‘GREEN’ SYNTHESIS OF SILVER NANOPARTICLES BY USING GRAPE (*Vitis vinifera*) FRUIT EXTRACT: CHARACTERIZATION OF THE PARTICLES & STUDY OF ANTIBACTERIAL ACTIVITY

BY
KAUSHIK ROY
Registration No.: 113811 of 2010-11
Exam Roll No.: M4NST12-14
M. Tech. 2nd Year
2012

Under the Guidance of
Dr. Pataki C. Banerjee

A Thesis Report
Submitted to
School of Material Science and Nanotechnology
Jadavpur University
For the degree of

MASTER OF TECHNOLOGY
IN NANO-SCIENCE AND TECHNOLOGY

School of Material Science and Nanotechnology
Jadavpur University
Kolkata-700032

Dedicated
to
My Parents

TO WHOM IT MAY CONCERN

This is to certify that KAUSHIK ROY (Registration No.: 113811 of 2010-11, Exam Roll No.: M4NST12-14), a student of M. Tech. 2nd year of School of Material Science and Nanotechnology, Jadavpur University has successfully carried out his Final Year Project and completed the Thesis report in the year 2011-12 under my guidance and supervision. This report describes the research work carried out on the topic of **GREEN' SYNTHESIS OF SILVER NANOPARTICLES BY USING GRAPE (*Vitis vinifera*) FRUIT EXTRACT: CHARACTERIZATION OF THE PARTICLES & STUDY OF ANTIBACTERIAL ACTIVITY.**

KAUSHIK ROY is a sincere hard-worker. I wish him success in his future endeavors.

Dr. Pataki C. Banerjee
Visiting Professor
School of Material Science and Nanotechnology
Jadavpur University
Kolkata-700032

Prof. G. C. Das
Director
School of Material Science and Nanotechnology
Jadavpur University
Kolkata-700032

Certificate of Approval

The foregoing thesis is hereby approved as a creditable study of an engineering subject and presented in a manner satisfactory to warrant acceptance as pre-requisite to the degree for which it has been submitted. It is understood that by this approval the undersigned do not necessarily endorse or approve any statement made, opinion expressed or conclusion drawn there in but approve the thesis only for which it is submitted.

Committee on final examination for the evaluation of the thesis

---------- ----------

Signature of
Internal Examiners

Signature of
External Examiners

ACKNOWLEDGEMENT

I take this opportunity to express my gratitude to **Dr. Pataki C. Banerjee,** Visiting Professor of Jadavpur University for his valuable suggestion and support at every stage of my work.

I am equally indebted to **Prof. G. C. Das**, Director of School of Material Science and Nanotechnology, Jadavpur University for his kind approval and providing all the facilities required for the fulfillment of this project.

I would also like to thank the Research Scholars of Jadavpur University and Indian Institute of Chemical Biology, Kolkata for their kind co-operation extended to me.

Date:
Place:

Kaushik Roy

Registration No.: 113811 of 2010-11
Exam Roll No.: M4NST12-14
School of Material Science and Nanotechnology
Jadavpur University
Kolkata-700032

Declaration of Originality and Compliance of Academic Ethics

I hereby declare that the thesis contains literature review and original research work by the undersigned candidate, as part of his Masters of Technology studies.
All information in this document have been obtained and presented in accordance with the academic rules and ethical conduct.
I also declare that as required by these rules and conduct, I have fully cited and referenced all material and result that are not original to this work.

Name : **Kaushik Roy**

Registration No.: 113811 of 2010-11

Exam Roll No.: M4NST12-14

Thesis Title: **GREEN' SYNTHESIS OF SILVER NANOPARTICLES BY USING GRAPE (*Vitis vinifera*) FRUIT EXTRACT: CHARACTERIZATION OF THE PARTICLES & STUDY OF ANTIBACTERIAL ACTIVITY**

Signature of the Candidate with date

CONTENTS

PREAMBLE

Research and developments in nanotechnology are leading to acceptance of this technology in day-to-day life as it continues to provide solutions and alternatives to technological, environmental, and health challenges. Nanostructures are the matter of interest for all applications of Nanotechnology wherein shape and size of the nanoparticles (NPs) determine their characteristic property. Due to the growing demand for various Nanoparticles, it is necessary to develop synthesis methods that are cost-effective and environment-friendly. The majority of the existing procedures used for nanoparticle synthesis rely upon physical and chemical methods that sometimes involve toxic and hazardous chemicals. Moreover, the specific requirement for size and shape of nanoparticles cannot be met with the physico-chemical methods. In this respect, biological methods involving microorganisms or plant extracts are more effective. The integration of the principles of green chemistry to nanotechnology toward the synthesis of "green" nanoparticles is a current requirement.

LITERATURE REVIEW

The discipline of nanotechnology is swiftly evolving as an interdisciplinary science, interfacing chemical, medical, environmental and physical sciences not leaving behind diverse engineering fields, with myriad of applications in the development of biosensors and biomedical devices, alternative energy generation and environmental restoration [1]. Various nanostructures such as thin films, nanospheres, nanorods, and a variety of nanoparticles (both metallic and non-metallic) are increasingly contributing to several innovative applications.

Industrial revolution in the twentieth century has led to the accumulation of huge quantities of harmful industrial wastes resulting in numerous health problems [3]. Since its advent, nanotechnology has been showing potential in many improbable areas creating them implemental in the form of Nanoparticles. Conventional procedures for nanoparticle synthesis incorporate physical or chemical routes, involving toxic chemicals as chemical precursors to transform macro or bulk materials into the nanoparticulate forms [2]. One of the objects of this research is to reduce the use of hazardous procedures through alternative route on NP synthesis. Application of green chemistry principles to the field of nanotechnology was introduced by researchers about a decade ago. Eco-friendly, "green" nanotechnological processes are assumed to have the capability to produce new products by utilizing eco-friendly materials [4]. Such processes have involved plant metabolites and plant extracts and products of biological macromolecules such as nucleic acids, peptides or proteins, carbohydrates, and lipids as well. It is now well-proven that the biological route for synthesis of nanoparticles saves energy and creates comparatively less amount of harmful waste.

Green nanotechnology encourages not only fundamental but also goal-oriented research in both the academic and industrial fields for the design and development of Green Nanoparticles (GNPs) [6] [7]. Green nanoparticles have already been used in the design of smart electronic devices, life-saving nano-pharmaceuticals, and in substitute green energy production devices as well.

In the present review, we are focusing on the various methods involved in the green synthesis of Nanoparticles using specific bio-molecules present in plant extracts as precursors with emphasis on the antimicrobial activity of NPs [9]. The limitations of green nanotechnology in development of green silver nanoparticles and its antimicrobial activity along with the strategies to improve the production of green antimicrobial drugs have also been discussed.

The size, shape, and surface morphology of NPs play a vital role in controlling their physical, chemical, optical, and electronic properties. The NPs that attract the attention of most researchers are produced from bulk silver and gold [11].

Due to the outbreak of multi-drug resistant microorganisms to common antimicrobial drugs available in the market, the focus of the researchers across the globe has shifted to the development of novel antimicrobial agents. Nano-materials, in the form of biosensors for pathogen detection and as a therapeutic tool against bacteria, fungi, and viruses have provided a promising alternative to deal with this subject of concern [8]. The properties of Nanoparticles are attributed to their high surface area to volume ratio at nano-dimensions.

Silver has been known for its antimicrobial activity since the ancient days. It was used for storing drinking water in ancient times. The formulation of silver has changed from bulk silver in coins, replaced by ionic silver to the current colloidal silver. Silver, whether in ionic or nanoparticle form, is highly toxic to microorganisms [15]. It is the most toxic metal known for its activity against microorganisms. Other metals follow silver in respect of antimicrobial activity as shown below:

Ag > Hg > Cu > Cd > Cr >Pb > Co > Au > Zn > Fe > Mn > Mo > Sn

Furthermore, silver in nanoparticle form is more efficient than silver ions in terms of its antimicrobial activity. Adding to that, it is also known to exert lower toxicity to mammalian cells as well [7].

Even though antibacterial properties have been known for a long time, the use of silver for infectious diseases was over-shadowed by the discovery of antibiotics [16]. However, the outbreak of infectious diseases caused by antibiotic-resistant pathogenic bacteria has brought the focus back on silver and its colloidal forms. At present, clothing,

respirators, household water filters, contraceptives, antibacterial sprays, cosmetics, detergent, dietary supplements, cell phones, laptop keyboards, and children's toys are among the products being marketed that purportedly exploit the antimicrobial properties of silver nanomaterials.

Apart from capitalizing the antimicrobial potential of the nano-form of silver, it is required to focus upon the green methods of synthesis of silver nanoparticles so as to counter the environmental peril being caused by the exhaustive use of chemicals for the implementation and fabrication of silver nanoparticles [18]. The precursors, which are being used presently for the production of Green silver nanoparticles, involve plant extracts, bio-macromolecules and peptides [17]. Synthesis of Green silver nanoparticles using plant extracts is a very simple and cost-effective way that satisfies the demand of the research community and eliminates the possibility of environmental hazards simultaneously.

Till date several groups had reported synthesis of Ag-nanoparticles by green method using extracts of plant tissues, fruits, vegetables, microorganisms etc. Some of these works, which used plant materials, are described.

Synthesis of silver nanoparticles (AgNPs) using *Polyalthia longifolia* leaf extract as reducing and capping agent along with D-sorbitol used to increase the stability of the nanoparticles has been reported by S. Kaviya et al [19]. The experiment was conducted with two different concentrations (10^{-3} M and 10^{-4} M) of silver nitrate. The effect of temperature on the synthesis of AgNPs was investigated by stirring at room temperature (25^{o}C) and at 60^{o}C. The UV-visible spectra of NPs showed a blue shift with increasing temperature at both concentrations. FT-IR analysis showed that the bio moieties played an important role in the reduction of Ag^{+} ions and the growth of AgNPs. The size and morphology of nanoparticles were determined by TEM. The synthesized silver nanoparticles were found to be highly toxic against Gram-positive bacteria than Gram-negative bacteria.

In another paper, the following report of study was found. A green rapid biogenic synthesis of silver nanoparticles (AgNPs) using *Terminalia chebula* (*T. chebula*) aqueous extract was demonstrated [14]. The formation of silver nanoparticles was confirmed by Surface Plasmon Resonance (SPR) at 452 nm using UV–visible spectrophotometer. The reduction of silver ions to silver nanoparticles by *T. chebula* extract was completed within

20 min which was evidenced potentiometrically. Synthesized nanoparticles were characterized using UV–vis spectroscopy, Fourier transformed infrared spectroscopy (FT-IR), powder X-ray diffraction (XRD), transmission electron microscopy (TEM) and atomic force microscopy (AFM). In addition, it showed good antimicrobial activity towards both Gram-positive bacteria (*S. aureus ATCC 25923*) and Gram-negative bacteria (*E. coli ATCC 25922*). Industrially it may be a smart option for the preparation of silver nanoparticles.

The design, synthesis and characterization of biologically synthesized nanomaterials have become an area of significant interest. It was reported that the extracellular synthesis of gold and silver nanoparticles using *Emblica officinalis* (amla, Indian Gooseberry) fruit extract could be a smart option for producing gold and silver nanoparticles through eco-friendly route [20]. On treating aqueous silver sulfate and chloroauric acid solutions with *Emblica officinalis* fruit extract, rapid reduction of the silver and chloroaurate ions is observed leading to the formation of highly stable silver and gold nanoparticles in solution. Transmission Electron Microscopy analysis of the silver and gold nanoparticles indicated that they ranged in size from 10 to 20 nm and 15 to 25 nm respectively.

In another experiment, five plant leaf extracts (Pine, Persimmon, Ginkgo, Magnolia and Platanus) were used and compared for their extracellular synthesis of metallic silver nanoparticles [23]. Stable silver nanoparticles were formed by treating aqueous solution of $AgNO_3$ with the plant leaf extracts as reducing agent of Ag(+) to Ag(0). UV-visible spectroscopy was used to monitor the quantitative formation of silver nanoparticles. Magnolia leaf broth was the best reducing agent in terms of synthesis rate and conversion to silver nanoparticles. Only 11 min was required for more than 90% conversion at the reaction temperature of 95 degrees C using Magnolia leaf broth. The synthesized silver nanoparticles were characterized with inductively coupled plasma spectrometry (ICP), energy dispersive X-ray spectroscopy (EDS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and particle analyzer. The average particle size ranged from 15 to 500 nm. The particle size could be controlled by changing the reaction temperature, leaf broth concentration and $AgNO_3$ concentration. This environmental-friendly method of biological silver nanoparticles production provided rates of synthesis faster or comparable to those of chemical methods and can potentially be used in various human contacting areas such as cosmetics, foods and medical applications.

The eco-friendly synthesis of nanoparticles through various biological means helped to explore various plants for their ability to synthesize silver nanoparticles (AgNPs) [22]. It was found in a report that AgNPs were synthesized by using rhizome extract of *Dioscorea batatas* at 80 degree Celsius as well as room temperature 25 degree Celsius. AgNPs were characterized under UV-vis spectrophotometer, SEM, FTIR, XRD, and EDX. The antimicrobial activity of AgNPs was evaluated on gram positive (*B. substilis* and *S. aureus*), gram negative (*E. coli*), and fungi (*S. cerivisae* and *C. albicans*). At room temperature, *S. cerivisae* and *C. albicans* were found to be more susceptible to AgNPs than at 80 degree Celsius [24].

A biogenic approach for green synthesis of silver nanoparticles using extract of *Foeniculum vulgare* and its activity against *Staphylococcus aureus* and *Escherichia coli* was reported. In this study, the synthesis of silver nanoparticles was detected by changing color from green to brown after treatment with AgNO3 (1mM) and the UV-visible spectrophotometer analysis showed the absorbance peak at about 427 nm indicating the synthesis of silver nanoparticles [27]. Nanoparticle Tracking and Analysis (NTA) by LM-20 was used for multi-parameter analysis, allowing for characterization of particle size and particle distribution of silver nanoparticles synthesized from the extract of *F. vulgare*. NTA revealed the poly-dispersed nanoparticles in the range of 18-83 nm. Phyto-synthesized silver nanoparticles showed antibacterial activity against the *Staphylococcus aureus* (ATCC-25923) and *Escherichia coli* (ATCC-39403). The silver nanoparticles also demonstrated remarkable antibacterial activity against two human pathogenic bacteria when used in combination with commercially available antibiotics. The bactericidal activity of the standard antibiotics was significantly enhanced in presence of silver nanoparticles against pathogenic bacteria.

The medicinal plants have also been used for this purpose. Green synthesis of silver nanoparticles using tobacco leaf extract was reported as well [26]. Synthesized nanoparticles were characterized using UV-Vis absorption spectroscopy, TEM, EDAX, FT-IR and photoluminescence study, respectively. UV-Vis absorption spectroscopy of prepared silver colloidal solution showed absorption maxima at 418 nm. Excitation maximum and emission maximum obtained from photoluminescence study were found at 414 and 576 nm, respectively. TEM analysis showed average particle size of 8 nm, while SAED pattern confirmed the crystalline nature of synthesized nanoparticles. EDAX analysis showed

proportion of silver (54.55%) among other elements in nanoparticle. *Pseudomonas aeruginosa* and *Escherichia coli* showed highest sensitivity towards silver nanoparticles.

Preparation of silver nanoparticles was performed using extract of leaves of lemon tree (*Citrus limon*) that can act as reducing agent for the silver nanoparticles [12]. These silver nanoparticles were used for durable textile finish on cotton and silk fabrics. Remarkable antimicrobial activity was observed in the treated fabrics. The antimicrobial activity of silver nanoparticles derived from lemon leaves showed enhancement in activity due to synergistic effect of silver and essential oil components of lemon leaves. This report showed the extracellular synthesis of highly stable silver nanoparticles by biotransformation using the extract of lemon leaves by controlled reduction of the Ag^{+} ion to Ag^{0}. Further the silver nanoparticles were used for antifungal treatment of fabrics which was tested by antifungal activity assessment of textile material by Agar diffusion method against *Fusarium oxysporum* and *Alternaria brassicicola*. Formation of the metallic nanoparticles was established by FT-IR, UV-Visible spectroscopy, transmission electron microscopy, scanning electron microscopy, atomic force microscopy [28].

OBJECTIVE

The principal objectives of this research work are:

1. Synthesis of silver nanoparticles from silver salts using biological route
2. Characterization of these synthesized silver nanoparticles
3. Determination of the size and shape of silver nanoparticles
4. Study of its antimicrobial activity against a few common bacteria

ABSTRACT

Nanotechnolgy is an expanding area of research where we use to deal with the materials in nano-dimensions. The conventional procedures for synthesizing metal nanoparticles need sophisticated and costly instruments or high-priced chemicals. Moreover, the techniques may not be environmentally safe. Therefore 'green' technologies for synthesis of nanoparticles are always preferred. In this dissertation a 'green' method for Silver nanoparticle synthesis is described. The method is simple, convenient and eco-friendly.

In the recent past, many researchers followed this biological route for nanoparticle synthesis. On the basis of their observations, it was inferred that plant bodies rich in ascorbic acid or Vitamin C, a reducing carbohydrate, might be useful for metal nanoparticle preparation. Hence a few locally available fruits including grape (*Vitis vinifera*), which are known to contain relatively high amount of ascorbic acid, were selected for silver nanoparticle synthesis. It was supposed that the fruit extract will act as capping agent as well. Silver nanoparticles were prepared from aqueous solution of silver nitrate using different volumes of fruit extract.

Usually change in colour of the aqueous salt solution of a metal is indicative of metal nanoparticle formation. In the present study also distinct color change of the silver nitrate solution after a certain period following addition of grape fruit (*Vitis vinifera*) extract was observed indicating formation of silver nanoparticles. The color change with addition of different volumes of silver nitrate solution was analyzed using a UV-Vis spectrophotometer, and the absorbance vs. wavelength curve showed a peak for silver. Later the nanoparticles were separated out from the mixture by ultra-centrifugation. The pellet formed at the bottom of the centrifuge tube was dispersed in a small volume of de-ionized water after aspirating the supernatant fluid carefully. The dispersed material was scanned under a high resolution Transmission Electron Microscope (TEM). The TEM images showed the size distribution of the nanoparticles and the average size was found to be 18-20 nm. By Dynamic Light

Scattering analysis, average size of the silver nanoparticles came out to be 19 nm; thus the DLS study corroborated exactly with that of TEM. Later the particles were dried using a Vacuum dryer and characterized by XRD and EDX. The results of these analyses confirmed formation of silver nanoparticles.

Antibacterial activity of the synthesized silver nanoparticles was tested with one Gram-positive (*Bacillus subtilis*) and one Gram-negative (*Escherichia coli*) bacterium. The nanoparticles exhibited antibacterial activity similar to that of the metal salt solution. Since the effective concentration of the metal was much less in the nanoparticle preparation, it may be concluded that the nanoparticles are more effective against tested microorganisms.

Keywords: 'Green' synthesis, Silver nanoparticles, Grapes (*Vitis vinifera*) fruit extract, UV-Vis spectroscopy, DLS, TEM, XRD, EDX, Antibacterial activity, *Bacillus subtilis*, *E. coli*

MATERIALS AND METHODS

MATERIALS:

CHEMICALS-

1. Silver Nitrate – Purchased from local chemical suppliers
2. Nutrient Agar Powder- Purchased from chemical suppliers

OTHER MATERIALS-

1. Fresh grapes (*Vitis vinifera*) – Collected from local market
2. De-ionized water – Collected from DI plant

BACTERIAL STRAINS-

1. *Bacillus subtilis* – Collected from Bidhan Nagar College, Kolkata
2. *Escherichia coli* – Collected from Bidhan Nagar College, Kolkata

OTHER APPARATUS-

1. Weight Machine
2. Micropipette
3. Petri dish of 11 cm diameter
4. Centrifuge machine
5. Heater
6. Stirrer
7. Ultracentrifuge machine
8. Autoclave
9. Culture tubes
10. Test tubes
11. Inoculating needle
12. Funnel
13. Refrigerator
14. Vacuum drier

METHODS

1. PREPARATION OF SILVER NANOPARTICLES

The synthesis procedure comprises of four simple steps:

1. Preparation of fruit extract of grapes (*Vitis vinifera*)
2. Preparation of silver nitrate solutions of different concentrations
3. Addition of the fruit extract to the silver nitrate solutions
4. Incubation at room temperature to allow nanoparticles formation

1.1 Preparation of grape fruit (*Vitis vinifera*) extract

A large number of plant extracts has already been used to synthesize metal nanoparticles from metal salts. But the preparation methods of those plant extracts were complicated. Here we had used a very easy and effective technique to prepare the required fruit extract.

a. Relatively fresh green grapes (*Vitis vinifera*) were purchased from the local market.
b. Grapes (200g) were thoroughly washed by sterile water.
c. The grapes were removed from the stalk, washed again and smashed inside a grinder.
d. The smashed grapes were then filtered to remove the debris.
e. At the end, the filtered juice was centrifuged at 5000 rpm for 15 minutes to obtain the liquid fruit extract.

The extract was preserved inside a refrigerator for future use.

Fig 1.Centrifuge Machine

1.2 Preparation of Silver Nitrate solutions

Analytical grade silver nitrate ($AgNO_3$), a costly chemical, was purchased from local laboratory chemical suppliers. A 50 mM stock solution of $AgNO_3$ in chloride-free distilled water was prepared according to the following calculations.

The molecular weight of $AgNO_3$:
[Ag-107.87, N-14, O-16]
= 107.87 + 14 + (16 × 3) = 169.87
Therefore, Molar mass of $AgNO_3$ = 169.87 g

Therefore, 50 ml of 50 mM solution will contain 420 mg or 0.42 g of $AgNO_3$:

Calculations:

Density	Volume	Required amount of $AgNO_3$
1000 mM	1000 ml	169.87 g
1 mM	1000 ml	(169.87/1000) g
50 mM	1000 ml	(169.87 x 50)/1000 g
50 mM	1 ml	(169.87 x 50)/(1000 x 1000) g
50 mM	50 ml	(169.87 x 50 x 50)/(10^6) = 420 mg or 0.42 g

Weighed amount of $AgNO_3$ was carefully transferred in a 50-ml volumetric flask and de-ionized water was added drop-wise while swirling to dissolve the salt up to the mark. The solution was diluted as required and all the solutions were kept away from light (the containers were wrapped with brown papers) and kept in dark.

1.3 Preparation of silver nanoparticles by adding grape fruit extract to $AgNO_3$ solutions

Before addition of fruit extract to the $AgNO_3$ solution, the volume of 50 mM $AgNO_3$ solution required to attain a specific concentration (5mM, 10mM, 20mM and 25mM) of the salt was calculated as follows.

Volume of stock solution	Volume of distilled H_2O	Volume of fruit extract*	Final concentration of $AgNO_3$
0.4 ml	1.6 ml	2 ml	5 mM
0.8 ml	1.2 ml	2 ml	10 mM*
1.6 ml	0.4 ml	2 ml	20 mM
2.0 ml	0.0 ml	2 ml	25 mM

*One blank (A) contained only the extract and no salt; another blank (B) contained no extract but 10 mM $AgNO_3$.

A set of six test tubes (Experimental 4, Control 2 – one for the juice only and another for $AgNO_3$ only) were taken and marked. In five test tubes (Experimental 4, Control B), requisite volumes of $AgNO_3$ solution and water were added one after another as shown in the table above; water (2 ml) was added in the rest tube (Control A). In five tubes (Experimental 4, Control A), 2 ml extract was poured and in the rest (Control B) only 2 ml water. The contents were mixed thoroughly and left at room temperature in dark.

Following image shows initial color of the mixtures in the six test tubes.

Fig 2.Color of the samples immediately after the mixing

1.4 Observation

After 24 hours, distinct change in the color of experimental samples was observed but colour of the controls remained unchanged. The colour of experimental samples (four mixtures of silver nitrate and fruit extract) turned light wine-red, which became deeper after 48 hr incubation. Change in colour in the experimental samples clearly indicated formation of silver nanoparticles (Figs. 1 & 2). Previous works in other laboratories also reported similar hue of colour change due to silver nanoparticle formation.

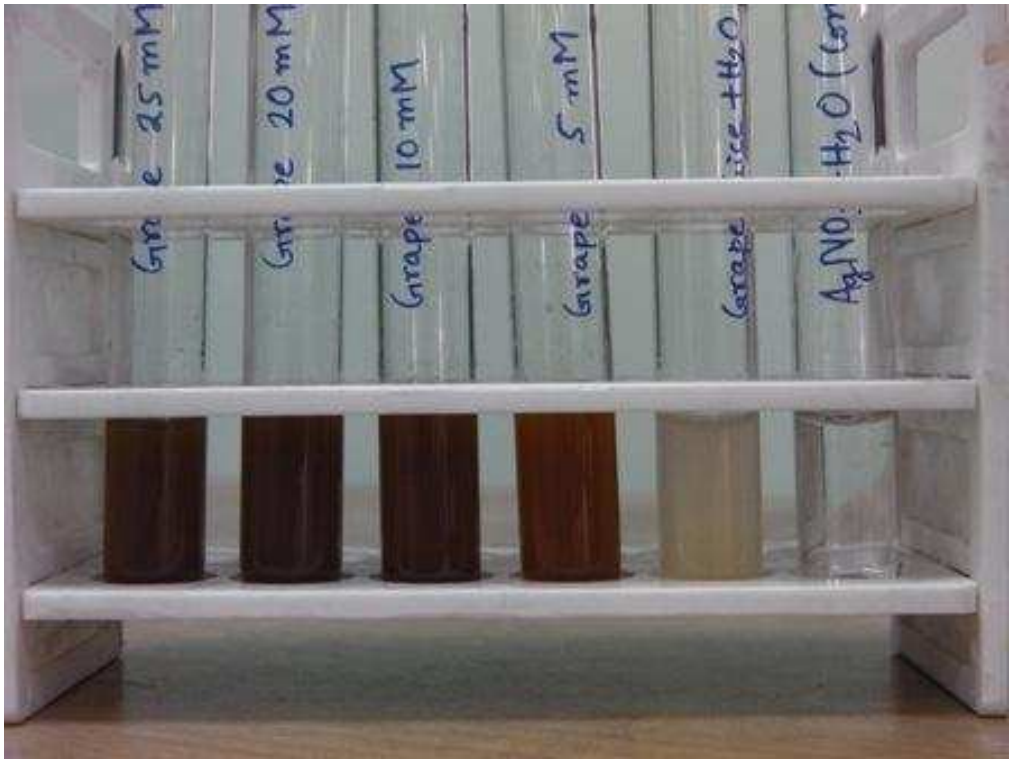


Fig.3.Color change of samples observed after 24 hours

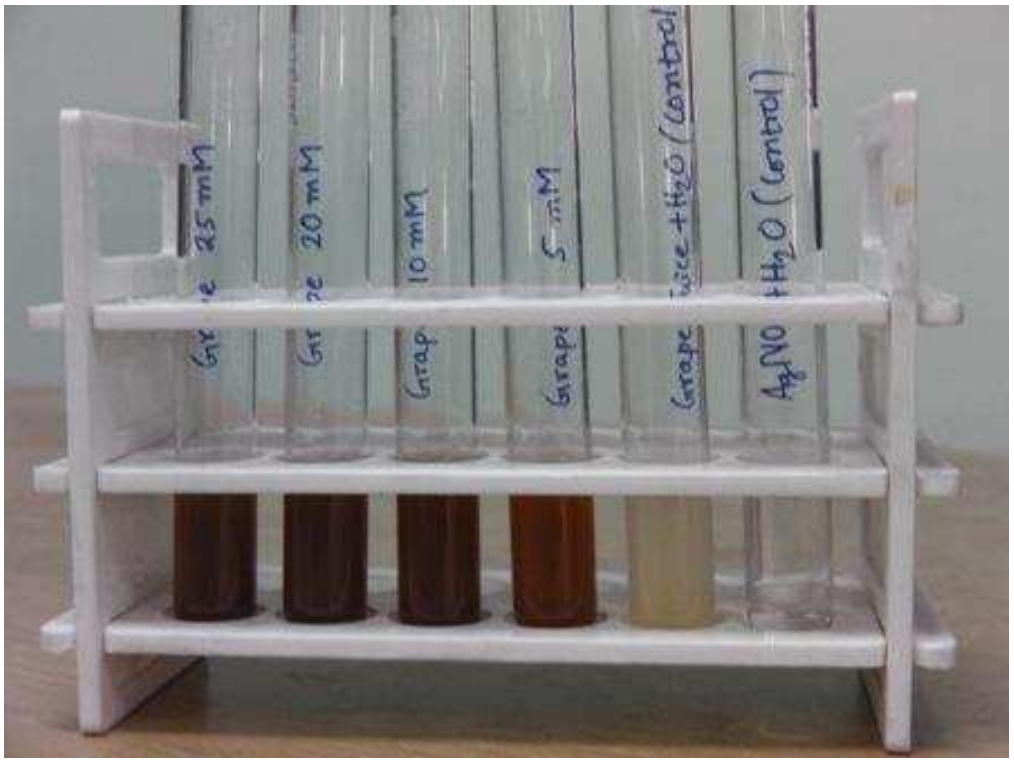


Fig.4.Color of samples became darker after 48 hours

All the four experimental samples were centrifuged at a low speed and the supernatants were taken out by aspiration. The clear wine-red liquids were analyzed by a UV-Vis spectrophotometer. The results of UV-Vis analysis revealed that the sample with 10 mM silver nitrate concentration exhibited maximum absorbance at ~450 nm – a feature indicative of silver nanoparticle formation.

To get a concentrated solution of the nanoparticles free from organic contents of fruit juice, the solution was subjected to ultracentrifugation at 30000 rpm for 12 hours. Thereafter the supernatant was aspirated out from the centrifuge tube and the loose precipitate formed at the bottom was dispersed in a small volume of deionised water. It was used in further analysis for determining various parameters like size, shape, chemical composition.

Fig 5.Ultracentrifuge Machine

After the ultracentrifugation, we removed the organic liquid from the centrifuge tube gradually and at the bottom of the centrifuge tube, we observed very small amount of brown precipitate (though there was no distinct button formation). We decanted the precipitate carefully using small amount of de-ionized water and stored it inside a small protected container. The particles remained in the suspension with a very high concentration.

2. DESCRIPTION OF CHARACTERIZATION METHODS

For characterization of the Green silver nanoparticles following methods were necessary by means of which we can confirm the production of Silver nanoparticles; we can have an idea of its size distribution profile and surface morphology and above all, we can determine the actual particle size as well. The following instruments were used to characterize the Green silver nanoparticles that we synthesized biologically.

1. **UV-VIS SPECTROSCOPY**
2. **DYNAMIC LIGHT SCATTERING**
3. **TRANSMISSION ELECTRON MICROSCOPY**
4. **X-RAY DIFFRACTION**
5. **SCANNING ELECTRON MICROSCOPY**
6. **ENERGY DISPERSIVE X-RAY SPECTROSCOPY**

2.1 UV-VIS SPECTROSCOPY

UV-Vis spectroscopy can be comprehended as absorption spectroscopy in the spectral region of ultra-violet and visible spectra. Generally, it uses light in visible and near-UV range. Ultraviolet and visible light are energetic enough to promote outer electrons to higher energy levels, and UV-Vis spectroscopy is usually applied to molecules in solution. The UV-Vis spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements. The concentration of an analyte in solution can be determined by measuring the absorbance at specific wavelength and applying the Beer-Lambert Law.

Since the UV-Vis range spans the range of human visual acuity of approximately 400 - 750 nm, UV-Vis spectroscopy is useful to characterize the absorption, transmission, and reflectivity of a variety of technologically important materials, such as pigments, coatings etc.

The light source is usually a deuterium discharge lamp for UV measurements and a tungsten-halogen lamp for visible and near infra-red measurements. The instruments automatically swap lamps when scanning between the UV and visible regions. Most

commercial UV-Vis absorption spectrometers use one of three overall optical designs: a fixed or scanning spectrometer with a single light beam and sample holder, a scanning spectrometer with dual light beams and dual sample holders for simultaneous measurement of baseline and sample of interest, or a non-scanning spectrometer with an array detector for simultaneous measurement of multiple wavelengths. In single-beam and dual-beam spectrometers, the light from a lamp is dispersed before reaching the sample cell. In an array-detector instrument, all wavelengths pass through the sample and the dispersing element is between the sample and the array detector. The dual beam spectrophotometer was used to obtain the absorbance vs wavelength curve of silver nanoparticles in this research.

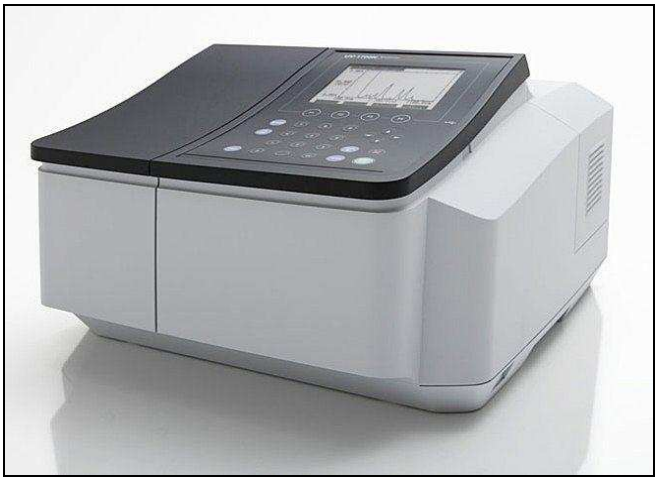


Fig 6.UV-Vis Spectrophotometer

Fig 7.UV Cuvette

2.2 DYNAMIC LIGHT SCATTERING

The dynamic light scattering (DLS) or photon correlation spectroscopy is a method frequently used in the study of material science and geology to obtain the size distribution profile of nanoparticles present in suspension or solution. Dynamic Light Scattering Analyzer is the instrument required for DLS analysis.

The working principle of DLS states that the particles, emulsions and molecules in suspension undergo Brownian motion. This is the motion induced by the bombardment by solvent molecules that themselves are moving due to their thermal energy.

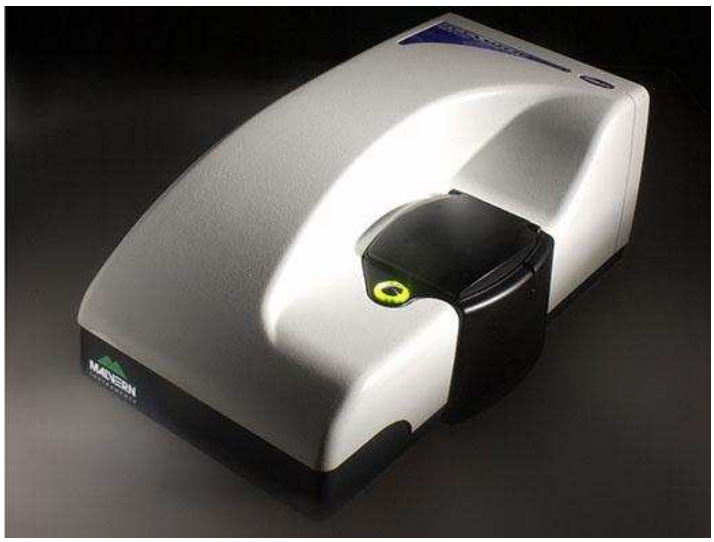


Fig 8.Dynamic Light Scattering Analyzer

If the particles or molecules are illuminated with a laser, the intensity of the scattered light fluctuates at a rate that is dependent upon the size of the particles as smaller particles are "kicked" further by the solvent molecules and move more rapidly. Analysis of these intensity fluctuations yields the velocity of the Brownian motion and hence the particle size using the Stokes-Einstein relationship. The diameter that is measured in Dynamic Light Scattering is called the hydrodynamic diameter and refers to how a particle diffuses within a fluid. The diameter obtained by this technique is that of a sphere that has the same translational diffusion coefficient as the particle being measured.

The translational diffusion coefficient will depend not only on the size of the particle "core", but also on any surface structure, as well as the concentration and type of ions in the medium. This means that the size can be larger than measured by electron microscopy. DLS analysis is a non-invasive, well-established technique for measuring the size of molecules and particles typically in the submicron region, and with the latest technology lower than 1 nm.

DLS study was performed to know about the size distribution profile of green silver nanoparticles produced through biological route.

2.3 TRANSMISSION ELECTRON MICROSCOPY

Transmission Electron Microscopy offers the highest resolution we can achieve. This method helps us to observe the particle size of a material in nano-dimension and study the crystal structure meticulously.

In a High Resolution Transmission Electron Microscope (TEM), a thin sample or specimen is irradiated with a sharp high-energy electron beam (usually in the range: 100-200 keV). The beam is strongly focused by magnetic lenses. The first few lenses before the specimen permit variation of the illumination aperture and the size of the illuminated area.

The electron intensity distribution of the beam after interaction with the specimen is imaged onto a fluorescent screen by the objective lens and the post-objective lens system. Images are recorded by a digital CCD camera and can be reproduced or displayed on a computer monitor as well.

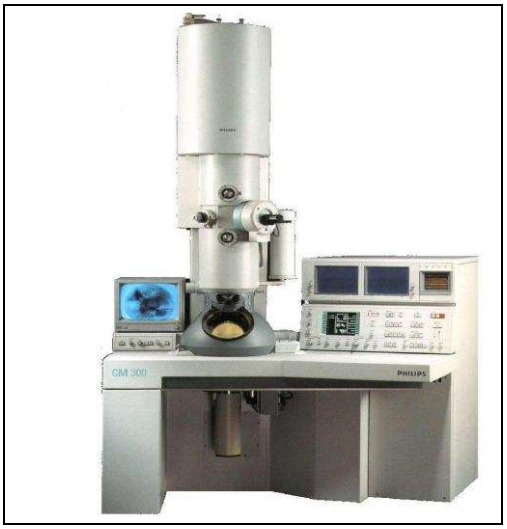


Fig 9.Transmission Electron Microscope

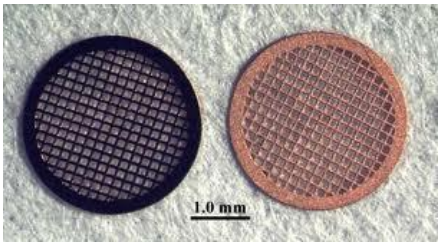


Fig 10.TEM grid

There are certain limitations of this technique too. Some of the materials may require extensive sample preparation steps for making a sample that is thin enough to be electron transparent, which makes this procedure relatively time consuming with comparatively low throughput of samples. The internal structure of the sample can be affected during the preparation process as well. Adding to that, the viewing field is relatively small, which raises the probability that the region studied may not be the characteristic of the entire sample. There is also possibility that the sample may be harmed by the powerful electron beam, mainly in the case of biological samples.

A few important applications of TEM include the observation of nanostructures and morphology, viewing of particle size in nano-dimension, study of the crystalline and nano-crystalline structure, defect and damage analysis, transmission electron diffraction (TED), phase identification etc. TEM analysis was done to know the particle size and other crystalline information in this study.

2.4 X-RAY DIFFRACTION

X-ray diffraction is an analytical technique is generally used for phase identification of a crystalline material and can provide information on unit cell dimensions as well. X-ray diffraction is now a common technique for studying crystal structures and atomic spacing.

Fig 11.X-ray Diffractometer

X-ray diffraction is based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The interaction of the incident rays with the sample produces constructive interference (and a diffracted ray) when conditions satisfy Bragg's Law ($n\lambda = 2d\sin\theta$). This law relates the wavelength (λ) of electromagnetic radiation to the diffraction angle (θ) and the lattice spacing (d) in a crystalline sample. These diffracted X-rays are then detected, processed and counted. By scanning the sample through a range of 2θ angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered material. Conversion of the diffraction peaks to d-spacings allows identification of a metal because each metal has a set of unique *d*-spacings. Typically, this is achieved by comparison of *d*-spacings with standard reference patterns.

XRD technique is widely used to identify unknown crystalline materials like minerals, inorganic compounds etc. Identification of unknown solids is necessary for the studies in geology, environmental science, material science, engineering and biology. Other important applications are determination of the dimensions of unit cell, crystalline material characterization, purity test of samples etc. For this experiment XRD helped to verify the presence of silver and its dominant planes.

2.5 SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy (SEM) is a technique that uses electrons instead of light to form an output image. Since their development in the early 1950's, SEMs have thrown lights in many new areas of research including material science and nanotechnology. The SEM has allowed researchers to examine a much larger variety of specimens.

Fig 12.Scanning Electron Microscope

Fig 13.SEM sample holder

The SEM has many advantages over traditional microscopes. The SEM has a large depth of field, which allows more of a specimen to be in focus at one time. The SEM also has much higher resolution; so closely spaced specimens can be magnified at much higher levels.

Since SEM uses electromagnets rather than lenses, the researchers have much more control in the degree of magnification. All of these advantages, as well as the actual strikingly clear images, make the SEM one of the most useful instruments in research today.

The SEM is an instrument that produces a largely magnified image by using electrons instead of light to form image. A beam of electrons is produced at the top of the microscope by an electron gun. The electron beam follows a vertical path through the microscope, which is held within a vacuum. The beam travels through electromagnetic fields and lenses, which focus the beam down toward the sample. Once the beam hits a sample, electrons and X-rays are ejected from the sample. Detectors collect these X-rays, backscattered electrons, and secondary electrons and convert them into a signal that is sent to a screen similar to a television screen. This produces the final image of the surface.

Because the SEM utilizes vacuum conditions and uses electrons to form an image, special preparations must be done to the sample. All water must be removed from the samples because the water would vaporize in the vacuum. All metals are conductive and require no preparation before use whereas all non-metals need to be coated by a thin layer of conductive material (using a "sputter coater)". The surface morphology of the biologically synthesized silver nanoparticles was studied by means of this.

2.6 ENERGY DISPERSIVE X-RAY SPECTROSCOPY

Energy Dispersive X-Ray Spectroscopy (EDX) is a technique that provides the elemental curve as output. This analytical technique is generally used in conjunction with the Scanning Electron Microscopy (SEM). EDX technique primarily detects the X-rays emitted from the sample during the process of bombardment by an electron beam for characterizing the elemental composition of the sample of interest.

Fig 14.Energy Dispersive X-ray Spectroscope

When a sample is bombarded by the electron beam of SEM, electrons are evicted from the atoms that comprise the surface of the sample. The resulting electron vacancies are filled by electrons from a higher energy state, and an X-ray is emitted to balance the energy difference between the two energy states of electrons. The energy of emitted X-ray is an important characteristic of the element from which it was emitted.

The EDS X-ray detector measures the relative abundance of emitted X-rays versus their energy. The detector is typically lithium-drifted silicon, solid-state device. When an incident X-ray strikes the detector, it creates a charge pulse that is proportional to the energy of the X-ray. The charge pulse is converted to a voltage pulse (which remains proportional to the x-ray energy) by a charge-sensitive preamplifier. The signal is then sent to a multichannel analyzer where the pulses are sorted by voltage. The energy (as determined from the voltage measurement) for each incident x-ray is sent to a computer for display and further evaluation of data. The spectrum of x-ray energy versus counts is evaluated to determine the elemental composition of the sampled volume.

The sample X-ray energy values from the EDS spectrum are compared with known characteristic x-ray energy values to determine the presence of an element in the sample. Elements with atomic numbers ranging from that of beryllium to uranium can be detected. Quantitative results can be obtained from the relative x-ray counts at the characteristic energy levels for the sample constituents. Some typical applications include alloy identification, foreign material analysis, coating composition analysis etc. EDX helped to verify the presence of silver in the sample and its percentage as well.

3. ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES

In previous chapters preparation and characterization of Ag-nanoparticles have been described. Since silver and its salts exhibit strong antibacterial activity, this property was evaluated for the Ag-nanoparticles prepared by us and presented in this chapter. Several groups had already studied the antimicrobial activity of chemically synthesized silver nanoparticles; but in this chapter we have described the antimicrobial activity of biologically synthesized (using grape fruit extract) silver nanoparticles.

3.1 Culturing and Preservation of Bacteria

The conventional cup-plate method was used to determine the antibacterial activity of biologically prepared Ag-nanoparticles is. It's a screening test generally done for verifying antimicrobial activity of samples. For performing the experiment, cultures of test bacterial strains- *Bacillus subtilis* (a Gram-positive bacterium) and *Eschirichia coli* (a Gram-negative bacterium), were collected from Microbiology Department, Bidhan Nagar College, Kolkata. The strains were grown and preserved in the culture media following standard procedures.

3.1.1 Preparing an enriched medium

The medium for selective growth of a bacterial strain may differ for various bacteria but each medium must contain such components which should support growth by providing the basic elements needed for growth and the source of energy. In this study, Nutrient Agar medium was used which supports growth of a wide range of bacteria including *Bacillus subtilis* and *Eschirichia coli*. The dehydrated medium, a product of Hi-Media (India) was constituted as prescribed. For 100 ml Nutrient Agar medium, 2.8 g Nutrient agar powder was added in 100 ml distilled water; for the cup-plate method, 0.5 g of agar powder was added in addition to the medium. The medium kept in cotton-plugged glass container was sterilized in an

autoclave at 121^{o}C for 15 mins. It was distributed inside a laminar hood either in culture tubes of on Petri dishes when hot (about 45^{o}C) and allowed to solidify.

Fig 15.Autoclave Machine

3.1.2 Serial subculture

Microorganisms should be preserved in a manner that will allow their long-term survival and genetic stability. The method of preservation depends on the organism. Subculturing in a suitable gelled medium after intervals is a popular method for the preservation of bacteria. Serial subculture in liquid medium is also a simple method where cultures are transferred to fresh medium of same type and allowed to grow in an incubator at 37^{o}C. We mentioned the bacterial strains in both liquid and gelled medium.

Fig 16.Bacterial Incubator

3.2 Evaluation of antibacterial activity of Ag-nanoparticles by cup-plate method

Nutrient agar medium (25 ml) was taken in two 100-ml Erlenmeyer flasks and sterilized. After cooling the medium at about 45^{o}C, freshly grown liquid culture (0.25 ml) of *Bacillus subtilis* and *Escherichia coli* was added in either of the flask, mixed thoroughly but quickly, and poured in four (two for each bacterium) Petri dishes equally. The dishes were kept at room temperature in a laminar hood. After the medium was solidified, three cups of ~0.5 cm diameter were made by a cork-borer at three corners of each Petri dish at about 1.5 cm away from the disk-wall (Fig 17). Occasionally some liquid may come out from the gelled medium in the cups, which is removed by aspiration by a Pasteur pipette or a micro-pipette. Thereafter samples were added in each well of each Petri dish.

Fig 17.The Petri dish after punching the cups

The Petri dishes were marked as D1, D2, D3 and D4; the cups were also marked as well. D1 and D2 were seeded with *B. subtilis* whereas D3 and D4 with *E. coli*.

As control, 0.1 ml of sterile fruit juice was added in one cup in D1; in two other cups of D1, 0.1 ml of silver nitrate and fruit extract mixture with 10 mM and 3.33 mM concentrations respectively were added. In D2, suspension of AgNPs was added in one cup and the mixture of silver nitrate and fruit extract with 10 mM and 3.33 mM respectively were added in two other cups. The same combination of samples was applied in D3 and D4 where *E. coli* was seeded. The suspension of Ag-nanoparticles was prepared by ultracentrifugation of the clear supernatant derived from the mixture of 10 mM $AgNO_3$ solution with the grape extract after 48 hours incubation as described before.

After adding the samples in the cups, the dishes were kept in a refrigerator for an hour for proper absorption of the samples into the surrounding medium from the well. The plates were then transferred into an incubator set at 37^oC to allow bacterial growth on the medium. After 24 hrs the plates were taken out of the incubator and observed for zone of bacterial growth inhibition around the cups.

3.3 Formation of the 'Zone of Inhibition'

After 24 hours of incubation at 37^{o}C, distinct zone of bacterial growth inhibition was observed around all the experimental and positive control cups whereas no such zone was formed around the cups containing grape juice only. The results strongly suggest that silver nanoparticles possess antibacterial activity similar to $AgNO_3$. The particles show activity against both Gram-positive and Gram-negative bacterial strains used in this study though the inhibition zone appears better in case of the Gram-negative bacterium, *E. coli*.

RESULTS & DISCUSSION

1. CHARACTERIZATION OF SILVER NANOPARTICLES

1.1 UV-Vis Spectroscopy

After addition of grape fruit (*Vitis vinifera*) extract to the aqueous solution of $AgNO_3$ of different concentrations, the mixture showed a gradual change in color at room temperature with time from yellowish to wine-red and the colour intensified after 48 hours. The color is characteristic of the surface plasmon resonance (SPR) of silver nanoparticles. The control sets showed no change in color under the same experimental conditions. The reduction of silver ion to silver nanoparticle was reflected in spectral data obtained by using a UV-Vis spectrophotometer. It shows an absorbance peak around 450 nm for all four samples (Fig18), which is specific for silver nanoparticles.

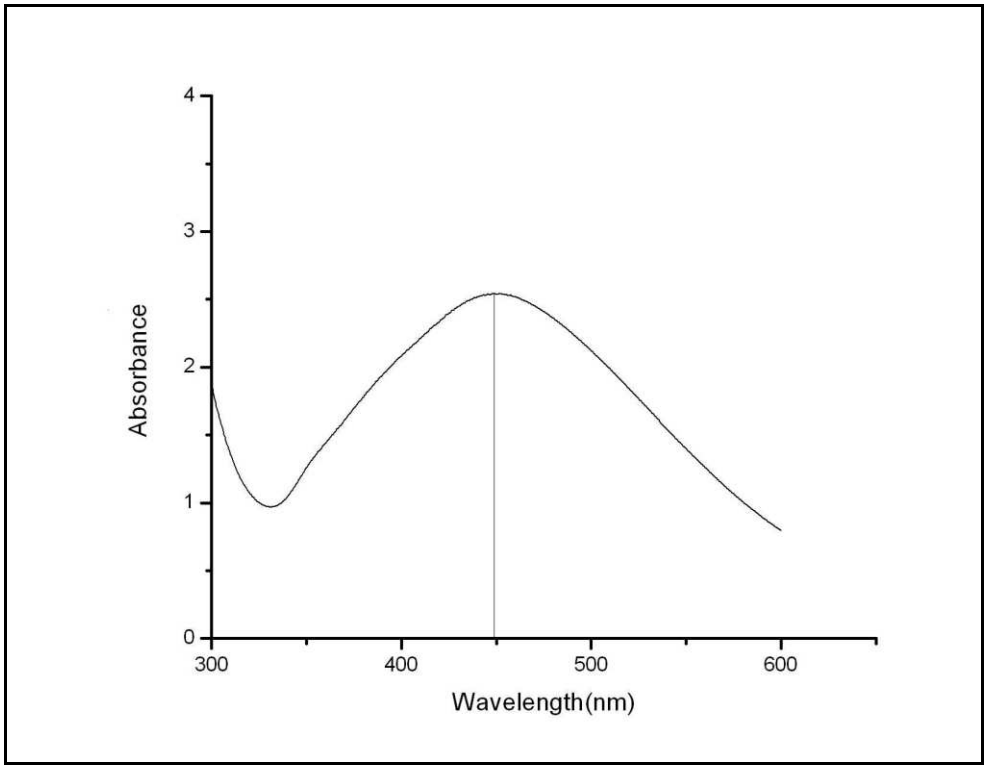


Fig 18.The absorbance spectrum of silver nanoparticles showing maximum absorbance near 450nm

1.2 Dynamic Light Scattering Analysis

Dynamic light scattering or Photon Correlation Spectroscopy is a technique used in material physics for determining the size distribution profile of nanoparticles in suspension or polymers in solution. Light scattering technique is used here to determine the size distribution profile of nanoparticles present in the final solution after ultracentrifugation. The study revealed that the average particle size of Ag nanoparticles range within 2-40 nm with average size of approximately 19-20 nm as shown in the Fig 19.

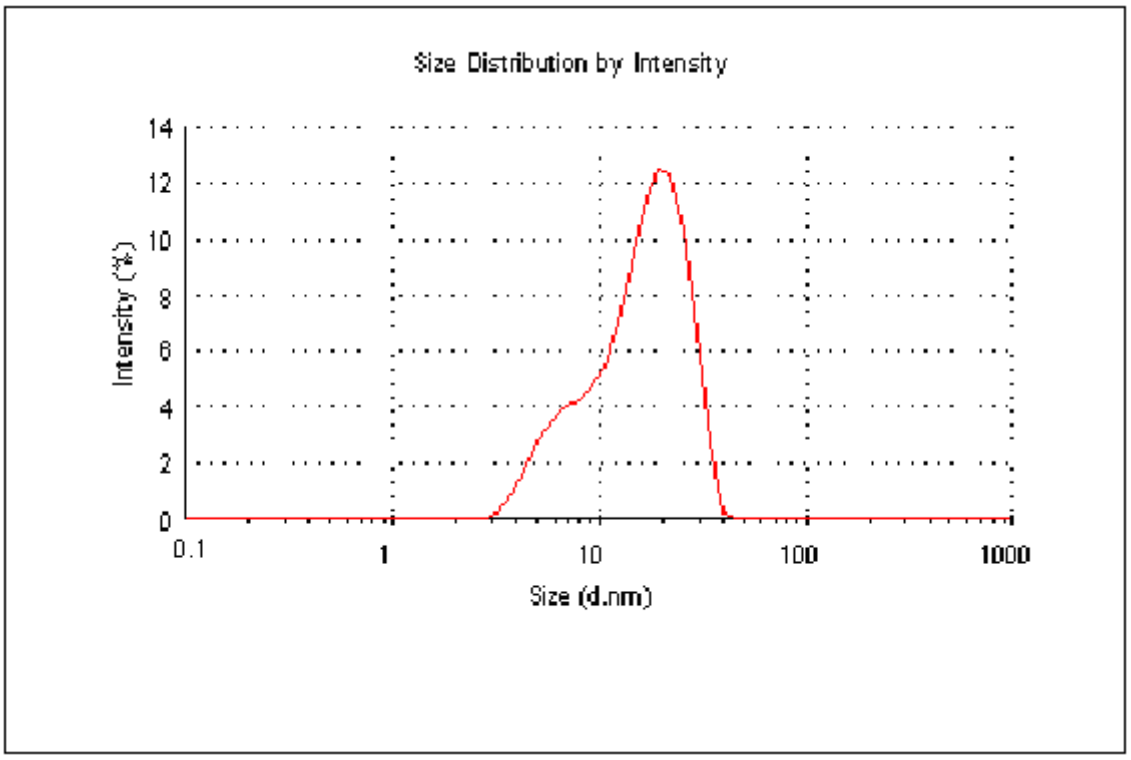


Fig 19.The DLS curve shows the average particle size as 19nm

1.3 Transmission Electron Microscopy

The grid for the TEM analysis of Ag-nanoparticles was prepared by placing a drop of the nanoparticles suspension on the carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer. Scanning under TEM (Philips CM-10) revealed that the average mean size of silver nanoparticles was 18-20 nm and the tiny particles were seemed to be spherical in morphology as shown in the following images (Fig 20). The images also show the existence of nano-crystalline structure in the particles.

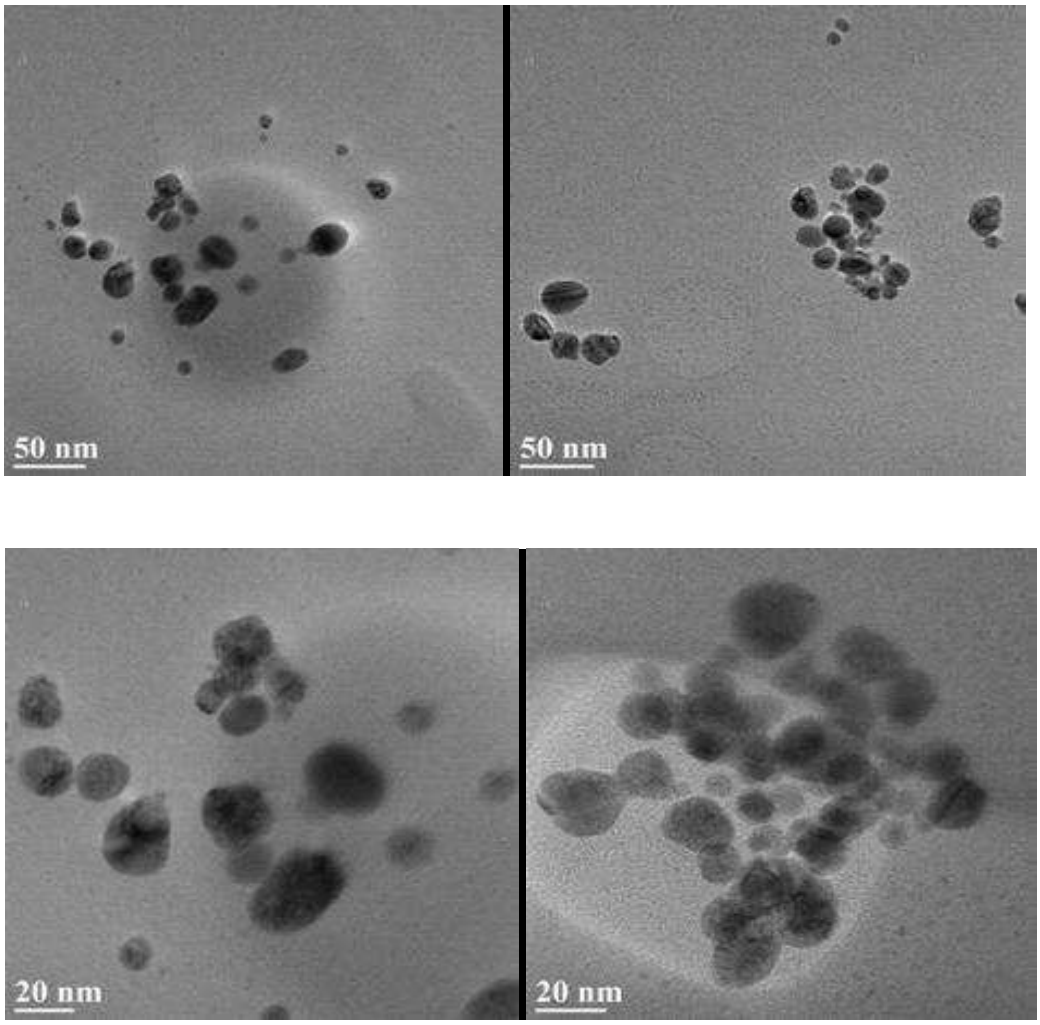
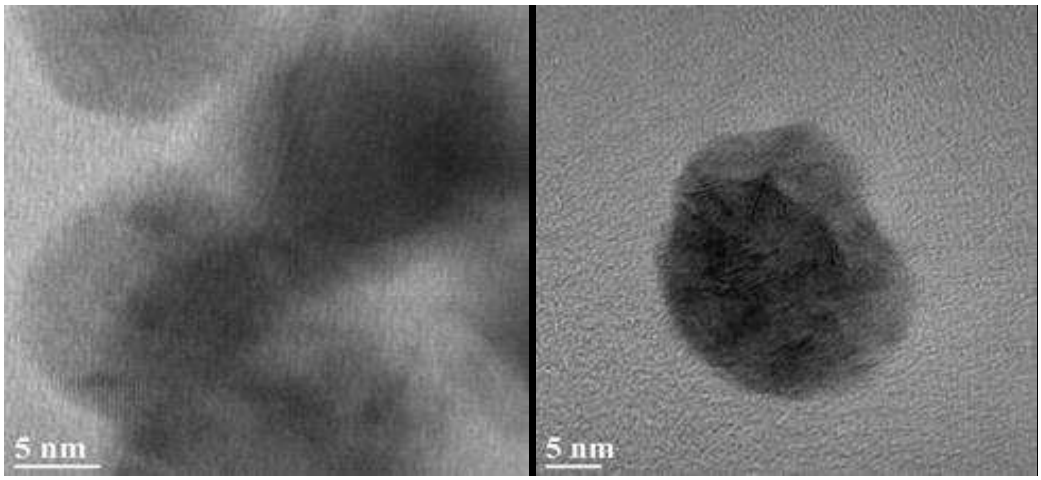
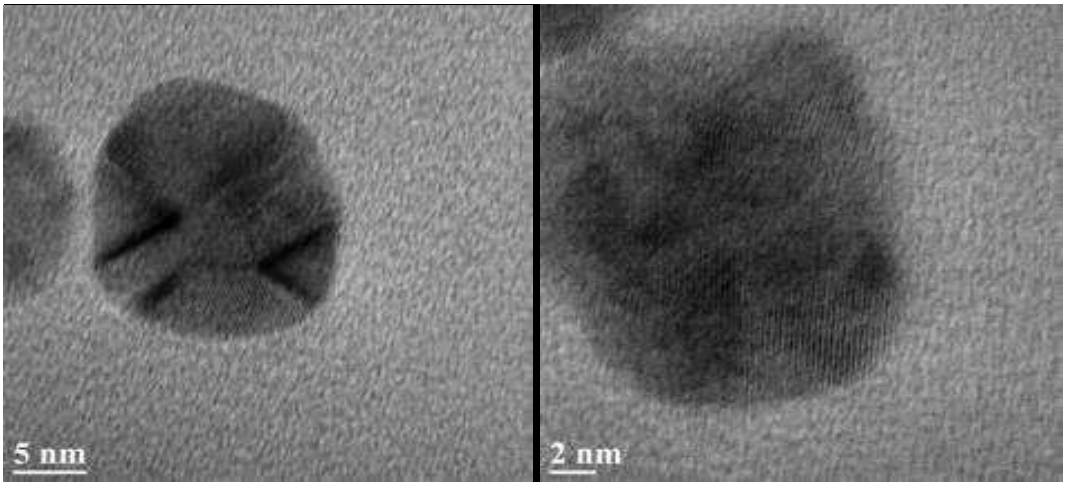


Fig 20.TEM images (A-D) showing average particle size as 18-20 nm





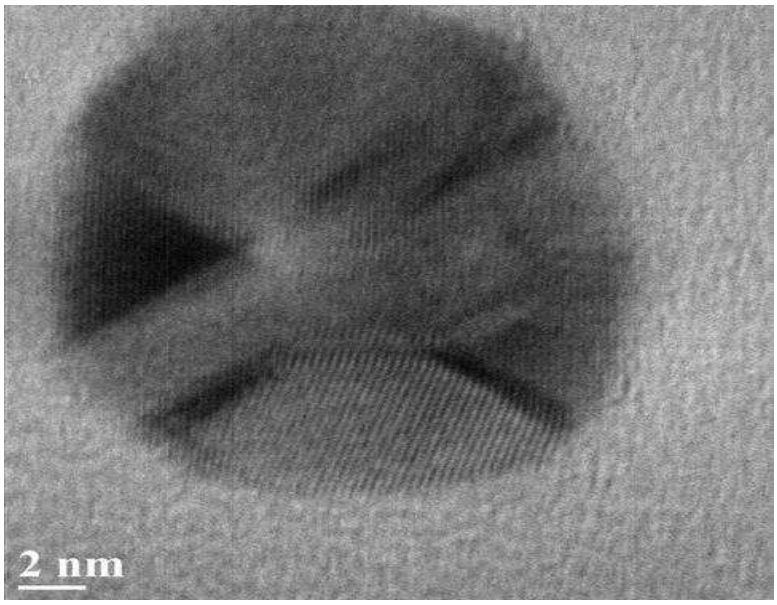


Fig 21.TEM images (A-E) showing nano-crystalline structure on the surface of Ag-nanoparticles.

1.4 X-Ray Diffraction

The suspension of silver nanoparticles was dried inside a vacuum chamber for 24 hours so that a small amount of dry silver nanoparticles can be obtained for X-ray diffraction (XRD) analysis. The XRD curve (Fig 22) confirmed that the nanoparticles are nothing but silver. Interpretation of this XRD pattern reveals the existence of diffraction lines at low angles (5° to 75°). The silver nanoparticles showed the two peaks of silver at $2\theta = 38°$ and 44° that can be assigned to the (111) and (200) facets of silver, respectively, which go very well with the

values manipulated for face centered cubic structure of silver nano-crystals (according to JCPDS: File No. 4-783).

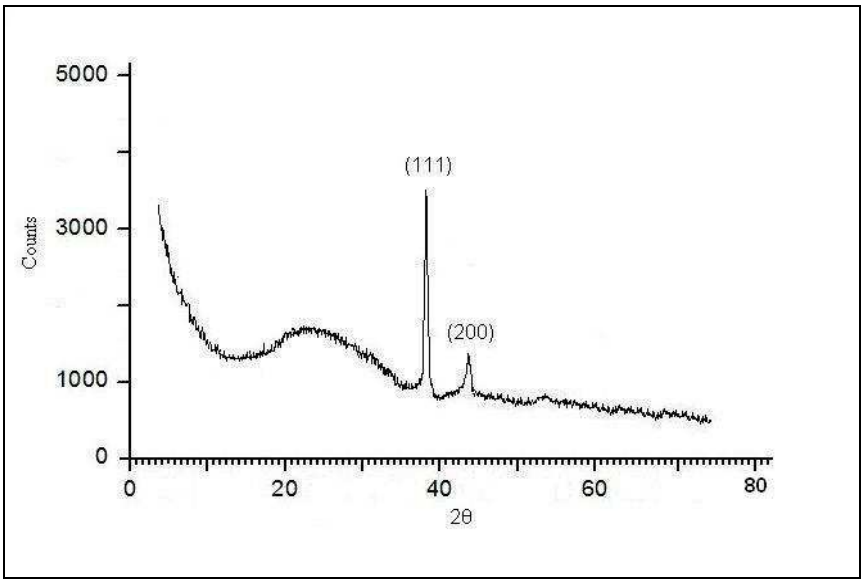


Fig 22.The XRD pattern shows two peaks assigned for (111) and (200) planes of silver

1.5 Scanning Electron Microscopy

Scanning Electron Microscopy is done for revealing the surface morphology of particles. Here, the bead for the SEM analysis was prepared by placing a drop of the silver nano-particle suspension on the carbon tape attached to the head of cylindrical bead and it was dried inside a vacuum dryer for a couple of hours. The particles on the top of the bead were scanned by Scanning Electron Microscope and the following image (Fig 23) was obtained.

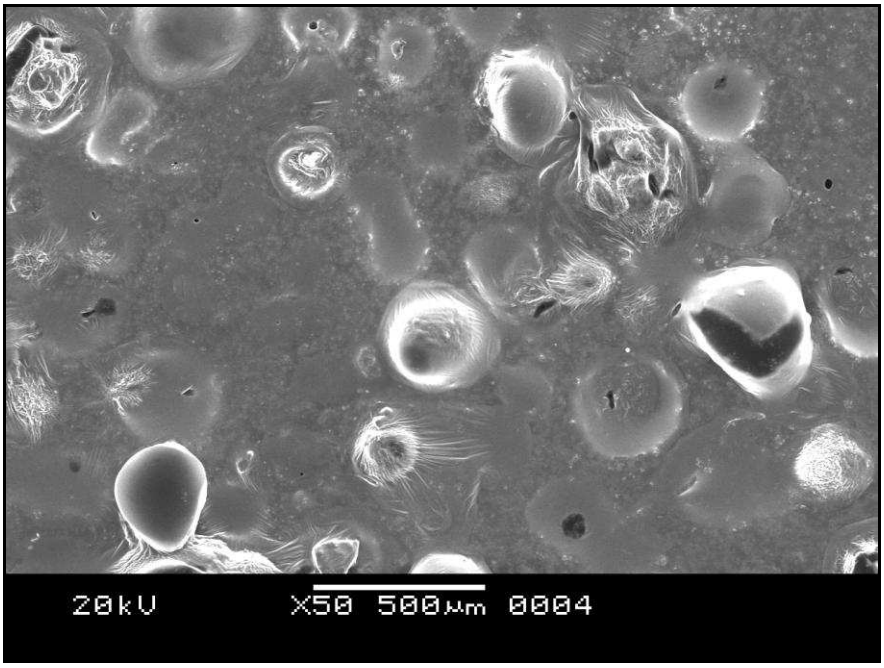


Fig 23 SEM image showing surface morphology of the silver nanoparticles

1.6 Energy Dispersive X-ray Spectroscopy

Energy Dispersive X-ray Spectroscopy or EDX is a technique that is mainly used to identify the presence of different elements in a sample. It is necessary to verify the presence of desired element in a sample. In the present study, this technique was used to verify the presence of Ag and the curve (Fig 24) showed a small peak of the element along with those of C and O.

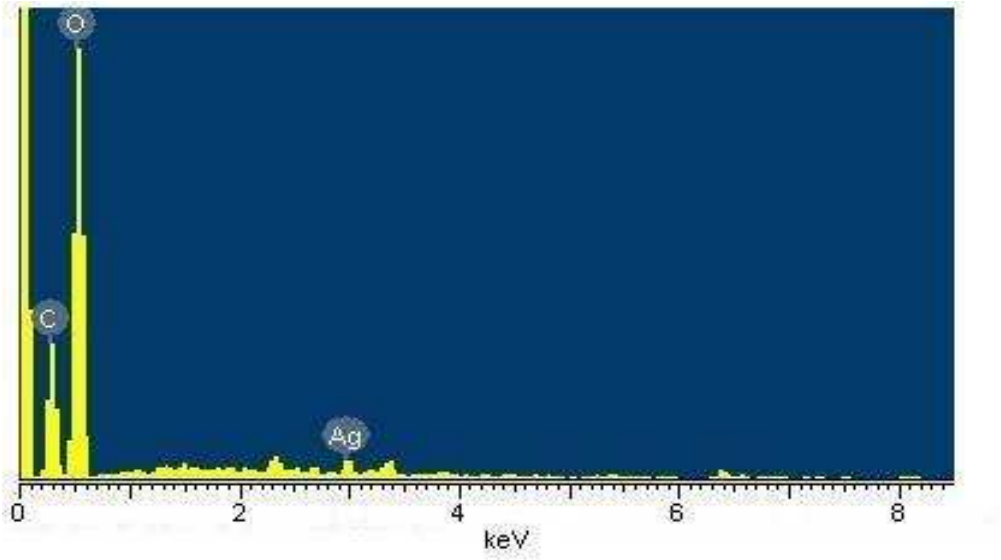


Fig 24. EDX curve of Ag-nanoparticles showing the presence of Ag and other elements (C and O).

Elements	Weight %	Atomic %
C K	25.44	31.9
O K	71.98	67.74
Ag L	2.58	0.36
Total	100	100

2. OBSERVATION OF ZONE OF INHIBITION

The following images show zone of inhibition as observed during the experiment

Fig 25.The disk D1 shows Inhibition Zone for silver nitrate with fruit extract where negative control shows no result

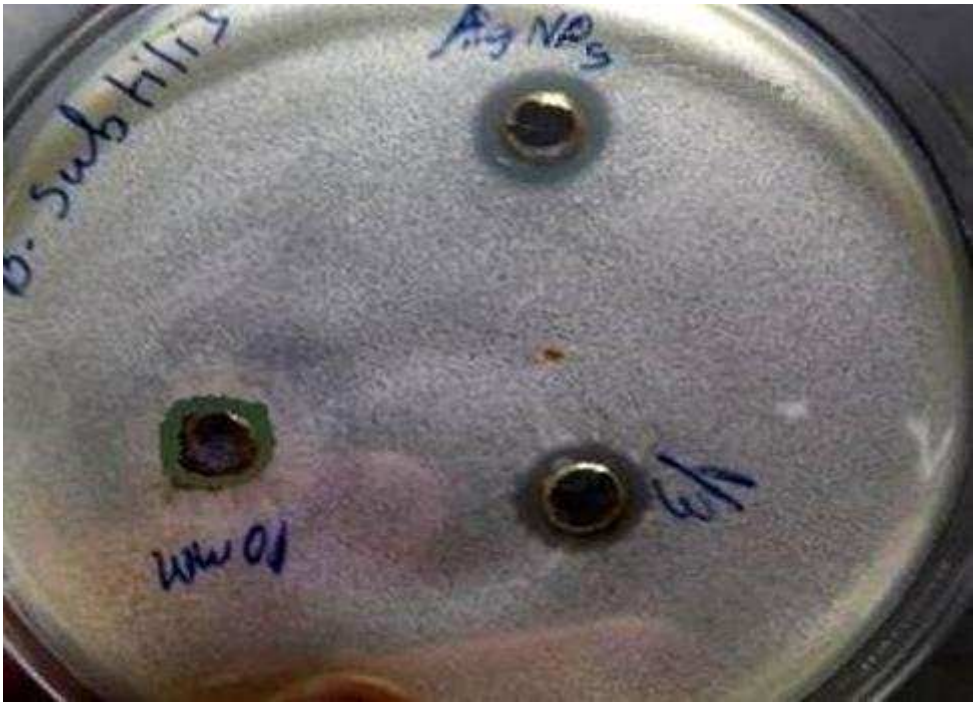


Fig 26.The disk D2 shows Inhibition Zone for silver nitrate with fruit extract where AgNPs shows the same result

Fig 27.The disk D3 shows Inhibition Zone for samples of silver nitrate with fruit extract where negative control shows no result

Fig 28.The disk D4 shows Inhibition Zone for silver nitrate with fruit extract where AgNPs shows the same result

The distinct Zone of Inhibition was observed around the cup wherein the suspension of AgNPs was applied as shown above. The mixtures of AgNO3 and fruit extract with 10 mM and 3.33 mM concentrations also prevented the bacterial growth. The sterile juice of grapes showed no result. The same microbiological test was performed against two common fungi- *A. niger* and *A. wentii* but the biologically synthesized silver nanoparticles did not show any positive result.

At the end of this antimicrobial screening test, it is confirmed that the biologically synthesized Silver Nanoparticles (SNP) possess effective antibacterial property. Therefore, applications of SNPs can cover a large domain of medical, leather and food technologies.

CONCLUSION: FUTURE SCOPE OF RESEARCH

The objective set for this study had been to prepare metal nanoparticles in a simple, cost-effective and eco-friendly way unlike chemical procedures. We used the extract of grape fruits (*Vitis vinifera*) as a reducing and capping agent. By this method of preparation, the problems of environmental pollution were avoided. We successfully characterized the biologically synthesized Ag-nanoparticles, which had an average size of 18-20 nm. These nanoparticles showed antibacterial activity against *B. subtilis* and *E. coli*.

A long term research is required to overcome these limitations and implement this procedure for large scale productions. A few applications of these Green Silver Nanoparticles are:

1. We proved the effective antibacterial property of these nanoparticles; hence we can think of its medicinal usage.
2. Due to the highest conductive properties, we can implement these Silver nanoparticles in advanced portable gadgets.
3. We can specifically use these nanoparticles in the production of clothing, leather items and coatings because it can protect these items from the attack of harmful microbes.

REFERENCES

[1] S. Silver, L. T. Phung, Anal. Rev. Microbiol. 50, 753-789 (1996).

[2] M. Catauro, J. Mat. Sci: Mat. in Med. 15, 831-837 (2004).

[3] J. H. Crabtree, Peritnl. Dial. Int. 23, 368-374 (2003).

[4] G. Cao, Nanostructures and Nanomaterials: Synthesis, Properties and Applications. Imperial College Press, London (2004).

[5] N. Kuyucak, B. Volesky, Biorecov. 1, 146-154 (1989).

[6] T. Klaus, R. Joerger, E. Olsson, C. G. Granqvist, J. Appl. Phys. Sci. Microbiol. 96, 13611–13614 (1999). 895

[7] A. R. Shahverdi, S. Minaeian, H. R. Shahverdi, H. Jamalifar, A. A. Nohi, Proc. Biochem. 42, 919–923 (2007).

[8] P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S. R. Sainkar, M. I. Khan, R. Parishcha, P. V. Ajaykumar, M. Alam, R. Kumar, M. Sastry, Nano Lett. 1, 515-519 (2001).

[9] A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M. I. Khan, R. Kumar, M. Sastry, Colld. Surf. B-Biointerfaces. 313-318 (2003).

[10] A. Ingle, M. Rai, A. Gade, M. Bawaskar, J. Nanopart. Res. 11, 2079-2085 (2009).

[11] A. K. Gade, P. Bonde, A. P. Ingle, P. D. Marcato, N. Durán, M. K. Rai, J Biobased Mater. Bioenrg. 2, 243-247 (2008).

[12] R. Sanghi, P. Verma, Biores. Technol. 100, 501-504 (2009).

[13] K. C. Bhainsa, S. F. D'Souza, Colld. Surf. B: Biointerfaces 47 160–164 (2006).

[14] K. Acharya, J. Sarkar, S. S. Deo, In: Bhowmik P.K., Basu S.K., Goyal A. (ed.) Bentham E-Books. Advances in Biotechnology, Bentham Science Publishers Ltd., p. 204-215 (2009).

[15] I. Sondi, B. Salopek-Sondi. J. Colld. Interf. Sci. 275, 177 (2004).

[16] M. Bosetti, A. Masse, E. Tobin, M. Cannas, Biomat. 23, 887 (2002).

[17] Z. Hu, J. Zhang, W. L. Chan, Y. S. Szeto, Mater. Res. Soc. Symp. Proc. 920, 0920-S02-03 (2006).

[18] C. V. Subramaniam, In: Hyphomycetes. Indian Council of Agricultural Research (ICAR). New Delhi (1971).

[19] M. B. Ellis, In: Dematiaceous Hyphomycetes. Commonwealth Mycological Institute (CMI) Kew, U. K. p. 608 (1971).

[20] M. B. Ellis, In: More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute (CMI) Kew, U. K. p. 507 (1976).
[21] A. Henglein, J. Phys. Chem. 97, 547-5471 (1993).

[22] P. Magudapathy, P. Gangopadhyay, B. K. Panigrahi, K. G. M. Nair, S. Dhara, Physics B. 299, 142-146 (2001).
[23] Catauro M, Raucci MG, De Gaaetano FD, Marotta A, J.Mater Sci Mater Med. 15(7), (2004).
[24] Crabtree JH, Brruchette RJ, Siddiqi Ra,Huen IT, Handott LL, Fishman A, Perit Dial Int. 23(4), (2003).
[25] Krolikowska A, Kudelski A, Michota A, Bukowska, J.Surf Sci 532, (2003).
[26] Zhao G, Stevens, J. Se. Biometals. 11, (1998).
[27] Hussain I, Brust M, Papworth A J, Cooper AI, Langmuir 19, (2003).
[28] Sharma Virender. K, Yngard Ria A., Lin Yekaterina, Colloid and Interface Science 145, (2009).
[29] Balantrapu Krishna; Goia Dan V, Journal of materials research 24, (2009).
[30] R.M.Tripathi, Antariksh Saxena, Nidhi Gupta, Harsh Kapoor, R.P.Singh, Digest journal of nanomaterials and Biostrucutres 5(2), (2010).
[31] Rodriguez-Sanchez.L, Blanco.M.C, Lopez-Quintela.M.A, J. Phys. Chem. 104, (2000).
[32] Taleb. A, Petit.C, Pileni .M.P, Chemistry of Materials 9, (1997).
[33] Begum Naznin Ara, Mondal Samiran, Basu Saswati, Laskar Rajibul A., MandDebabrata, Colloids and Surfaces B: Biointerfaces. 71(1), (2009).
[34] Lengke Maggy, Southam Gordon, Acta 70 (14), (2006).
[35] Binoj Nair and T. Pradeep, Crystal Growth & Design, 2, (2000).
[36] Shiying He, Zhirui Guo, Yu Zhang, Song Zhang, Jing Wang and Ning Gu, Materials Letters 61, (2007).
[37] Holmes J.D., Smith P.R, Evans-Gowing R., Richardson D.J., Russel D.A., Sodeau J.R, Arch. Microbiol.163, (1995).
[38] Mukherjee P, Senapati S, Mandal D, Ahmad A, Khan M.I, Kumar R, Sastry M, J. Nanotechnology 5, (2005).
[39] Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan M. I,Kumar R and Sastry M, Colloids and Surfaces B: Biointerfaces 28, (2003).
[40] Kuber C. Bhainsa and S.F. D'Souza, Colloids and Surfaces B: Biointerfaces 47, (2006).

[41] Shankar S. Shiv, Rai Akhilesh, Ahmad Absar and Sastry Murali, Colloid and interface science 275, (2004).
[42] Gardea-Torresdey J.L, Parsons J.G, Gomez E, Peralta-Videa J, Troiani H.E, Santiago P and Jose Yacaman M, Nano Letters 2, (2002).
[43] Raffi, M. Hussain, F. Bhatti, T.M. Akhter, J .I.,Hameed, A. Hasan, Journal of Material Sciences and Technology 24 (2), (2000).

[44] Monica Heger, A Silver Coating in the Fight Against Microbes, Silver nanoparticles could be the next step forward in antibacterial products, New York University's Science, Health and Environmental Reporting Program, (2008).
[45] S. Pal, Y. T. Tak, J. M. Song, Does, Applied and environmental Microbiology, 73 (6), (2007).
[46] M. Singh, S. Singh, S. Prasad, I. S. Gambhir, Digest Journal of Nanomaterials and Biostructures, 3(3), (2008).
[47] R. E. K.León, Study of Silver Nanoparticles Biocidal Impact on *Escherichia coli* Using Optical and Atomic Force Microscopy, Research Accomplishments, (2007).
[48] R. G. Cutler, E. J. Evans, Journal of Bacteriology, 9(2), (1996).
[49] Y. Li, X. Duan, Y. Qian, L. Yang, H. Liao, J. Colloid Interf. Sci. 209 (1999) 347G.
[50] P.V. Kamat, J. Phys. Chem. B 106 (2002) 7729–7744.
[51] Schmid, Chem. Rev. 92 (1992) 1709.
[52] S. Mann, G.A. Ozin, Nature 382 (1996) 313.
[53] M.C. Daniel, D. Astruc, Chem. Soc. Rev. 104 (2004) 293.
[54] L.N. Lewis, Chem. Rev. 93 (1993) 2693.
[55] S. Link, M.A. El-Sayed, Annu. Rev. Phys. Chem. 54 (2003) 331–366.
[56] J.J. Mock, M. Barbic, D.R. Smith, D.A. Schultz, S.J. Schultz, Chem. Phys. 116 (2002) 6755.

[57] H. Hovel, S. Fritz, A. Hilger, U. Kreibig, M. Vollmer, Phys. Rev. B 48 (18) (1993) 178.
[58] (a) A. Ahmad, S. Senapati, M.I. Khan, R. Kumar, M. Sastry, Langmuir 19 (2003) 3550;
(b) A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M.I. khan, R. Kumar, M. Sastry, Colloids Surf. B 28 (2003) 313;
(c) A. Ahmad, P. Mukherjee, D. Mandal, S. Senapati, M.I. Khan, R. Kumar, M. Sastry, J. Am. Chem. Soc. 124 (2002) 12108.
[59] T. Kluas, R. Joerger, E. Olsson, C.G. Granqvist, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 13611.

APPENDIX

ABBREVIATIONS

AFM	:	Atomic Force Microscope
DLS	:	Dynamic Light Scattering
EDX	:	Energy Dispersive X-ray Spectroscope
FESEM	:	Field Emission Scanning Electron Microscope
FTIR	:	Fourier Transform Infra-red
GSNPs	:	: Green Silver Nanoparticles
NIR	:	Near Infra-red
NPs	:	Nanoparticles
SEM	:	Scanning Electron Microscope
SNPs	:	Silver Nanoparticles

TED : Transmission Electron Diffraction
TEM : Transmission Electron Microscope
UV-Vis : Ultra Violet Visible
XRD : X-ray Diffraction

Notes