Green Synthesis of Silver Nanoparticles by Azadirachta Indica Plant

A dissertation submitted in partial fulfillment of the requirements for the degree of

Master of Technology

In

Nano Science and Technology

By

Mayank Kumar

(2K15/NST/04)

Under the guidance
Of

Dr. Mohan Singh Mehata

Assistant Professor

Department of Applied Physics

Delhi Technological University

Shahbad Daulatpur, Main Bawana Road, Delhi-110042

CERTIFICATE

This is to certify that the work which is being presented in the dissertation entitled **'Green Synthesis of Silver Nanoparticles by Azadirachta Indica Plant'** submitted by Mayank Kumar (2K15/NST/04) student of final year M. Tech in the DEPARTMENT OF APPLIED PHYSICS with specialization in NANO SCIENCE AND TECHNOLOGY is a record of student work carried out by him under my guidance and supervision during the year 2016-17.

Signature of Supervisor

Dr. Mohan Singh Mehata
Assistant Professor
Department of Applied Physics
Delhi Technological University
Delhi-110042

Head of Department
Department of Applied Physics
Delhi Technological University
Delhi -110042

DECLARATION

I hereby declare that the work which is being presented in this dissertation entitled **"Green Synthesis of Silver Nanoparticles by Azadirachta Indica Plant"** is my own work carried out under the guidance of Dr. Mohan Singh Mehata, Assistant Professor, Delhi Technological University, Delhi. I further declare that the matter embodied in this dissertation has not been submitted for the award of any other degree or diploma.

Date: **Mayank Kumar**

Place: **(2K15/NST/04)**

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Mayank Kumar

(2K15/NST/04)

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Abstract:

Silver Nanoparticles (AgNPs) were synthesized from plant extracts of Azadirachta Indica (Neem) and silver salt ($AgNO_3$) following the purely green approach. In this study we synthesized and analyzed the controllable synthesis of nanoparticles by changing different physico-chemical parameters like time reaction, plant extract concentration, pH and temperature. Synthesized AgNPs were also analyzed by absorption/photoluminescence spectroscopy, fourier transform infrared spectroscopy (FTIR), Energy Dispersive X-Ray Analysis (EDX) and scanning electron microscopy (SEM). The synthesized nanoparticles showed a strong absorption peak at around 400 to 450 nm due to strong surface plasmon resonance (SPR) exhibited by the silver nanoparticles. From the results obtained it can be concluded that the green synthesis approach is simple, economic, time saving and environment friendly as compared to the chemical and physical methods of synthesis. The AgNPs synthesized using this approach can be used in wide range of applications such as biomedical, sensor technology, optoelectronics, space technology and nanotechnology.

Chapter-1

INTRODUCTION

1.1. Nanotechnology

Over the past few years nanotechnology has been gaining appreciable attention towards the modern research and one of the most important field in modern research dealing with size approximately 1-100 nm in at least one dimension. In the year 1974 term “nanotechnology” was first coined by Professor Norio Taniguchi of Tokyo Science University. Nanotechnology is a field that makes a powerful effect in all domains of human life and rapidly gaining importance due to its small size, synthesis, characterization properties as these properties differ from bulk material to single atoms and molecules.

There is a growing interest in nanoparticles as they have most promising and remarkable applications specially in the medical field, biomedical sciences ,drug delivery, gene delivery (***Song and Kim.,2009***) , DNA sequence detection (***Hua, L. et al.,2007***), cosmetics, dietary food intake, environmental safe keeping, biomechanics, optics, aerospace industries, energy source, catalysis, single electron tunneling transistors(SET), light emitters, chemical industries, electrical equipment’s, nonlinear optical devices, nanoelectronics like nano connectors (***S.H. Kim et al.,2007***) and sensor technology applications (***Harekrishna et al., 2009***).

Nanoparticles due its unique property change from bulk materials to molecular or atomic level has shown great interest to researchers. As the size changes the physical, chemical, biological properties changes fundamentally hence the properties of bulk material to individual atoms/molecules also changes.

Green synthesis approach have encouraged the researchers to further finding solutions to many technological and environmental challenges to reduce toxic waste in the field of drug delivery, nano therapeutics effect, gene delivery, solar energy conversion, biomedical, sensors and waste water treatment and global efforts has been continuously going on to increase the demand of green synthesized nanomaterials.

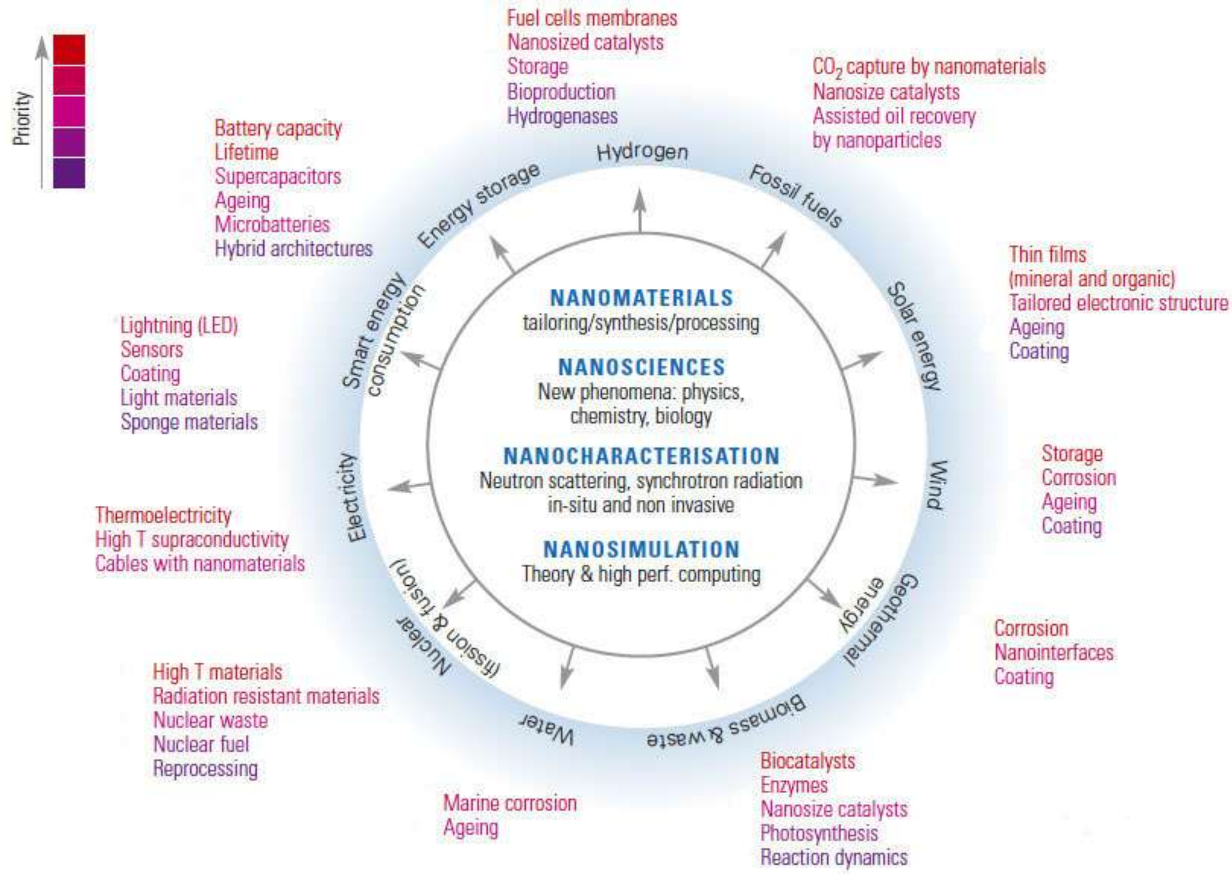


Fig. 1. Wide Applications of Nanomaterials. (*Source: Gennesys White Paper*).

1.2. Nanoparticles and their types.

A particle with size ranging from 1-100 nm is term as nanoparticles in which at least one of the three possible dimension is in nanometer range. Nanoparticles probe is currently an area of strong scientific research due to a wide diversity of probable applications in biomedical, optical, and electronic fields. Nanoparticles due its unique property change from bulk materials to molecular or atomic level has shown great interest to researchers. As the size changes the physical, chemical, biological properties changes fundamentally hence the properties of bulk material to individual atoms/molecules also changes. A bulk material which is metal or semiconductor can behave as an insulator in the nano state or vice-versa. This vast change in behavior observed due to large surface area to volume ratio of nanoparticles and lattice constriction. Different types of nanoparticles can be formed by varying chemical and physical parameters like metals nanoparticles (gold and silver), metal oxides (zinc oxide), ceramics ($BaTiO_3$), polymers, silicates, organic (carbon nanotubes, grapheen sheets, carbon rod) and non-organic materials (gold, silver, ZnO), and biomolecules such as DNA. Nanoparticles composed of different elements having several morphologies such as squares, spheres, triangular, cylinders, sheets, tubes or even quantum dots etc. Quantum dots having the size range from 1-10 nm.

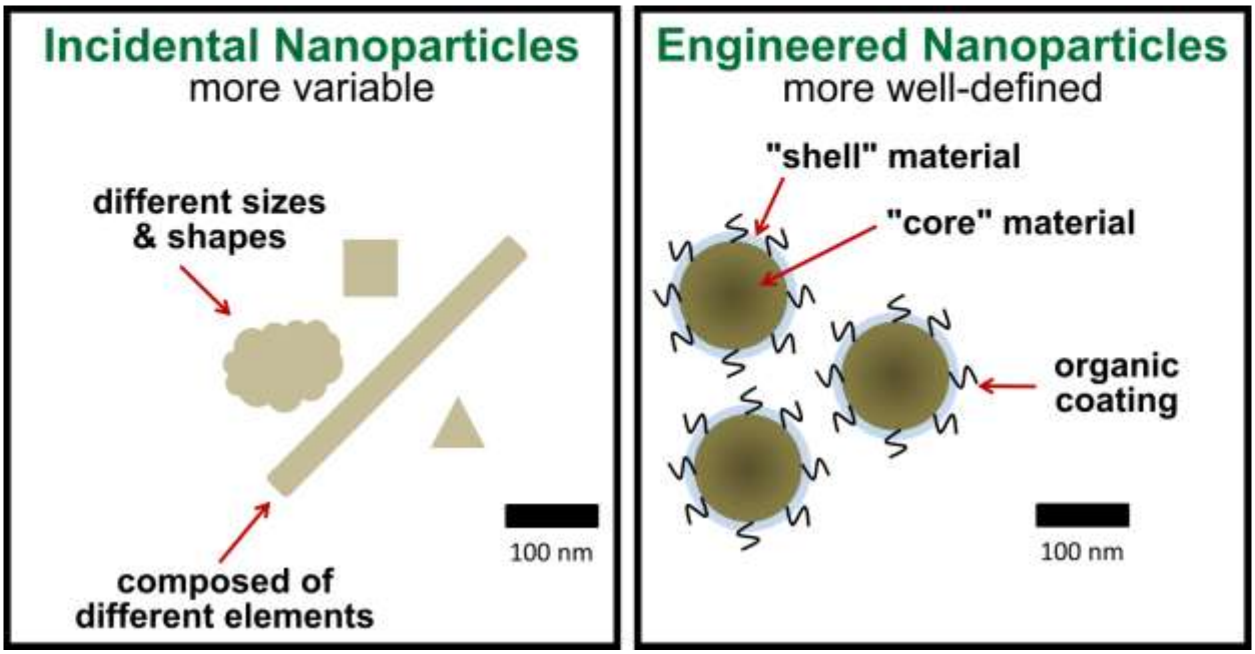


Fig. 2. Surface modified Nanoparticles. (Source: *Sustainable nano.com*).

Nanoparticles can also be categorized into generally two types **organic nanoparticles** which includes carbon nanoparticles such as fullerenes, graphene sheets, carbon nanotubes and **inorganic nanoparticles** which include metal nanoparticles like gold(AuNPs) and silver(AgNPs) and semi-conductor nanoparticles like zinc selenide (ZnS), lead selenide (PbS), cadmium Selenide (CdS), titanium oxide(TiO_2) and zinc oxide(ZnO). There are also some natural nanoparticles which includes magnetic nanoparticles like in magnetotactic bacteria found near the magnetite ores mines. They are basically found near the anaerobic zone of sea bed. There is an emergent curiosity in development of inorganic nanoparticles like gold and silver as gold and silver as they provide higher quantifiable properties over inorganic nanoparticles also with efficient adaptability. Due to their salient features and advantages of nano size over existing chemical imaging drugs inorganic particles has been studied as a potential tool for bio-medical diagnosis imaging as well as for diagnosing and treating diseases like cancer. Inorganic nanomaterial have been extensively used for drug delivery due to their efficient adaptability like easy and varied obtainability, rich functionality, very good compatibility and proficiency of targeted delivery and controlled release of drugs (***Xu et al., 2006***).

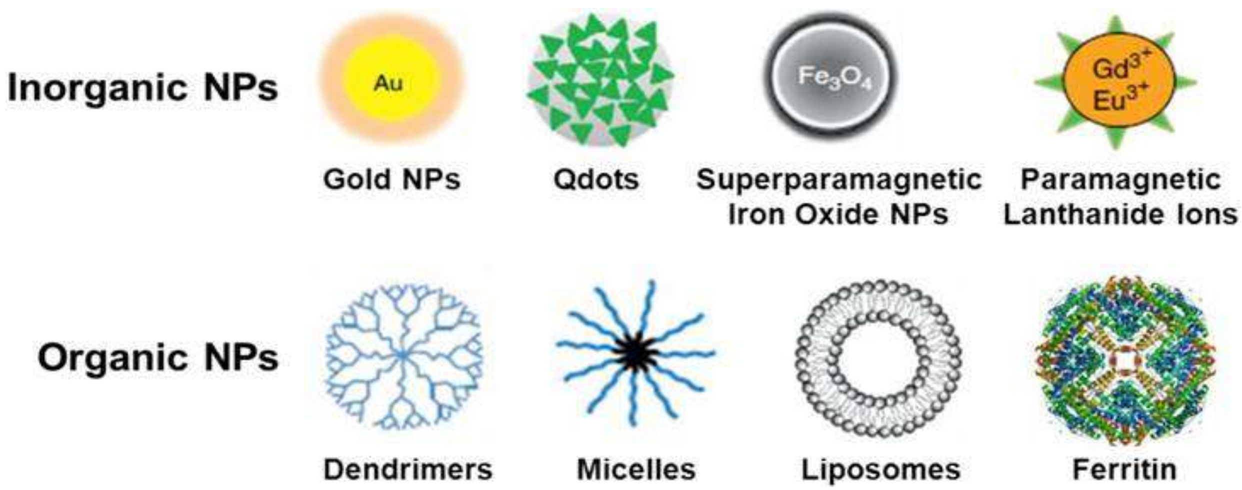


Fig. 3. Types of nanoparticles (Source*: Xing et al., 2014, Theranostics*).

1.3. Elemental Silver

Silver is a naturally occurring valuable metallic element placed at the 47th element in the periodic table and having an atomic weight of 107.8 and two natural isotopes 106.90 Ag and 108.90 Ag. It is a d block, group 11 element which is lustrous white in appearance. Metallic silver has some very good properties like high electrical conductivity and thermal conductivity (**Nordberg and Gerhardsson, 1988**). It has been used in a wide variety of applications like antimicrobial applications, nano drug delivery, sensor materials, composite fibers, packaging industry, cryogenic, superconducting materials, cosmetics, food and beverages industry and optoelectronic devices. There are so many different methods for synthesis of AgNPs has been developed comprising physical, chemical and green (biological) synthesis resulting in different shapes, sizes and morphologies. Mainly, there are different synthesis methods are categorized into two sub-categories namely top down approach and bottom up approach. In top-down approach, lithography and laser ablation methods are used to synthesize the size of silver metal in its bulk form reduces mechanically to the nano-scale. Self-assembly technique i.e. bottom-up approach comprises of dissolution of silver salt like $AgNO_3$ into a solvent like distill water, reduction of silver ions (Ag^+) to their element using addition of a reducing agent and then stabilization of the forming AgNPs using a become stable agents to prevent agglomeration of nanoparticles (***Tolaymat et al.,2010***).

1.4. Methods for AgNPs synthesis.

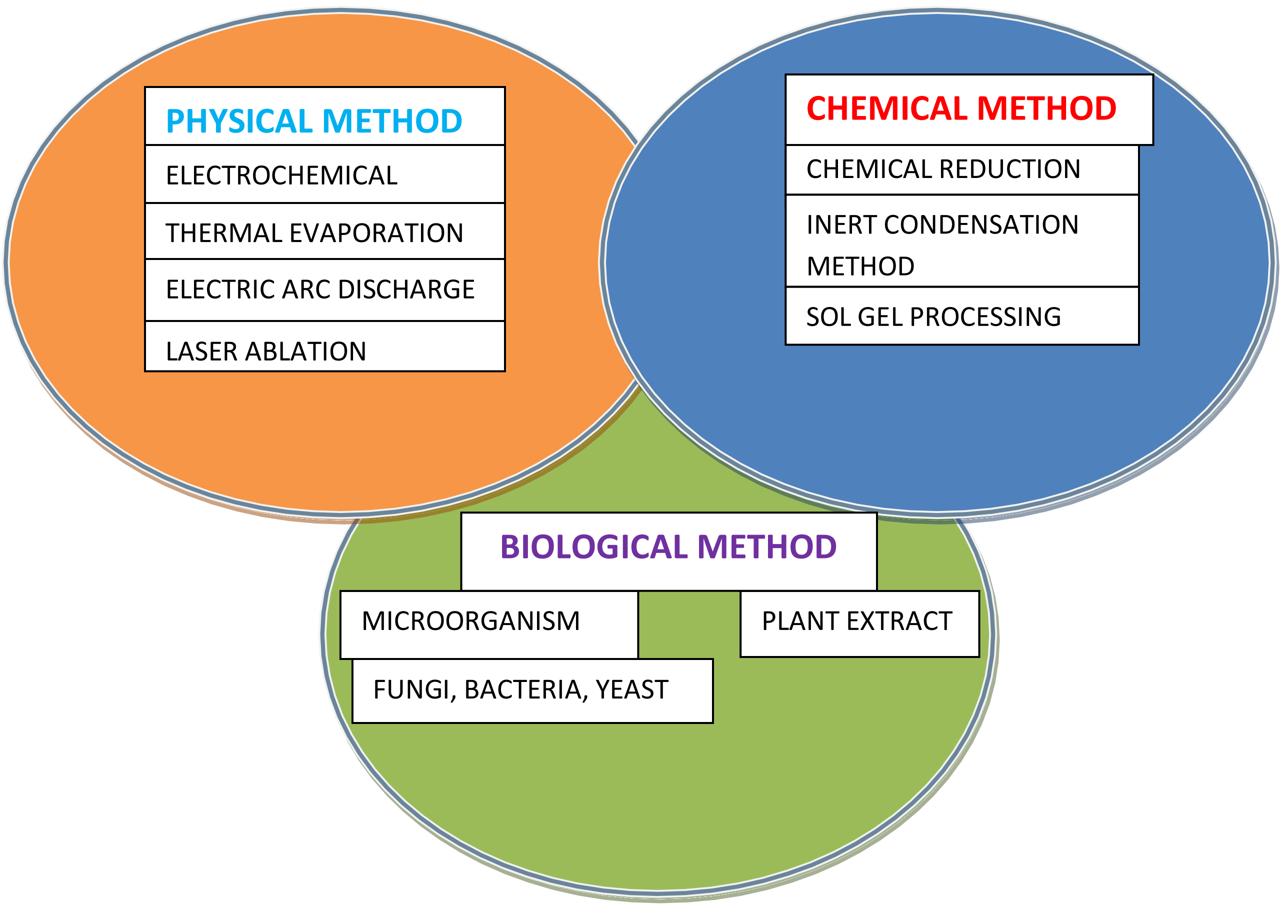


Fig.4. Various methods of silver nanoparticle synthesis.

1.4.1. Physical methods.

The most common and important physical approaches for synthesis of AgNPs comprise evaporation-condensation, arc discharge and laser ablation. The main advantage of these methods are nonexistence of chemical reagents in solutions. From these above methods various metal nanoparticles can be synthesized such as silver (Ag), gold (Au), lead sulfide (PbS), cadmium sulfide (CdS), and fullerene (***Gurav et al.,1994; Magnusson et al., 1999; Kruise et al.,2000***).

1.4.2. Chemical methods.

The most common chemical approach for AgNPs synthesis is chemical reduction by organic and inorganic chemical reducing agents. There are several reducing agents were used for the reduction of silver ions (Ag^+) to metal silver (Ag) in aqueous or non-aqueous solutions like sodium citrate ($Na_3C_6H_5O_7$), ascorbic acid, ethylene glycol, ethanol, sodium borohydrid ($NaBH_4$), elemental hydrogen, Tollen's reagent, N, N-dimethylformamide (DMF) etc. These above reducing agents can reduce silver ions (Ag^+) to the formation of metallic silver nanoparticles (Ag^0), which may later on followed by agglomeration and turns into oligomeric (a molecular complex monomer units) clusters (***Iravani et al., 2013***). As the time passes on these clusters eventually leads to the formation of metallic colloidal silver particles (***Evanoff et. al., 2004, Wiley et. al., 2005; Merga et al., 2007)***. It is important to use capping/shielding agents to stabilize dispersive NPs and avoiding their agglomeration during the course of metal nanoparticles preparation (***Evanoff et. al., 2004***). Although from these chemical reducing agents nano particles can be formed but they are expensive and toxic to environment.

1.4.3. Biological methods.

There is a growing concept of green approach is introduced in which different nanoparticles can be synthesized from plant extract and microorganisms like bacteria, fungi, yeast etc. These biological methods provides a meaningful symbiosis between natural science including plant, microorganism and environment and this connection provides green approach to green synthesis nanotechnology. Generally this method is eco-friendly, easily available, safe and do not use any toxic, hazardous and expensive chemical substance or apparatus. For the reduction of metal ions to metal nanoparticles large number of phytochemicals or biomolecules are responsible in case of plant extract and in case of microorganisms reduction is done by biological parameters such as protein ,amino acids, DNA, electron donor such as glucose and fructose . These phytochemicals found in the plant extracts of neem, aloe vera, tulsi and many herbal plants. The main phytochemicals present in plant extract are terpenoids, flavonones, ketones, aldehydes, amides and carboxylic acids.

The synthesis of AgNPs can be obtained by different plant extract such as Jatropha curcas seeds, Banana peel, Oscimum sanctum stems and roots (***Bar et. al., 2009; Bankar et. al., 2009***; ***Ahmad et. al., 2010***). The synthesis of AgNPs from microorganisms like bacteria, yeast, fungi, algae is also achieved. The synthesis of silver nanoparticles from bacteria named *Pseudomonas stutzeri*AG259 strain, E. Coli, Lactobacillus strains (***Haefeli et al., 1984; Shahverdi et.al., 2007; Korbekandi et.al. 2012***) has been reported.

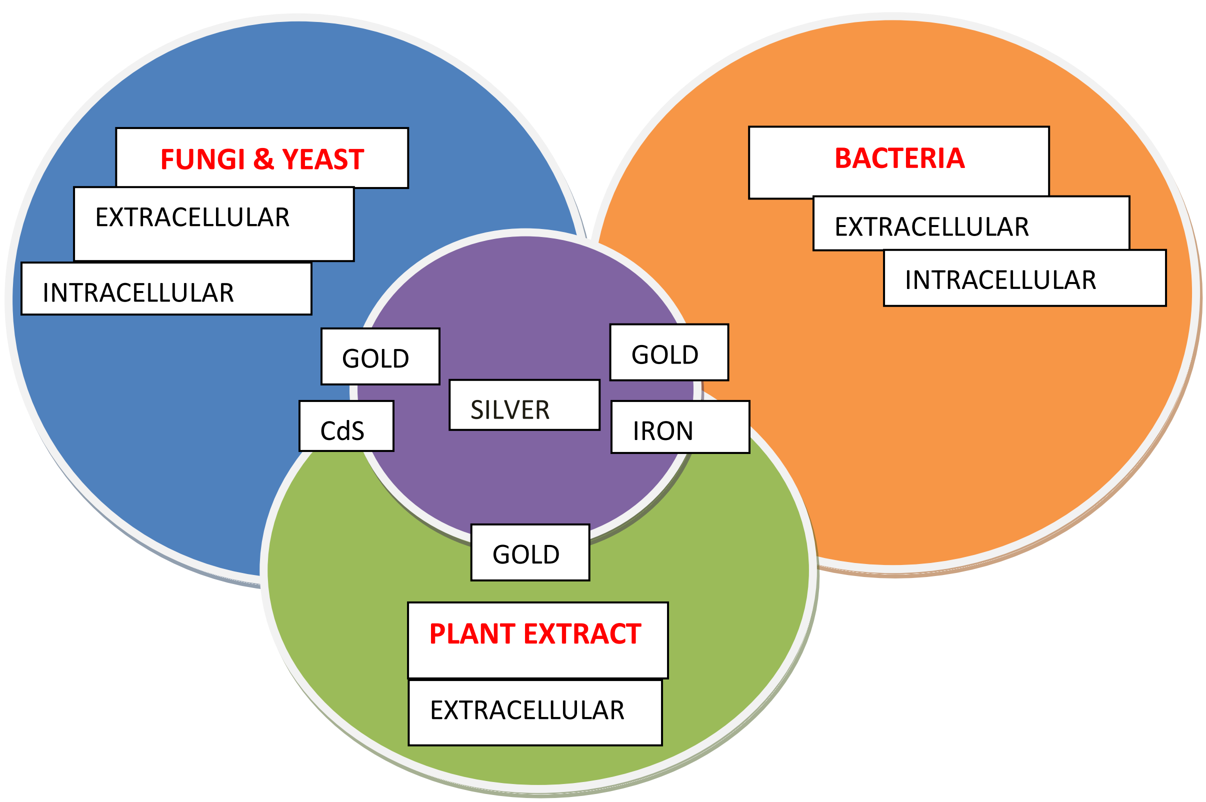


Fig.5. Biological synthesis of nanoparticles

Biological based synthesis is highly stable and well characterized nanoparticles. Their size and shape can be control by physicochemical parameters such as pH, concentration, light, temperature, time, buffer strength and by biological parameters such as protein, amino acids, DNA, electron donor such as glucose and fructose.

1.5. Need for green synthesis.

The concept of green synthesis is brought to diminish or eliminate the toxic and harmful effects towards the environment. The advantages of green synthesis is clean and eco-friendly approach as there is no toxic chemical is used and overall reduction of cost. In this method, the biological component itself act as reducing and capping agent. This approach can also be used for large scale nanoparticles as no external experimental conditions like high energy, temperature, pressure and toxic chemicals required. Green approach can be performed using both plant extract and microorganism. It is a process of bottom up approach where redox reaction takes place. As the physical and chemical processes were costly and the by-products of the synthesis are toxic for both man and environment may restrict their use in the medical applications (***WHO, 2002)***. When it comes to nanoparticles synthesized via green approach toxicity is not an issue and in order to cheaper the synthesis process, researchers used microorganisms, enzymes, DNA and plant extracts (phytochemicals) for the bio-reduction of nanoparticles. As this method is nontoxic and biocompatible enhance their potential in medical diagnosis (***Ingale et. al.; 2013***).

1.6. Nanosilver.

Silver is one of the rare naturally occurring element. It has been used in extensive variety of applications as it has exceptional properties such as high electrical conductivity, thermal conductivity and has lowest contact resistance. It is lightly harder than gold and very high grade malleability & ductility means it can be forged into thin sheets and wires. Nano silver means nano-sized particles of range 1-100 nm. Ag nano has additional active surface area and better porosity than commercial silver (***Alt et. al.; 2004***). Metallic silver salts are easily soluble in water like silver nitrate ($AgNO_3$) and Silver Chloride (AgCl) ,but metallic silver itself is insoluble in water (**WHO,2002**).Metallic silver is used from ancient civilization in jewelry, coins, utensils, surgical prosthesis and splints, fungicides containers and as a purifier for water and human septicity. Metallic silver when taken in small amount has no bad effect on the human body and it has natural antimicrobial action (***Margaret et al. 2006***) towards many pathogens like bacteria, viruses, fungi, yeast (***Hill and Pillsbury 1939***).

The nanosilver is showing strong biocidal effects on bacteria, fungi, virus, yeast and highly toxic to microorganisms.Due to high specific surface area to volume ratio of nanosilver increases their contact with microorganism and hence increases biocidal effectiveness (***Vijaykumar et. al., 2013***). The AgNPs antimicrobial effect depends strongly on different parameters which includes pH, temperature, $AgNO_3$ concentration and type of microorganisms. Nanosilver has a unique properties like large surface area to volume ratio, absorption in the visible range, surface functionalization, high electrical conductivity, thermal conductivity and controlled drug release which makes them valuable for human life (***Mittal et al., 2004***).It has a wide of applications such as clothing, respirators, household, water filters, electronics, food packaging materials, cosmetics, detergents, antibacterial sprays, space technology, drug delivery, gene delivery etc. Despite of several applications and has powerful antimicrobial properties but it could have serious negative consequences for human health. Silver nanoparticles are extremely harmful to mammalian cells, brain cells, liver cells (***Hussain et al; 2006***).

1.7. Action of AgNPs on microbes.

It is well identified that silver and silver based compounds showing robust antimicrobial effects towards microorganisms such as bacteria, virus, fungi and yeast. However, it has proven that silver showing strong biocidal effects against several types of viruses like influenza A/H1N1, H1N5, SARS-cov, hepatitis B, HIV, dengue virus and herpes simplex virus (***Sondi et al.,2004;Lara et al., 2012***). The exact mechanism of AgNPs antimicrobial effect is not known clearly. The silver nanoparticles antibacterial property is also related to the quantity of silver and the degree of silver released. Metallic silver is inert but when gets moisture in the skin, gets ionized which results as a highly reactive ionized silver as it fixes to tissue proteins and transports structural variations in the bacterial cell wall and nuclear membrane leading to cell distortion and death. There is an accumulation of the nanoparticles of the nanoparticles and formation of pits on the cell surface.

There is another innovation of electron spin resonace spectroscopy that revealed that creation of free radicals by silver nanoparicle, when comes in interaction with bacteria have capability to rupture the cell membrane and create it to porous which can ultimately lead to cell death. Another mechanism is that the DNA strand composed of soft bases like sulfur and phosphorus as its main components when interacts with silver nanoparticles can lead to problems on these soft bases and damage the DNA which would terminate the microbes. It is also reported that silver ions interaction with thiol group rupture the enzymes of bacterial cell which leads to cell death (***Chaudhari et. al., 2016***).

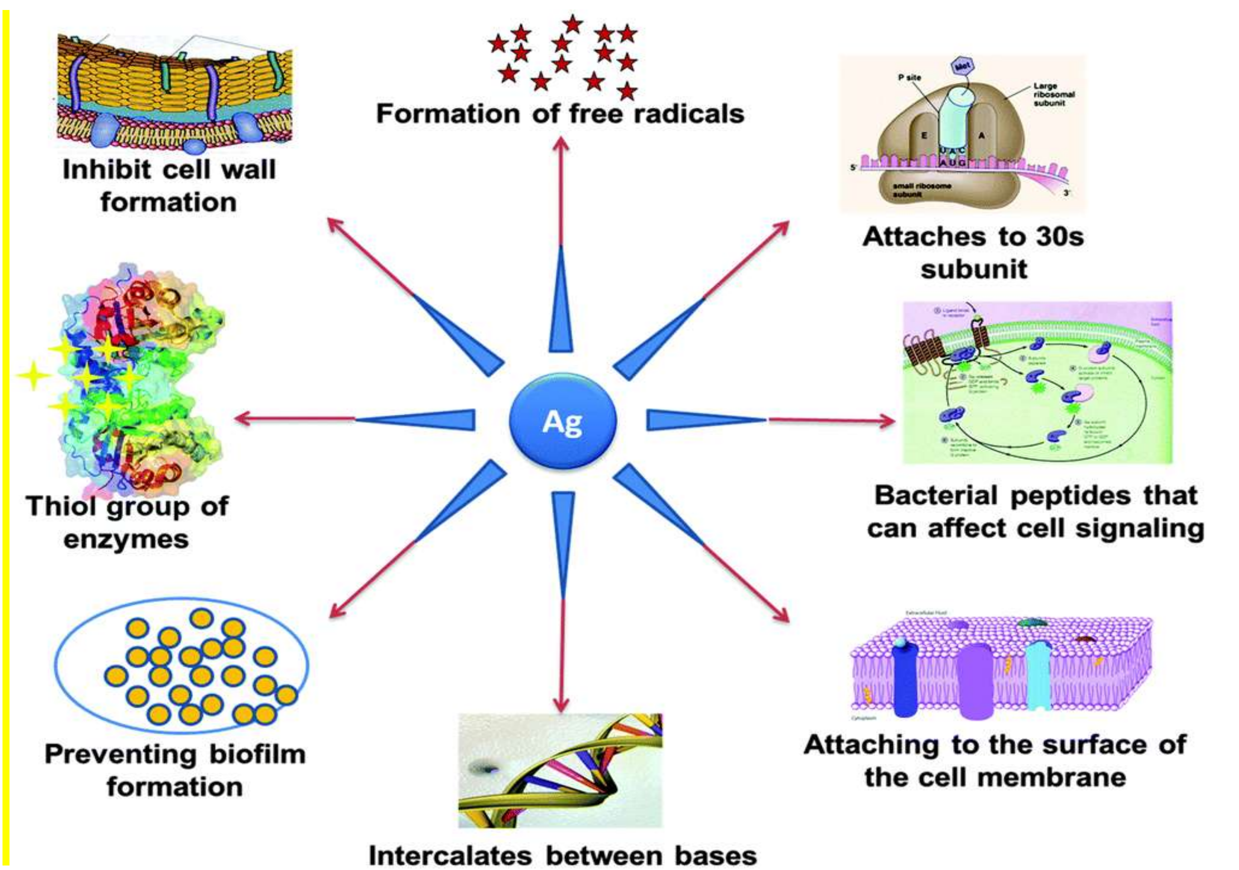


Fig.6. Various modes of action of AgNPs on microbes.(Source: *Chen et al., 2014, chemical communications).*

1.8. Applications of Silver nanoparticles

Nanoparticles put forward essentially wide ranging applications due to implausible properties like optical, electrical, and mechanical. The exclusive properties and usefulness of nanoparticles are arise due to similar size of nanoparticles and phytochemicals or biomolecules like DNA, protein and comparable size of microorganisms like virus, bacteria, fungi, yeast. Nanoparticles newly develop imaging techniques for in vivo diagnosis, cell imaging, drug delivery leads to more effective medication with less unfavorable effects (***Gao et al., 2008***). Nanoparticles also present a diverse range in agriculture applications like safety and preserve food packaging, biosensors, nanosensors which are able to sense the existence of plant virus, nanocapsules containg herbicides, chemical ,genes to enhance the properties of plants or herbs. AgNps are also used for purifying of water, antibacterial sprays, cosmetics, dietary supplements, detergents, ointments and creams.

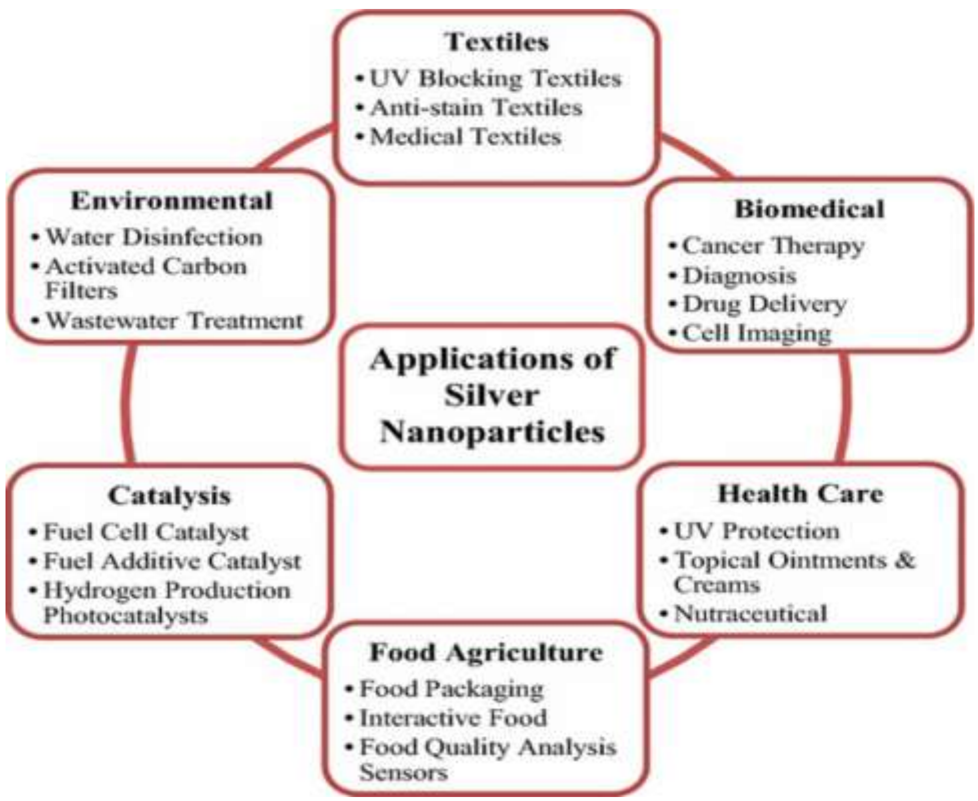


Fig.7. Applications of Silver Nanoparticles. (Source: *Keat et al., 2015, Bioresour.and Bioprocess).*

1.9. Toxicity of Silver nanoparticles.

Nanotechnology is rapidly growing and already having an impact on various applictions specially in medical field due to its unique size dependent properties which makes these nanomaterials superior and indispensable. AgNPs have great potential antimicrobial and anti-inflammatory activities towards many pathogens. AgNPs show powerful antimicrobial activity but has negative impact also occurs on human health and the environment. It is reported in many research papers that AgNPs not only toxic to bacteria but also highly toxic to mammalian cells and stem cells (***Braydich-Stolle et al., 2005***). AgNPs also shown damage to brain cells, liver cells (***Hussain et al., 2006***).High exposure to silver salt can cause skin diseases like argyria and argyrosis. It is also reported in (***McAuliffe et al., 2007***) that nanoparticles affected male reproductive organs by crossing the blood –testes barrier and which may deposited in the testes which cause damage to sperm cells. AgNPs also accumulated in the nails, kidneys, skin, liver, mucous membranes and spleen. Long term exposure of AgNPs accumulate in the skin which turns skin discoloration to an ashen-grey color known as argyria and in eyes known as argyrosis. These nanoparticles in higher concentrations can damage the cell membranes. It has been reported that AgNPs can enter through the cardiovascular system, pulmonary system and lung associated lymph nodes mainly on the alveolar walls. In respiratory system ultrafine particles like nanoparticles accumulated and deposited on alveolar space(***Panyala et al. 2008***).Long term exposure of AgNPs accumulate in the skin which turns skin discoloration to an ashen-grey color known as argyria and in eyes known as argyrosis.

Nanosilver also distrupts denitrification process which leads to damage eco-system. Nitogen fixation bacteria like rhizobium and ammonifying bacteria produce crucial nutrients and form symbiotic relationships with legumes plants but nanosilver exposure affects these bacteria and harms the soil microbial communities. Denitrification is important environmental phenomenon in which nitrates are converted to nitogen gas(N_2)in soils, swamps and ecosystem. Excess nitrates also causes eutrophication in rivers, lakes and marine ecosystems which reduce the plant productivity.

1.10. Literature Review

Nanotechnology has now become a buzz of modern scientific world has been witnessed an appreciable interest in metal nanoparticles like silver and gold because of their unique optical, electrical, physical, mechanical properties. Nowadays researchers are focused more to develop a synthesis route which is economic, cost effective, non-toxic and productive. Metal and alloy nanoparticles due to its size dependent properties like electrical, optical and catalytic properties have fascinated much consideration over the few years. Alloy nanoparticles have received special attention due to the possibility of tuning the optical and electronic properties over a broad range by simply varying the alloy composition ***(Raveendran et al.***, **2006)**. The surface Plasmon Resonance (SPR) phenomena which gives nanoparticles their unique optical properties is one of the important characteristic property**.** Metal and alloy nanoparticles are used effectively in medical fields, space technology, electronics etc. (***Lu & Lieber, 2007; Shen et al., 2000; Karni et al., 2012***).

Till date several researchers had reported the synthesis of silver nanoparticles using plant extract such as aloevera **(*Zhang et al., 2010*)**, Azadirachta indica (neem) (***Verma & Mehata, 2015***), geranium (***Shankar et al., 2003*)**, Ocimum tenuiflorum (black Tulsi) (***Banerjee et al., 2014***), Ficus Benghalensis (Banyan tree) (***Saware et al., 2014***) etc and many weeds (***Roy and Barik , 2010; Parashar et al.,2009***). From other parts like stem, roots etc metal nanoparticles can also be synthesized (***Jha et al., 2009***). Various microorganisms like bacteria, fungi, yeasts (***Narayanan & Sakthivel, 2010***) and DNA (***Sohn, Kwon, Jin & Jo, 2011***) are also used for green approach synthesis of silver nanoparticles.

AgNPs can be synthesis through various physical and chemical methods but these methods require high pressure and temperature. Metal nanoparticles synthesized by chemical methods are harmful to human beings and environment as they are highly toxic and flammable. Thus the new concept of Green approach had been developed for their synthesis which not only eliminate the toxic chemical methods but creates meaningful symbiosis between natural/plant science and nanotechnology. This green nanotechnology has its own advantages of easy availability, cost effectiveness and environment friendly nature.

In this work, we have done a study of green synthesis of AgNPs using Azadirachta Indica (Neem) leaf extracts by varying different physical and chemical parameters like reaction time, concentration, pH and temperature. The plant based phytochemicals like terpenoids, ketones, aldehydes, amides, carboxylic acids and flavanones are present in neem and tulsi plant extract, which are responsible for reduction of silver salt ($AgNO_3$) to AgNPs. Silver nanoparticles show yellowish-brown color in aqueous solution due to the excitation of surface plasmon vibrations **(Shankar et al., 2004**).

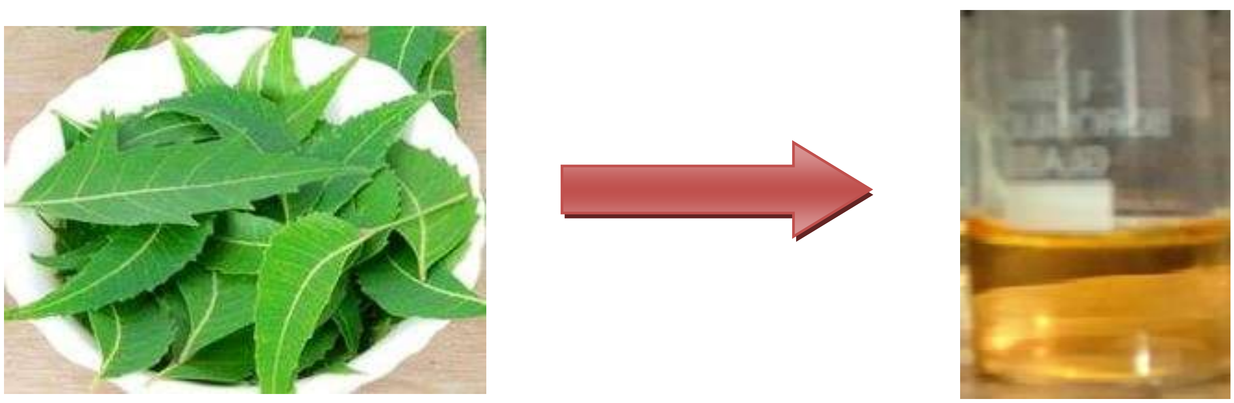
It has been well-known that plant extract have potential to reduce metal ions into nanoparticles **(Makarov et al.,2014).** Terpenoids and flavanones found in many medicinal plants like neem, tulsi, ginger, giloy, aloe vera, fruits and vegetables. It can be used as an ingredient in supplements, beverages & foods. The AgNPs obtained by the reaction of Silver salt and neem plant extracts at different environmental conditions were characterized by different techniques.

Chapter-2

Materials and Methods

2.1. Preparation of Plant Extract.

Firstly, wash all the fresh neem leaves, sample bottles, petri-dish, and agar properly. Neem leaves are collected from the campus only. Before using the perti-dish and agar first auto claved them so that no moisture will be present. Silver Nitrate ($AgNO_3$) salt from Sigma-Aldrich Chemical Co is 99% pure. In the preparation of plant extract, leaves of Azadirachta Indica (neem) was first wash properly with normal tap water nearly 2-3 times and then wash properly with distill water in order to remove all the dust particles and unwanted particles. Now the leaves were completely dried in order to remove the water from the leaves. In next step take 2.5 gm of crushed leaves and then boiled in 50 ml of distill water at 50°C for 15 minutes and then filtered it with using whatman filter paper No.1 to remove the particulate matter. Finally we get pale yellow color solution which can be stored at 5-10°C**.**



Neem Leaves **Extract**

Fig.8. Neem leaves converted into its extract

2.2. **Synthesis of AgNPs**

Now first prepare silver nitrate solution by dissolving 0.017 gm of $AgNO_3$ in 25 ml of distill water. Then add 5ml of $AgNO_3$ solution with 1 ml of neem extract solution; here we observed a change in color from pale yellow to brown color which indicates the formation of silver nanoparticles as shown in Fig.8. After that by changing various parameters like pH, concentration, reaction time and temperature on the synthesis of silver nanoparticles is performed.

Fig.9. Change in the yellow to brown color solution after adding $AgNO_3$ solution to extract

2.3. Characterization of AgNPs.

2.3.1. UV-Vis & PL spectra analysis.

The absorption spectra were recorded with UV/VIS/NIR spectrometer (Perkin Elmer Lambda 750) and photoluminescence spectra were recorded with Fluorolog-3 Spectrofluorometer (Horiba Jobin Yyon) equipped with double-grating at excitation and emission monochromators (1200 grooves/mm) and R928P photomultiplier tube (PMT). The excitation source was a 450 watt CW Xenon lamp.

Effect of time was studied at intervals of 1hr, 2hr, 3 hr and 4 hr in case of leaf extract . Effect of temperature was also studied by varying the temperature between 30-55°C in Neem plant extracts with an accuracy of ±2°C. Effect of pH was studied by varying from 7-12 pH of neem plant extract . The pH of$AgNO_3$ solution is not varied.

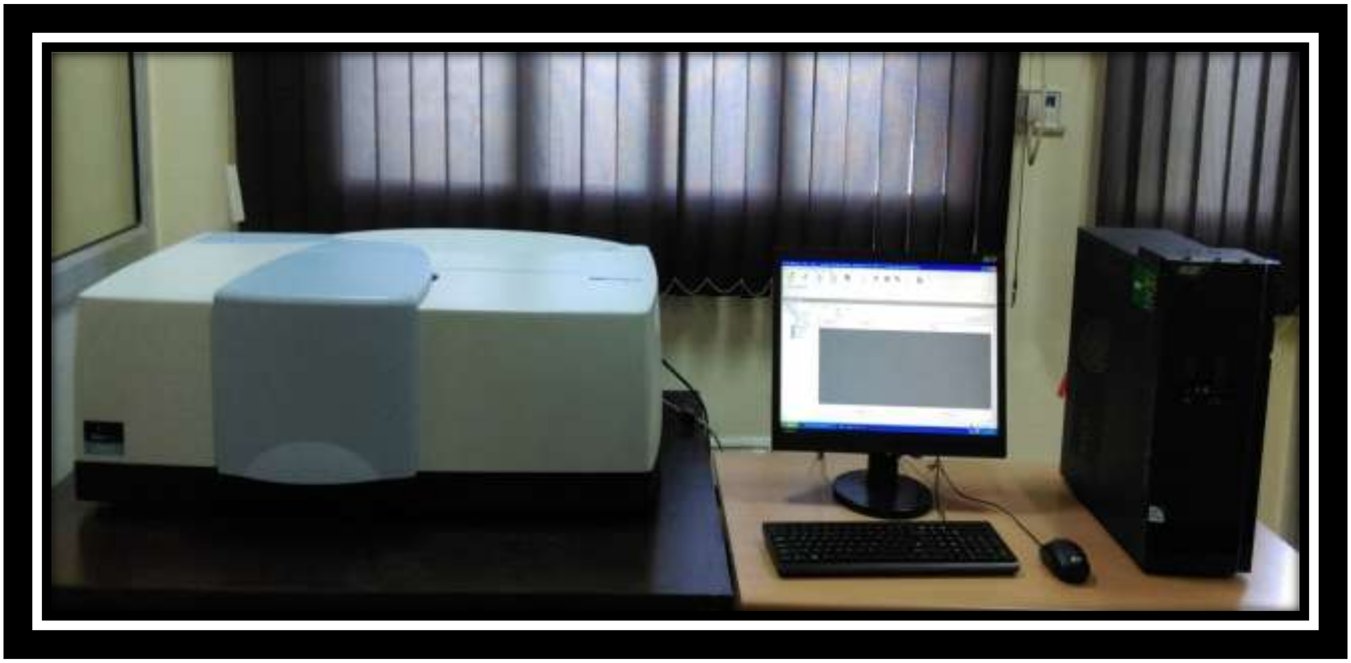


Fig. 10. Perkin Elmer Lambda 750 Spectrophotometer at Laser Spectroscopy Lab, DTU.

Features of the instrument are:

- Automated polarizer/depolarizer drive in the large sample compartment provides further depolarization or allows study of oriented samples with polarized light
- Compatible with transmission and reflectance measurements.
- DEUTERIUM and TUNGSTEN halogen Light sources.
- High-Sensitivity R928 Photomultiplier and Peltier-cooled PBS Detectors.

Fig. 11. Fluorolog-3 Spectrofluorometer (Horiba JobinYyon) at Laser Spectroscopy Lab, DTU.

Features of this instrument are.

- Automated polarizer/depolarizer drive in the large sample compartment provides further depolarization or allows study of oriented samples with polarized light.
- Consists of double grating monochromators in excitation and emission positions, and a red-sensitive photomultiplier.
- Powerful 450 W Xenon (Xe) arc lamp for high intensity, broad spectrum excitation.
- Pulsed Xe flash lamp for phosphorescence measurements.
- Peltier system for temperature variations
- Double monochromators provide the highest stray light rejection, highest spectral resolution, and highest light throughput.
- Front face optics for efficient collection of light from solid samples, powders, films, and opaque solutions.

2.3.2. EDX Analysis.

Energy Dispersive X-Ray Analysis (EDX), known as EDS or EDAX, is an x-ray technique employed to identify the elemental composition of materials. EDX systems are generally attached to Electron Microscopy instruments (Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM)) where the imaging capability of the microscope identifies the specimen of interest. The data obtained by EDX analysis consist of spectra showing peaks corresponding to the elements making up the composition of the sample being analyzed.

Benefits from EDX analysis:

- Advanced quality control and process optimization
- Quick identification of contaminant and source
- Full control over environmental factors etc
- Greater on-site confidence, higher production yield.
- Identifying the source of the problem in process chain

2.3.3. SEM Analysis.

SEM is a microscope assisted imaging technique in which the beam of electrons is allowed to transmit through a very thin specimen; the beam of electrons interacts with the specimen as it passes through it. An image is then produced by the interaction of the electrons with the sample which when transmitted through the specimen, then the image is magnified by a number of magnifying lenses in a set up and focused onto an imaging device, like a fluorescent screen, or upon a layer of a film coated with photographic material, or it can be detected using a sensor. These microscopes are capable of imaging at a much higher resolution compared to other optical microscopes, due to very small de Broglie wavelength of the electrons. At smaller magnifications SEM image contrast is poor because of absorption of electrons inside the material, or due to high thickness of the material. At higher magnifications complex wave interactions alters the intensity of the formed image, requiring experienced manual analysis of produced images. Alternate modes of operating allows the electron microscope to observe alterations in the crystal orientation, electronic structure, chemical composition and sample induced electron phase shift and regular absorption based imaging. SEM provides a important analysis methodology in a number of scientific fields, both physical as well as biological sciences.

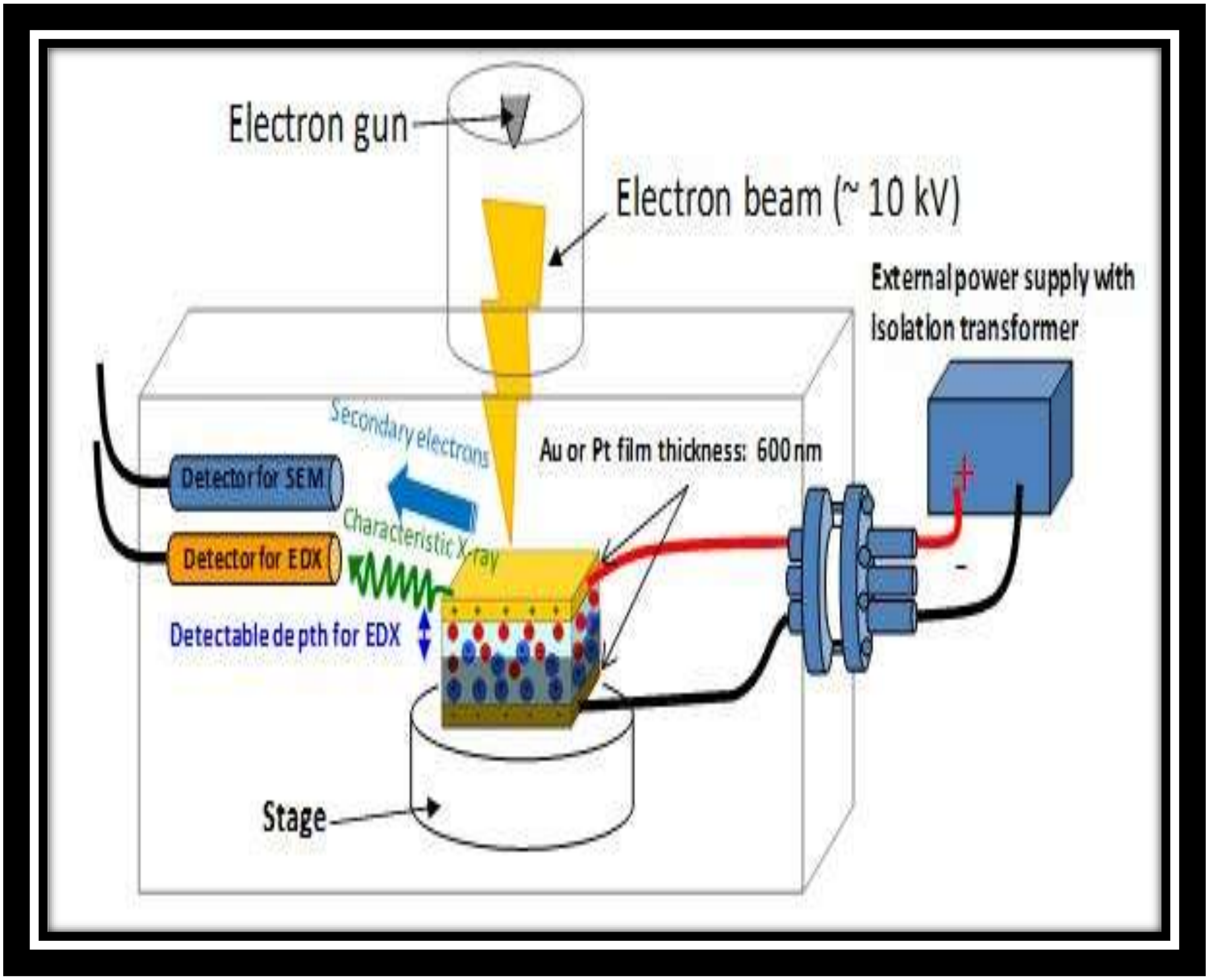


Fig. 12. Schematic of EDX incorporated Scanning Electron Microscope (SEM). (Source: *Kuwabata et al., 2013, Intech*).

2.3.4 FTIR Analysis.

Fourier transform infrared spectroscopy (FTIR) is a characterization technique used to obtain an infrared spectrum of absorption and emission of solid, liquid or gas. An FTIR spectrometer simultaneously collects data at high spectral resolution over a wide range of spectrum. This provides a significant advantage over any dispersive spectrometer which measures the intensity between a narrow range of wavelength at a time. FTIR offers quantitative as well as qualitative analysis for organic and inorganic samples. Fourier Transform Infrared Spectroscopy (FTIR) identifies the chemical bonds (especially covalent bonds) in a molecule by producing an infrared absorption spectrum. The spectra produce a profile of the sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components. FTIR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information.

Benefits of FTIR spectroscopy.

- Quantitative and qualitative scans
- Solids, Liquids, Gases
- Organic Samples, Inorganic Samples
- Unknowns Identification
- Impurities Screening
- Formulation, Deformulation

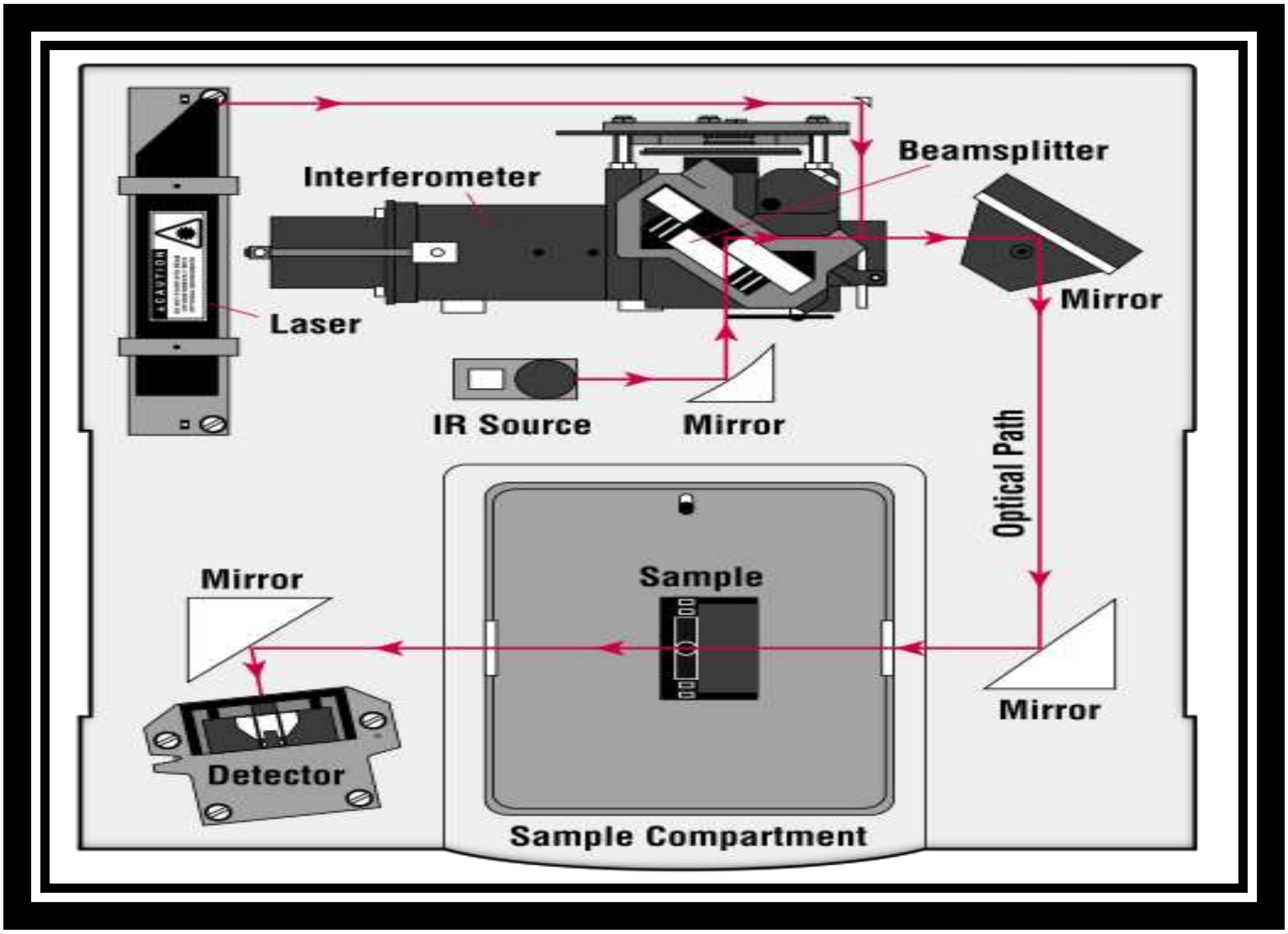


Fig. 13. Schematic showing the internal functioning of FTIR Spectrometer. (Source: *Chromacademy.com*).

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Chapter- 3

Results and Discussion

3.1. Effect of reaction time on the formation of AgNPs.

When the Neem plant leaf extract is mixed with the $AgNO_3$ solution, there is change in a color is observed within few minutes from pale yellow to dark yellow and to colloidal brown. As the time passes on there is an increase in absorbance and hence indicates an enhancement of formation of AgNPs. The UV-Vis absorbance of reaction time of AgNPs will be shown in the range between 400-450 nm. Brown color presence may be an indication for formation of AgNPs but UV-Vis absorbance of AgNPs at 400-450 nm, which exhibit strong surface plasmon resonance confirms the formation of AgNPs. Plant extract act as a natural reducing agent which contains phytochemicals such as flavonoids, terpenoids, aldehydes, phenolic compounds, ketones etc., which can be responsible for the reduction of silver ions to silver metal nanoparticles. The formation of silver nanoparticles was examined by UV-Vis spectroscopy at different time intervals of 1hour, 2hour, 3hour and 4hour in the range between 300-600 nm. Fig. 19 clearly shows as the time passes, in the interval of hours there is an increase in the absorbance intensity of synthesized AgNPs .

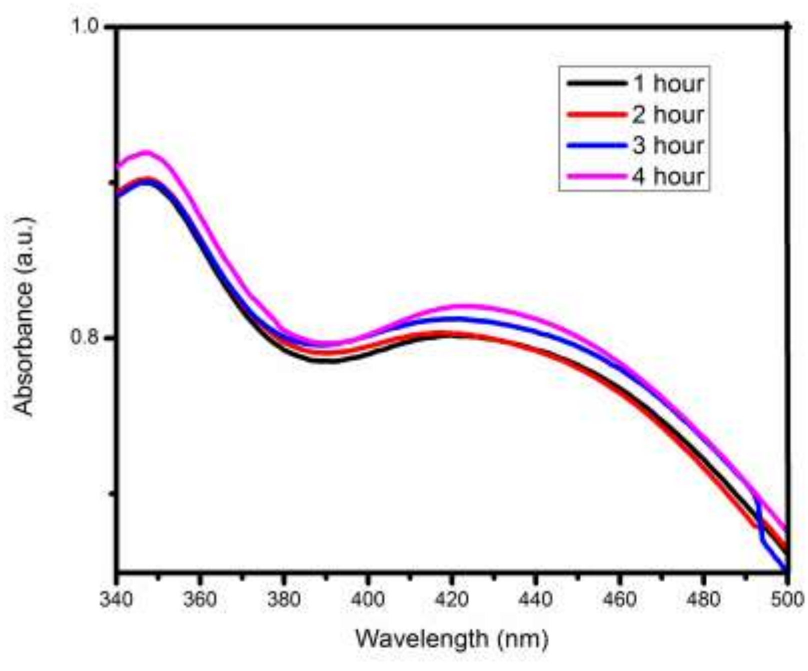


Fig.14. Absorption spectra of AgNPs performed at different time intervals.

3.2. Effect of change in volumetric concentrations of plant extract on formation of AgNPs

The effect of change in volumetric concentration on the formation of AgNPs by varying plant extract is performed by varying the plant extract at different volumes, without changing the concentration of $AgNO_3$. It was found that on increasing the concentration or we can say volume of Neem plant extract into silver solution $AgNO_3$, there will be a change in absorption band which is shifted to longer wavelength from 435 to 460 nm, which also indicates there will be a red shift and hence increase in the particle size.

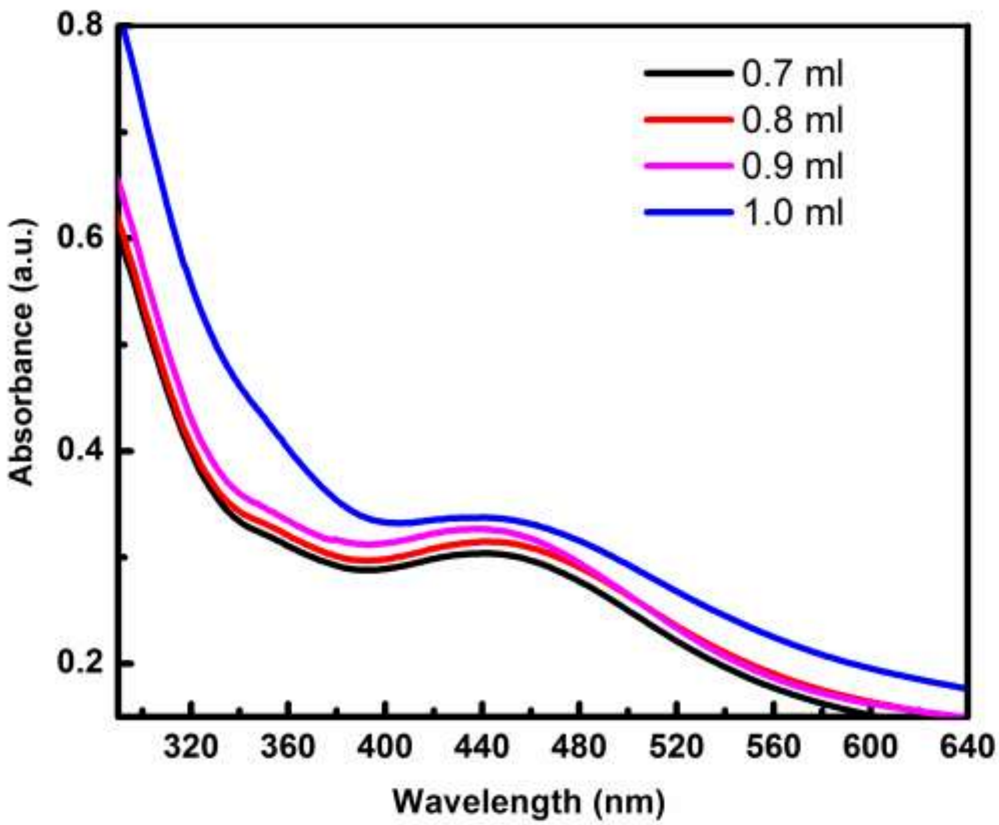
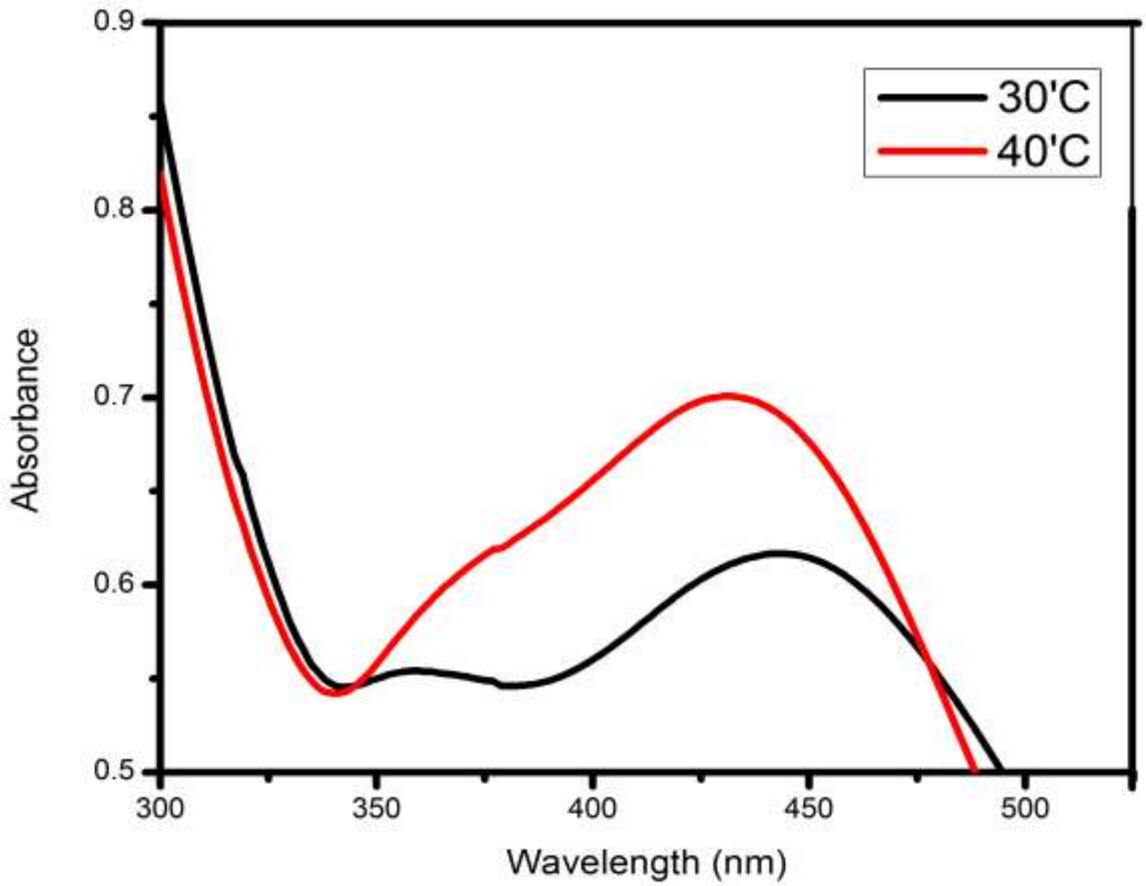


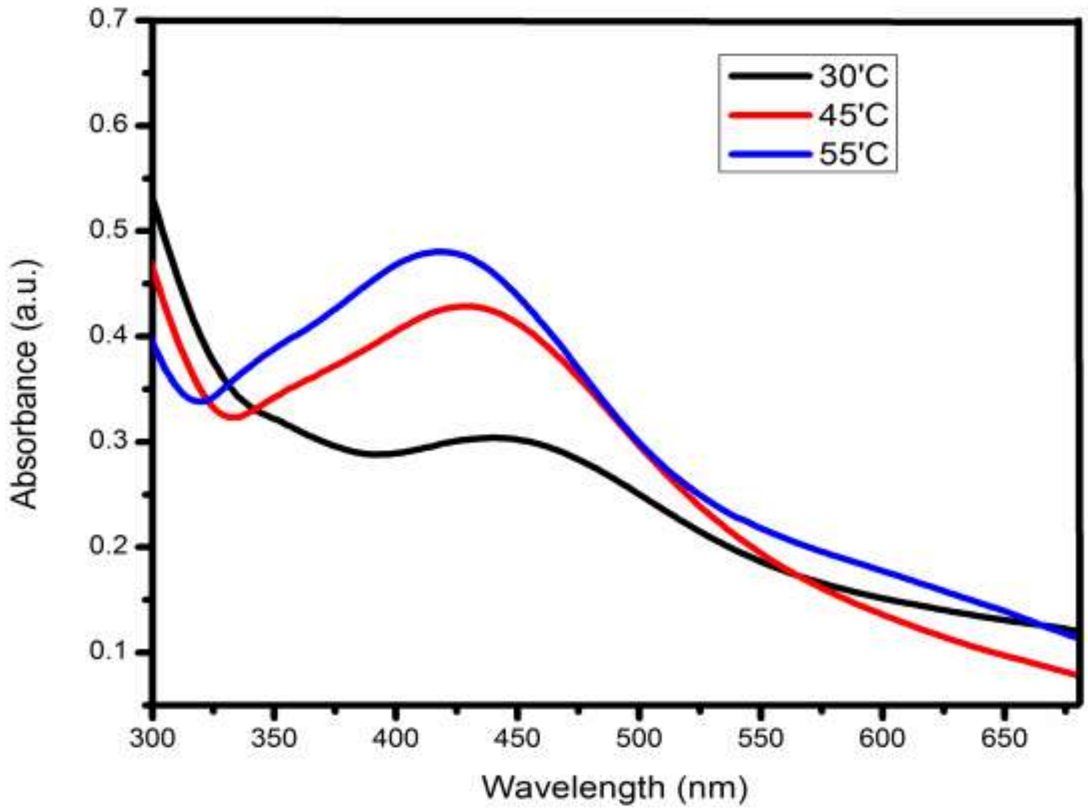
Fig. 15. Absorption spectra of AgNPs at different volumetric concentrations of Neem plant extract and $AgNO_3$.

3.3. Effect of Temperature on the formation of AgNPs

Temperature plays an important role while synthesizing the AgNPs. Effect of temperature was observed while going from 30 to 55°C, as shown in Fig. 16. Upon variation temperature from 30 - 40°C there is a blue shift in absorbance peak, i.e., shifted from 440 to 430 nm. As the reaction temperature increases from 30 to 55°C, the absorption peak shifted towards blue, i.e., toward lower wavelength from 450 to 420 nm, as shown in Fig. 16(b). Hence, from above results, we concluded that upon increasing the temperature the absorption shifted towards to the lower wavelength as a result of the decrease in particle size of AgNPs with increase in temperature.



(a)



(b)

Fig. 16 (a, b) Absorption spectra of AgNPs performed at different temperatures.

3.4. Effect of pH on the formation of AgNPs

Effect of pH on the formation of AgNPs also plays an important role. By changing the pH of solution from 9 to 12, it is observed that there is blue shift in absorption peak or the absorption peak shifted towards the lower wavelength. The blue shifted absorption indicates that the size of AgNPs is decreases as the wavelength shifts from 430 to 390 nm. It is observed that alkaline nature favors the formation of AgNPs, as the pH increases the color turns brown more quickly.

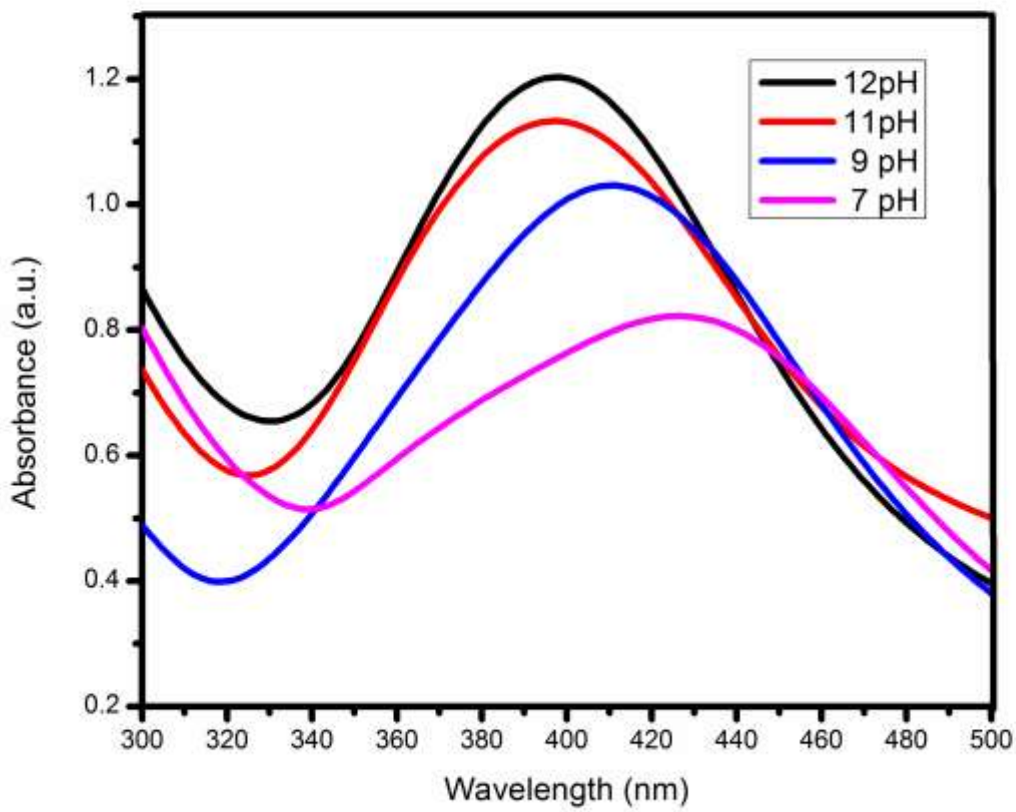


Fig.17. Absorption spectra of AgNPs performed at different pH using Neem plant extract

3.5. EDX and SEM analysis of AgNPs.

The EDX spectrum (Fig. 18 and Table 1) shows different elements like carbon, oxygen, silicon and silver present in a sample. The sample had been analyzed at an accelerating voltage of 20 KV, and shows yield of silver nanoparticles 4.62% by unnormalized concentration in weight % of Ag, 6.94% by normalized concentration in weight % of Ag and 1.17% by atomic weight % Ag in L series (the EDX of the sample have been performed at Centre Nanoscience and Nanotechnology, Jamia Millia Islamia, New Delhi).

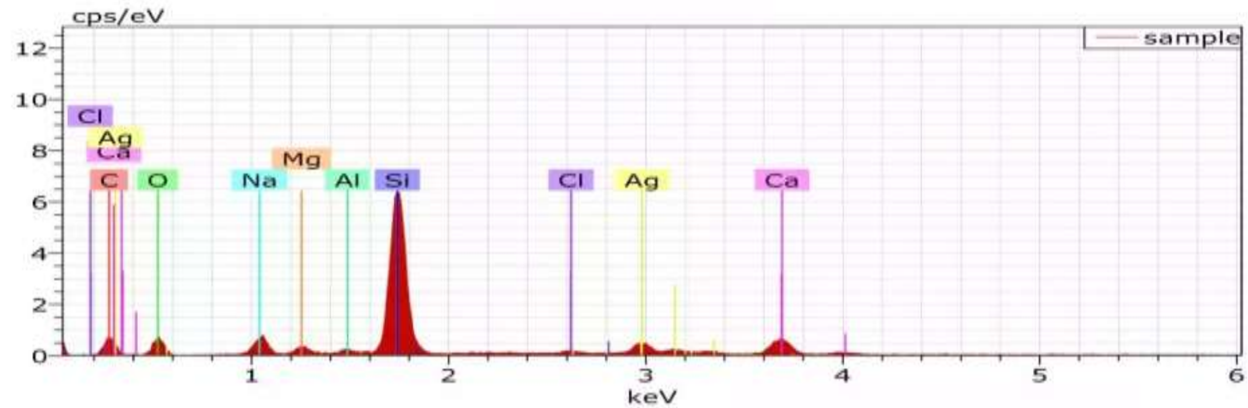


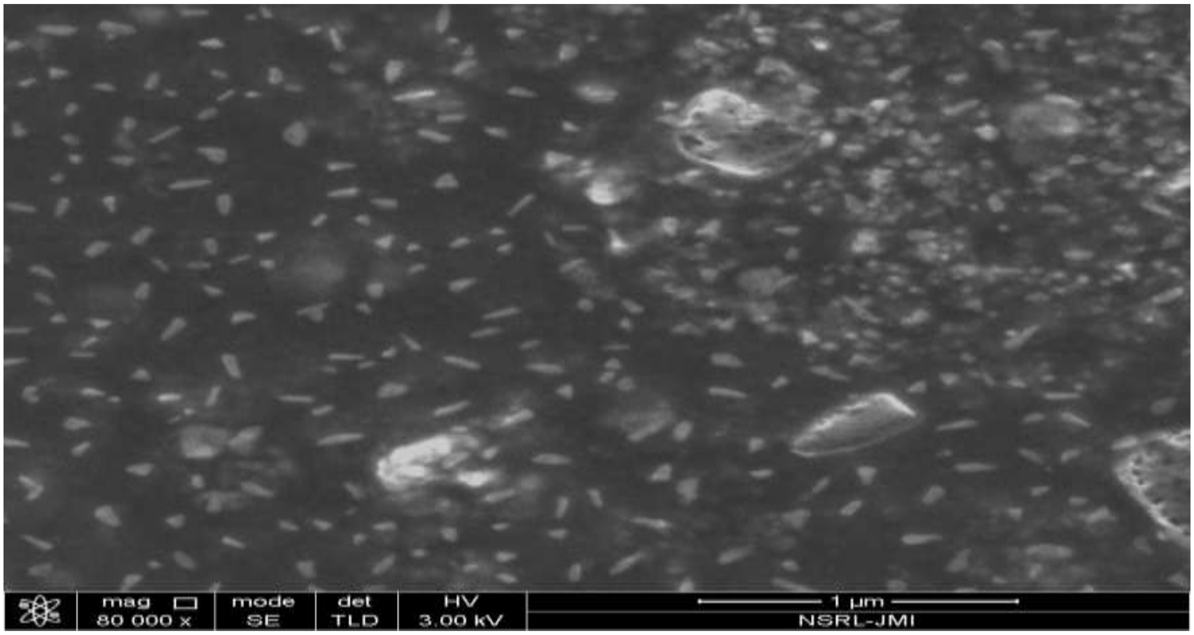
Fig.18. EDX Spectra of synthesized AgNPs

Spectrum: sample

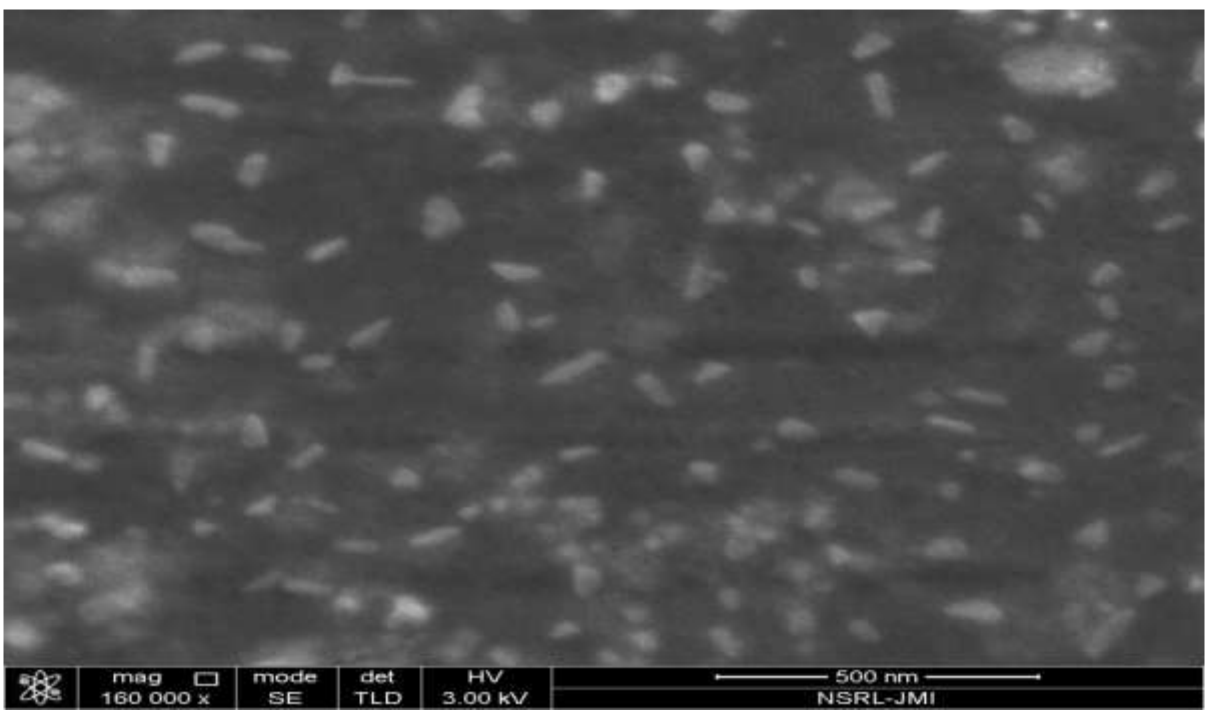
El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
C	6	K-series	22.02	33.09	50.22	4.43
O	8	K-series	13.78	20.71	23.60	2.78
Si	14	K-series	17.22	25.88	16.80	0.78
Na	11	K-series	3.21	4.83	3.83	0.27
Ca	20	K-series	4.24	6.37	2.90	0.18
Ag	47	L-series	4.62	6.94	1.17	0.21

Table.1. Elemental composition of Synthesized AgNPs.

From the SEM images (Fig. 19), it is clearly visible that silver nanoparticles of different sizes had been formed. Synthesized silver nanoparticles from Neem plant extract have anisotropic shape or non-spherical shape like triangular structure or nanoprism shape (the SEM of the sample has been performed at Centre for Nanoscience and Nanotechnology, Jamia Millia Islamia, New Delhi)



(a)



(b)

Fig. 19.(a,b) SEM images of silver nanoparticles using Neem plant extract have triangular shape or nanoprism shape

3.6 Fourier Transform Infrared study of AgNPs synthesized using Neem extract

Fourier Transform Infrared study is done to identify different functional groups present in plant extract. Mainly different types of biomolecules present in plant extract and are responsible for the formation of AgNPs were identified. Fig. 20 shows the FRIR spectrum of AgNPs containing solution and the peaks are observed at 1640.66 cm^{-1}, 2112.12 cm^{-1} and 3366.96 cm^{-1}.These peaks are resembled to the peaks of different functional groups like ketonic or carboxylic group, i.e., C=O (around 1641 cm^{-1}), C≡C group (around 2110 cm^{-1}) and amine N-H group (around 3365 cm^{-1}). Hence from FTIR study it can be concluded that the biomolecules present in Neem plant extract are responsible for the synthesis of silver nanoparticles.

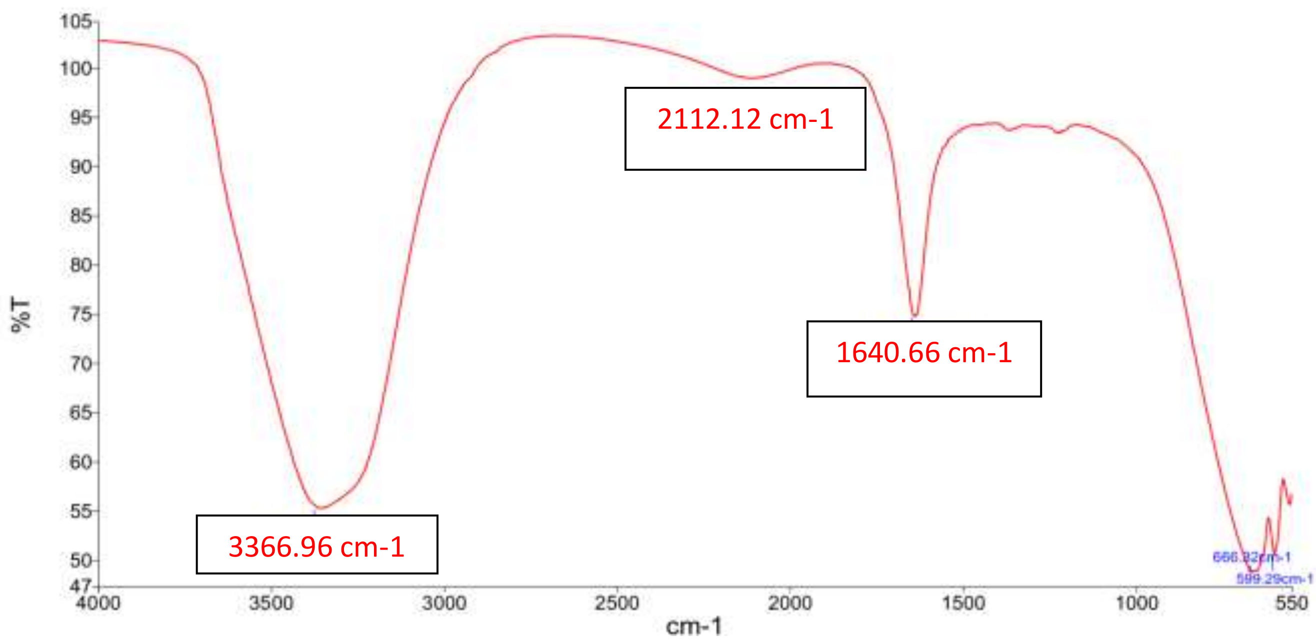


Fig. 20. FTIR spectra of AgNPs synthesized using Neem extract

3.7. Photoluminescence study of synthesized AgNPs.

PL spectra of AgNPs containing solution is shown in Fig. 21. A strong peak observed at around 440 nm with 320 nm excitation wavelength, which may be due to the presence of biomolecules.

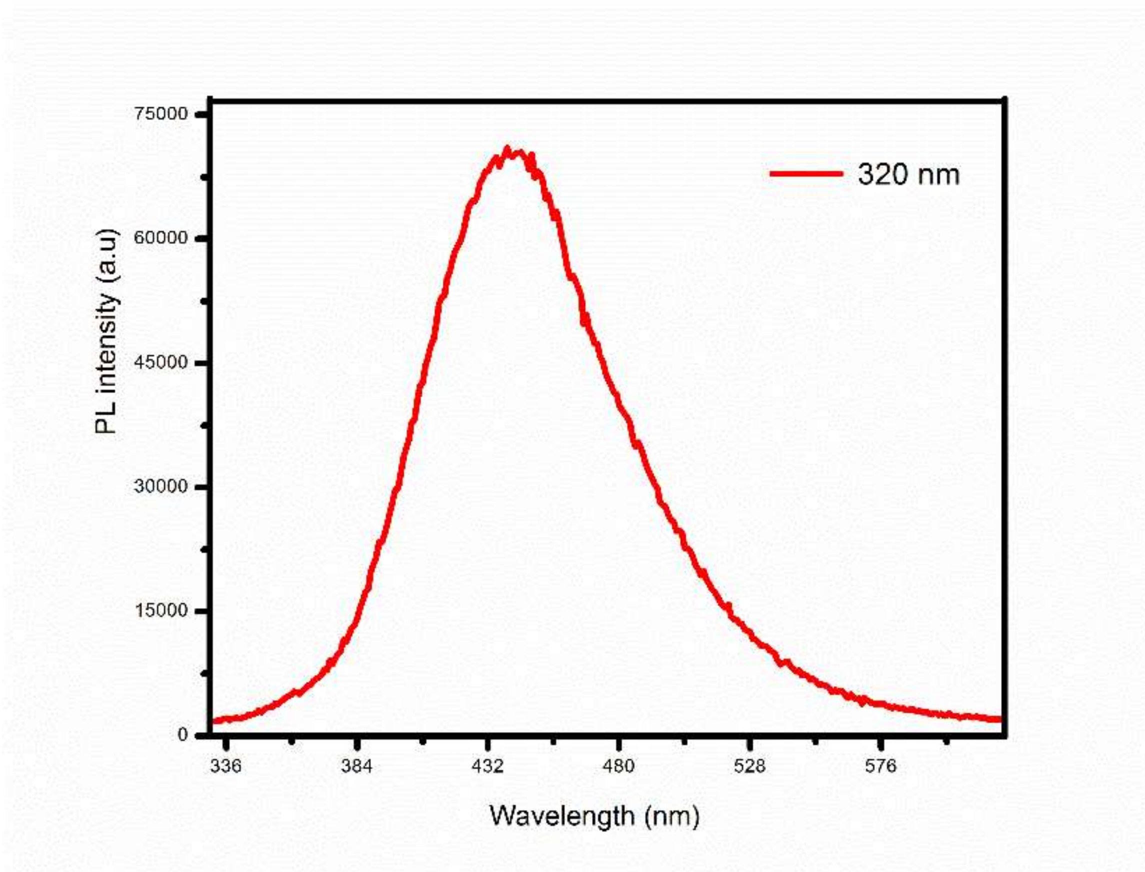


Fig. 21. PL spectra of AgNPs obtained using neem extract and silver salt.

3.8. Stability analysis of synthesized AgNPs.

Stability is another important parameter. The samples were kept for several days and to examine the photo stability of the prepared AgNPs. After 10 days, the absorption intensity of silver nanoparticles decreases and at the same time the absorption peak shifted towards the longer wavelength, indicating the increase of the size of AgNPs.

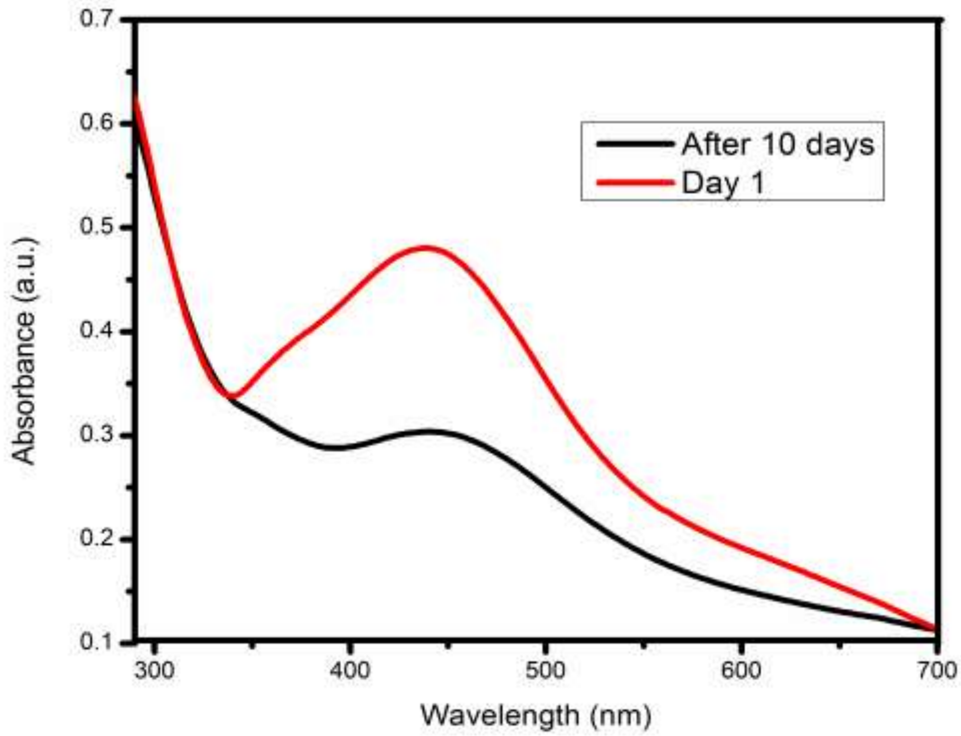


Fig.22. AgNPs absorption spectra synthesized using neem extract taken after 10 days.

Chapter-4

Conclusion

Here, we have synthesized silver nanoparticles by changing various parameters like reaction time, temperature, pH and plant extract concentration. Natural plant (Neem) extract is used as a reducing agent and when mixed with $AgNO_3$ solution, then there is a change in color from pale yellow to brown which confirms the formation of silver nanoparticles. The obtained nanoparticles show strong absorption between 400 and 450 nm. Further, we could control the shape and size of AgNPs significantly by varying these parameters. This is purely a green approach method and the most efficient in order to control the size and shape of nanoparticles. Green approach is simple, cost effective, ecofriendly and it eliminates the use of toxic chemicals which effects the human beings and environment. This green approach can be used as a boom in the nano drug delivery industry and nano genetics industry as it eliminates the harmful chemicals during diagnosis. Hence, we can say this method is time saving, cost effective, ecofriendly, non-toxic and large scale production can be done.

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