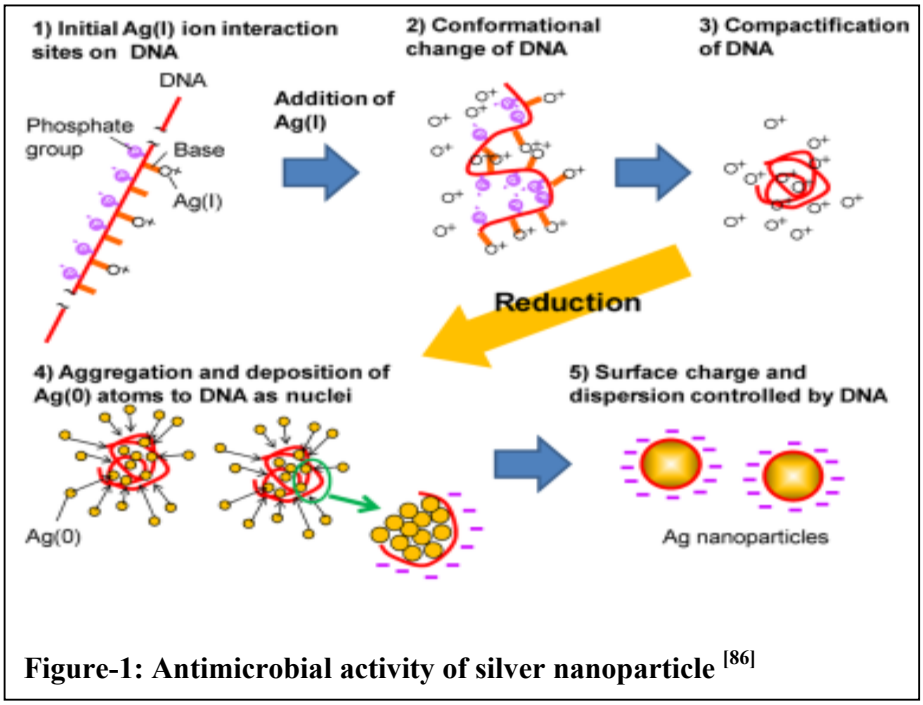



**Figure-1: Antimicrobial activity of silver nanoparticle [86]**

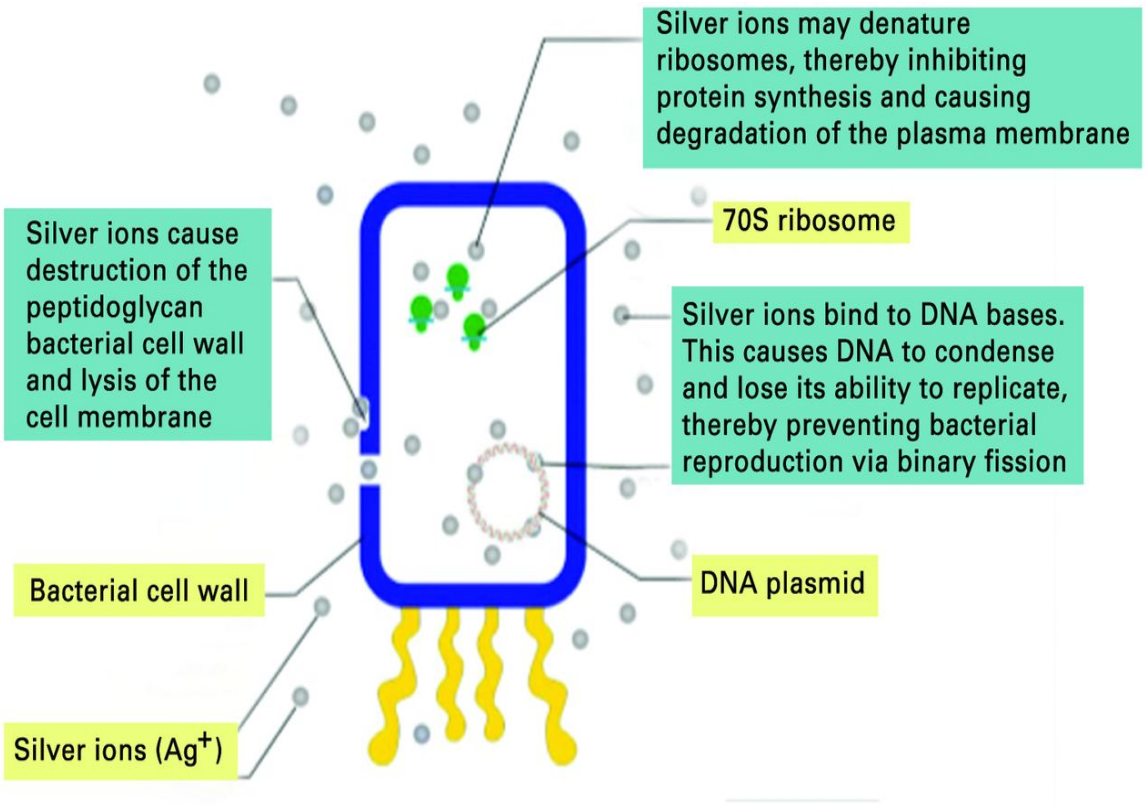



**Figure-2: Antibacterial activity of silver nanoparticle** [87]

# Chapter2: Aims and Objectives

## Aims and Objectives

1. To synthesize silver nanoparticles by green protocol using dextrose as a reducing and stabilizing agent

2. To investigate the kinetics of the formation of nanoparticles

3. To study the effect of temperature on the kinetics of reduction process and morphology of silver nanoparticle.

4. To characterize the as-synthesized silver nanoparticle by spectroscopy.

5. To study the stability of silver nanocolloid stored at different temperatures.

6. To study the antimicrobial efficacy of as-synthesized silver nanoparticles.

# Chapter 3: Materials and Methods

# 3. MATERIALS AND METHODS:

## 3.1 Green synthesis of Silver nanoparticles

### I. Chemicals and reagents:

1. Dextrose ($C_6H_{12}O_6$)
2. Silver nitrate($AgNO_3$)
3. Double distilled water
4. Sodium hydroxide (NaOH) from Qualigens fine chemicals

### II. Instruments used

1. METTLER Digital weighing balance
2. PERKIN-ELMER UV-Visible Spectrophotometer (Lambda- 25)
3. REMI Magnetic Stirrer set at 1150 rpm
4. PERKIN-ELMER fluorescence Spectrophotometer(LS- 45)
5. BHATTACHARYA & CO. hot air oven
6. pH meter

### III. Equipments used

1. Pipettes (0.1ml, 1ml and 5ml)
2. Round bottom flask (50ml)
3. Conical flasks(100ml and 250ml)
4. Plastic bottles
5. Spatula

### IV. Preparation of 10mM $AgNO_3$ stock solution:

0.0338gm of $AgNO_3$ (99.99% purity from MERCK) was added to a 20ml of double distilled water in a round bottom flask. The flask was gently rotated manually to dissolve and to obtain a clear solution. The flasks were covered with brown paper and placed in a dark place to prevent reaction of silver nitrate with light.

### V. Preparation of NaOH stock solution:

1 NaOH pallet was added to a 10ml volumetric flask and the flask was gently rotated manually to dissolve and to obtain a clear solution.

### VI. Preparation of sample:

Dextrose-silver sample solutions of different temperature (5°C, 32°C, 40°Cand 60°C) were prepared with aqueous solutions. The sample solutions were prepared in 20ml plastic bottles. There were two batches samples. One of sample contains 18ml H2O, 2ml AgNO3 and 5gm dextrose with pH 5.4. Other sample contains 18ml H2O, 2ml AgNO3 and 5gm dextrose with NaOH. The pH of the solutions was adjusted to 10.6 using reagents NaOH. These way four batches of these samples were prepared and that were stored under different temperature conditions (5ºC, 32ºC, 40ºC and 60ºC). First batch was stored at 5ºC, second batch was stored

at 32ºC, Third batch was stored in incubator at 40ºC and fourth batch was also stored in incubator at 60ºC.

**Table-4: Samples subjected to different synthesis conditions**

| Composition | Storage temperature(°C) | pH | Stirring speed(rpm) |
|---|---|---|---|
| 2ml $AgNO_3$, 5g dextrose, 18ml$H_2O$ | 5 | 10.6 | 1150 |
| | 32 | | |
| | 40 | | |
| | 60 | | |
| 2ml $AgNO_3$, 5g dextrose, 18ml$H_2O$ | 5 | 5.4 | |
| | 32 | | |
| | 40 | | |
| | 60 | | |

## 3.2 Characterization of Ag Nanoparticles

### I. UV-Visible Spectrophotometer

The absorption spectrum of the Ag-NPs synthesized was determined by this characterization technique. The Surface Plasmon Resonance (SPR) of the synthesized Ag nanoparticles can be measured using this method. The Spectrophotometer (Perkin-Elmer Lambda-25) was employed for carrying out the ultraviolet-visible (UV-Vis) Spectroscopy in a range of 200 to 800 nm. The sample was directly poured into quartz cuvettes without any further dilution.

### II. Fluorescence Spectrophotometer

The Fluorescence spectrophotometer (Perkin-Elmer LS-45) was employed to determine whether the synthesized Ag nanostructure produced any fluorescence spectrum within a wavelength range of 200 to 600nm. The sample solutions were directly poured into quartz cuvettes without further dilution.

## 3.3 Antimicrobial analysis

### I. Reagents Used

1. Beef extract
2. Peptone
3. Agar agar
4. Distilled water
5. Silver nanoparticle solution

### II. Equipments Used

1. Conical flasks
2. Petri dishes
3. Pipettes
4. Spreader

**III. Instruments Used**

1. Autoclave
2. pH meter
3. BOD incubator

**IV. Methodology**

- Preparation of nutrient broth (for bacterial growth): The following chemicals were weighted (g/ml):
  - Peptone 0.5g%
  - Beef extract 0.3g%
  - Agar 1.5g%
- Required amount of beef and peptone were dissolved in distilled water
- pH was adjusted to 7.2 by adding NaOH
- After that required amount of agar was added to it and allowed for dissolving
- Prepared media was autoclaved at 121ºC for 15 psi
- Along with media other equipments (Petri dishes, pipettes and spreader) are also autoclaved
- After autoclaving, those were allowed to cool
- Then those were kept in laminar flow chamber for 30 minutes under UV-radiation

**V. Addition of Ag nanoparticles**

25µg/ml, 50µg/ml and 100µg/ml Ag-Dextrose sample solution (pH-5.4) were added in three plates and in other three plates 25 µg/ml, 50µg/ml and 100µg/ml Ag-Dextrose sample solution (pH-10.6) were added.

**VI. Inoculation of Test Plates**

*Escherichia coli* (Gram-negative) and *Bacillus subtilis* (Gram-positive) were used in the antibacterial tests.

- Molten agar was poured in each of the six sterile plates containing Ag-Dextrose sample solution and in one more sterile petri plate without Ag-Dextrose sample solution (Control) and then it was allowed to solidify at room temperature
- After solidification, 0.1 ml of microbial inoculums were taken aseptically in each of the seven petri plates and were spread properly by using a spreader

**VII. Incubation**

All petri plates were incubated at 37ºC for 24 hours.

# Chapter4: Results and Discussions

# 4. RESULTS AND DISCUSSIONS:

## 4.1 Green synthesis of silver nanoparticles

### 4.1.1 Characterisation of the as-synthesised Ag-NPs:

Following the protocol mentioned in materials and methods the silver nanoparticles were prepared. In this synthesis method the dextrose acted as both reducing and stabilizing agent. Dextrose reduces silver cations to metallic silver and itself get oxidised to gluconic acid. In this reaction colour of $Ag^{+}$/dextrose solutions with pH of 10.6 changes from colourless to light brown and then dark brown and the other set of samples with pH of 5.4 changes their colour from colourless to light yellow and then to dark yellow as the reaction time increases, indicating the formation of dextrose reduced Ag-NPs of different particle sizes. The silver nanoparticles formed were confirmed using UV-Visible spectroscopy. Using UV-Vis spectroscopy, gradual formation and growth of silver nanoparticles at different reaction times were studied using UV-Vis spectroscopy. UV-Visible spectroscopy is one of the most noted characterization methods to determine the particle.

### 4.1.2 Kinetic Study of Synthesized Silver Nanoparticles

Figure-3, Figure-4 and Figure-5 represent the absorbance intensity at $\lambda_{max}$ of Ag-NPs with dextrose solutions plotted against wavelength for various reduction temperatures (5ºC, 32ºC and 40ºC). The sample solutions have maintained pH of 5.4. It is quite evident from the figures that the time taken for completion of reaction decreases with increase in reduction temperature. In case of the sample solution that has been reduced at 5ºC, the reaction completed after 648 hrs. The reaction completed after 432 hours and 312 hours respectively for sample solutions subjected to thermal reduction at 32°C and 40°C.

Figure-6, Figure-7 and Figure-8 represent the absorbance intensity at $\lambda_{max}$ of Ag-NPs with dextrose solutions plotted against wavelength for various reduction temperatures (5ºC, 32ºC and 40ºC). The sample solutions are treated with NaOH to maintain the pH 10.6. In case of the sample solutions reduced at 5ºC and 32ºC, the reaction completed after 0.83 hrs. In case of sample that has been subjected to thermal reduction at 40ºC; the reaction took 1.83 hours to complete. Higher intensity peaks were obtained for sample that has been reduced at 40ºC. Samples that are treated with NaOH took less time to complete the reaction. Thus it is fast reaction in case of samples with pH of 10.6. Refer to figure-9 and figure-10.

Data of time vs. absorbance is in good match in log scale ($R^2$>0.8). Therefore change of pattern is logarithmic in nature. Representative graphs are shown in figure-11.

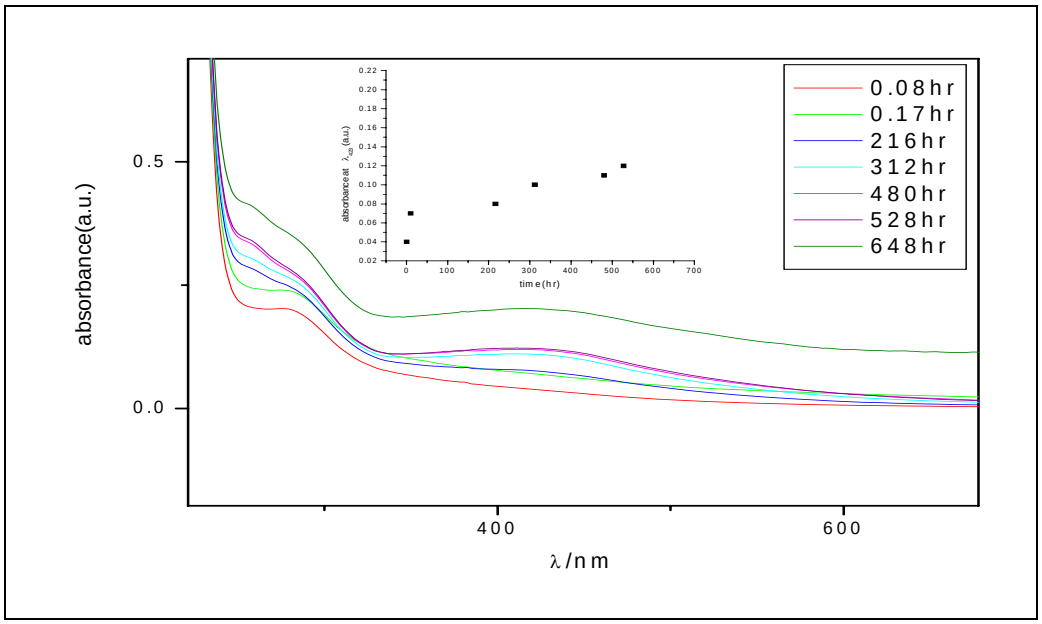



**Figure-3: Time dependent UV-Visible spectra of the aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^+$ to Dextrose 1:$10^3$. Temperature-5°C, pH-5.4, Inset-plot of absorbance at $\lambda_{max}$ (423nm) vs. time**

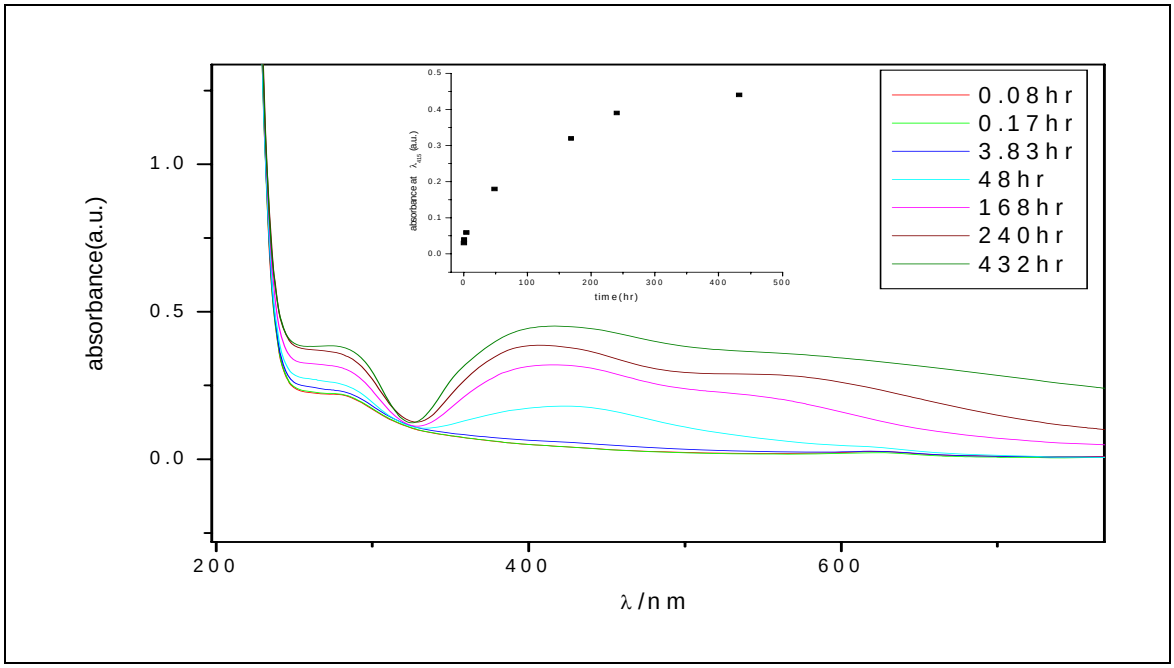



**Figure-4: Time dependent UV-Visible spectra of the aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^+$ to Dextrose 1:$10^3$. Temperature-32°C, pH-5.4, Inset-plot of absorbance at $\lambda_{max}$ (415nm) vs. time**

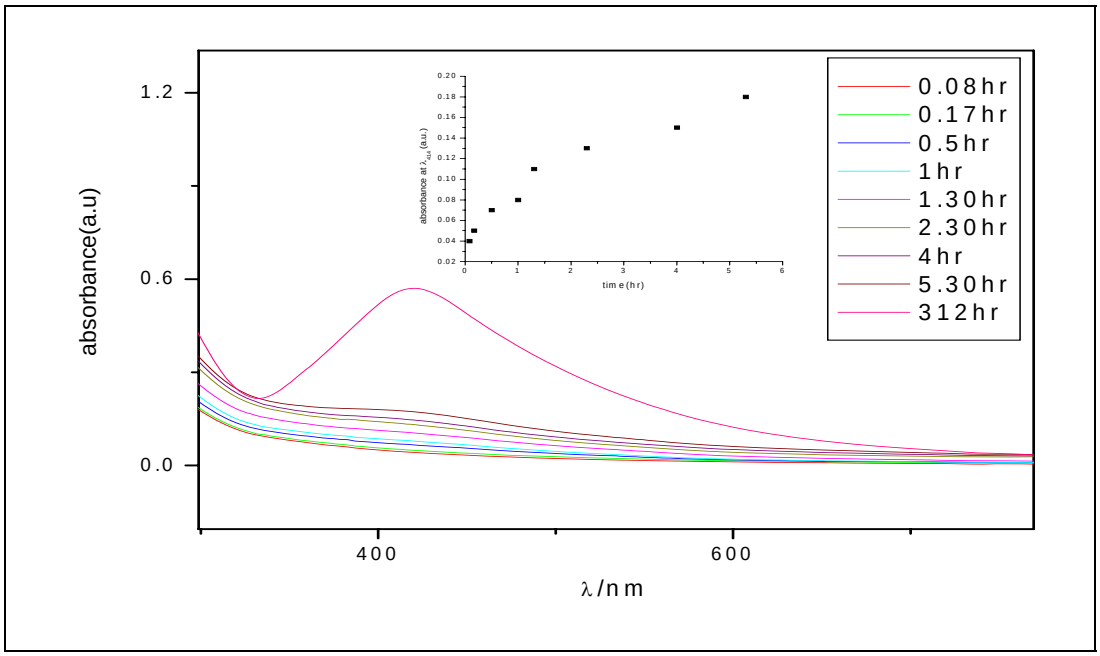



**Figure-5: Time dependent UV-Visible spectra of the aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^+$ to Dextrose 1:$10^3$. Temperature-40°C, pH-5.4, Inset-plot of absorbance at $\lambda_{max}$ (414nm) vs. time**

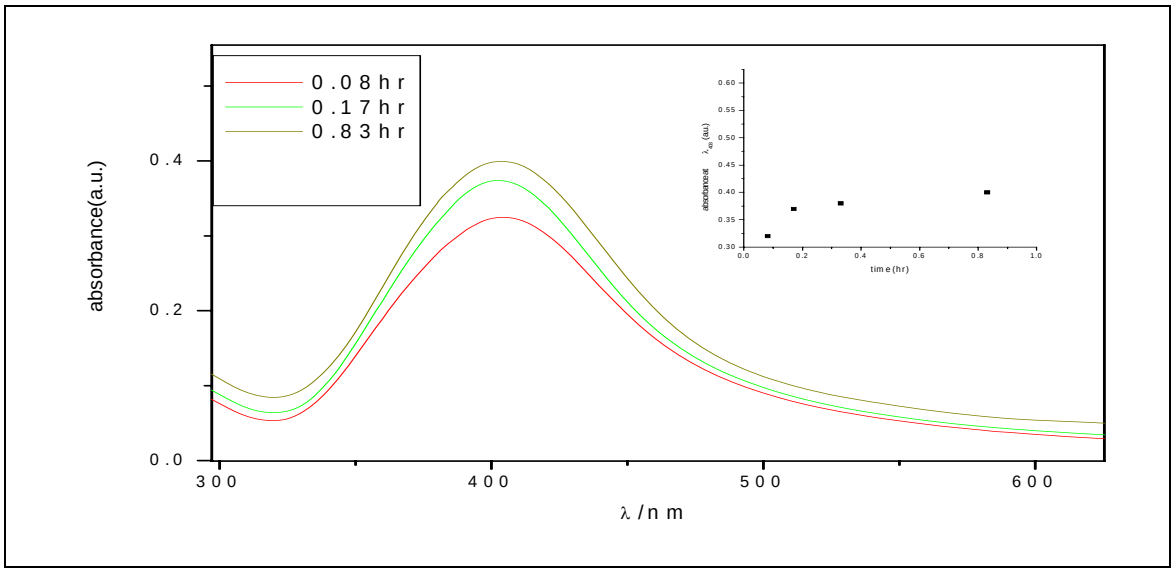



**Figure-6: Time dependent UV-Visible spectra of the aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^+$ to Dextrose 1:$10^3$. Temperature-5°C, pH-10.6, Inset-plot of absorbance at $\lambda_{max}$ (403nm) vs. time**

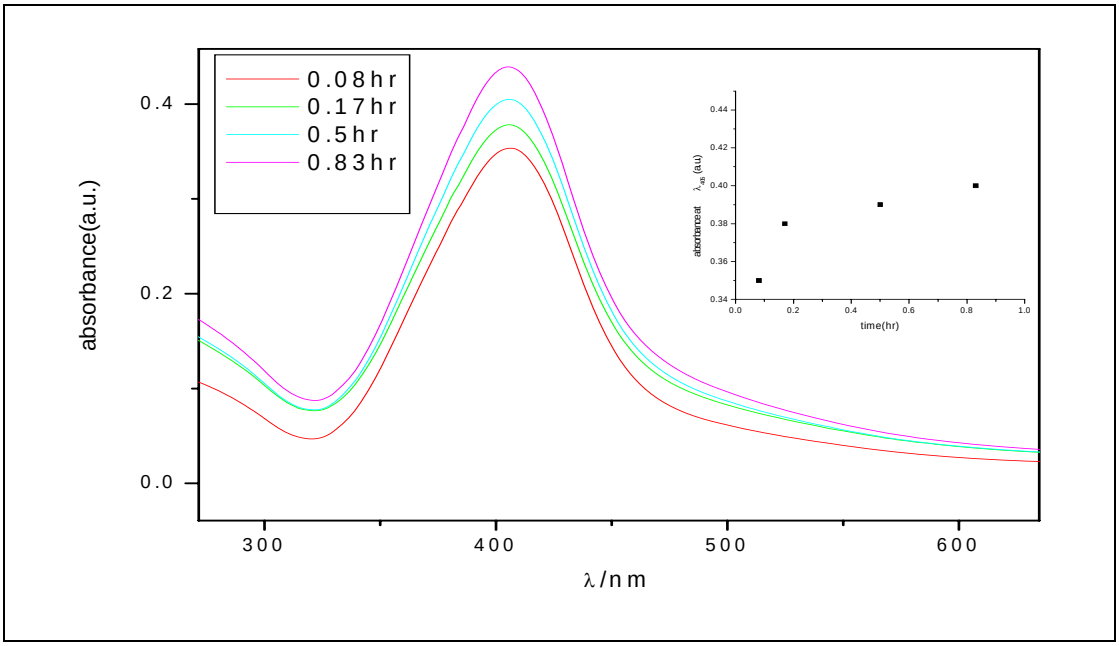



**Figure-7: Time dependent UV-Visible spectra of the aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^{+}$ to Dextrose $1:10^{3}$. Temperature-32°C, pH-10.6, Inset-plot of absorbance at $\lambda_{max}$ (405nm) vs. time**

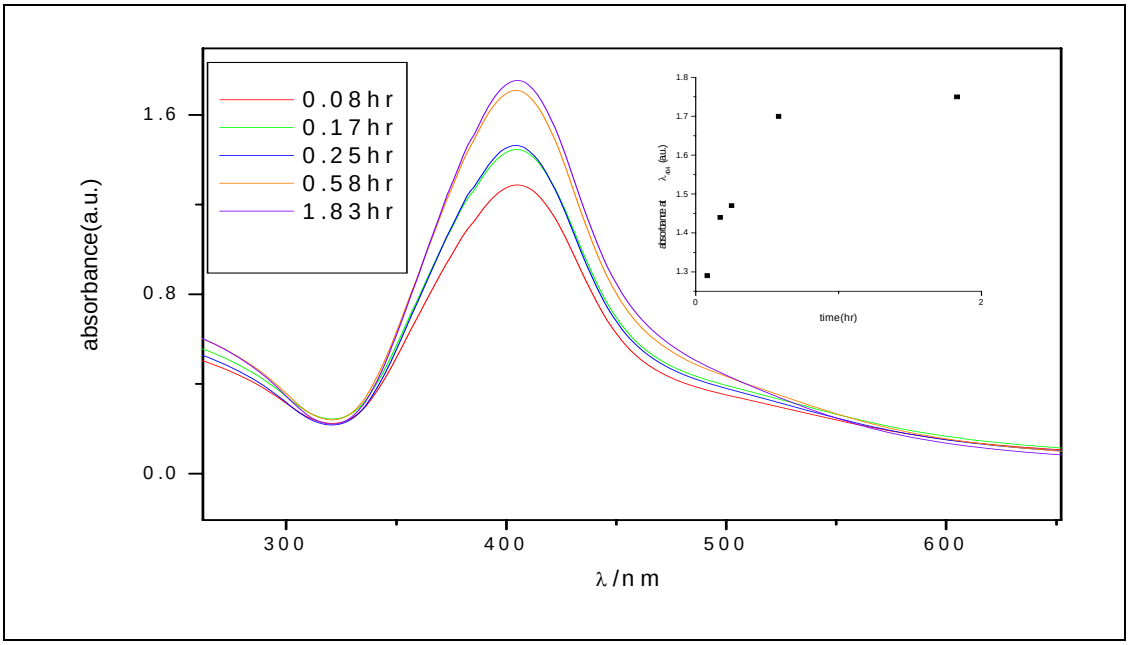



**Figure-8: Time dependent UV-Visible spectra of the aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^{+}$ to Dextrose $1:10^{3}$. Temperature-40°C, pH-10.6, Inset-plot of absorbance at $\lambda_{max}$ (404nm) vs. time**

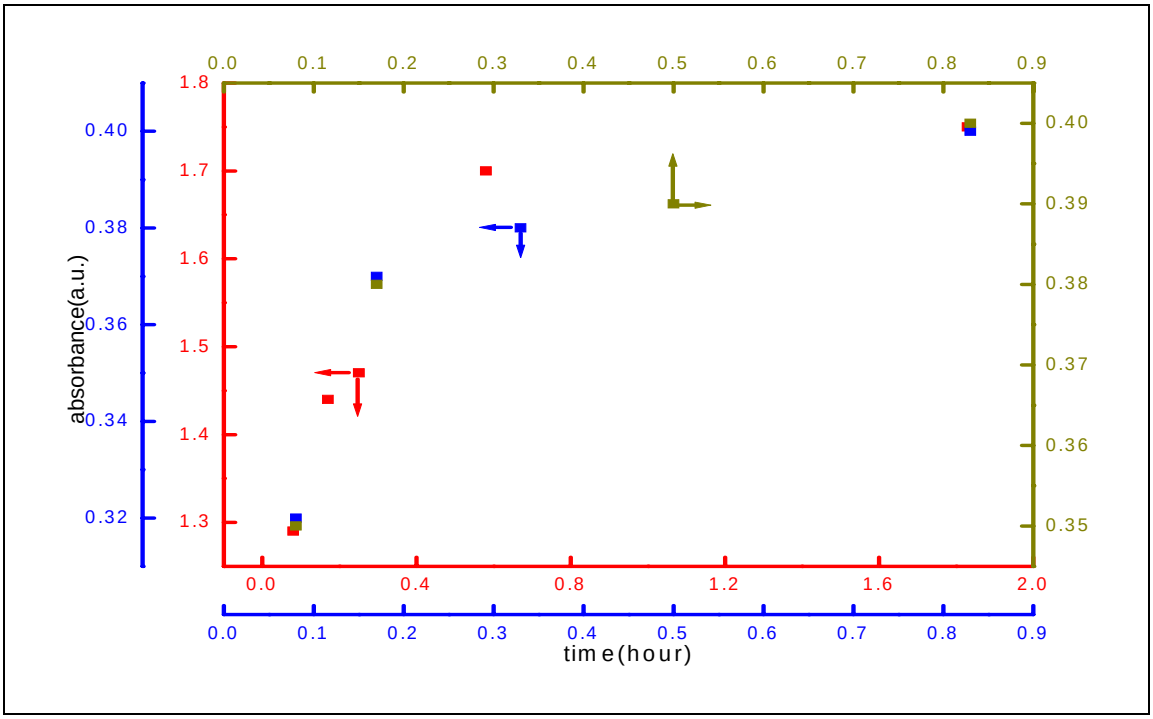



**Figure-9: Plot of absorbance vs. time of the reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^{+}$ to dextrose $1:10^{3}$, pH-10.6 at 5°C, 32°C and 40°C respectively.**

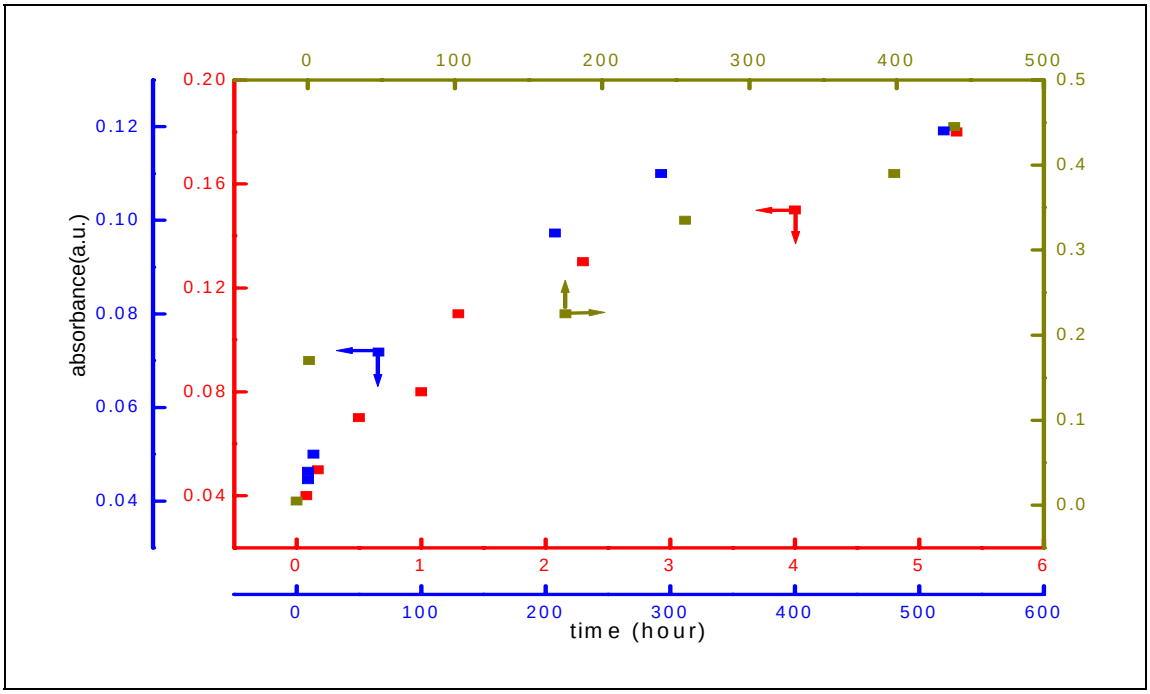



**Figure-10: Plot of absorbance vs. time of the reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^{+}$ to dextrose $1:10^{3}$, pH-5.4 at 5°C, 32°C and 40°C respectively.**

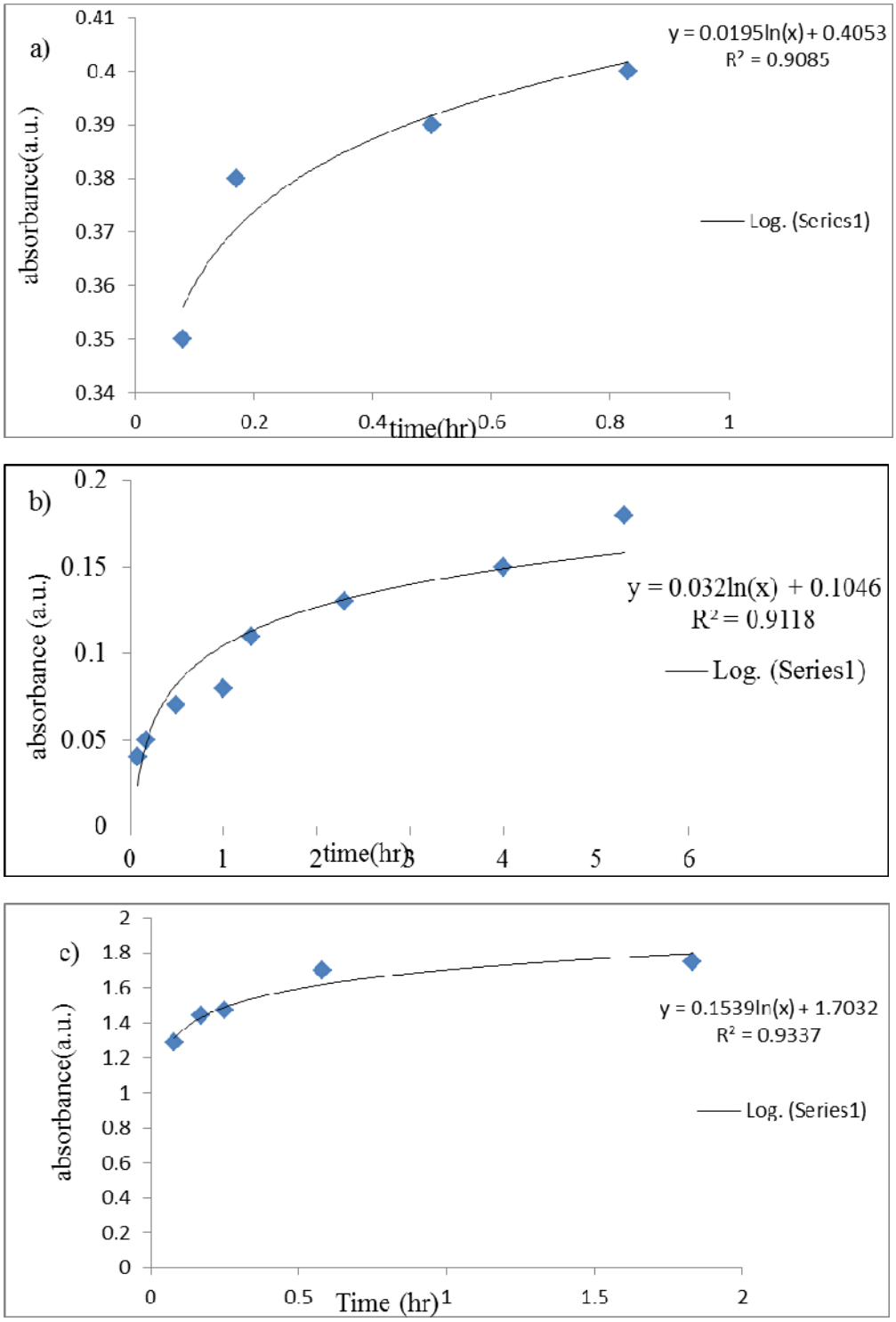



**Figure-11: Time vs. absorbance profile of Ag-Dextrose sample solution subjected to thermal reduction at a) temp-32°C, pH-10.6; b) temp-40°C, pH-5.4; c) temp-40°C, pH-10.6**

### 4.1.3 Effect of Temperature

Effect of temperature on characteristics of synthesized silver nanoparticles was studied. This study was conducted on nanoparticles synthesized at different reduction temperatures (5ºC, 32ºC and 40ºC). Keeping the other parameters constant and varying the temperature nanoparticles were synthesized and the effect of temperature on characteristics of these synthesized nanoparticles was studied. This study was conducted for two sets of samples reduced at different temperatures. One of the set is having pH of 5.4 and the other set is having pH of 10.6. Figure-3 represent UV-Visible spectra of sample reduced at 5ºC having pH of 5.4. Here the reaction is slow. The reaction was completed after 648 hrs. At lower temperature rate of reduction and rate of decomposition of dextrose is slow. SPR peaks obtained in these spectra gave broader peaks, which in turn can be concluded as the nanoparticles formed are highly poly-dispersed in nature. SPR absorbance is at $\lambda_{max}$ 423nm. This particular wavelength 423nm is the characteristic Surface Plasmon Resonance absorption of spherical Ag-NPs (S. Shankar et al). Thus we can say that the nanoparticles formed are spherical in shape. Even the wavelength of peaks obtained is higher compared to other higher temperatures, indicating that increase in silver nanoparticle size at this temperature. Figure-4 represent UV-Visible spectra of sample reduced at room temperature (32ºC) having pH of 5.4. It took 432 hr for the completion of reaction. Compared to reaction at 5ºC, this reaction is faster. SPR peaks obtained are broad, which indicates that Silver nanoparticles obtained are poly-dispersed in nature. Intensity of SPR peaks of this sample is higher than that of sample reduced at 5ºC. Here absorbance value is almost 0.5, but in case of sample reduced at 5ºC have an absorbance value less than 0.5. Spectra show peak at 415nm, which indicates formation of smaller nanoparticles. Figure-5 represent the sample reduced at 40ºC having pH of 5.4. It took 312 hr for the completion of reaction. This reaction is faster compared to sample reduced at 5ºC and 32ºC. Absorbance value is 0.6. Absorbance increased with higher temperature. Spectra show peak at 414nm, indicating that the nanoparticles formed are smaller. Figure-6 represent the sample reduced at 5ºC having pH of 10.6. UV-Vis spectra shows broad peak. Bur not as broad as sample with pH 5.4. As the pH increase there is shift from poly-dispersity. Absorbance value is 0.4. Spectra show peak at 403nm. With the influence of higher pH peak here shifted to somewhat shorter wavelength. Figure-7 represent the sample reduced at room temperature (32ºC) having pH of 10.6. At this temperature UV-Vis spectra show narrow peak at lower wavelength region (405nm) which indicates that the nanoparticles formed are more or less mono-disperse in nature. Figure-8 represent the sample reduced at 40ºC having pH of 10.6. UV- Visible spectra show sharp and narrow peak at lower wavelength (404nm) which indicates that the nanoparticles formed are mono-disperse in nature. Absorbance value is more than 1.6. Overall conclusions for effect of temperature are as follows:

- With increase in temperature intensity of SPR peak increased.
- Increase in reaction temperature UV spectra show sharp narrow peaks at lower wavelength region, which indicate the formation of smaller nanoparticles.
- At lower reaction temperature, the peaks observed at higher wavelength regions which clearly indicate increase in silver nanoparticle size.

- At higher temperature silver nanoparticle aggregate very quickly.

#### 4.1.4.1 Stability

Stability of silver nanoparticles synthesized at different temperatures (5ºC, 32ºC, 40ºC and 60ºC) was studied. Figure-12 represents the UV-Vis spectra of sample that has been synthesized at 5ºC with pH of 10.6. In case of this sample the reaction completed after 5 days. After that the stability of this sample was checked by analysing the surface Plasmon resonance (SPR) peaks obtained, it was found that the sample was stable for more than 30 days. After $40^{th}$ day the peak got shifted. After $5^{th}$ day the intensity of the peaks obtained found decreasing. Intensity decreased due to oxidation and agglomeration. Figure-13 represents the UV-Vis spectra of sample synthesized at 32ºC with pH of 5.4. For this sample, the reaction was completed on $11^{th}$ day. After that the stability of the sample was studied. SPR peaks obtained were analysed and found out that the sample was stable for more than 30 days. Peak shifted after $50^{th}$ day. Intensity of the peaks was found decreased after $11^{th}$ day due to oxidation and agglomeration. Figure-14 represent the UV-Vis spectra of sample that has been synthesized at 40ºC having pH of 5.4. Highest peak was obtained on $30^{th}$ day. Reaction completed on $30^{th}$ day. After that stability was checked by analysing the SPR peaks obtained and it was found that the sample was stable for another 30 days. Peak got shifted after $60^{th}$ day. Intensity of peaks was found decreasing gradually after $30^{th}$ day. This decrease in intensity was due to oxidation and agglomeration. Figure-15 represent the UV-Vis spectra of sample synthesized at 60ºC having pH of 10.6. For this sample the reaction completed on $2^{nd}$ day itself. This was very fast reaction. And then the stability of sample was checked. SPR peak got shifted after $14^{th}$ day. Sample was stable for 12 days. In this graph, UV spectra of dextrose were clearly visible at about wavelength of 270 nm. The intensity of the peaks formed by dextrose was also found increasing. From these observations it was inferred that the decomposition of dextrose happens faster at higher temperature. At higher temperature sample is not stable. Background scattering was observed on $26^{th}$ day. This is clearly visible in the graph that shows higher intensity due to background scattering on $26^{th}$ day. Scattering happens due to turbidity, which in turn increase the background scattering.

Stability of samples with pH 5.4 reduced at different temperatures (32ºC, 40ºC and 60ºC) was studied. Figure-16 represents the UV-Vis spectra of sample reduced at 32ºC with pH of 5.4. Reaction was completed on $20^{th}$ day. After that stability of sample was checked and it was found that the sample was stable for 20 days. On $40^{th}$ day UV-Vis spectra did not give any SPR peak. Figure-17 represents the UV-Vis spectra of sample that has been thermally reduced at 40ºC with pH of 5.4. Reaction was completed on $30^{th}$ day. After that stability was checked. SPR peaks were analysed and found out that the sample was stable for 30 days. After 60th day peak got shifted.

Figure-18 represent the UV-Vis spectra of sample reduced at 60ºC having pH of 5.4. Reaction was completed on $2^{nd}$ day. Stability was checked for this sample. This sample was stable for 3 days. From this graph it is also evident that decomposition of dextrose happened faster at higher temperature. Thus it can be concluded that at higher temperature sample is not stable.

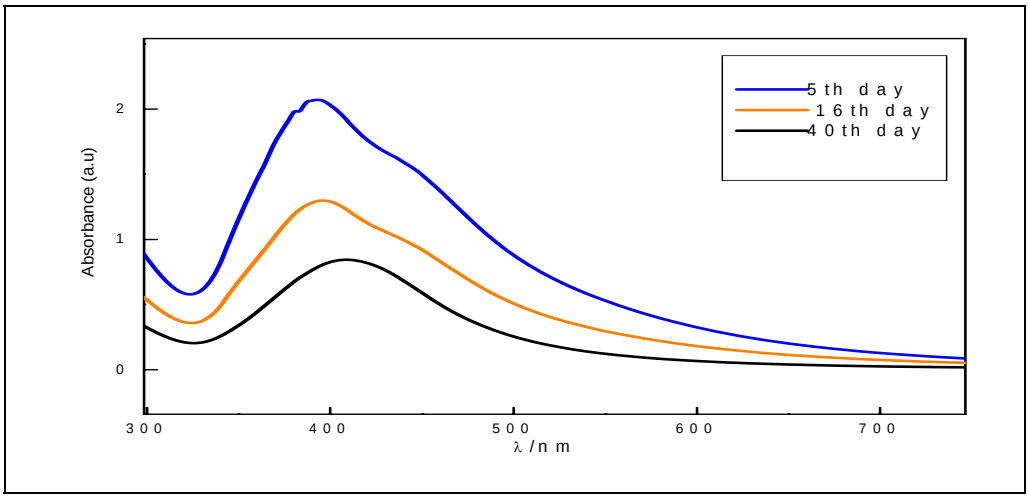



**Figure-12: UV-Vis spectra of aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^{+}$ to Dextrose $1:10^{3}$ .Temperature-5ºC, pH-10.6**

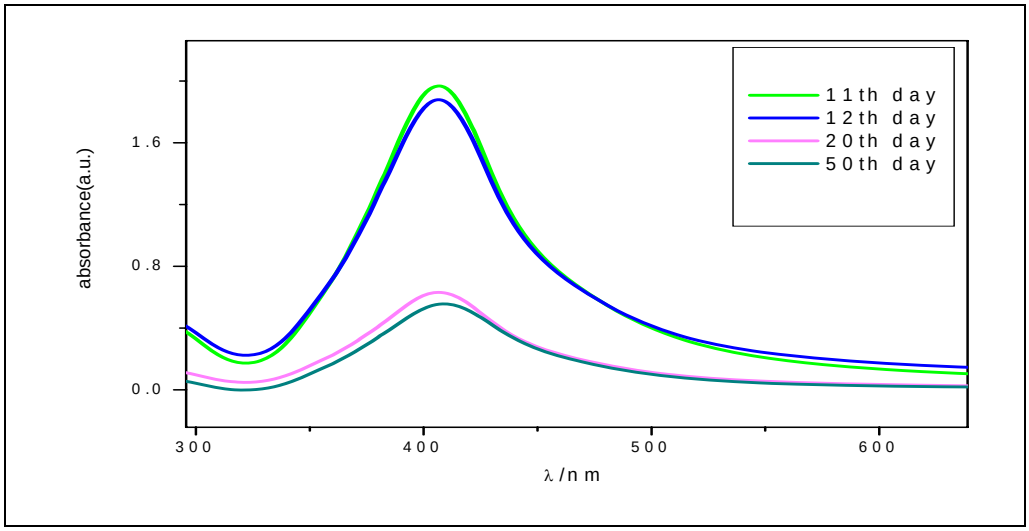



**Figure-13: UV-Vis spectra of aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^{+}$ to Dextrose $1:10^{3}$ .Temperature-32ºC, pH-10.6**

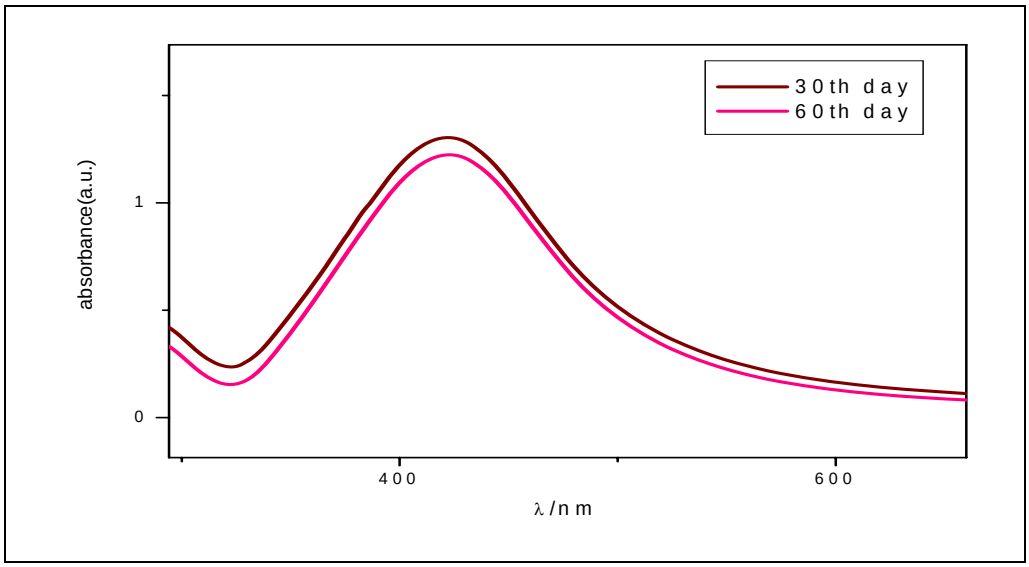



**Figure-14: UV-Vis spectra of aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^+$ to Dextrose $1:10^3$ .Temperature-40ºC, pH-10.6**

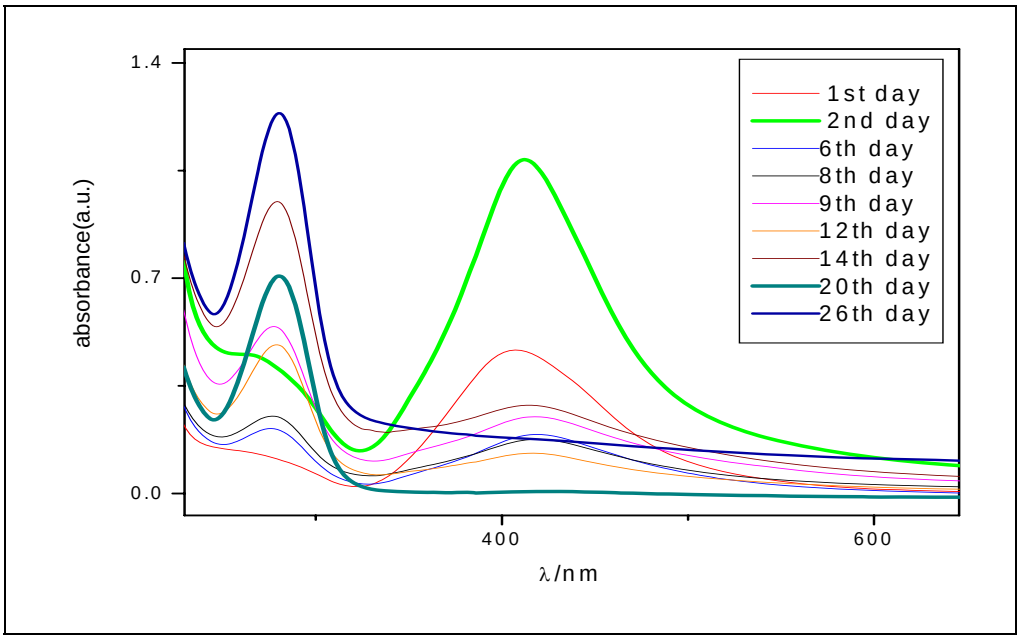



**Figure-15: UV-Vis spectra of aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^+$ to Dextrose $1:10^3$ .Temperature-60ºC, pH-10.6**

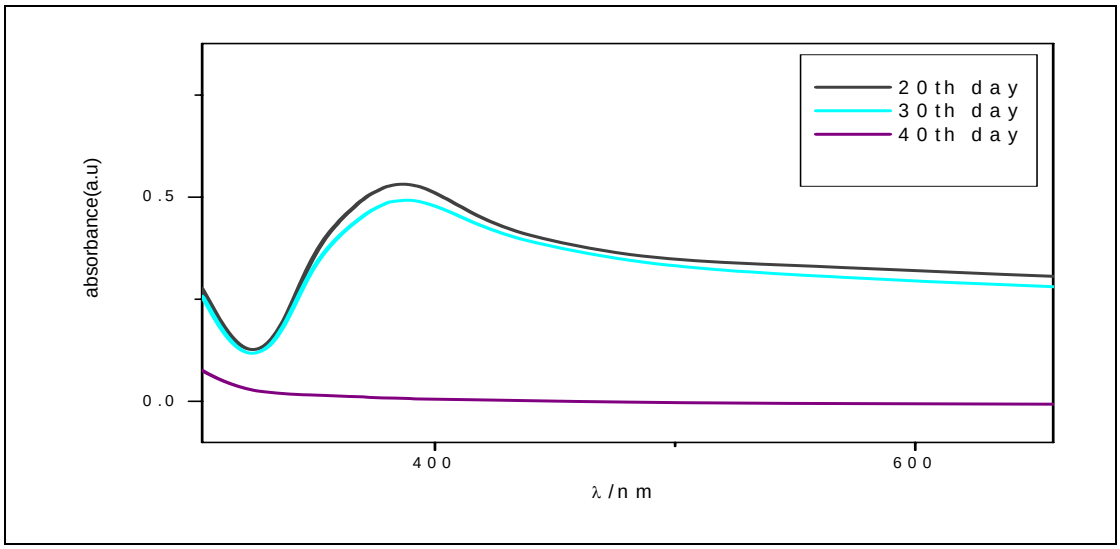



**Figure-16: UV-Vis spectra of aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^+$ to Dextrose $1:10^3$ .Temperature-32ºC, pH-5.4**

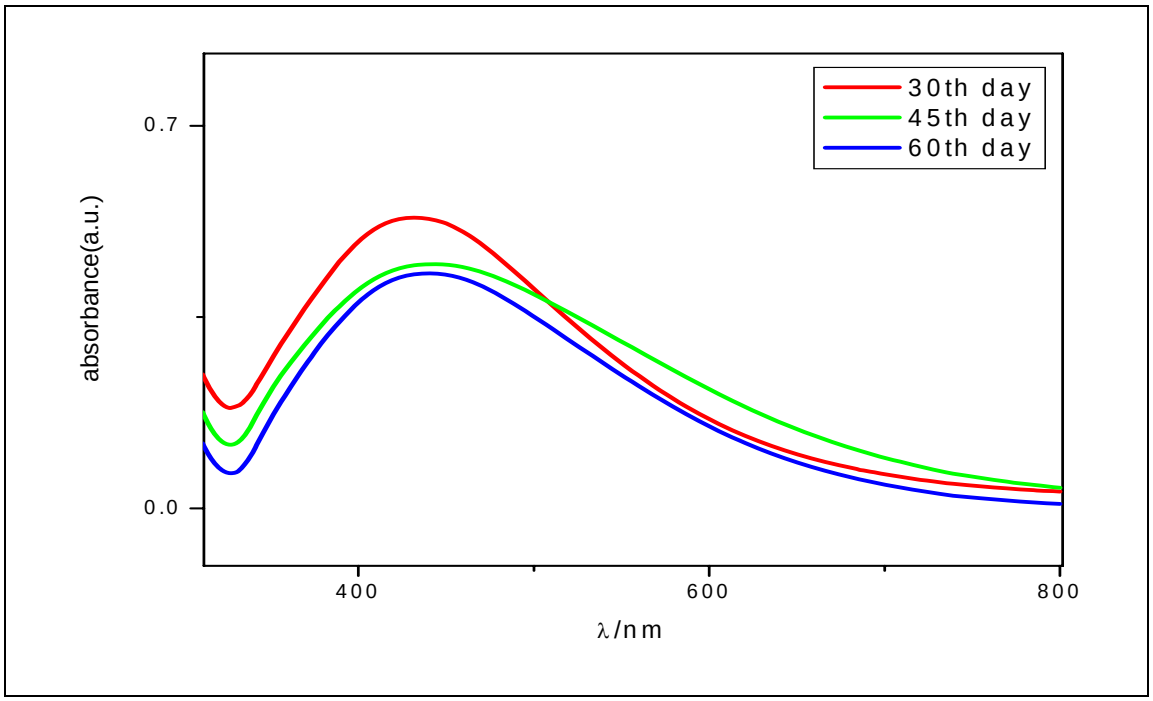



**Figure-17: UV-Vis spectra of aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^+$ to Dextrose $1:10^3$ .Temperature-40ºC, pH-5.4**

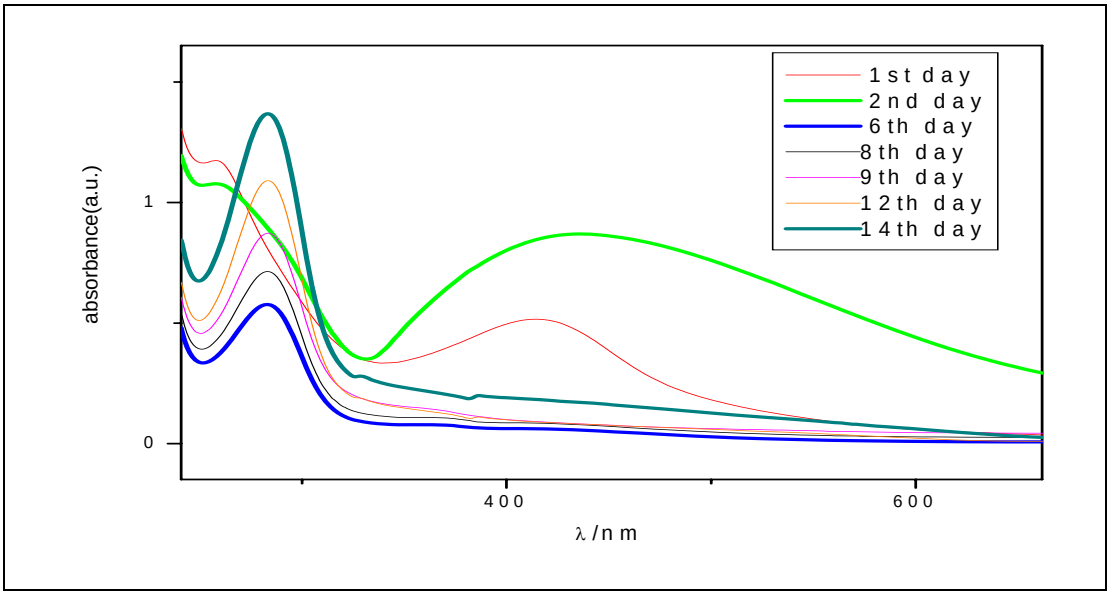



**Figure-18: UV-Vis spectra of aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^+$ to Dextrose $1:10^3$ .Temperature-60ºC, pH-5.4**

**Table-5: Study of Stability**

| Composition | Storage Temperature(°C) | Initial Plasmon peak position(nm) | Final Plasmon peak position(nm) | Stability |
|---|---|---|---|---|
| 2ml $AgNO_3$, 5g dextrose, 18ml$H_2O$, pH-10.6 | 5 | 392 | 409 | More than one month |
| | 32 | 405 | 408 | More than one month |
| | 40 | 421 | 423 | 30 days |
| | 60 | 411 | 415 | 12 days |
| 2ml $AgNO_3$, 5g dextrose, 18ml$H_2O$, pH-5.4 | 32 | 385 | 387 | 20 days |
| | 40 | 432 | 439 | 30 days |
| | 60 | 414 | 434 | 3 days |

### 4.1.4.2 Stability of silver nanoparticles after two months

Figure-19 represents the UV-Visible spectra of sample synthesized at 5ºC having pH of 5.4. The intensity of the peak increases even after two months. The SPR peaks obtained were broad, indicating that the nanoparticles formed are poly-dispersed in nature. The increase of the absorbance of the Ag-Dextrose sample solution after 2 months of storing means that the reaction was continued at 5ºC temperature until all silver ions are converted to silver nanoparticles.

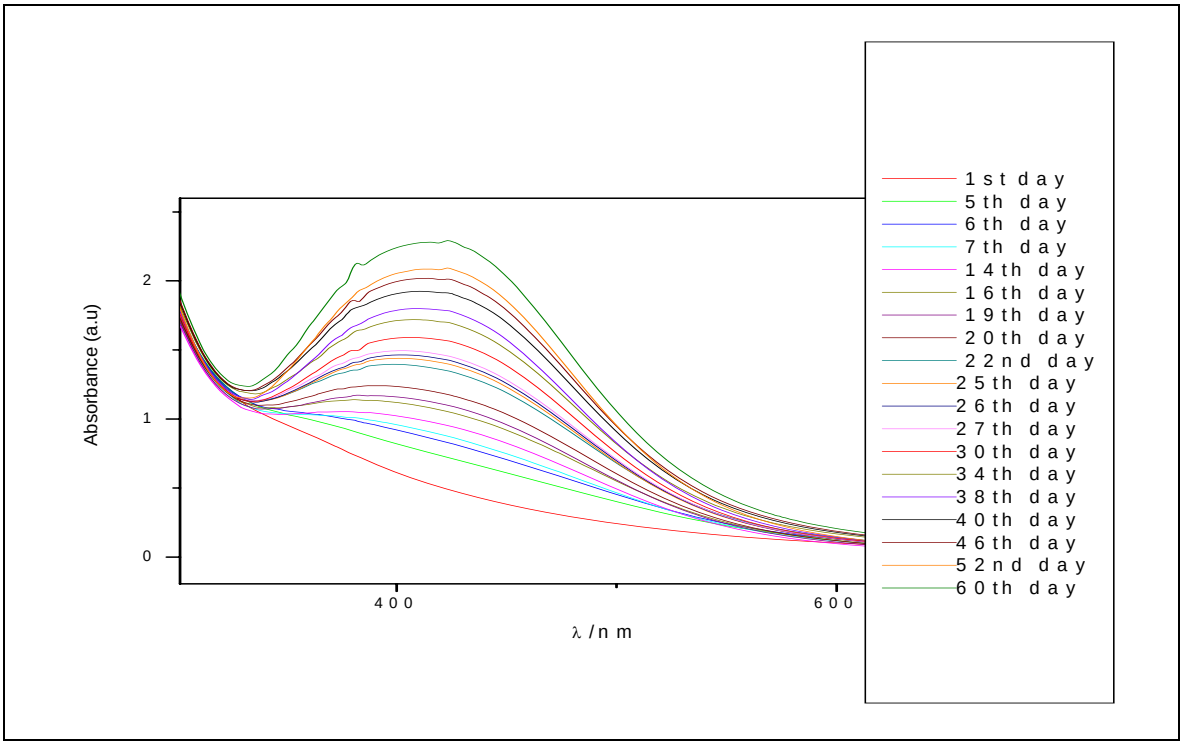



**Figure-19: UV-Visible spectra of aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^{+}$ to Dextrose $1:10^{3}$ .Temperature-5ºC, pH-5.4**

### 4.1.5 Fluorescence spectroscopic observations

Ag-Dextrose sample solutions were found to generate fluorescence spectrum. Figure-20 represents the spectral data for a) Ag-Dextrose sample solution of ratio $1:10^{3}$ and b) dextrose solution having pH 5.4. An emission spectrum is observed at 424 nm when dextrose solution was excited at 340 nm. The corresponding excitation spectrum was obtained at 331 nm when this solution was emitted at 450 nm. But for Ag-Dextrose sample solution an emission peak is observed at 431 nm when sample solution was excited at 340 nm. The corresponding excitation spectrum obtained at 335 nm when sample solution was emitted at 450 nm. That is a mirror image of emission spectrum. The decreasing of peak height and shifting of peak position possibly is due to interaction of dextrose and Ag-NPs surface.

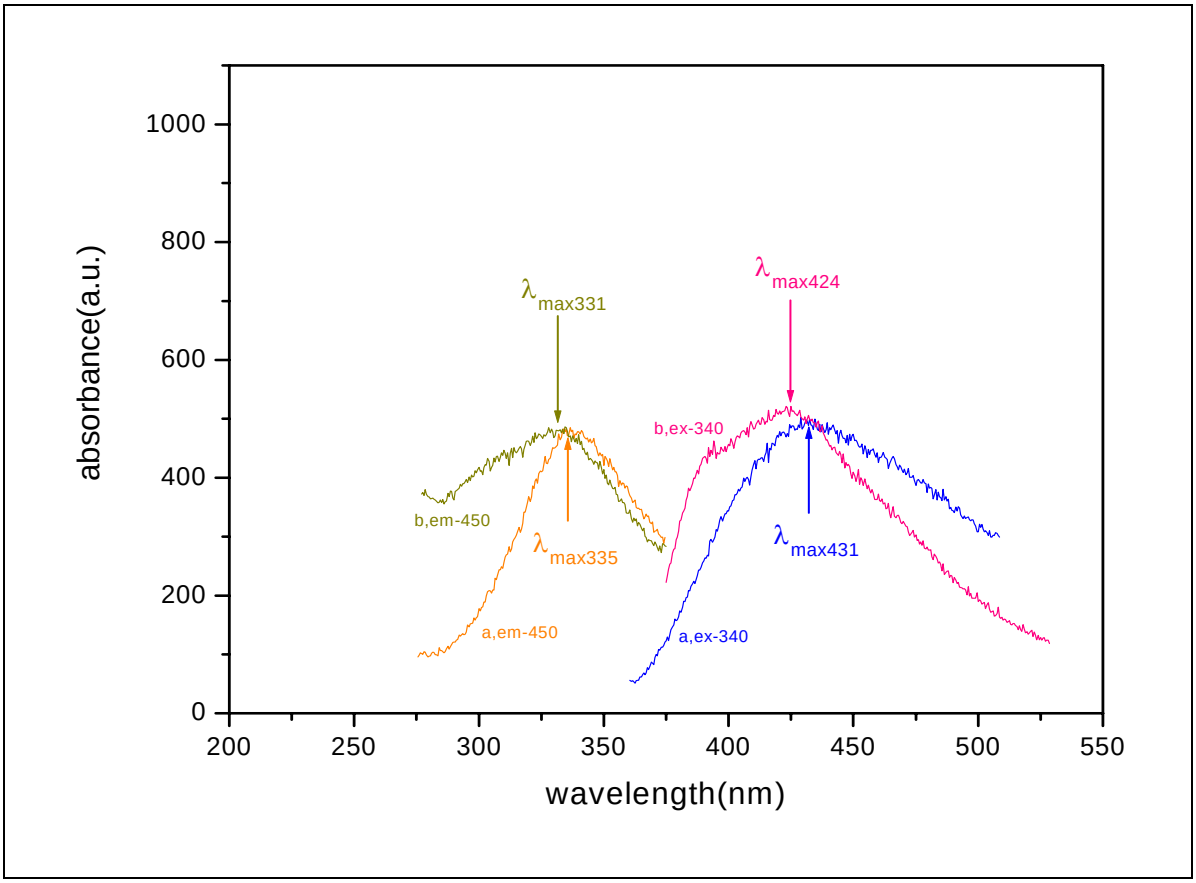


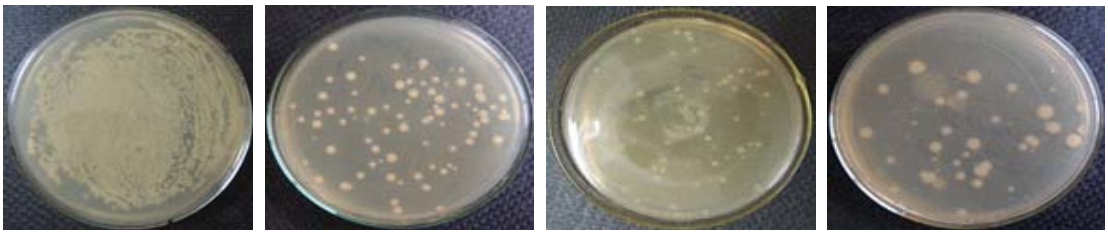

**Figure-20: Emission and excitation peaks of fluorescence spectrum of the aqueous reaction mixture containing silver nitrate and dextrose with molar ratio $Ag^+$ to Dextrose $1:10^3$. Temperature-5°C, pH-5.4, a) Ag-dextrose sample solution; b) dextrose**

### 4.2 Antimicrobial activity

After 24 hours incubation, each plate was examined. The antimicrobial efficacy of the materials were investigated by supplementing nutrient-agar media with an aqueous suspension of Ag-Dextrose sample soluton at various concentrations. The presence of Ag-Dextrose sample solution in the nutrient-agar plates was able to inhibit the formation of colonies for both types of bacteria (gram-negative and gram-positive). The effect was more noticeable for the ***E. coli*** compared to the ***B. subtilis***, as the nanoparticles were able to substantially reduce the number of colonies at a final Ag-Dextrose sample solution concentration of 25 µg/ml. The formation of colonies for ***E. coli*** strains was fully inhibited when 100 µg/ml of the particles were added. Even for 50 µg/ml Ag-Dextrose sample solution (pH-10.6) ***E. coli*** growth was inhibited totally. At the same concentration of Ag-dextrose sample solution (50 µg/ml, pH-10.6) effective inhibition was shown for ***B. subtilis***. Thus it can be concluded that silver nanoparticles having pH 10.6 have shown higher antibacterial

activity at 100 µg/ml and 50 µg/ml concentration than the silver nanoparticles having pH 5.4(100 µg/ml and 50 µg/ml concentration).

**A) *E.Coli***



A1-control A2-25 µg/ml, pH-5.4 A3-25 µg/ml, pH-10.6 A4-50 µg/ml, pH-5.4

A5-50 µg/ml, pH-10.6 A6-100 µg/ml, pH-10.6 A7-100 µ/ml, pH-10.6

**Figure-21: Petri dishes with nutrient-agar inoculated with A) *E. coli*, showing variable numbers of colonies when supplemented with different amounts of nanoparticles.**

**B) *Bacillus subtilis***

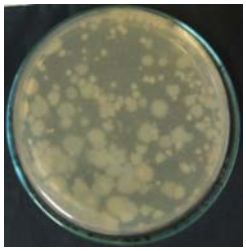
B1-control

B2-25 µg/ml, pH-5.4

B3-25 µg/ml, pH-10.6

B4-50 µg/ml, pH-5.4

B5-50 µg/ml, pH-10.6



B6-100 µg/ml, pH-5.4

B7-100 µg/ml, pH-10.6

**Figure-22: Petri dishes with nutrient-agar inoculated with B) *B. subtilis*, showing variable numbers of colonies when supplemented with different amounts of nanoparticles.**

**Table-6: Number of colonies formed**

| **Bacteria name** | **Control** | **25µg/ml Ag-NPs** | | **50µg/ml Ag-NPs** | | **100µg/ml Ag-NPs** | |
|---|---|---|---|---|---|---|---|
| | | **pH-5.4** | **pH-10.6** | **pH-5.4** | **pH-10.6** | **pH-5.4** | **pH-10.6** |
| A)*Escherichia coli* | 670 | 82 | 81 | 40 | 25 | nil | nil |
| B)*Bacillus subtilis* | 737 | 250 | 234 | 178 | 138 | 100 | 30 |

# Chapter5- Conclusion

## Conclusion

In summary complete green synthesis of silver nanoparticles using dextrose has been achieved. Dextrose acted as both reducing as well as stabilizing agent in this synthesis process. Kinetics study of the reduction of $Ag^+$ ions and the formation of Ag-NPs at different temperatures (5ºC, 32ºC and 40ºC) was carried out. Less time was taken for completion of reaction at higher temperature. Data of time vs. absorbance is in good match in log scale ($R^2$>0.8). Temperature effect on UV-Vis spectra of Ag-NPs was studied. It was observed that 1) Increase in reaction temperature UV spectra show sharp narrow peaks at lower wavelength region, which indicate the formation of smaller nanoparticles, 2) At lower reaction temperature, the peaks observed at higher wavelength regions which clearly indicate increase in silver nanoparticle size, 3) At higher temperature silver nanoparticle aggregate very quickly. Stability of silver nanoparticles stored at different temperature conditions (5ºC, 32ºC, 40ºC and 60ºC) was studied. Nanocolloids stored at lower temperature were stable for more than 1 month. At higher temperature (60ºC) stability was very less. 4) For Ag-NPs formation emission and excitation spectrum were shifted in Ag-NPs sample solution. The as-synthesized Ag-NPs show good antibacterial activity against Gram-negative bacteria *E. coli.* It also showed antibacterial activity against Gram-positive bacteria *B. subtilis* at higher concentration. These nanoparticles with antibacterial potency may be exploited for various biomedical applications, food packaging and food processing equipment and so on.

# Chapter6- References

## References


1. Q. Chaudhry, L. Castle and R. Watkins; Nanotechnologies in Food; RSC Nanoscience & Nanotechnology; 2010; vol. 14; pp. 1-4.

2. J. L. Clement and P.S. Jarrett; "Antimicrobial silver"; Metal Based Drugs Journal; 1994; vol. 1(5–6); pp. 467-482

3. A. B. G. Lansdown; "Silver in healthcare: Its Antimicrobial Efficacy and Safety in Use; Royal Society of Chemistry; 2010; pp. 159.

4. J. Y. Maillard and P. Hartemann; "Silver as an antimicrobial: facts and gaps in knowledge"; Critical Reviews in Microbiology; 2013; vol. 39; pp. 373–383.

5. H. J. Johnston, G. Hutchison, F. M. Christensen, S. Peters, S. Hankin and V. Stone; "A review of the in vivo and in vitro toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity"; Critical Reviews in Toxicology; 2010; vol. 40(4); pp. 328–346.

6. V. L. Colvin, M. C. Schlamp, A. P. Alivisatos; "Light-emitting diodes made from cadmium selenide nanocrystals and a semiconducting polymer"; Nature; 1994; vol. 370; pp. 354–357.

7. Y. Wang and N. Herron; "Nanometer-Sized Semiconductor Clusters: Materials Synthesis, quantum Size effects, and Photophysical Properties"; The Journal of Physical Chemistry; 1991; vol. 95; pp. 525–532.

8. G. Schmid; "Large clusters and colloids. Metals in the embryonic state"; Chemical Reviews; 1992; vol. 92; pp. 1709–1727.

9. A. J. Hoffman, G. Mills, H. Yee and M. R. Hoffman; "Q-sized cadmium sulfide: Synthesis, Characterization, and Efficiency of Photoinitiation of Polymerization of Several Vinylic Monomers"; The Journal of Physical Chemistry; 1992; vol. 96; pp. 5546–5552.

10. J. F. Hamilton and R. C. Baetzold; "Catalysis by Small Metal Clusters"; Science; 1979; vol. 205; pp. 1213–1220.

11. H. S. Mansur, F. Grieser, M. S. Marychurch, S. Biggs, R. S. Urquhart, D. Furlong; "Photoelectrochemical properties of 'qstate' cds particles in arachidic acid langmuir-blodgett films"; Journal of Chemical Society, Faraday Transactions; 1995; vol.91; pp. 665–672.

12. S Senapati; Ph.D. Thesis; India: University of pune; Biosynthesis and immobilization of nanoparticles and their applications; 2005; pp. 1–57.

13. T. Klaus-Joerger, R. Joerger, E. Olsson, C. Granqvist; "Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science"; Trends in Biotechnology; 2001; vol. 19; pp. 15–20.

14. M. Sastry, A. Ahmad, M. I. Khan, R. Kumar; "Biosynthesis of metal nanoparticles using fungi and actinomycete"; Current Science; 2003; vol. 85; pp. 162–170.

15. S. Iravani; “Green synthesis of metal nanoparticles using plants”; Green Chemistry; 2011; vol. 13; pp. 2638–2650.

16. H. Korbekandi, S. Iravani and S. Abbasi; “Production of nanoparticles using organisms”; Critical Reviews in Biotechnilogy; 2009; Vol. 29; pp. 279–306.

17. F. Kruis, H. Fissan and B. Rellinghaus; “Sintering and evaporation characteristics of gas-phase synthesis of size-selected PbS nanoparticles”; Materials Science and Engineering B; 2000; vol. 69; pp. 329–334.

18. M. H. Magnusson, K. Deppert, J. O. Malm, J. O. Bovin and L. Samuelson; “Gold nanoparticles: Production, reshaping, and thermal charging”; Journal of Nanoparticle Research; 1999; vol.1; pp. 243–251.

19. J. Jung, H. Oh, H. Noh, J. Ji and S. Kim; “Metal nanoparticle generation using a small ceramic heater with a local heating area”; Journal of Aerosol Science; 2006; vol. 37; pp. 1662–1670.

20. T. Tsuji, K. Iryo, N. Watanabe and M. Tsuji; “Preparation of silver nanoparticles by laser ablation in solution: influence of laser wavelength on particle size”; Applied Surface Science; 2002; vol. 202; pp. 80–85.

21. T. Tsuji, T. Kakita and M. Tsuji; “Preparation of nano-size particle of silver with femtosecond laser ablation in water”; Applied Surface Science; 2003; vol. 206; pp. 314–320.

22. D. C. Tien, K. H. Tseng, C. Y. Liao, J. C. Huang and T. T. Tsung; “Discovery of ionic silver in silver nanoparticle suspension fabricated by arc discharge method”; Journal of Alloys and Compounds; 2008; vol. 463; pp. 408–411.

23. J. Siegel, K. Ondoej, U. Pavel, K. Z, PSlepieka and V Svoreik; “Progressive approach for metal nanoparticle synthesis”; Materials Letters; 2012; vol. 89; pp. 47–50.

24. B. Wiley, Y. Sun, B. Mayers and Y. Xi; “Shape-controlled synthesis of metal nanostructures: the case of silver”; Chemistry - A European Journal; 2005; vol. 11; pp. 454–463.

25. D. D. Evanoff, Jr. and G. Chumanov; “Size-controlled synthesis of nanoparticles measurement of extinction, scattering, and absorption cross sections”; The Journal Physical Chemistry B; 2004; vol. 108; pp. 13957–13962.

26. G. Merga, R. Wilson, G. Lynn, B. Milosavljevic and D. Meisel; “Redox Catalysis on “naked” silver nanoparticles”; The Journal of Physical Chemistry C; 2007; vol. 111; pp. 12220–12206.

27. M. Oliveira, D. Ugarte, D. Zanchet and A. Zarbin; “Influence of synthetic parameters on the size, structure, and stability of dodecanethiol-stabilized silver nanoparticles”; Journal of Colloid and Interface Science; 2005; vol. 292; pp. 429–435.

28. D. Kim, S. Jeong and J. Moon; “Synthesis of silver nanoparticles using the polyol process and the influence of precursor injection”; Nanotechnology; 2006; vol. 17; pp. 4019.

29. Y. Zhang, H. Peng, W. Huang, Y. Zhou and D. Yan; “Facile preparation and characterization of highly antimicrobial colloid Ag or Au nanoparticles”; Journal of Colloid and Interface Science; 2008; vol. 325; pp. 371–376.

30. Y. Krutyakov, A. Olenin, A. Kudrinskii, P. Dzhurik and G. Lisichkin; “Aggregative stability and polydispersity of silvernanoparticles prepared using two-phase aqueous organic systems”; Nanotechnology in Russia; 2008; vol. 3; pp. 303–310.

31. W. Zhang, X. Qiao and J. Chen; “Synthesis of nanosilver colloidal particles in water/oil microemulsion”; Colloids and Surface A: Physicochemical and Engineering Aspects; 2007; vol. 299; pp. 22–28.

32. H. Huang and Y. Yang; “Preparation of silver nanoparticles in inorganic clay suspensions”; Composites Science and Technology; 2008, vol. 68, pp. 2948–2953.

33. Y. Socol, O. Abramson, A. Gedanken, Y. Meshorer, L. Berenstein and A. Zaban; “Suspensive electrode formation in pulsed sonoelectrochemical synthesis of silver nanoparticles”; Langmuir; 2002; vol. 18; pp. 4736–4740.

34. D. G. Shchukin, I. L. Radtchenko and G. Sukhorukov; “Photoinduced reduction of silver inside microscale polyelectrolyte capsules”; ChemPhysChem; 2003; vol. 4; pp. 1101–1103.

35. C. Johans, J. Clohessy, S. Fantini, K. Kontturi and V. J. Cunnane; “Electrosynthesis of polyphenylpyrrole coated silver particles at a liquid-liquid interface”; Electrochemistry Communication; 2002; vol. 4; pp. 227–230.

36. H. Ma, B. Yin, S. Wang, Y. Jiao, W. Pan, S. Huang, S. Chen and F. Meng; “Synthesis of silver and gold nanoparticles by a novel electrochemical method”; ChemPhys Chem; 2004; vol. 24; pp. 68–75.

37. J. P. Abid, A. W. Wark, P. F. Brevet and H. H. Girault; “Preparation of silver nanoparticles in solution from a silver salt by laser irradiation”; Chemical Communication-Royal Society of Chemistry; 2002; pp. 792–793.

38. M. N. Nadagouda, T. F. Speth and R. Varma; “Microwave-assisted green synthesis of silver nanostructures”; Accounts of Chemical Research; 2011; vol. 44; pp. 469–478.

39. V. Polshettiwar, M. N. Nadagouda and R. Varma; “Microwave assisted chemistry: A rapid and sustainable route to synthesis of organics and nanomaterials”; Australian Journal of Chemistry; 2009; vol. 62; pp. 16–26.

40. H. Huang and X. Yang; “Synthesis of polysaccharide-stabilized gold and silver nanoparticles: A green method”; Carbohydrate Research; 2004; vol. 339; pp. 2627–2631.

41. Y. Yin, Z. Y. Li, Z. Zhong, B. Gates and S. Venkateswaran; “Synthesis and characterization of stable aqueous dispersions of silver nanoparticles through the Tollens process”; Journal of Materials Chemistry; 2002; vol. 12; pp. 522–527.

42. P. Mohanpuria, N. K. Rana and S. K. Yadav; "Biosynthesis of nanoparticles: technological concepts and future applications"; Journal of Nanoparticle Research; 2008; vol. 10; pp. 507–517.

43. S. Senapati; Ph.D. Thesis, India: University of pune; "Biosynthesis and immobilization of nanoparticles and their applications"; 2005; pp. 1-57.

44. B. Debaditya and K. Rajinder; "Nanotechnology and potential of microorganisms"; Critical Reviews in Biotechnology; 2005; vol. 25; pp. 199-204.

45. S. Iravani, H. Korbekandi, S.V. Mirmohammadi and B. Zolfaghari; "Synthesis of silver nanoparticles: chemical, physical and biological methods"; Research in Pharmaceutical Science; 2014; vol. 9(6); pp. 385-406.

46. Shahverdi, A. R., Minaeian, S., Shahverdi, H. R., Jamalifar, H., Nohi, A.; Biosensors Nanotechnology; Process Biochemistry; 2007; vol. 42; pp. 919.

47. D. Jain, S. Kachhwaha, R. Jain, G. Srivastava, S. L. Kothari; "Novel microbial route to synthesize silver nanoparticles using spore crystal mixture of *Bacillus thuringiensis*"; Indian Journal Experimental Biology; 2010; vol. 48; pp. 1152-1156.

48. K. Kalishwaralal, V. Deepak, S. Ramkumarpandian, H. Nellaiah and G. Sangiliyandi; "Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*"; Materials Letters; 2008; vol. 62; pp. 4411.

49. R. Joerger, T. Klaus and C. G. Granqvist; "Biologically produced silver carbon composite materials for optically functional thin-film coatings"; Advanced Materials; 2000; vol. 12; pp. 407.

50. H. Zhang, Q. Li, Y. Lu, D. Sun, X. Lin, et al; "Biosorption and bioreduction of diamine silver complex by Corynebacterium"; Journal of Chemical Technology and Biotechnology; 2005; vol. 80; pp. 285-290.

51. A. Nanda and M. Saravanan; "Biosynthesis of silver nanoparticles from *Staphylococcus aureous* and its antimicrobial activity against MRSA and MRSE"; Nanomedicine; 2009; vol. 5; pp. 452.

52. S. S. Shankar, A. Ahmad and M. Sastry; "Geranium leaf assisted biosynthesis of silver nanoparticles"; Biotechnology Progress; 2003; vol. 19; pp. 1627-1631.

53. P. Daizy; "Green synthesis of gold and silver nanoparticles using *Hibiscus rosa*sinensis"; Physica E; 2010; vol. 42; pp. 1417-1424.

54. J. Huang, Q. Li, D. Sun, Y. Lu, Y. Su, X. Yang, H. Wang, Y. Wang, W. Shao, N. J. Hong and C. Chen; "Biosynthesis of silver and gold Nanoparticles by Novel Sundried Cinnamomum camphora Leaf"; Nanotechnology; 2007; vol. 18; pp. 105104-105115.

55. S. S. Shankar, A. Rai, A. Ahmad and M. Sastry; "Biosynthesis of silver and gold nanoparticles from extracts of different parts of the Geranium plant"; Application in Nanotechnology; 2004; vol. 1; pp. 69-77.

56. B. Ankamwar, C. Damle, A. Absar and S. Mural; "Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution"; Journal of Nanoscience and Nanotechnology; 2005; vol. 10; pp. 1665-1671.

57. H. Bar, D. K. Bhui, G. P. Sahoo, P. Sarkar, S. P. De and A. Misra; "Green synthesis of silver nanoparticles using latex of *Jafropha curcas*"; Colloids and Surface A: physicochemical and engineering aspects; 2009; vol. 339; pp. 134-139.

58. S. P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad and M. Sastry; "Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract"; Biotechnology Progress; 2006; vol. 22; pp. 577-583.

59. A. Ahmad, P. Mukherjee, D. Mandal, S. Senapati, M. I. Khan, R. Kumar and M. Sastry; "Enzyme mediated extracellular synthesis of CdS nanoparticles by the fungus, *Fusarium oxysporum*"; Journal of American Chemical Society; 2002; vol. 124; pp. 12108-12109.

60. A Mourato, M Gadanho, AR Lino, R Tenreiro; " Biosynthesis of crystalline silver and gold nanoparticles by extremophilic yeasts"; Bioinorganic Chemistry and Application; 2011; pp. 546074.

61. M. Koushik, S. Ashtaputre, S. Kharrazi, W. Vogel, J. Urban, et al; "Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3"; Nanotechnology; 2003; vol. 14; pp. 95-100.

62. A. K. Gade, P. Bonde, A. P. Ingle, P. D. Marcato, N. Duran, et al; "Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles"; Journal of Biobased Materials and Bioenergy; 2008; vol. 2; pp. 243-247.

63. N. Duran, P. D. Marcato, G. I. H. De Souza, O. L. Alves and E. Esposito; "Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment"; Journal of Biomedical Nanotechnology; 2007; vol. 3; pp. 203-208.

64. K. C. Bhainsa, S. F. D'Souza; "Biosensor nanotechnology"; Colloid Surface B; 2006; vol. 47; pp. 160.

65. A. Ingle, M. Rai, A. Gade and M. Bawaskar; "Fusariumsolani: a novel biological agent for the extracellular synthesis of silver nanoparticles"; Journal of Nanoparticle Research; 2009; vol. 11; pp. 2079-2085.

66. P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S. R. Sainkar, M. L. Khan, R. Parishcha, P. V. Ajaykumar, M. Alam, R. Kumar and M. Sastry; "Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis"; Nano Letters; 2001; vol. 1; pp. 515-519.

67. N. Vigneshwaran, N. M. Ashtaputre, P. V. Varadarajan, R. P. Nachane, K. M. Paralikar, R. H. Balasubramanya; "Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*"; Material Letters; 2007; vol. 61; pp. 1413-1418.

68. L. Guo, J. Nie, B. Du, Z. Peng, B. Tesche and K. Kleinermanns; "Thermoresponsive polymer-stabilized silver nanoparticles"; Journal of Colloid and Interface Science; 2008; vol. 319; pp. 175-181.

69. G. A. Khahrilas, M. L. Wally, S. J. Fredrick, M. Hiskey, A. L. Prieto and J. E. Owens; "Microwave assisted green synthesis of silver nanoparticles using orange peel extract"; ACS Sustainable chemical & engineering; 2014; vol. 2; pp. 367-376.

70. A. A. Kajani, A. K. Bordbar, S. H. Z. Esfahani, A. R. Khosropour and A. Razmjou; "Green synthesis of anisotropic silver nanoparticles with potent anticancer activity using Taxus baccata extract"; RSC Advances; 2014; vol. 4; pp. 61394-61403.

71. P. K. Singh, K. Bhardwaj, P. Dubey and A. Prabhune; "UV-assisted size sampling and antibacterial screening of Lantana camara leaf extract synthesized silver nanoparticles"; RSC Advances; 2015; vol. 5; pp. 24513-24520.

72. E. Roy, S. Patra, S. Saha, R. Madhuri and P.K. Sharma; "Shape-specific silver nanoparticles prepared by microwave-assisted green synthesis using pomegranate juice for bacterial inactivation and removal"; RSC Advances; 2015; vol. 5; pp. 95433-95442.

73. P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S. R. Sainkar, M. I. Khan, R. Parishcha, P. V. Ajaykumar, M. Alam, R. Kumar and M. Sastry; "Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis"; Nano Letters; 2001; vol. 1(10); pp. 515-519.

74. B. Ramalingam, T. Parandhaman and S. K. Das; "Antibacterial effects of biosynthesized silver nanoparticles on surface ultrastructure and nanomechanical properties of gram-negative bacteria viz. *Escherichia coli* and *Pseudomonas aeruginosa"*; ACS Applied Matererials & Interfaces; 2016; vol. 8; pp. 4963-4976.

75. H. Sowani, P. Mohite, H. Munot, Y. Shouche, T. Bapat, A.R. Kumar, M. Kulkarni and S. Zinjarde; "Green synthesis of gold and silver nanoparticles by an actinomycete Gordonia amicalis HS-11: Mechanistic aspects and biological application"; Process Biochemistry; 2016; vol. 51; pp. 374-383.

76. P. Manivasagan, K. H. Kang, D. G. Kim and S. K. Kim; "Production of polysaccharide-based bioflocculant for the synthesis of silver nanoparticles by Streptomyces Sp."; International Journal of Biological Macromolecules; 2015; vol. 77; pp. 159-167.

77. S. Mohan, O. S. Oluwafemi, S. C. George, V. P. Jayachandran, F. B. Lewu, S. P. Songca, N. Kalarikkal and S. Thomas; "Completely green synthesis of dextrose reduced silver nanoparticles, its antimicrobial and sensing properties"; Carbohydrate Polymers; 2014; vol. 106; pp. 469-474.

78. W. Haggren, G. A. Kahrilas, R. L. Read, L. M. Wally, S. J. Fedrick, M. Hiskey, A. L. Prieto and J. E. Owens; "Investigation of antibacterial activity by silver nanoparticles prepared by microwave-assisted green synthesis with soluble starch, dextrose, and arabinose"; ACS Sustainable Chemistry & Engineering; 2014; vol. 2; pp. 590-598.

79. B. Xia, F. He and L. Li; “Preparation of bimetallic nanoparticles using facile green synthesis method and their application”; Langmuir; 2013; vol. 29; pp. 4901-4907.

80. O. S. Oluwafemi, Y. Lucwaba, A. Gura, M. Masabeya, V. Ncapayi, O. O. Olujimi and S. P. Sogca; “A facile complete green size tunable synthesis of maltose-reduced silver nanoparticles without the use of any accelerator”; Colloids Surface B; 2013; vol. 102; pp. 718-723.

81. H. J. Klasen; "Historical review of the use of silver in the treatment of burns. I. Early uses"; Burns; 2000; vol. 26 (2); pp. 117–130.

82. Q. L. Feng, J. Wu, G. Q. Chen, F. Z. Cui, T. N. Kim and J. O. Kim; "A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*"; Journal of Biomedical Materials Research; 2000; vol. 52 (4); pp. 662–668.

83. M. Yamanaka, K. Hara and J. Kudo; "Bactericidal Actions of a Silver Ion Solution on *Escherichia coli*, Studied by Energy-Filtering Transmission Electron Microscopy and Proteomic Analysis"; Applied and Environmental Microbiology; 2005; vol. 71 (11); pp. 7589–7593.

84. A. R. Shahverdi, A. Fakhimi, H. Q. Shahverdi and S. Minaian; "Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*"; Nanomedicine; 2007; vol. 3 (2); pp. 168–171.

85. P. Pradeep and T. Jain; "Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter"; Biotechnology and Bioenggineering; 2005; vol. 90 (1); pp. 59–63.

86. T. Takeshima, Y. Tada, N. Sakaguchi, F. Watari and B. Fugetsu; “DNA/Ag Nanoparticles as Antibacterial Agents against Gram-Negative Bacteria”; Nanomaterials; 2015; vol. 5(1); pp. 284-297.

87. S. A. Brennan, C. N. Fhoghlu, B. M. Devitt, F. J. O’Mahony, D. Brabazon and A. Walsh; “INSTRUCTIONAL REVIEW: GENERAL ORTHOPAEDICS-Silver nanoparticles and their orthopaedic applications”; The Bone and Joint Journal; 2015; vol. 97-B; pp. 582–589.