# *Medicinal Plant Leaf mediated Green Synthesis of Silver Nanoparticles*

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

**Master of Technology**

In

**Nano Science and Technology**

*By*

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**(2K14/NST/17)**

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DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)

# CERTIFICATE

I hereby certify that the work which is being presented in the M.Tech thesis entitled **"Medicinal Plant Leaf mediated Green Synthesis of Silver Nanoparticles",** in partial fulfillment of the requirements for the award of the **Master of Technology in Nano Science & Technology** submitted to the Department of Applied Physics, Delhi Technological University, Delhi-110042 is an authentic record of my own work carried out during the period July 2015 to June 2016 under the supervision of **Dr. Mohan Singh Mehata**, Assistant Professor, Department of Applied Physics .

The matter presented in this report has not been submitted by me for the award of any other degree or diploma elsewhere.

***Signature of the Candidate***

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

***Signature of the Supervisor*** ***Signature of the HOD***

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# DECLARATION

I hereby declare that the work which is being presented in this thesis entitled **"Medicinal plant leaf mediated green synthesis of Silver nanoparticles"** 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 thesis has not been submitted for the award of any other degree or diploma.

**Date:** **Siddhant Jain**

**Place:** **(2K14/NST/17)**

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# Abstract

Silver Nanoparticles (AgNPs) were synthesized by green approach using plant extracts of Ocimum Sanctum (Holy Basil or Tulsi) and silver salt. In this study we analyzed the synthesized nanoparticles using absorption & photoluminescence (PL) spectroscopy, X-Ray diffraction analysis (XRD), Fourier transform infrared spectroscopy (FTIR), energy dispersive X-Ray (EDX) and Transmission Electron Microscopy (TEM). The synthesized nanoparticles showed a strong absorption peak at around 420 nm due to strong surface plasmon resonance (SPR) exhibited by the metal nanoparticles. The variation of size and surface morphology of synthesized nanoparticles is due to different physico-chemical parameters such as pH, temperature, concentration, reaction time. Formation of silver nanoparticles by flavonoid reduction is also studied briefly. Quercetin, a flavonoid present in Tulsi, ginger and many other plants was used as reducing agents. The results obtained using plant extracts and quercetin showed prominent similarities implying that the bio-molecules present in plants are mainly responsible for the reduction of silver ions to silver nanoparticles. From the results obtained, it can be concluded that the green 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 and nanotechnology.

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# Chapter-1

## INTRODUCTION

## 1.1. Literature Review

Over the past few decades there has been an increased emphasis on metal nanoparticles because of their unique optical and electrical properties. There is always a need to develop a synthesis route which is economic, cost effective, non-toxic and productive. Green approach is a technique for controllable synthesis of nanoparticles of well-defined size and shape. The surface Plasmon Resonance (SPR) exhibited by the nanoparticles is one of their important characteristic which gives them their unique optical properties. Metal Nanoparticles proved to be very efficient and useful in the field of electronics, Photonics and medicine (***Lu & Lieber, 2007; Shen et al., 2000; Karni et al., 2012***) The properties of metal Nanoparticles vary according to their size, shape and morphology (***Khlebtsov, 2005***). These applications emerge from the fact that nanoscale dimensions show different properties as compared to their bulk counterpart due to their high surface to volume ratio. This is the main reason for the development of various nanostructures in 0D, 1D, 2D and 3D arrays by controlled synthesis.

Recently, researchers have developed a great interest in the synthesis of silver Nanoparticles due to their enhanced antimicrobial activity (***Verma & Mehata, 2015***) and their use as anticancer agents (***Ashanrani et al., 2009***). AgNPs can be synthesized through various physical and chemical methods. On one hand, these methods are efficient for AgNPs synthesis but on the other hand they are harmful to the environment due to the use of toxic chemicals. A relatively new approach i.e.; the Green approach had developed for their synthesis which not only overcomes the cons of physical and chemical method and also has its own advantages of accessibility, cost effectiveness and environment friendly. Leaves of various plants such as Azadirachta indica (neem) (***Verma & Mehata, 2015***), Ocimum tenuiflorum (black Tulsi) (***Banerjee et al., 2014***), Ficus Benghalensis (Banyan tree) (***Saware et al., 2014***) etc. can be used for the synthesis of AgNPs. Not only leaves but other parts of the plants such as stem, roots etc (***Jha et al., 2009***) can also be used for synthesis. Various microorganisms like bacteria, fungi, yeasts (***Narayanan & Sakthivel, 2010***) and DNA (***Sohn, Kwon, Jin & Jo, 2011***) are also used for green synthesis of AgNPs. Besides silver nanoparticles these microorganisms and DNA can be

used for the synthesis of Quantum dots like synthesis of CdS Quantum dots using fungi (***Ahmad et al., 2002***).

In this work we have done a comparative study of green synthesis of AgNPs using Ocimum Sanctum (Tulsi) leaf extracts and quercetin. Quercetin is a polyphenolic flavonoid found in many fruits, vegetables, leaves and grains. It can be used as an ingredient in supplements, beverages & foods. It has Molecular formula $C_{15}H_{10}O_7$ & is a good antioxidant. Tulsi leaf extract and quercetin solution were separately used as a reducing agent. The AgNPs obtained by the reaction of Silver salt and plant extracts and also by reduction of silver ions by Quercetin at different environmental conditions were characterized by different techniques. The results obtained were compared to verify whether the Bio-molecules present in Plant extracts are responsible for the reduction of silver ions to AgNPs.

## 1.2. Nanotechnology

Nanotechnology is one of most important field of modern research dealing with design, synthesis, characterization and application of particles ranging from approximately 1-100 nm in at least one dimension. Nanotechnology is rapidly gaining importance in a number of fields such as health care, cosmetics, dietary food intake, environmental safe keeping, biomechanics, optics, biomedical sciences, chemical industries, electronics, Aerospace industries, drug delivery, Energy source, optoelectronics, catalysis, single electron tunneling transistors, light emitters, nonlinear optical devices and sensor technology applications (***Harekrishna et al., 2009***) Nanomaterials can be seen as solutions to many technological and environmental challenges in the field of solar energy conversion, medicine, and waste water treatment. In the process of global efforts to reduce hazardous waste, there is a continuously increasing demand of green synthesized nanomaterials.

Nanotechnology is definitely changing the way in which particles or materials are analyzed, synthesized and devices are fabricated. Incorporating nanoscale building blocks into functional assemblies and further into multifunctional state of the art devices can be achieved through a “bottom-up approach”. Research on the synthesis of nano dimensional material is of great

interest because of their unique optoelectronic, magnetic and mechanical properties which differs from their bulk counterpart (***Kaviya et al., 2011***).

## 1.3. Nanoparticles and their types

The term Nanoparticles is used to describe a particle with size ranging from 1-100 nm, at least in one of the three possible dimensions. At this size range, the physicochemical and biological properties of the nanoparticles changes in fundamentally from the properties of both individual atoms/molecules of their corresponding bulk material. A material which is metal or semiconductor in bulk state can become an insulator in the nano state or vice-versa. This vast change occurs due to large surface to volume ratio of nanoparticles. Nanoparticles can be made of diverse materials with varying chemical and physical nature; the most common are metals, metal oxides, ceramics, polymers, silicates, organic and non-organic materials, carbon and biomolecules such as DNA. Nanoparticles exist in several morphologies such as squares, spheres, triangular, cylinders, sheets, tubes etc. Generally the nanoparticles are designed with surface modifications in order to meet the needs for specific applications.

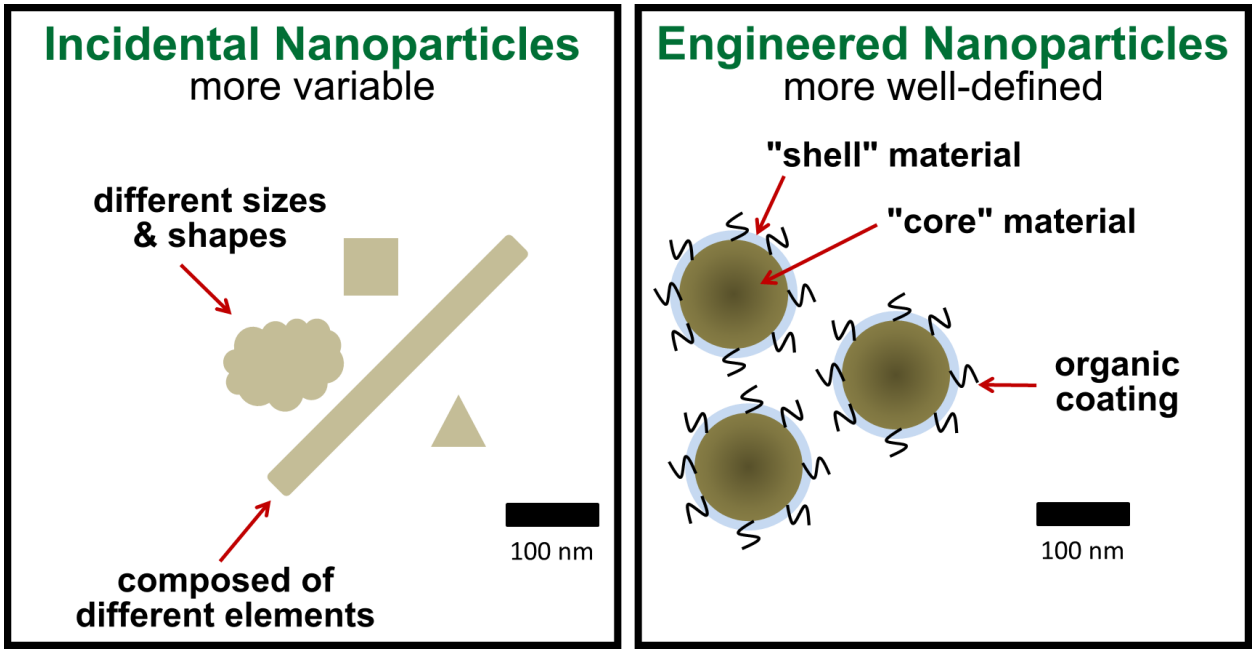



**Fig. 1. Surface modified Nanoparticles. (**Source: *Sustainable nano.com*).

Nanoparticles can be broadly classified into two, namely, **organic nanoparticles** which includes carbon nanoparticles such as fullerenes while, on the other hand some of the **inorganic nanoparticles** which includes magnetic nanoparticles like Fe, Ni etc, noble metal nanoparticles like gold and silver and semi-conductor nanoparticles like Zinc Selenide, cadmium Selenide titanium oxide and Zinc Oxide. There is a growing interest in development of inorganic nanoparticles i.e. of noble metal nanoparticles such as Gold and silver as they provide superior material properties over Inorganic nanoparticles also with functional versatility. Due to their variable size features and advantages over available chemical imaging drugs inorganic particles have been examined as a potential tool for Bio-medical imaging as well as for diagnosing and treating diseases. Inorganic nanomaterial have been widely used for drug delivery due to their versatile features like easy and wide availability, rich functionality, very good compatibility and capability of targeted delivery and controlled release of drugs (***Xu et al., 2006***).

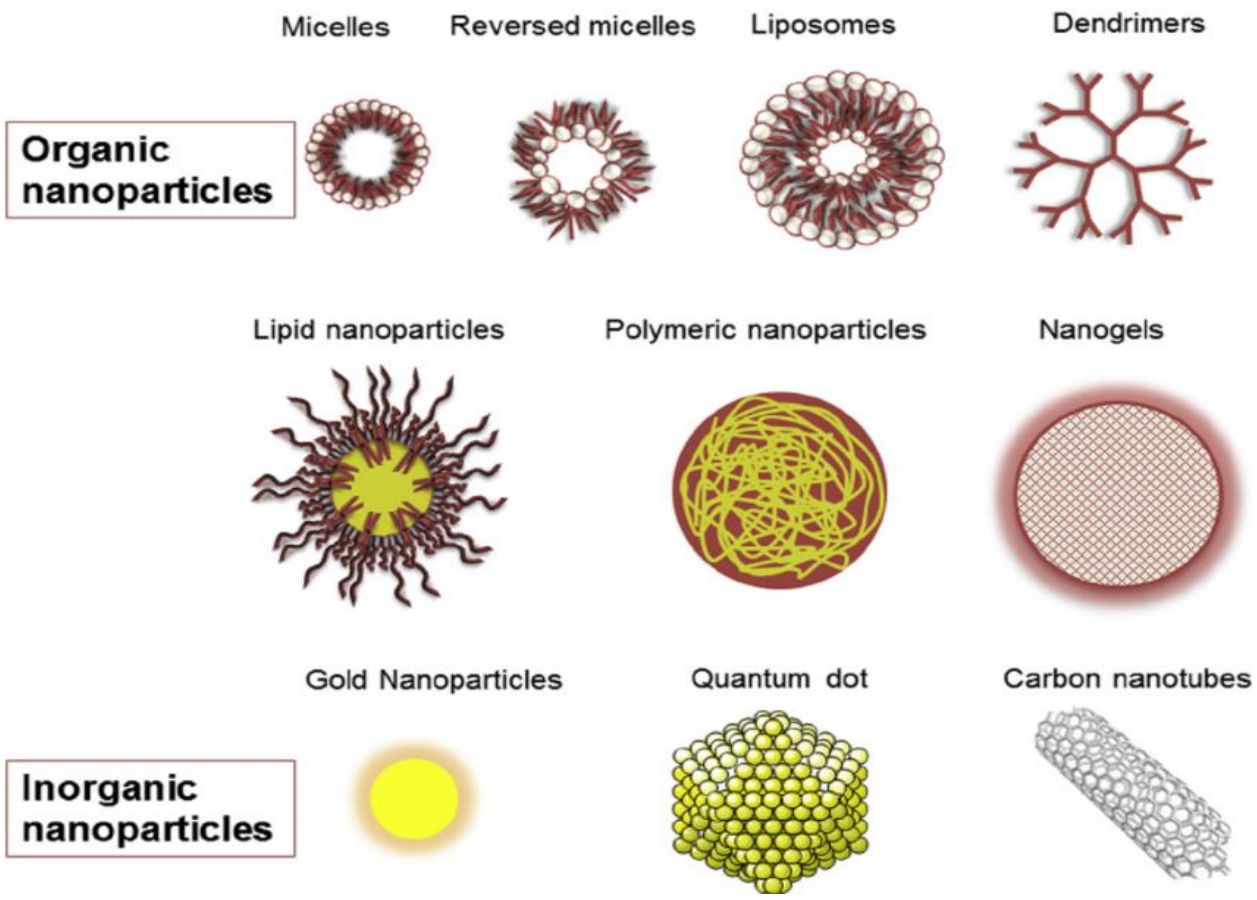



**Fig. 2. types of Nanoparticles.** (Source: Andre et al., 2016, Biomaterials).

## 1.4. Silver nanoparticles (AgNPs)

Bulk Silver (Ag) is a transition metal with Atomic No. 47. It is a group 11, d block element which is lustrous white in appearance. It has wide Physical, chemical and electrical properties which makes it a noble metal. Silver is one of the basic element that makes up our planet. It is a rare, but naturally occurring element, slightly harder than gold and very malleable & ductile means it can be forged into thin sheets and wires. Silver in pure form has the highest electrical and thermal conductivity of all metals and has lowest contact resistance ***(Nordberg and Gerhardsson, 1988)***. Metallic silver itself is insoluble in water, but metallic salts such as Silver Nitrate ($AgNO_3$) and Silver Chloride (AgCl) are soluble in water (**WHO, 2002**). Metallic silver is used for the surgical prosthesis and splints, fungicides and coinage. Soluble silver compounds such as silver slats, have been used in treating mental illness, epilepsy, nicotine addition, gastroenteritis and infectious diseases including syphilis and gonorrhea (***Lara et al., 2011***). The wide variety of uses of silver allows exposure through various routes of entry into the body. Ingestion is the primary route for entry for silver compounds and colloidal silver proteins. Dietary intake of silver is estimated at 70-90 µg/day. Since silver in any form is not thought to be toxic to the immune, cardiovascular, nervous or reproductive system as it is not considered to be carcinogenic, therefore silver is relatively non-toxic (***Ramya & Subapriya, 2012***).

There is continuously increasing interest in nanoscale Silver because of their unique properties (*e.g.,* optical, electrical, and magnetic properties depending on their size and shape and morphology) which can be incorporated into antimicrobial applications, biosensing materials, composite fibers, cryogenic, superconducting materials, cosmetics and electronic devices. Various chemical, physical and biological methods have been used for synthesizing and stabilizing AgNPs (***Klaus et al., 1999***; ***Senapati, 2005***).

Most chemical approaches include chemical reduction using a variety of organic and inorganic reducing agents, electrochemical reduction, physicochemical techniques, which are widely used for the synthesis of AgNPs. Recently, there is growing attention to synthesize nanoparticles using environmentally friendly methods (green approach).

## 1.5. Methods for nanoparticle synthesis

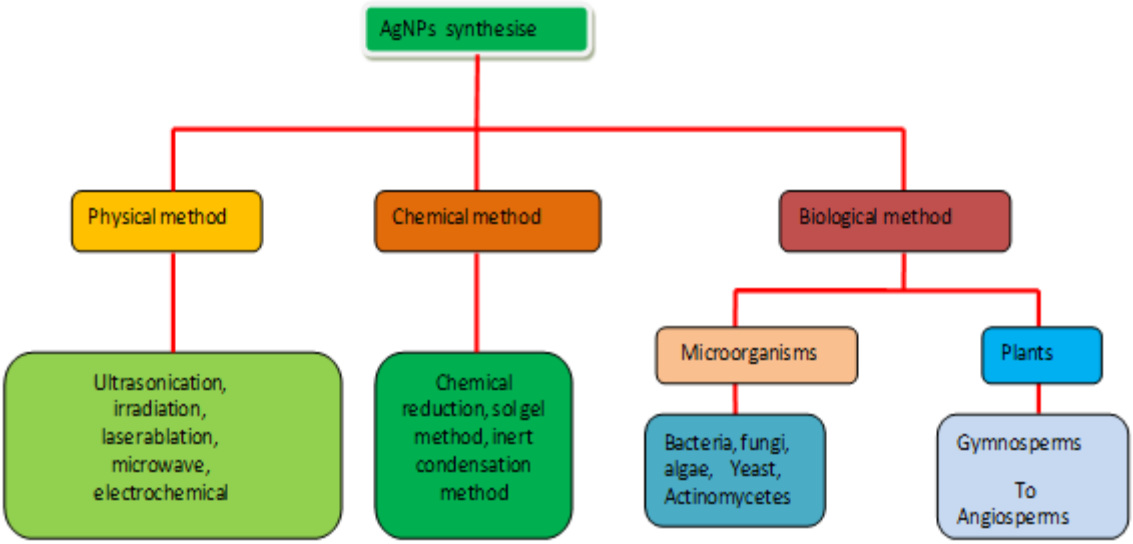



**Fig. 3. various methods of silver nanoparticle synthesis.** (Source: Kumar et al., 2015, Pharmatutor).

### 1.5.1. Physical methods

Most common physical approaches include evaporation-condensation and laser ablation. Various metal nanoparticles such as silver (Ag), gold (Au), lead sulfide (PbS), cadmium sulfide (CdS), and fullerene have been previously synthesized using the above methods. The solvent contamination is absent in the prepared thin films using physical approach and the uniformity of nanoparticles distribution are the advantages of physical approaches (***Kruis et al., 2000***).

### 1.5.2. Chemical methods

The most important approach for synthesis of AgNPs is chemical reduction by organic and inorganic reducing agents. Different reducing agents like sodium citrate ($Na_3C_6H_5O_7$), sodium borohydride ($NaBH_4$), elemental hydrogen, Tollen's reagent, N, N-dimethylformamide (DMF) etc. have been used for the reduction of silver ions ($Ag^+$) in aqueous or non-aqueous solutions. The above reducing agents reduce silver ions ($Ag^+$) and lead to the formation of silver nanoparticles ($Ag^0$), which may be followed by agglomeration into oligomeric (a molecular

complex that consist of a few monomer units) clusters. As time passes these clusters leads to the formation of metallic colloidal silver particles (***Wiley et al., 2005***) It is important to use capping/shielding agents to stabilize dispersive nanoparticles during metal nanoparticle preparation, avoiding their agglomeration. The presence of surfactants containing functionalities such as (thiols, amines, acids, and alcohols) interacts with the particle surface and can restrict particle growth, and can protect particles from agglomeration, or losing their surface emerged properties (***Evanoff et al., 2004***).

### 1.5.3. Biological methods

Biological methods are being used to synthesize AgNPs without using any toxic and expensive chemical substance or apparatus. The reduction of metal ions by large number of biomolecules found in the extracts of certain organisms (enzymes, proteins, amino acids, polysaccharides, and vitamins) is environmental friendly, but chemically complex.

The synthesis of AgNPs by bacteria is also achieved. The first reported synthesis of silver nanoparticles using bacteria was established using *Pseudomonas stutzeri* AG259 strain that was isolated from silver mine (***Haefeli et al., 1984***) There are some microorganisms that can survive high metal ion concentrations and can also grow under adverse conditions. There is also another aspect that though these microorganisms can grow at lower metal ion concentrations, their exposure to higher concentrations can induce toxicity.

Comparing to nanoparticle synthesis using bacteria, fungi can produce comparatively higher amounts of nanoparticles as they secrete higher amount of protein which reduced metal ions to metal nanoparticles. Fungi use a unique mechanism of silver nanoparticle synthesis. They trap the metal ions on their cell surfaces and secrete enzymes present in fungal system for the reduction (***Kumar et al., 2007***).

Bioreduction of metal ions to metal nanoparticles using plant extract as reducing agent is also possible. Leaves, roots, stems etc. of various plants such as Neem, Tulsi, Ginger etc can be used as a reducing agent (***Jha et al., 2009***). The main advantage of nanoparticle synthesis using plant extract is that they are easily available and there is no need for culture growth of

microorganism for nanoparticle reduction which is requires in case of bacteria, fungi and yeast assisted nanoparticle synthesis.

## 1.6. Need for green synthesis

Use of biological methods for the synthesis of nanoparticles is a kind of bottom up approach where reduction/oxidation or redox is the main occurring reaction. The need for synthesis of nanoparticles using bioreduction arises as the physical and chemical processes were costly and the by-products of the synthesis are toxic for both man and environment. Chemical methods of synthesis lead to absorption of some of the toxic chemical on the surface that may restrict their use in the medical applications (***parasharu et al., 2009***). Toxicity is not an issue when it comes to nanoparticles synthesized via green approach. In order to cheaper the synthesis process, scientist used microbes, enzymes, DNA and plant extracts (phyto-chemicals) for the bioreduction of nanoparticles. Their antioxidant or reducing properties are mainly responsible for the reduction of metal complexes into their respective nanoparticles. Green synthesis proves to be an advancement over chemical and physical methods of synthesis as it is cost effective, ease of availability, environment friendly, it can be easily scaled up for large scale synthesis and in this method there is no need of high pressure, energy, temperature and toxic chemicals.

## 1.7. Nanosilver

One of the most preferred substances used in synthesis of nanostructures is silver (Nanosilver). Due to its enhanced antimicrobial properties, nanosilver has also been used in water filters to purify drinking water and clean stagnant water. To obtain nanosilver, metallic silver has been transformed into ultrafine nano particles by several techniques; which includes electric arc discharge, electrochemical reduction, microwave irradiation and cryo-chemical synthesis. Nanosilver particles are usually smaller than 100 nm in diameter and consist of about 20-15,000 silver atoms (***J, 2008***). In addition to spherical particles, nanostructures can be synthesized as tubes, wires, or films. At the nano level, the silver particles exhibit varying physico-chemical properties (like pH, temperature and other environmental conditions) and biological activities

compared with the bulk silver. This is due to the higher surface to volume ratio, allowing a larger amount of surface atoms to interact with their surroundings. Due to the unique properties of silver at the nanoscale, nanosilver is nowadays used very often in huge number of consumable and medical products. Their strong antimicrobial activity is the major reason for development of nano-silver products (***C, 2006***).They are used in various fields such as food packaging materials, food supplements, textiles, electronics, household appliances, cosmetics and medicine, water disinfectants and room sprays.

## 1.8. Action of silver nanoparticles on microbes

The exact mechanism which causes antimicrobial effect in AgNPs is not clearly known. There are however various reports on the action of silver nanoparticles on microbes to cause the antibacterial effect (***Sondi et al., 2004***). Silver nanoparticles have the power to penetrate the bacterial cell wall and subsequently causing structural changes in the cell membrane like the permeability of the semi permeable membrane and death of the cell tissue. The formation of free radicals by the AgNPs can be considered to be another mechanism by which the cell death occurs. The electron spin resonance spectroscopy studies suggested that there is production of free radicals by the AgNPs when in contact with the microbe, and these free radicals have the ability to destroy the membrane and turn it porous which will ultimately lead to the death of cell tissue (***Kim et al., 2007***). It has also been explained that there may be release of silver ions by the nanoparticles, and these ions can react with the thiol (-SH) group of many important enzymes and inactivate them. The microbial cells in contact with AgNPs take in silver ions, which restricts several metabolisms in the cell and damage the cell membrane. The cells are mainly composed of sulfur and phosphorus which are soft base. The action of these nanoparticles on the cell entities may cause the reaction to take place and may lead to necrobiosis or cell death. Another fact is that the DNA strand has sulfur and phosphorus as its main components; the nanoparticles can attack on these soft bases and damage the DNA which would definitely lead to cell impairment (***Matsumura et al., 2003***).

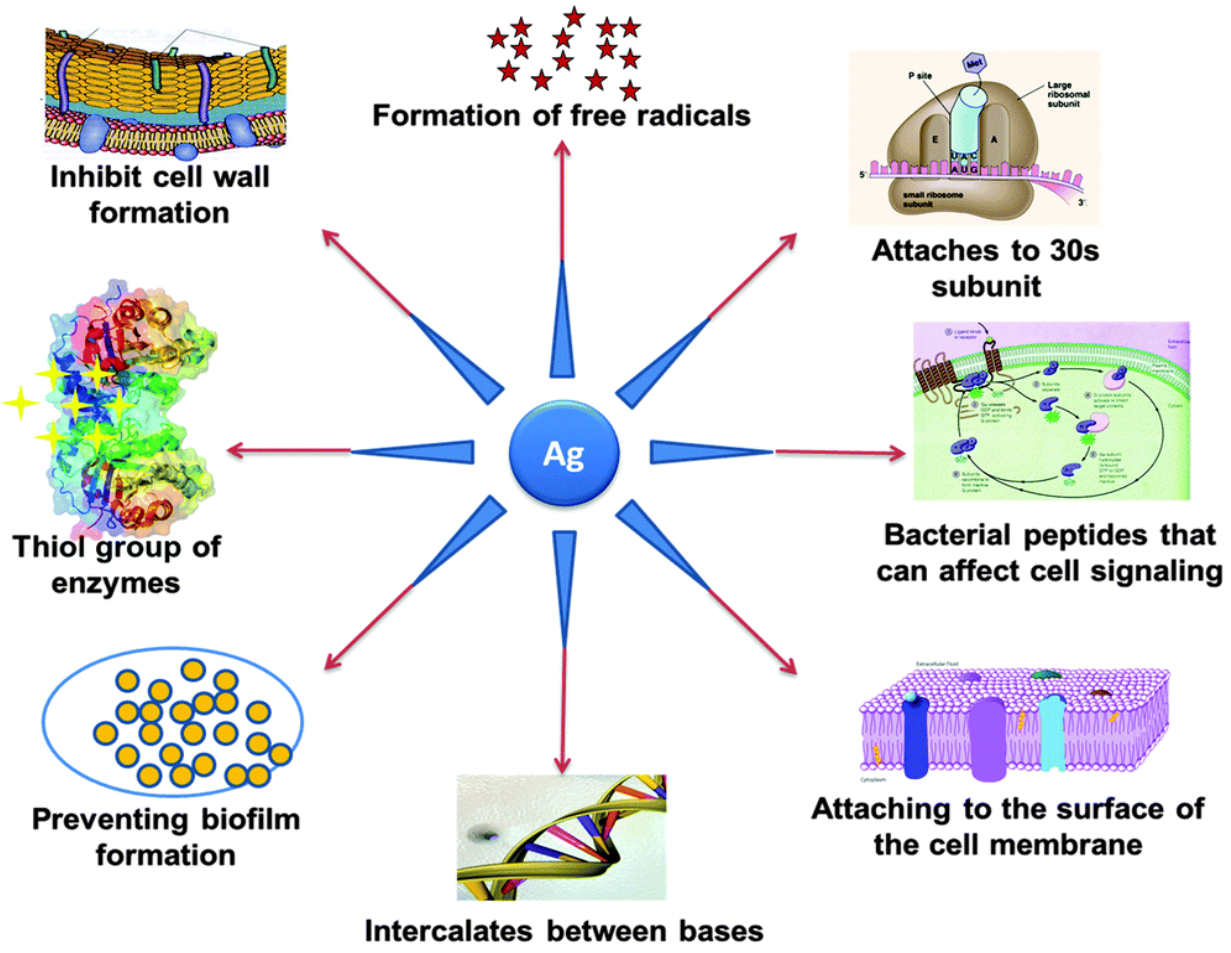



**Fig. 4. various modes of action of AgNPs on microbes.** (Source: Chen et al., 2014, chemical communications).

## 1.9. Applications of Silver nanoparticles and their incorporation into other materials

Nanoparticles are of great importance due to their extremely small size and high surface to volume ratio, which leads to chemical and physical variation in their properties when compared to their bulk counterpart, such as mechanical, biological and electrical properties. Therefore, designing and synthesis of materials with novel applications can be achieved by controlling the shape and size at nano dimensions. Nanoparticles exhibit size and shape and morphology dependent properties which are of great use in applications ranging from biosensing and

catalysts to optics, antimicrobial activity (***Hong et al., 2006***) single electron transistors, electrodynamometers, chemical and biological sensors, and wireless electronic logic and memory systems. These particles also have many applications in different medical fields such as cell imaging, drug delivery, inhibition of unwanted cell growth. AgNPs have drawn attention of scientists and researchers because of their extensive applications in electro-mechanical integrated circuits, sensors technologies, bio-labeling, low-cost very small size batteries (silver nano-wires) and antibacterial. Silver nanoparticles have also been extensively used as antimicrobial agents in health and cosmetic industry, food storage, waste water treatment, textile coatings and a number of other environmental applications. (***Jain et al., 2005***).

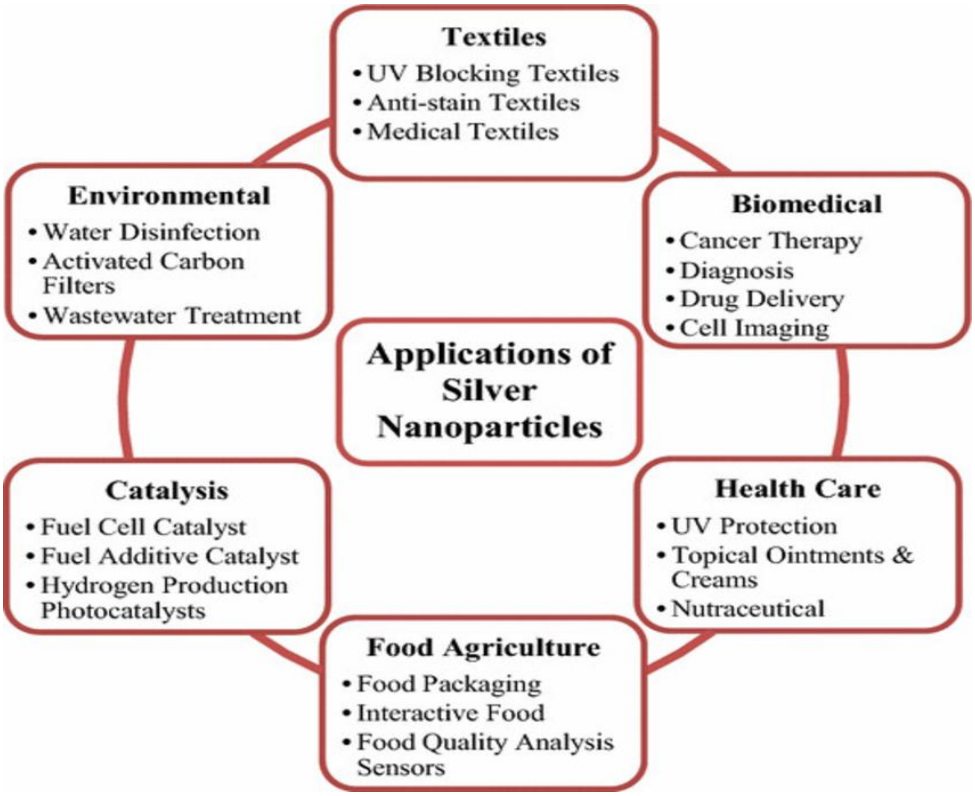



**Fig. 5. Applications of Silver Nanoparticles.** (Source: Keat et al., 2015, Bioresour. and Bioprocess).

## 1.10. Toxicity of Silver nanoparticles

The unique physical and chemical properties of AgNPs make them excellent constituents for a number of day-to-day activities, and also their antimicrobial and anti-inflammatory properties

make them noble candidates for many applications in the medical field. However, there are studies and reports that nanosilver can evidently have adverse effects on humans as well as on the environment. It is estimated that huge quantities of silver are being released into the environment through industrial wastes, and it is believed that the toxicity of silver in the environment is mainly due to free Ag ions in aqueous phase. The adverse effects of these free radicals on all living beings and environment include permanent bluish-gray discoloration of the epidermis (argyria) or the eyes (argyrosis), and exposure to soluble silver compounds can produce toxic effects on liver, lungs and kidney (***Panyala et al., 2008***). Since from the beginning of the 21$^{st}$ century, AgNPs have been gaining popularity and are now being used in almost every area, especially in the medical field. However, there have been studies on how AgNPs can discriminate between different strains of bacteria and hence cannot destroy microbes beneficial for ecological welfare. There are very few researches conducted to study the toxicity of nanosilver. In a study, the in-vitro toxicity assay of AgNPs in rat liver cells has shown that even low-level exposure of silver nanoparticles to cells may result in oxidative stress and impaired mitochondrial function (***Hussain et al., 2005***). There is evidence that shows that Ag ions cause alteration in the permeability of the cell membrane to potassium (K) and sodium (Na) ions at very low concentrations. Nanosilver is also reported to show severe toxic effects on the male and female reproductive system (***Kone et al., 1998***). Research shows that AgNPs can penetrate the blood-testes barrier and can deposit in the testes where they adversely affect the sperm cells. In-vivo studies on the oral toxicity of AgNPs on rats have showed that the target organ in the mouse AgNPs was the liver. Studies have also indicated that there is release of Ag when the nanoparticles are stored over a definite period of time. Hence, it can be said that aged nanosilver is more toxic than newly produced AgNPs. AgNPs with their antimicrobial property can hinder the growth of many eco-friendly bacteria present in the soil. Showing toxic effects on denitrifying bacteria, AgNPs can restrict the denitrification process, which involves the conversion of useless nitrates into nitrogen gas which is very essential for the plants. Loss of environmental nitrogen can lead to eutrophication of oceans, lakes, and marine ecosystems and disrupt the functioning of ecosystem (***Drake et al., 2005***). To understand the toxic potential AgNPs have on the freshwater environment, the Daphnia magna 48-h immobilization test was

conducted, and the results indicated that the silver nanoparticles have to be classified under category “acute 1” according to the Globally Harmonized System of Classification and Labeling of Chemicals, indicating that the release of AgNPs into the environment has to be delft carefully.

# Chapter-2

## Materials and Methods

## 2.1. Preparation of Plant Extract

Silver Nitrate ($AgNO_3$) salt (99% purity) was obtained from Sigma-Aldrich Chemical Co. All the glass wares were properly cleaned. The petri-dish and agar were autoclaved before use. Leaves of Ocimum Sanctum (Tulsi) were first rinsed with tap water and then distill water to remove all the dust and unwanted visible particles. Then the leaves were dried at room temperature to remove the water from the surface of the leaves. About 2 g of finely incised dried Tulsi leaves were boiled in 40 ml distill water at 60°C for about 10 min. The supernatant was filtered using whatman filter paper No.1 to remove the particulate matter. A pale yellow clear solution is obtained and stored at 4-8°C**.**

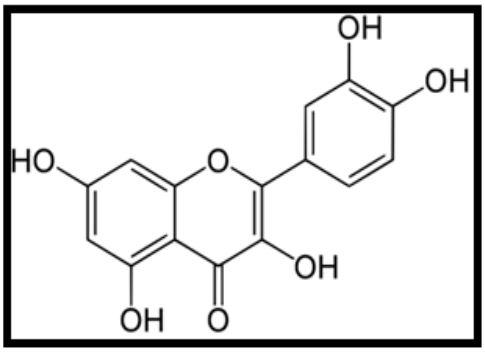
**Tulsi Leaves** **Extract**

**Fig.6**

## 2.2. Preparation of flavonoid solution

The stock solution is prepared by taking about 0.003 gm of Quercetin (yellow powder) and dissolving it in 10 ml mixture of distill water and ethyl alcohol to obtain 1 mM solution. Quercetin does not dissolve in normal distill water. For analysis with variation in pH it was dissolved in 1mM solution of sodium hydroxide. At normal pH, the solution is pale yellow in colour but at higher pH the colour of the stock solution turns red. The stock solution is stored at room Temperature in colored glass bottle (or wrap aluminum foil around transparent glass bottle) to prevent the photo-degradation of Quercetin molecule ***(Dall' Acqua S et al., 2012)***.

**Quercetin (Yellow powder)**




**Chemical structure of Quercetin**

Fig. 7

## 2.3. Synthesis of AgNPs

A 2 mM solution of silver nitrate is prepared by dissolving 0.0085 gm of $AgNO_3$ in 25 ml of distill water. 1 ml extract of Tulsi and Ginger was mixed with 5 ml of 2 mM $AgNO_3$ solution. Similarly about 50 μL of Flavonoid (Quercetin) solution is mixed with 5 ml of 2 mM $AgNO_3$ Solution. A Colour change from pale yellow to colloidal brown indicates the formation of silver Nanoparticles (Fig.8). The effects of various parameters such as pH, concentration, reaction time and temperature on the synthesis of silver Nanoparticles were examined.

**Fig. 8. Change in the colour of the solution with time when Plant extracts are added to silver salt.**

## 2.4. Characterization of AgNPs.

### 2.4.1. 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 50 watt CW Xenon lamp.

Effect of time was studied at intervals of 1hr, 2hr, 3 hr and 4 hr in case of leaf extract and 1 min, 10 min, 30 min, 1 hr and 2 hr in case of Quercetin. Effect of pH was studied by varying the pH of leaf broth and Quercetin solution. The pH of $AgNO_3$ solution is not varied. 0.1 N NaOH and 0.1 N HCl were used to adjust the pH of the leaf extract and Quercetin solution. The pH variation was observed from pH 7-12 with an accuracy of ±0.2. Effect of temperature was studied by varying the temperature between 25-55°C in case of Plant extracts and between 5-35°C in case of Quercetin with an accuracy of ±2°C.

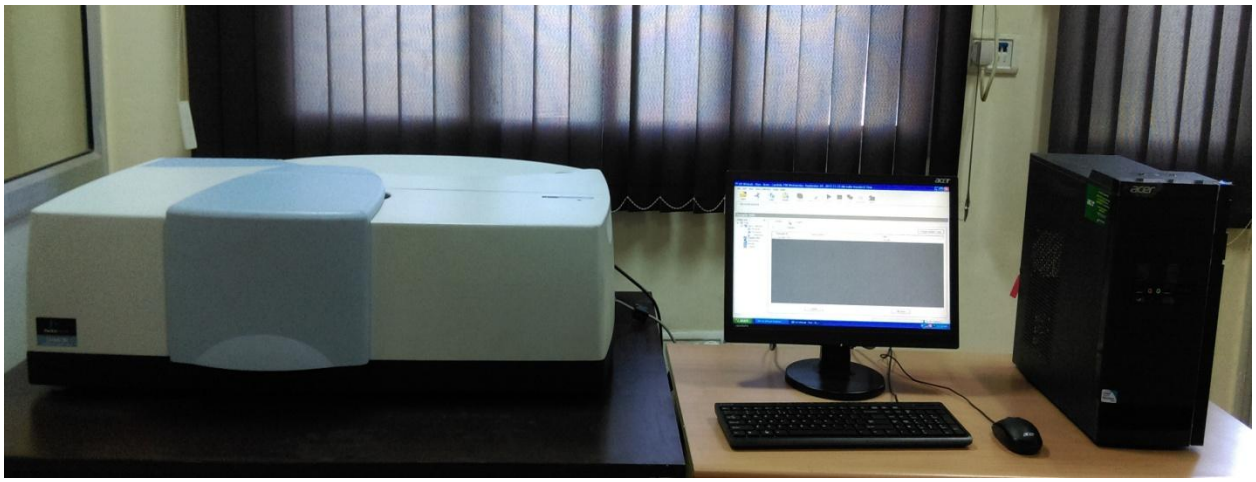


**Fig. 9. Perkin Elmer Lambda 750 Spectrophotometer at Laser Spectroscopy Lab, DTU.**

**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.
- Twice the flexibility with two sampling compartments.

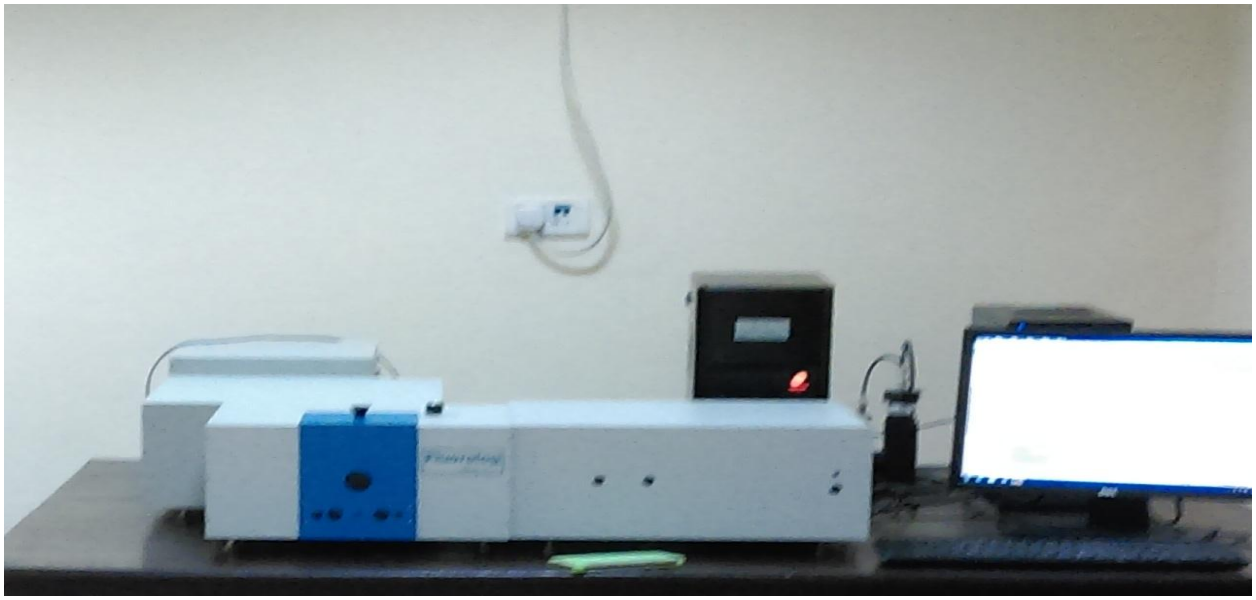


**Fig. 10. Fluorolog-3 Spectrofluorometer (Horiba Jobin Yyon) at Laser Spectroscopy Lab, DTU.**

**Features of this instrument are:**

- 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 measurement.
- Peltir 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.

**2.4.2. XRD Analysis**

The sample for XRD analysis is prepared by depositing a thin film of silver nanoparticles on a glass slide by heating the AgNPs solution drop by drop at 60°C allowing the water to evaporate. The synthesized silver nanoparticles were studied with X-ray diffractometry (XRD, Bruker D8 advanced) Cu Kα radiation at voltage of 30 kV and current of 20 mA. Different phases present in the synthesized samples were determined by JCPDS software with search and match facility. The particle size of the prepared samples were determined by using Scherrer's equation as follows $D \approx 0.94\lambda/\beta\cos\theta$ Where D is the crystal size, λ is the wavelength of X-ray, θ is the Braggs angle in radians and β is the full width at half maximum of the peak in radians.

This instrument uses the spatial distribution and intensities of X-ray radiation scattered by the sample under keep and helps in the analysis of the structure of the sample. X-ray diffraction analysis technique is used to establish the crystalline samples because some crystals have a non flexible periodicity in their structure and consist of naturally produced Bragg diffraction gratings for X rays. When X-ray interacts with the electrons of any sample, the X rays are diffracted according to the atomic arrangement of that sample. The diffraction pattern depends upon the wavelength (λ) of the X-rays and the type of structure the sample posses. Crystals can be understood as periodic arrays of atoms, and X-rays are considered as short waves of electromagnetic spectrum.. X-ray scattering is a sort of elastic scattering; so the outgoing X-rays will have the same energy, and hence the same wavelength, as that of the incoming X-rays, only there is change in direction. These particular directions can be visible as spots on the diffraction pattern called reflections. Hence, X-ray diffraction results striking X-ray on a regular arrangement of scatterers (the periodic arrangement of atoms within the crystal). X-rays is used to generate the diffraction pattern and the reason is because of their wavelength λ which is typically the order of (1–100 angstroms) which is the same as that of spacing d between

planes in any crystal. As the wavelength of x-ray is accordance with the lattice parameter of the crystals, diffractions might occur when X-ray interact with the sample's surface. According to the Bragg's law, diffraction happens only if the stated condition is verified

$$n\lambda = 2d\sin\theta$$

This is known as Bragg's law of diffraction. Where λ is the x-ray wavelength, the diffraction angle (θ) is related to the distance between planes of crystals (d), as shown in the figure. Now from the diffraction spectrum, the information about the crystal structure can be produced. One of the advantages of X-ray diffraction is that it is a reconstructive analytical method which can be used to detect the phase as well as the orientation of the crystal lattice, determining the structural properties of the sample, to calculate the thickness of thin films, measure the size of nanoparticles, etc.

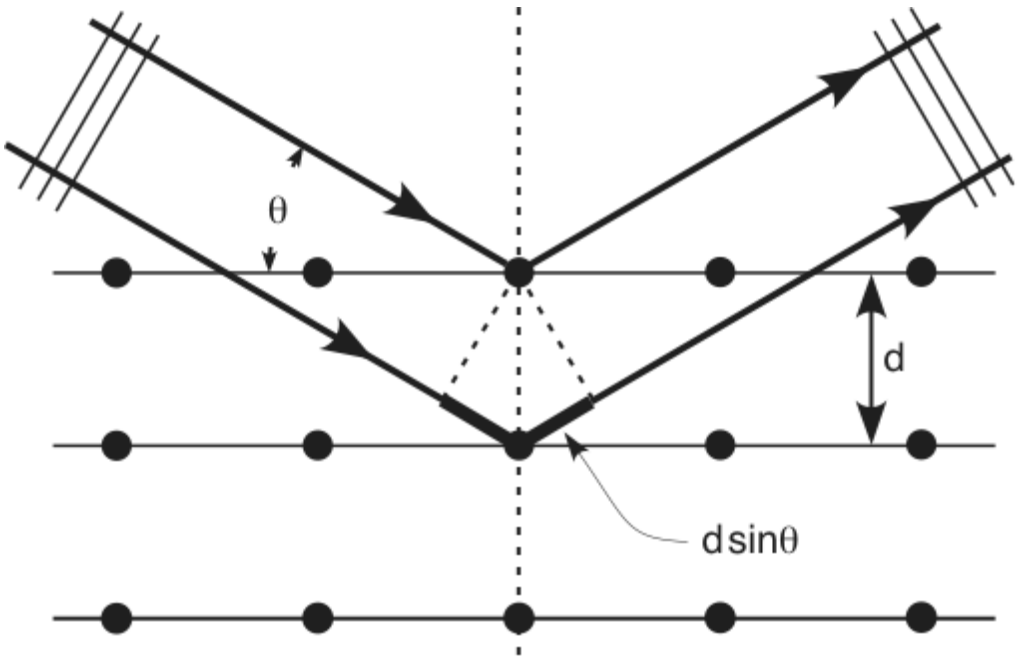



**Fig. 11. Bragg's reflection.**

**Fig. 12. X-ray diffraction Instrument at Advanced Instrumentation Center, DTU.**

### 2.4.3. 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.
- Greater on-site confidence, higher production yield.
- Identifying the source of the problem in process chain

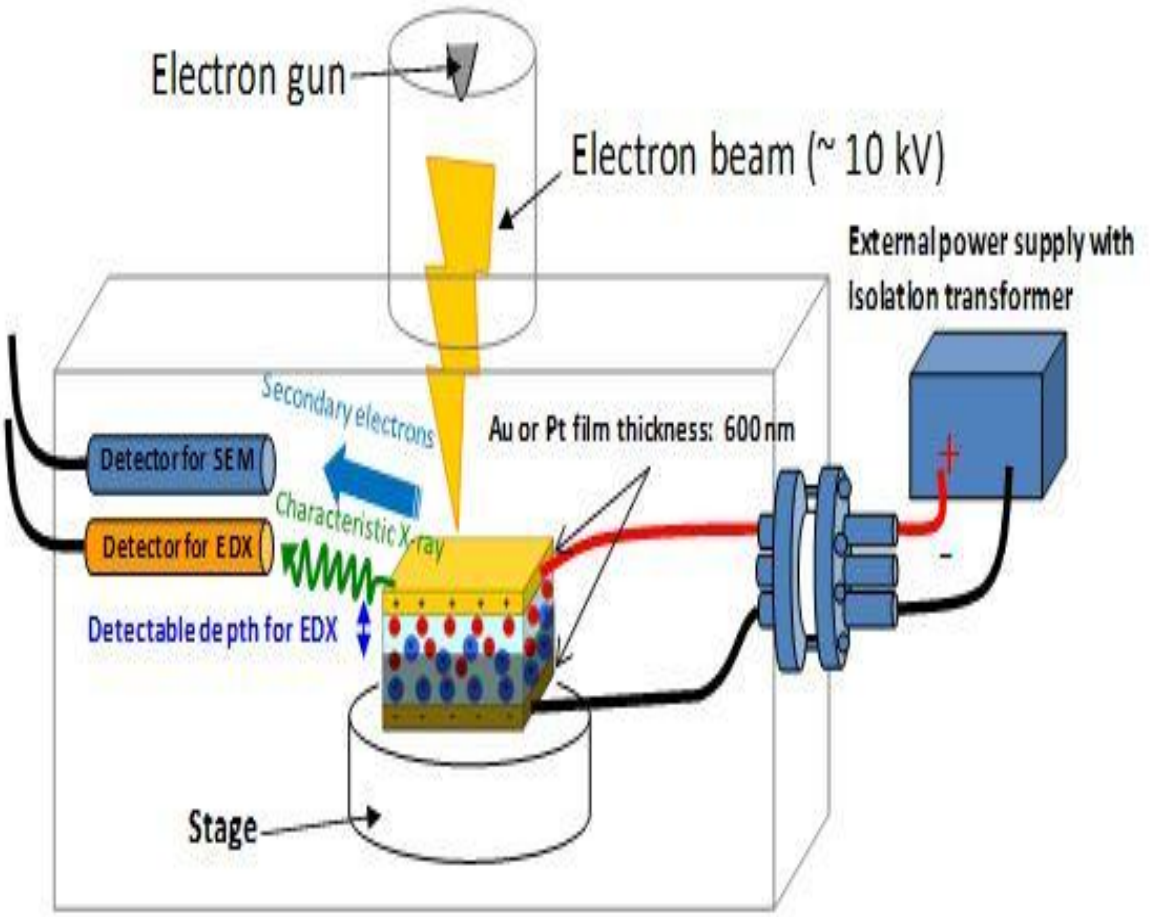



**Fig. 13. Schematic of EDX incorporated Scanning Electron Microscope (SEM).** (Source: Kuwabata et al., 2013, Intech).


**Fig.14. Scanning Electron Microscope (SEM) with attached EDX systems at Advanced Instrumentation Center, DTU.**

### 2.4.4. 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

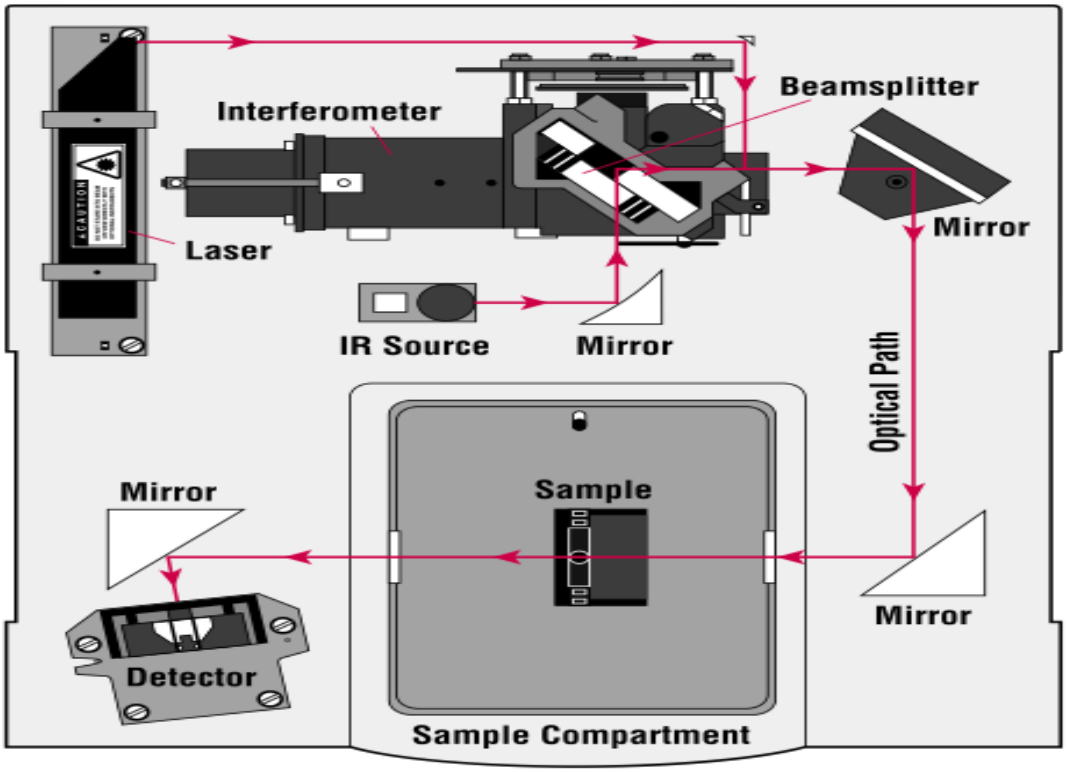



**Fig. 15. Schematic showing the internal functioning of FTIR Spectrometer. (**Source: Chromacademy.com).

### 2.4.5. TEM Analysis.

TEM 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 TEM 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. TEM provides a important analysis methodology in a number of scientific fields, both physical as well as biological sciences.

Transmission electron microscopy (TEM) uses electrons accelerated with very high energy to penetrate through an ultrathin (≤100 nm) sample. This process increases the spatial resolution (down to individual atoms) and the possibility of carrying out diffraction from nano-sized entities. When electrons are accelerated upto a high energy levels (few hundred KeV) and when focused upon a material, they might scatter or backscatter elastically or non-elastically, or may produce many interaction source of different signals such as X-rays, Auger electrons or light. Many of them can be used in transmission electron microscopy (TEM).

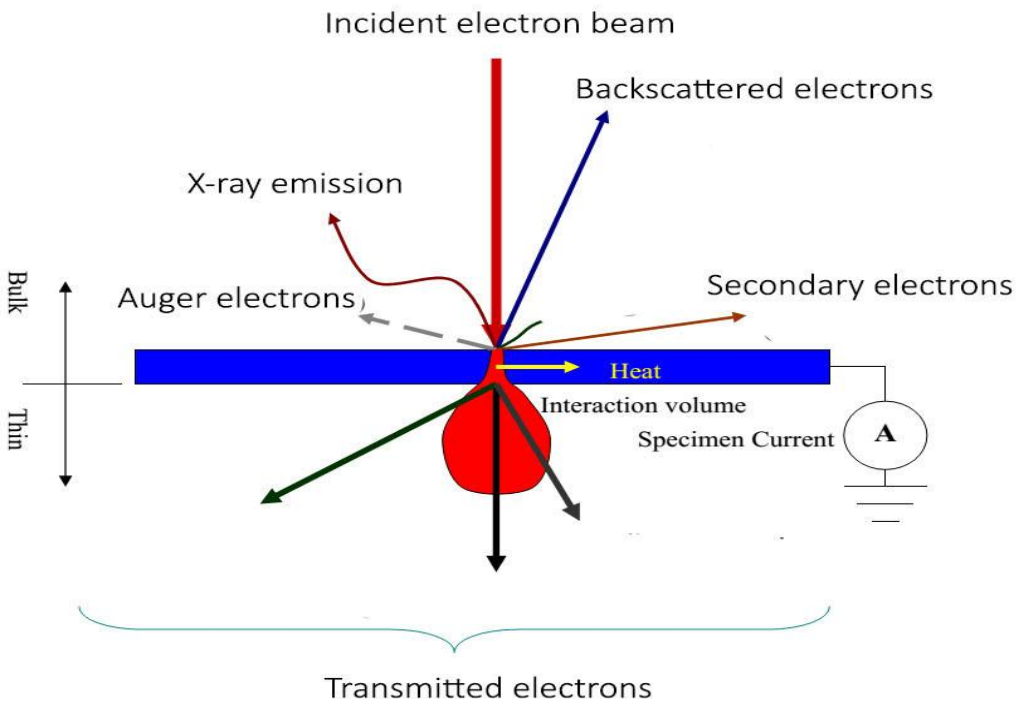



**Fig. 16. Different types of signals produced during TEM imaging**. (Source: Ninithi.com, Electron microscopy in nanotechnology).

A transmission electron microscope is composed of:

(1). Two to three condenser lenses which focus the electron beam upon the sample.

(2). an objective lens which forms the diffraction in the back focal plane and the image of the sample on the image plane.

(3). One or two intermediate lenses which magnify the image and the diffraction pattern on the screen.

TEM operates mainly in two modes, namely,

(A) **Imaging Mode**: If the sample is thin (< 200 nm) and made up of early periodic table chemical elements, the image presents a very low contrast when it is focused. To get an amplitude contrasted image, an objective diaphragm has to be inserted in the back focal plane to select the transmitted beam (and possibly few diffracted beam): the crystalline parts in Bragg

orientation appears to be dark and not Bragg oriented parts or the amorphous appear bright. This type of imaging mode is called *bright field* mode BF.

(B) **Diffraction Mode**: The selected area diaphragm is used only to select one part of imaged sample for example a particle or a precipitate. This mode is known as selected area diffraction SAED. The spherical aberrations of the objective lens restrict the area of the selected object to few hundreds of nanometers.

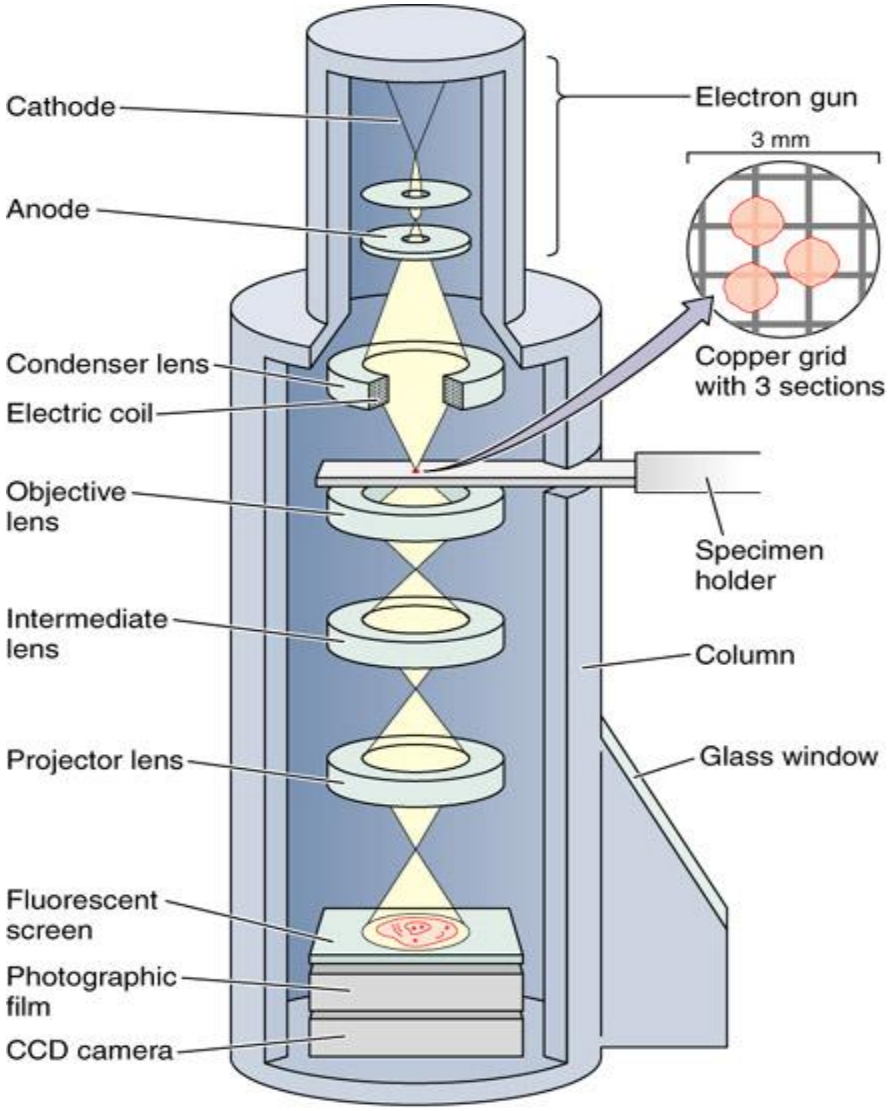



**Fig. 17. Schematic showing the internal assembly of a Transmission Electron Microscope.**

(Source: Intranet.tdmu.edu.ua).

The Transmission Electron Microscope (TEM) imaging for the synthesized silver nanoparticles was performed at Sophisticated Analytical Instrumentation Facility (SAIF), All India Institute of Medical Science (AIIMS), New Delhi.

The sample for imaging was prepared by carefully placing single drop of synthesized AgNPs (aqueous) on a copper coated grid and the sample was imaged on TECNAI (TEM) Electron Microscope.


**Fig. 18. Tecnai (TEM) Electron Microscope at SAIF, AIIMS New Delhi.**

# Chapter- 3

## Results and Discussion

The effect of various environmental conditions such as temperature, pH, reaction time, reactants concentration on the formation of silver nanoparticles has been discussed briefly and the results obtained using Tulsi extract and quercetin separately has been compared.

## 3.1. Effect of Reaction time on the formation of AgNPs.

As the plant leaf extract is mixed with the $AgNO_3$ solution a colour change from pale yellow to dark yellow and colloidal brown is observed within few minutes. The colour change is due to the formation of AgNPs. In aqueous solution silver nanoparticles exhibit strong Surface Plasmon Resonance (SPR) (***Shankar et al., 2004***). The obtained AgNPs emits light between 400-700 nm ***(Vigneshwaran et al., 2007).*** The bio-molecules such as Flavonoid, Terpenoids, Phenolic compounds present in Tulsi are responsible for the reduction of silver ions to AgNPs. After few hrs, there is no further change in the colour of the solution indicating that the whole silver salt present in the solution has been reduced. The formation of silver nanoparticles was examined by UV-Vis & PL spectroscopy at different time intervals. Fig.19 shows the absorption spectra of synthesized AgNPs obtained from the reaction of silver salt and Tulsi extract at different time intervals in the range of 250-600 nm. Similarly, Fig.20 shows the absorption spectra in the range of 250-700 nm at different time intervals using Quercetin as a reducing agent for the synthesis of AgNPs. An increase in absorbance is observed with the passage of time indicating an enhancement in the formation of AgNPs. It is also observed that the time required for the reduction of silver ions to silver nanoparticles is much less in case of Quercetin as compared to Plant extract, this fact can be explained by taking into account the structural property of Quercetin. The structure of quercetin includes an extended system of conjugated double bonds and contains 5 hydroxyl groups which provides the high reductive ability ***(Terenteva et al., 2015).***

Reports show that Plant leaf extracts of Tulsi, Neem, Ginger etc contains variable amounts of Phenolic compounds. A high-Performance thin layer chromatographic (HPTLC) method was applied for the quantization of quercetin in Tulsi and found to be 0.74 mg/mL of extract. The total Phenolic and Flavonoid content of Ocimum Sanctum is 82.02±8.17

mg GAE/g and 74.6±5.1 mg/g, respectively (***Gupta et al., 2012***). The size of AgNPs was calculated using the formula given by Eq. (1). **(*GArnold, 1975; Rozra et al., 2012)***.

$$\boldsymbol{d = \frac{h v_f}{\pi \Delta E_{1/2}}} \quad \text{.....} \ (1)$$

where, $d$ is the diameter of the particle, $h$ is the Planck's constant, $v_f$ (1.39x10$^6$ m/sec) is the Fermi velocity of electrons in bulk silver and $\Delta E_{1/2}$ is the FWHM (Full Width Half Maxima) of the absorption band. The above equation is valid as long as the size of the silver nanoparticles is smaller than the mean free path of the electrons in the bulk silver ***(Kreibig & Vollmer, 1995; Karthikeyan, 2008)***.

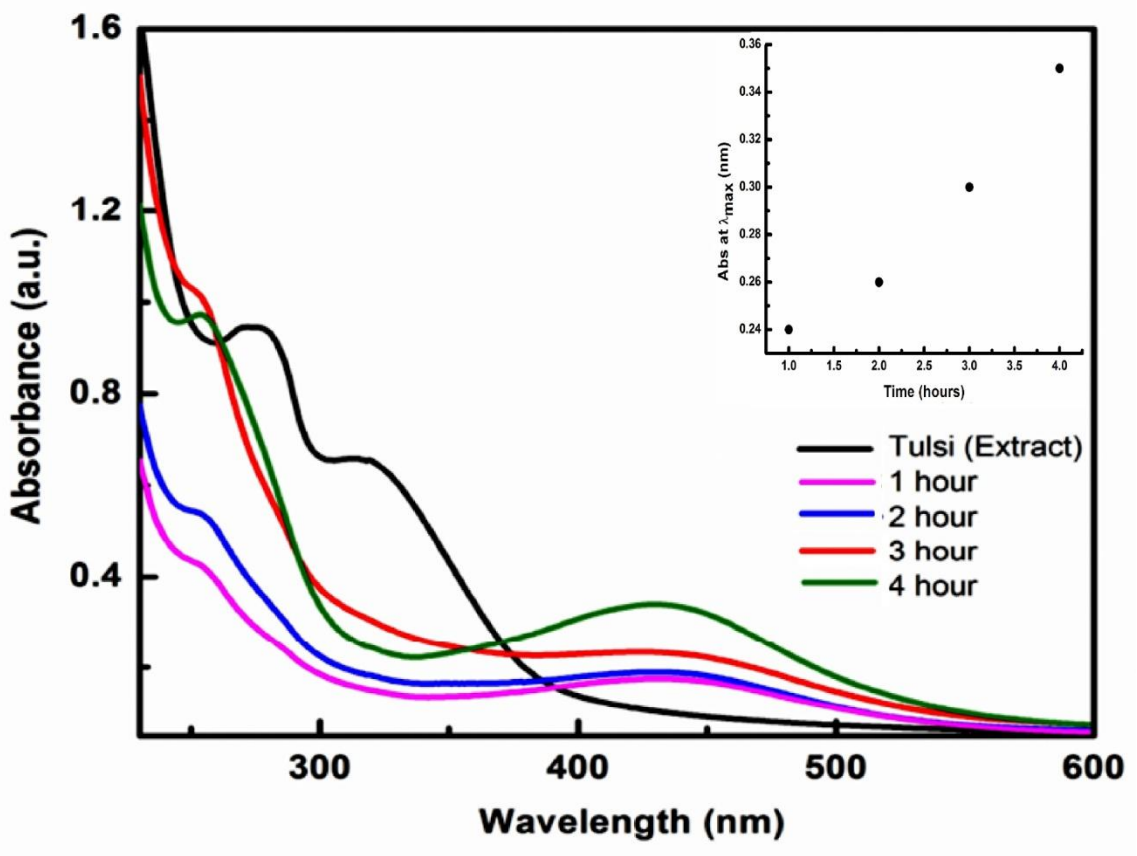



**Fig. 19. Absorption spectra of AgNPs observed at different time intervals by mixing Tulsi leaf extract in 2mM aqueous solution of silver nitrate. Inset shows the variation of optical intensity with time.**

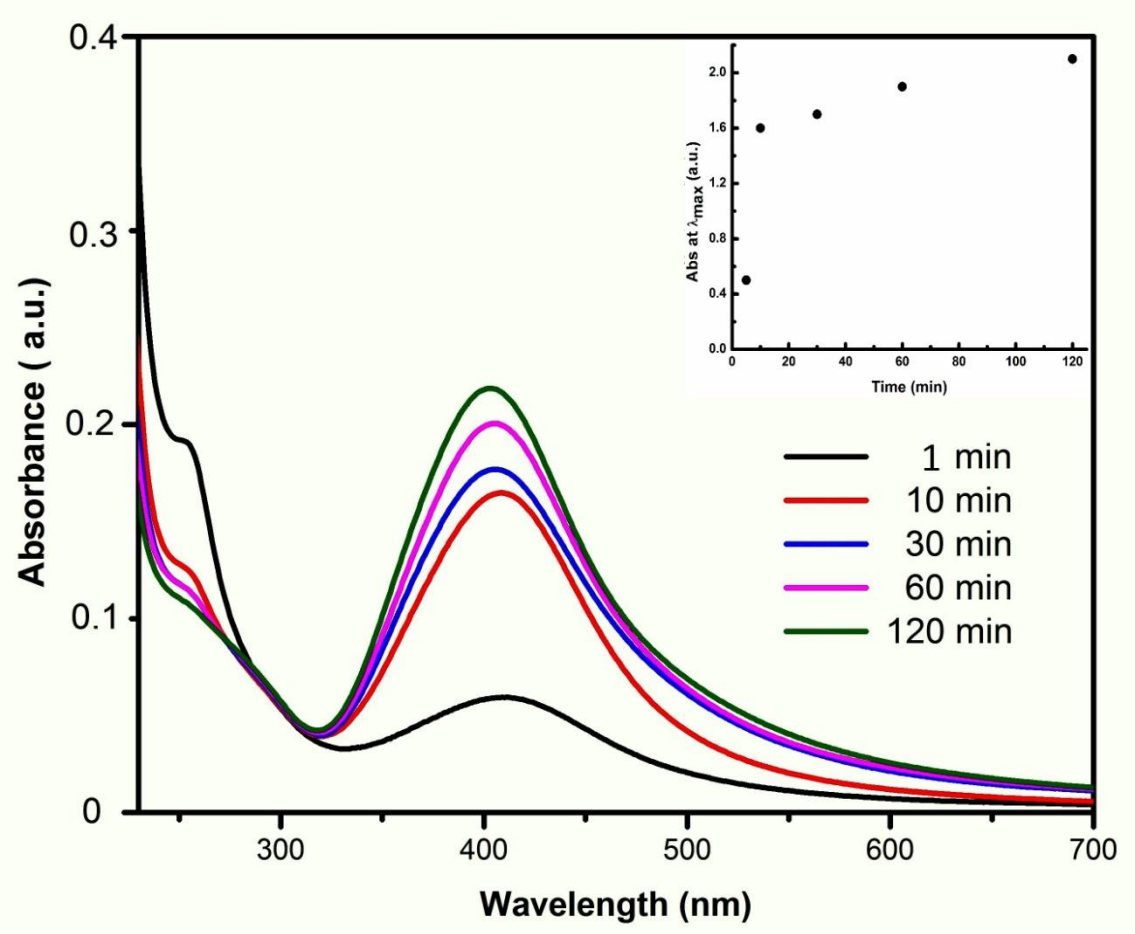



**Fig. 20. Absorption spectra of AgNPs observed at different time intervals by mixing aqueous quercetin in 2mM aqueous solution of silver nitrate. Inset shows the variation of optical intensity with time.**

## 3.2. **Effect of concentration of plant extract & quercetin on formation of AgNPs**

The effect of concentration of Plant extract and quercetin on the formation of AgNPs is studied systematically by mixing the Plant extract and $AgNO_3$ solution in different concentration ratios. Similarly, a solution of silver nitrate and quercetin was prepared by mixing quercetin solution to aqueous $AgNO_3$ in different concentration ratios. It was found that on increasing the concentration of plant extract into $AgNO_3$ solution a shifts in the absorption band to higher wavelength (from 430 to 455 nm in case of Tulsi extract & from 420 to 425 nm in case of

quercetin) i.e., red shift occurs, which indicates that the particle size is increasing with increasing the ratio of leaf extract and quercetin separately to $AgNO_3$ solution. In case of quercetin, the red shift is less compared to that with Tulsi extract because quercetin is a pure bio-molecule, may be due to the its low concentration in $AgNO_3$ solution. If the concentration of quercetin is further increased, the colour of the solution turns blackish grey and the formation of AgNPs is restricted. Also the Absorption peak gets narrower with increase in concentration, as shown in Fig. 21 & Fig. 22. According to Mie theory the size of nanoparticles is inversely proportional to the FWHM as explained above in Eq. (1), hence narrow absorption spectra means less FWHM indicating an increase in the size of synthesized AgNPs.

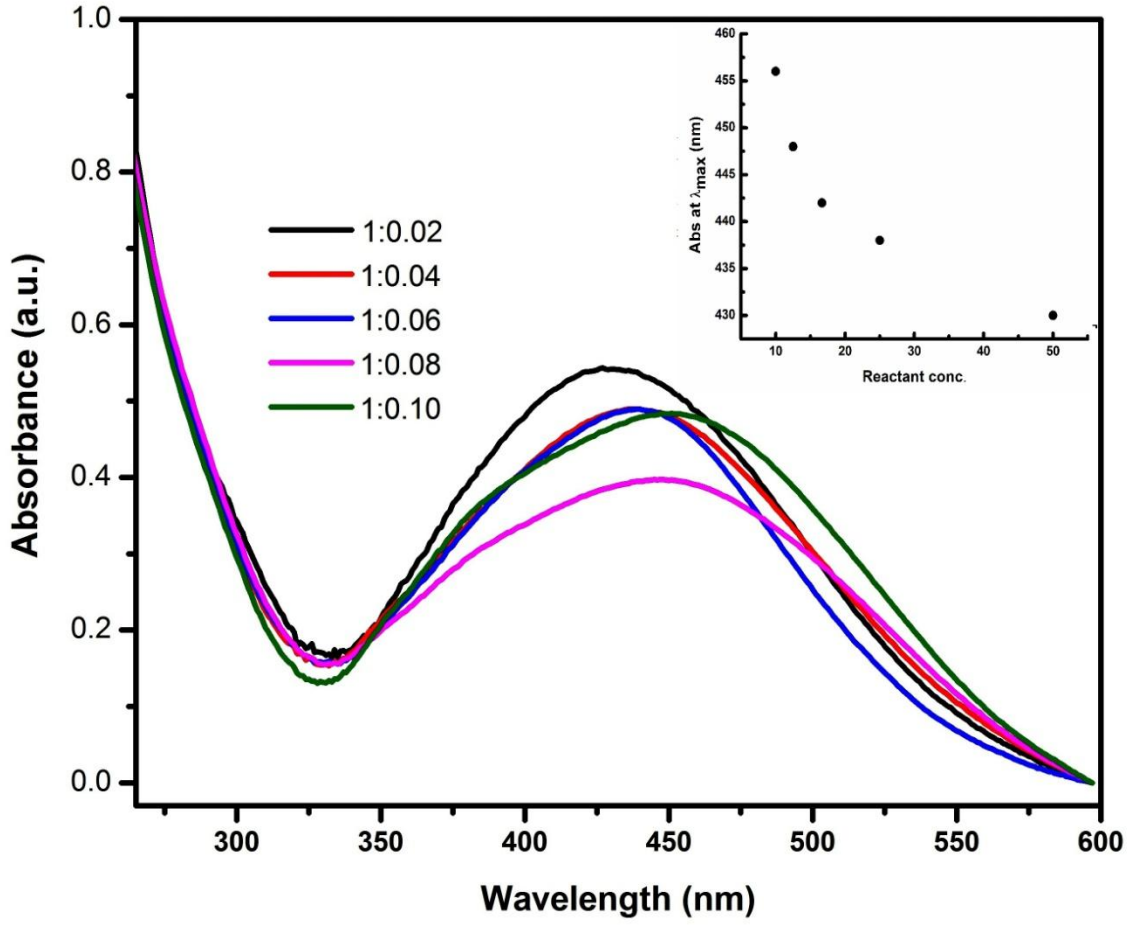



**Fig. 21. Absorption spectra of AgNPs at various concentrations of silver salt and Tulsi broth. Inset figure shows the shift in absorption peak with reactant concentration.**

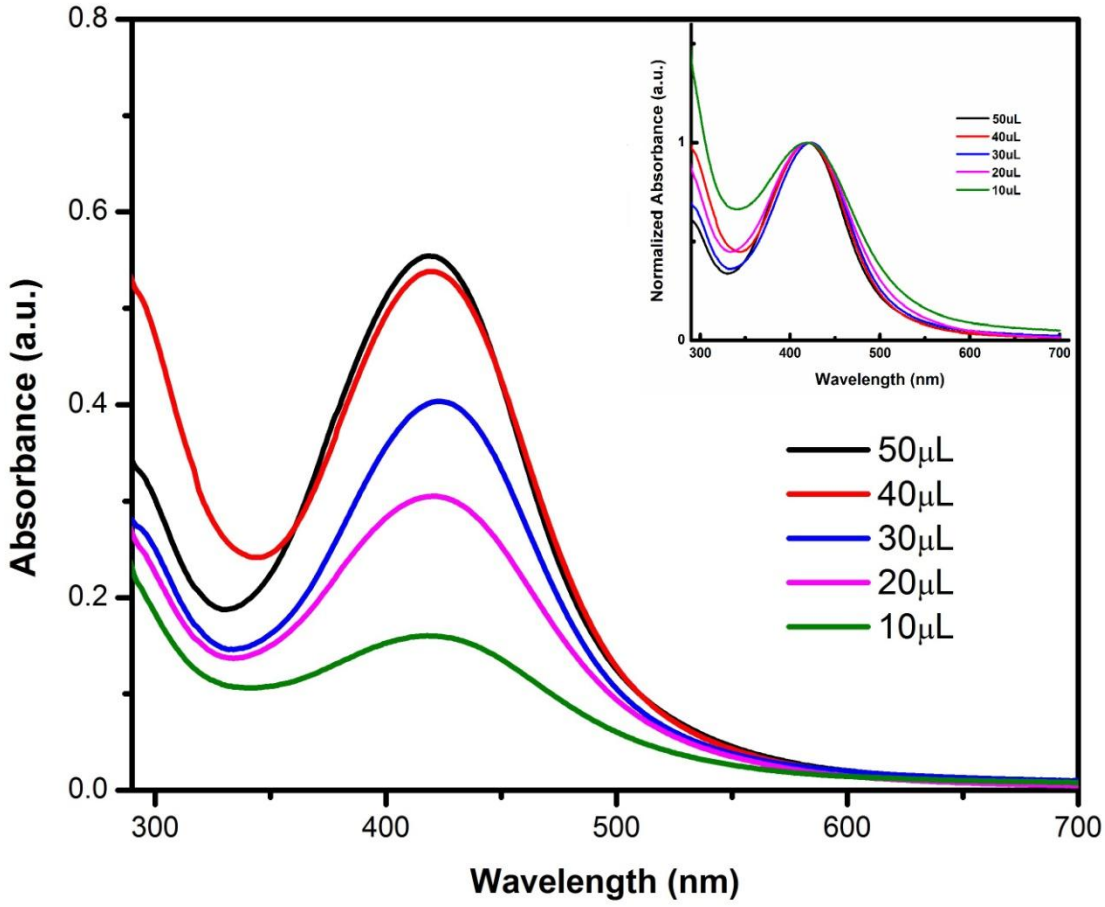



**Fig. 22. Absorption spectra of AgNPs at different amount of quercetin mixed in 5 ml aqueous of $AgNO_3$ (concentration 2mM). Inset shows the variation in spectral width with change in concentration.**

## 3.3. **Effect of Temperature on the formation of AgNPs**

The reaction temperature also has a great effect on the size and morphology of the synthesized AgNPs. Figures 23 & 24 show the variation in the absorption spectra of AgNPs synthesized at different temperature using aqueous $AgNO_3$ & Tulsi extract as well as aqueous $AgNO_3$ & quercetin, respectively. The reaction temperature was varied from 25-55°C in case of AgNPs synthesized using Tulsi extract, whereas from 5-35°C in case of AgNPs synthesized using quercetin. In both cases it was observed that with increase in the reaction temperature the

absorption peak shifts towards lower wavelength or a blue shift occurs (from 455 to 436 nm in case of Tulsi and from 429-405 nm in case of quercetin), which indicates a decrease in particle size with increase in temperature ***(Saware et al., 2014)***. At 5°C there is no significant peak in the absorption spectra indicating there is no formation of AgNPs. The shift in the absorption peak is due to localization of Surface Plasmon Resonance (SPR) of the AgNPs. This indicates that the size of the synthesized AgNPs decreases with increasing temperature, which is probably due to the faster reaction rate at higher temperature. At high temperature, the kinetic energy of the molecules increases and silver ions gets consumed faster, thus leaving less possibility for particle size growth. Thus, smaller particles of uniform size distribution are formed at higher temperature (***Verma & Mehata, 2015***).

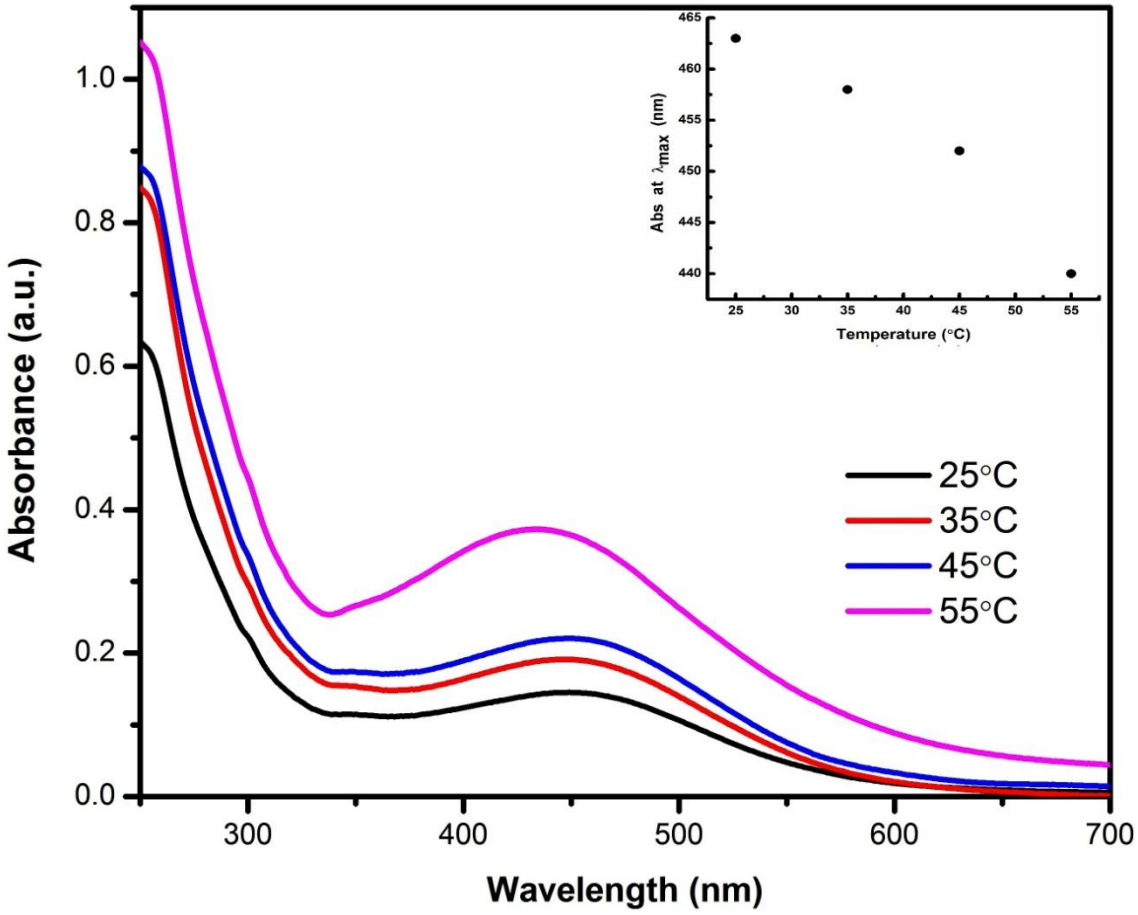



**Fig. 23. Absorption spectra of AgNPs obtained at different reaction temperatures using Tulsi extract as reducing agent. Inset figure shows the relation between temperature and peak absorption wavelength.**

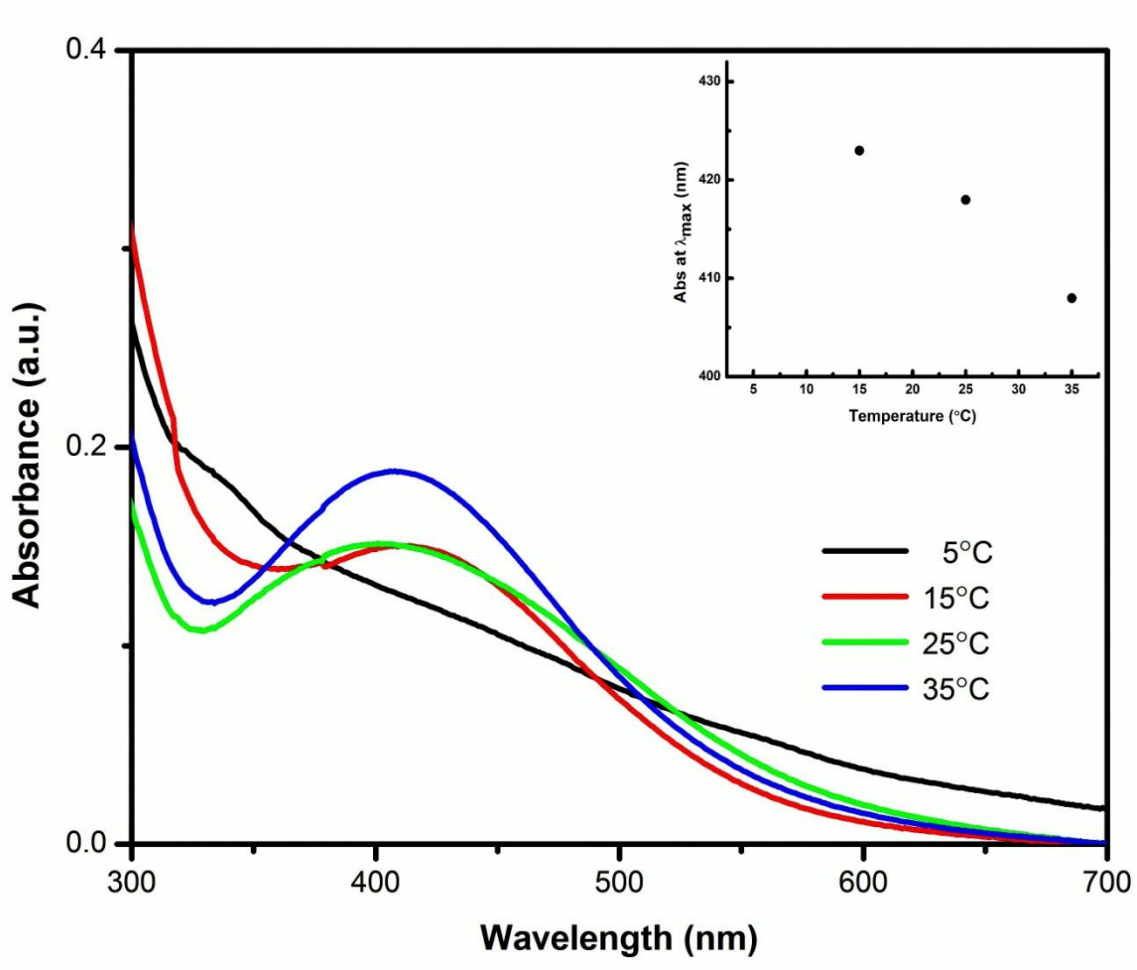



**Fig. 24. Absorption spectra of AgNPs obtained at different reaction temperatures using quercetin as reducing agent. Inset figure shows the relation between temperature and peak absorption wavelength.**

## 3.4. Effect of pH on formation of AgNPs

pH is another important factor which affects the size, shape and morphology of the synthesized AgNPs. Figures 25 & 26 show the effect of pH on the absorption spectra of AgNPs synthesized using plant extract and quercetin, respectively. In both cases, It is observed that with increase in pH the absorption peak shifts towards higher wavelength (from 396 to 411 nm in case of Tulsi extract & from 409 to 420 nm in case of quercetin) indicating an increase in the size of synthesized AgNPs. As the diameter of the particle increases, the energy required to excite the

Surface Plasmon electrons decreases. As a result the absorption maxima shift towards longer wavelength. In addition to the shift the spectral intensity also increases with increase in pH. Further, it is observed that higher pH enhances the rate of reduction as the colour of the solution turns colloidal brown more quickly as compared to solution with lower pH. Hence alkaline pH is favorable for the synthesis of AgNPs.

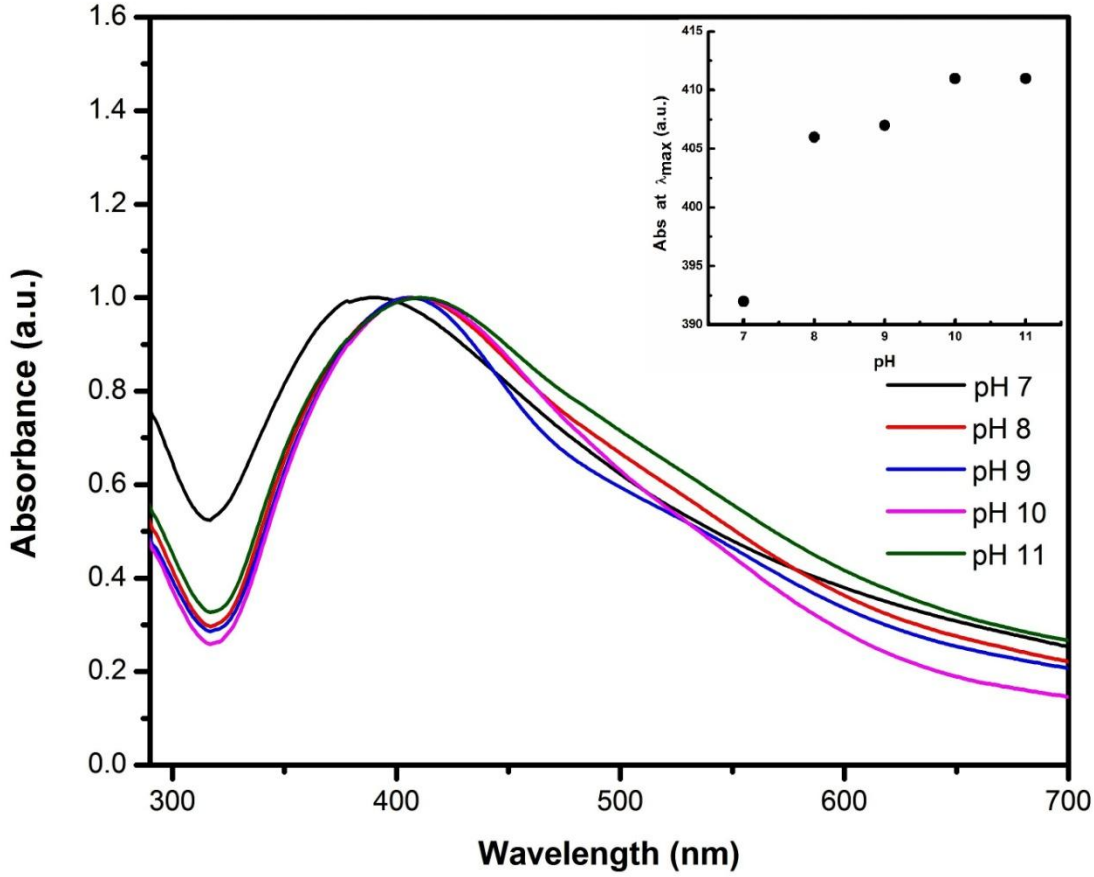



**Fig. 25. Normalized absorption spectra of AgNPs obtained at different pH values of reaction mixture using Tulsi extract. Inset figure shows the relation between pH and peak absorption wavelength.**

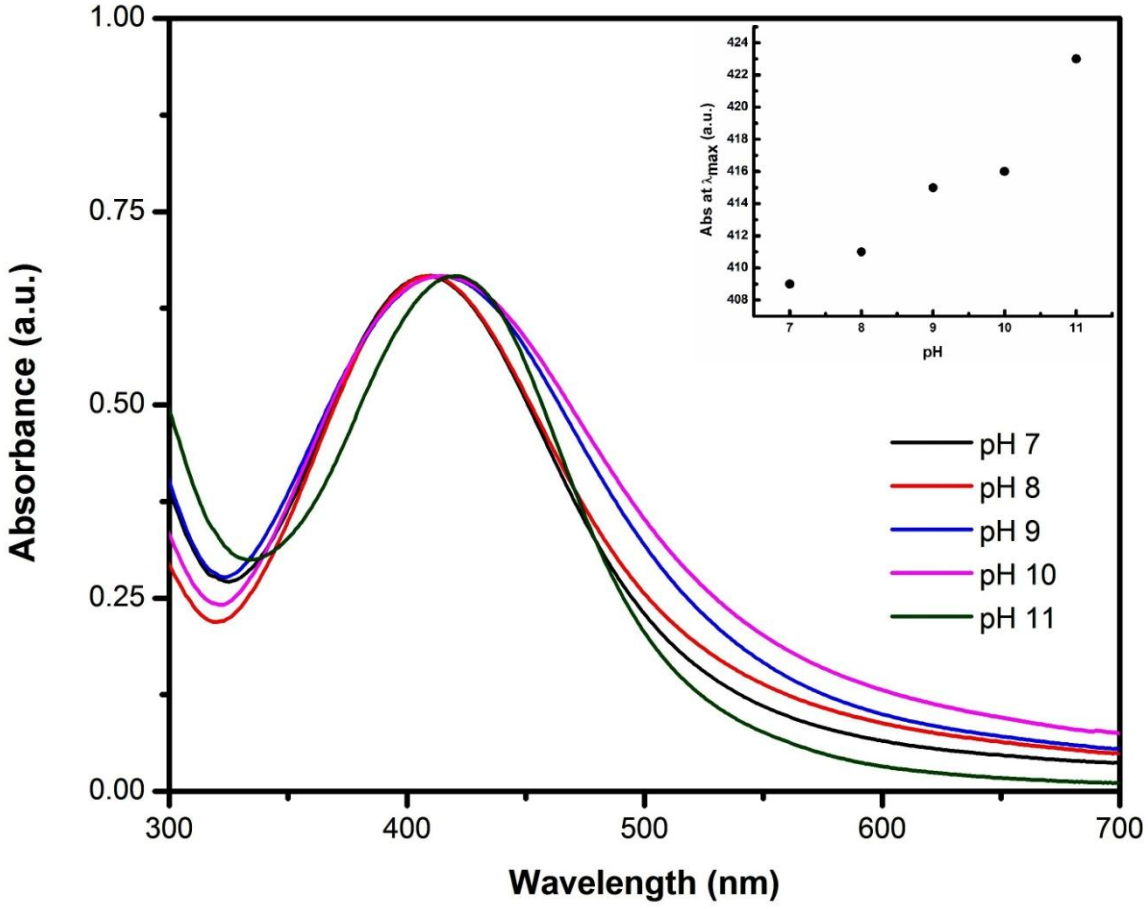



**Fig. 26. Normalized absorption spectra of AgNPs obtained at different pH values of reaction mixture using Quercetin. Inset figure shows the relation between pH and peak absorption wavelength.**

## 3.5. Surface morphology of AgNPs

To identify the crystalline nature and surface morphology of the synthesized AgNPs XRD analysis was performed in the range of 30-70° at 2θ angles. Fig. 27 Shows the XRD spectra of AgNPs synthesized by the reduction of aqueous silver salt using Tulsi leaf extract and Quercetin respectively. The high intensity peaks of AgNPs synthesized using quercetin were observed at around 38°, 44° and 64°corresponding to (111), (200) and (211) Bragg reflections, respectively, which are exactly the same peak positions as given for face center cubic (FCC) lattice structure of silver and in case of AgNPs obtained using Tulsi leaf extract as reducing agent the peak

positions are at 32°, 38°, 46° and 57°. The unassigned peaks are marked as (*) which may be due to the impurities present in the sample or may be related to crystalline and amorphous organic phase ***(Awwad et al., 2013)***. It can be seen from Fig. 27 that on increasing pH of the solution the width of XRD peak decreases indicating an increase in the size of synthesized AgNPs (as size of nanoparticles is inversely proportional to FWHM) which is in accordance with the absorption spectra of AgNPs at different pH. The average size obtained from XRD spectra using Debye-Scherrer equation is approximately 14 nm in case of AgNPs synthesized using quercetin and 17 nm in case of AgNPs synthesized using Tulsi extract. The XRD analysis was not performed at the same time of synthesis therefore the size of AgNPs calculated from XRD spectra is bigger as compared to that calculated from absorption spectra.

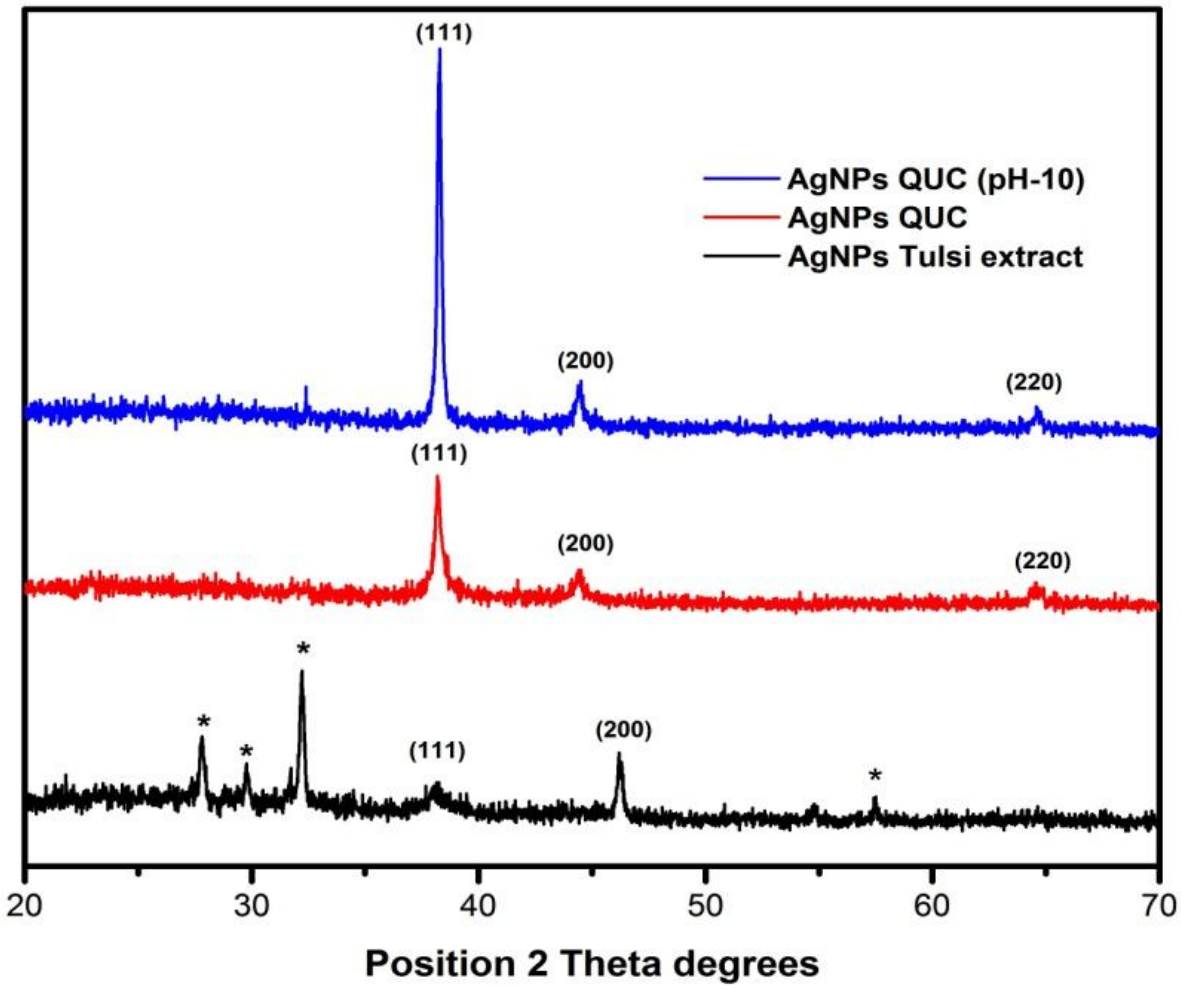



**Fig. 27. XRD spectra of AgNPs obtained using Tulsi extract and Quercetin as reducing agents.**

The EDX analysis was performed at an accelerating voltage of 15 KV and take off angle of 66.6°. The quantitative results of EDX spectra of AgNPs showed yield of 91.36 (weight %) of elemental Ag in L line. Fig. 28 shows the EDX spectra of synthesized AgNPs.

**Table. 1. Elemental composition of Synthesized AgNPs.**

| *Element Line* | *Net Counts* | *Int. Cps/nA* | *Weight %* | *Weight % Error* | *Atom %* | *Atom % Error* | *Formula* | *Standard Name* |
|---|---|---|---|---|---|---|---|---|
| ***O K*** | 228 | --- | 5.11 | +/- 0.65 | 24.73 | +/- 3.15 | O | |
| ***Si K*** | 836 | --- | 3.53 | +/- 0.23 | 9.73 | +/- 0.64 | Si | |
| ***Si L*** | 0 | --- | --- | --- | --- | --- | | |
| ***Ag L*** | 9809 | --- | 91.36 | +/- 2.35 | 65.54 | +/- 1.68 | Ag | |
| ***Ag M*** | 0 | --- | --- | --- | --- | --- | | |
| ***Total*** | | | 100.00 | | 100.00 | | | |

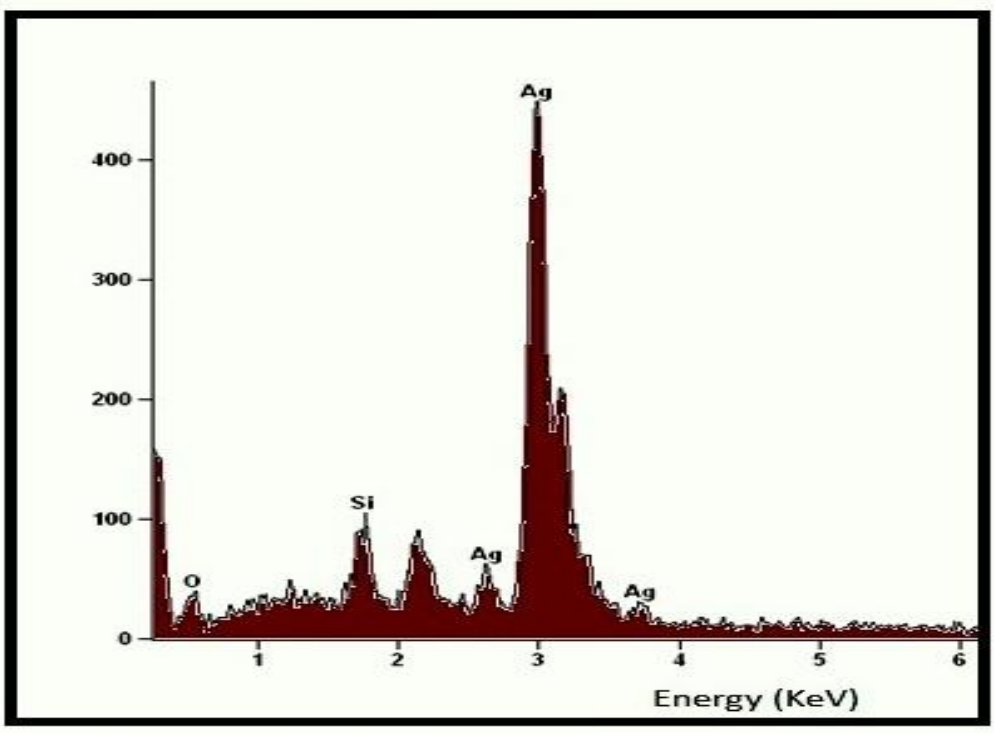



**Fig. 28. EDX Spectra of synthesized AgNPs.**

The bio-molecules present in plant extract responsible for the reduction of silver ions to AgNPs were identified using FTIR analysis. Fig. 29 shows the FTIR spectra of pure leaf extract, quercetin solution and AgNPs synthesized using both plant extract and quercetin as reducing agent. The FTIR spectra of fresh Tulsi extract shows peaks around 1636, 2132 and 3336 $cm^{-1}$ which corresponds to the groups C=C (around 1635 $cm^{-1}$), C≡C (around 2100 $cm^{-1}$) and amine N-H stretch (around 3300 $cm^{-1}$), whereas neat quercetin shows FTIR peaks at around 1639, 2105 and 3264 $cm^{-1}$ which corresponds to the same groups as that for Tulsi extract. This shows that quercetin is a major bio-molecule present in Tulsi, which is responsible for the reduction of silver ions to AgNPs. The silver nanoparticles synthesized using both Tulsi extract and quercetin show similar peaks at around 1640, 2112 and 3370 $cm^{-1}$ corresponding to the functional groups of amide C=O (around 1640$cm^{-1}$), C≡C stretch (around 2100 $cm^{-1}$) and amine N-H stretch (around 3370 $cm^{-1}$). In case of AgNPs synthesized using Tulsi extract the peaks are at around 1631, 2118 and 3345 $cm^{-1}$ and represents the same functional groups.

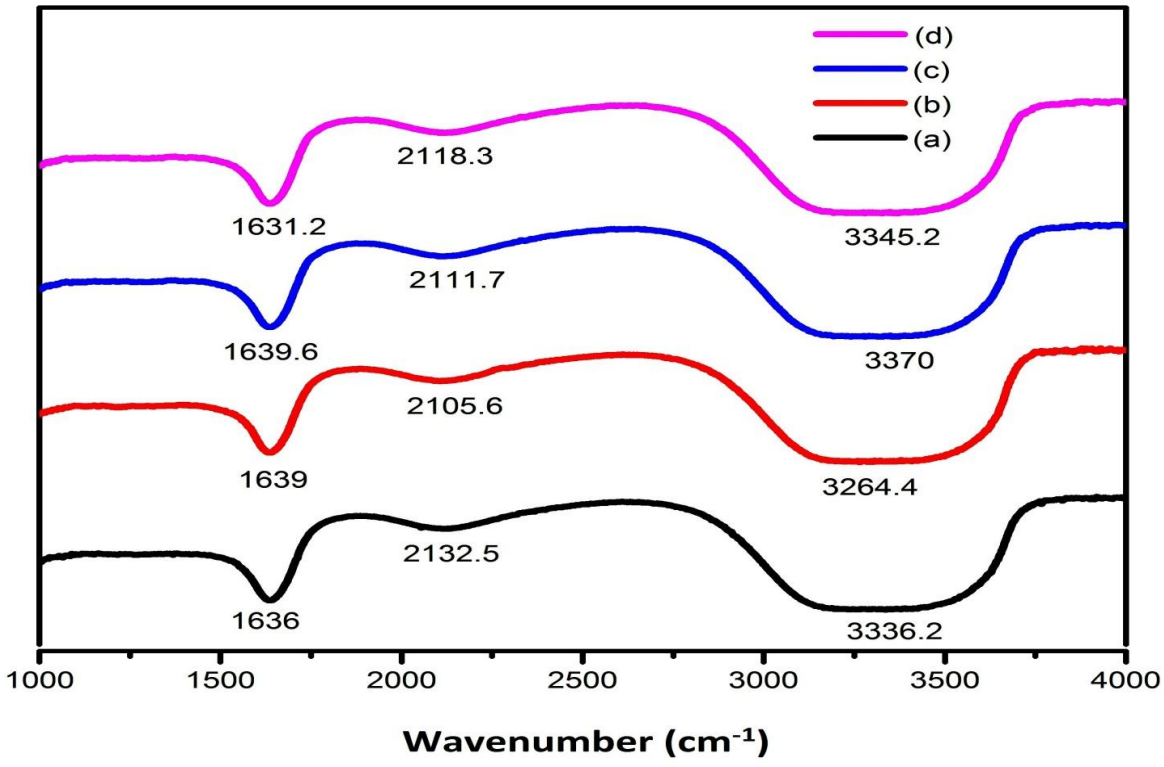



**Fig. 29. FTIR spectra of (a) Tulsi extract, (b) quercetin solution in water, (c) AgNPs synthesized using Tulsi extract and (d) AgNPs synthesized using quercetin.**

Figure 30 (a, b &c) shows the TEM images of AgNPs synthesized using both Tulsi leaf extract and quercetin. The prepared particles are nearly spherical in shape and uniform in size distribution. The mean particle size obtained using Tulsi extract and quercetin as reducing agents are 14.6 and 11.35 nm, respectively. It is confirmed by TEM analysis that on increasing the pH the size of AgNPs increases, as the mean particle size obtained at pH 10 is 18 nm, which is in good correlation with absorption and XRD spectra. The particles are agglomerated in an overnight as can be seen in the micrographs; therefore the agglomerated particles are neglected in calculating the mean particle size of AgNPs. Not only spherical particles but all kinds of other shapes such as trigonal, square, cubic, rhombic, hexagonal etc can be obtained by altering the capping agent.

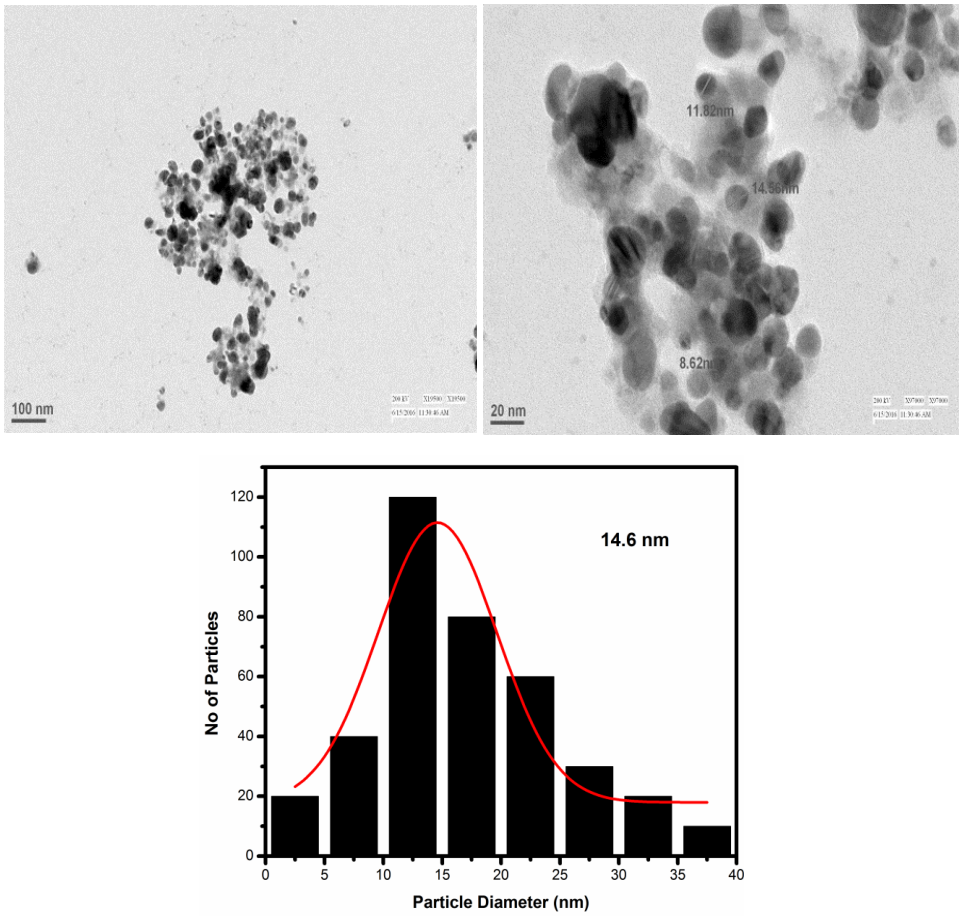
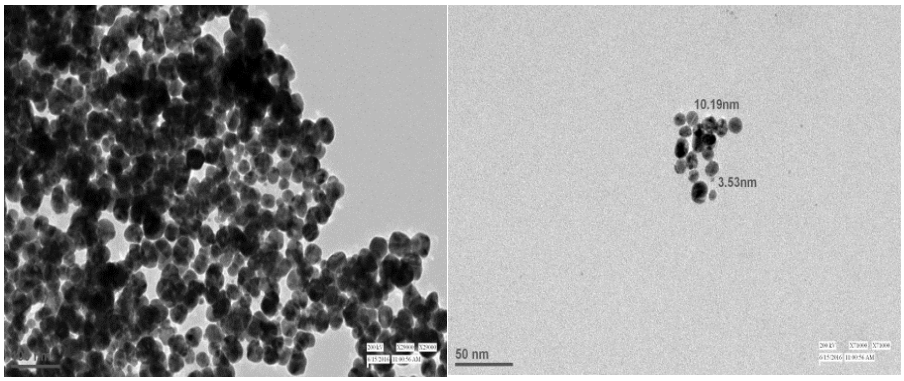



**Fig. 30 (a).**



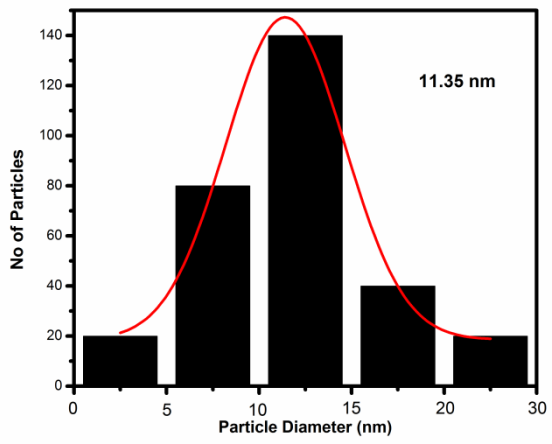
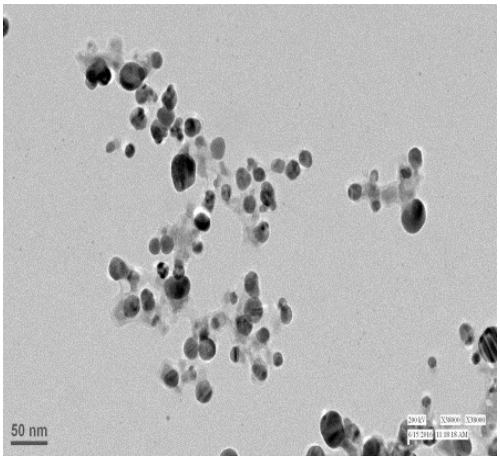




**Fig. 30 (b).**



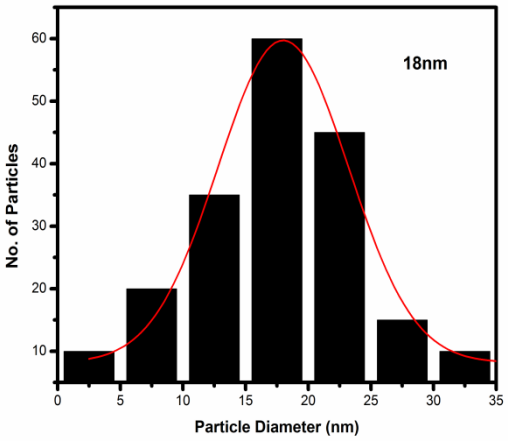




**Fig. 30 (c).**

**Fig. 30. TEM micrograph and particle size distribution histogram of AgNPs synthesized using (a) Tulsi extract, (b) quercetin and (c) quercetin at pH 10 of reaction mixture.**

## 3.6. Photoluminescence study of synthesized AgNPs

Figure 31 shows the PL spectra of AgNPs obtained using Tulsi extract at different time interval with 320 nm excitation wavelength. It can be seen that the PL intensity increases with time and a well-defined peak is obtained at around 440 nm. The small peak observed at around 360 nm is due to the water Raman, as shown in inset fig. The PL spectra of AgNPs obtained using quercetin as reducing agent with excitation wavelength of 340 nm is shown in Fig. 32. The figure in Inset shows the PL spectra of quercetin solution in water and in ethanol, respectively. The PL spectra of quercetin in ethanol shows no small peak whereas the PL spectra of quercetin in water show small peak around 370 nm which confirms that the small peak corresponds to water Raman.

Since bulk silver is metal, it has very narrow band gap due to which there is no photoluminescence (PL) form silver. However, when it is top downed at nano scale a band gap must have been created which may produce PL. In case of AgNPs both interband and intraband transitions occurs between the electronic states, the PL of AgNPs is due to excitation of electrons from occupied d band into the states above the Fermi level ***(Vasireddy et al., 2012)***. When surface plasmon electrons absorbs light at resonant frequency a part of this energy is transferred into heat and part of this energy is radiated as PL and recombination occurs between electrons of sp band with hole in d band ***(Parang et al., 2012)***.

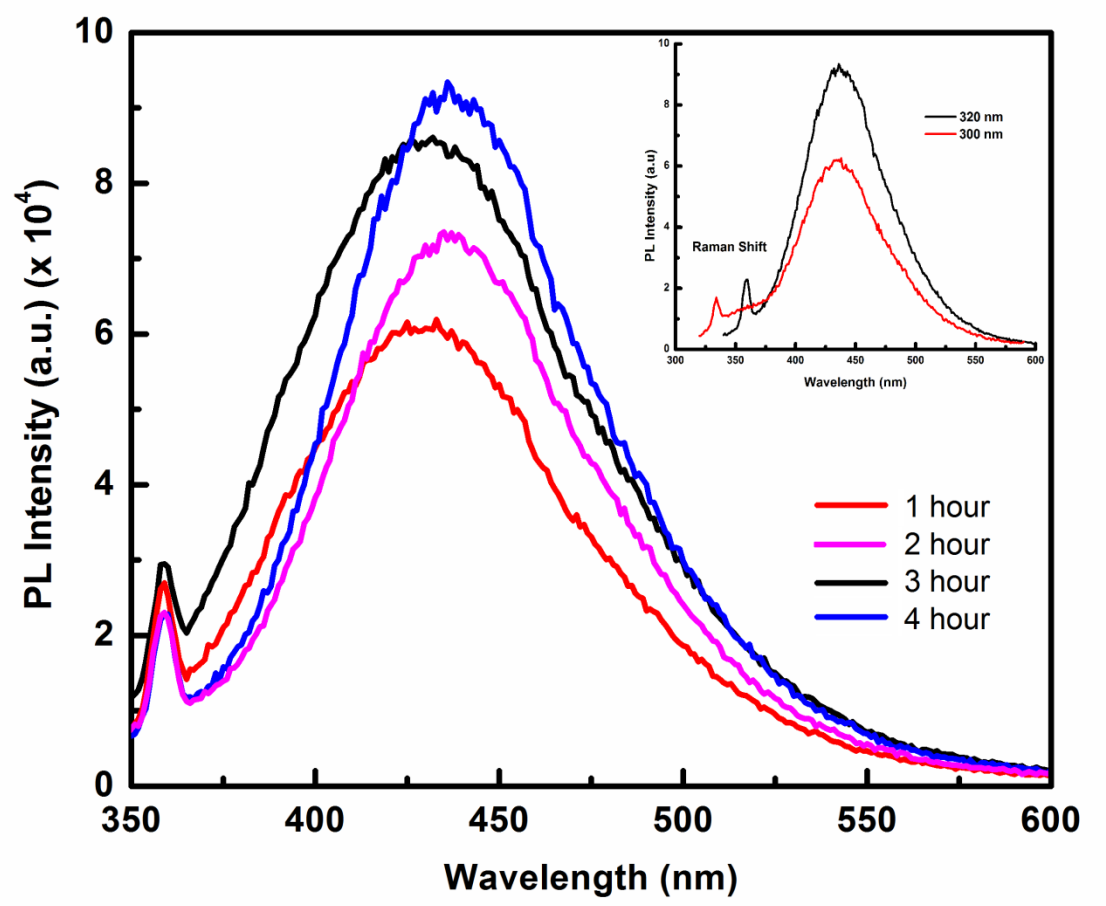



**Fig. 31. PL spectra of AgNPs at different time interval obtained using Tulsi extract and silver salt. Inset figure shows the Raman shift of water peak.**

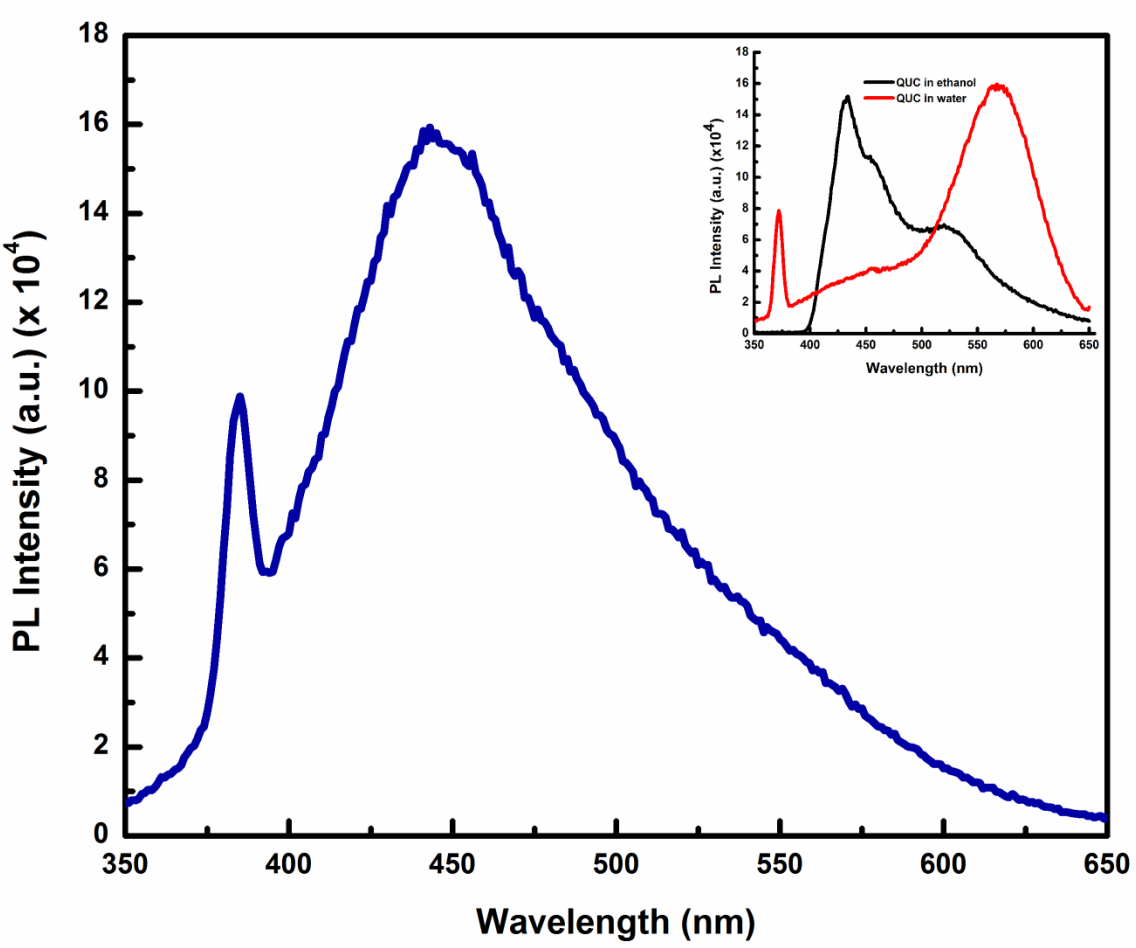



**Fig. 32. PL spectra of AgNPs obtained using Quercetin solution and silver salt. Inset shows the PL spectra of Pure Quercetin in water & ethanol respectively.**

## 3.7. Stability analysis of synthesized AgNPs

Stability is another important parameter ensuring the area of application in which nanoparticles can be incorporated based on their stability. The stability of silver nanoparticles varies according to the synthesis technique and also depends upon the stabilizing and capping agent used in the synthesis process. Figures 32 and 33 show the change in absorption spectra of AgNPs taken after 5 days, indicating the shift in peak towards higher wavelength and a decrease in the absorption intensity. However, the change in absorption spectra of AgNPs synthesized using quercetin showed little variation compared to the absorption spectra of AgNPs synthesized using Tulsi leaf extract, which shows that the quercetin alone act as a better

stabilizer than Tulsi extract. However, Tulsi extract also contains quercetin as a main biomolecule but other biomolecules present in Tulsi may have low capping potential, which hinders the overall capping ability of Tulsi extract.

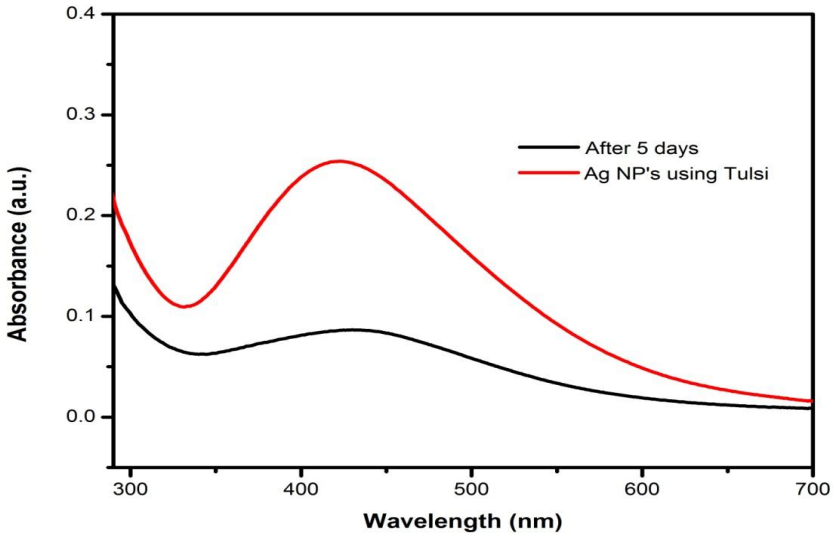



**Fig. 33. Absorption spectra of same sample of AgNPs synthesized using Tulsi extract taken after 5 days.**

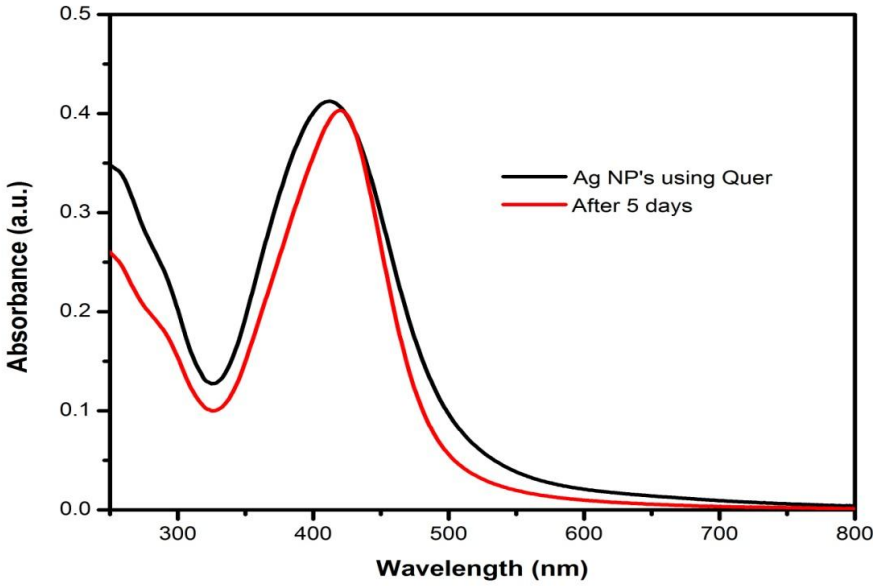



**Fig. 34. Absorption spectra of same sample of AgNPs synthesized using Quercetin taken after 5 days.**

## 3.8. Probable mechanism for synthesis of AgNPs

There is no proper literature explaining the mechanism for the flavonoid reduction and stabilization of AgNPs. According to ***(Makarov et al., 2014)*** the various –OH groups present in flavonoids such as Quercetin may be responsible for the reduction of silver ions to AgNPs. It is possible that the tautomeric transformation of flavonoids from enol form to keto form may release reactive hydrogen atom that reduces silver ions to silver nanoparticles. ***(Zhang et al., 2011)*** reported that Quercetin has high reduction potential therefore it acts as a reducing agent. Fig. 34 shows the mechanism of synthesis of AgNPs by flavonoid, reduction of silver ions to AgNPs. This redox reaction shown in figure indicates the production of two protons per catechol ***(T. Venkata Rajesh Kumar et al., 2016)***.

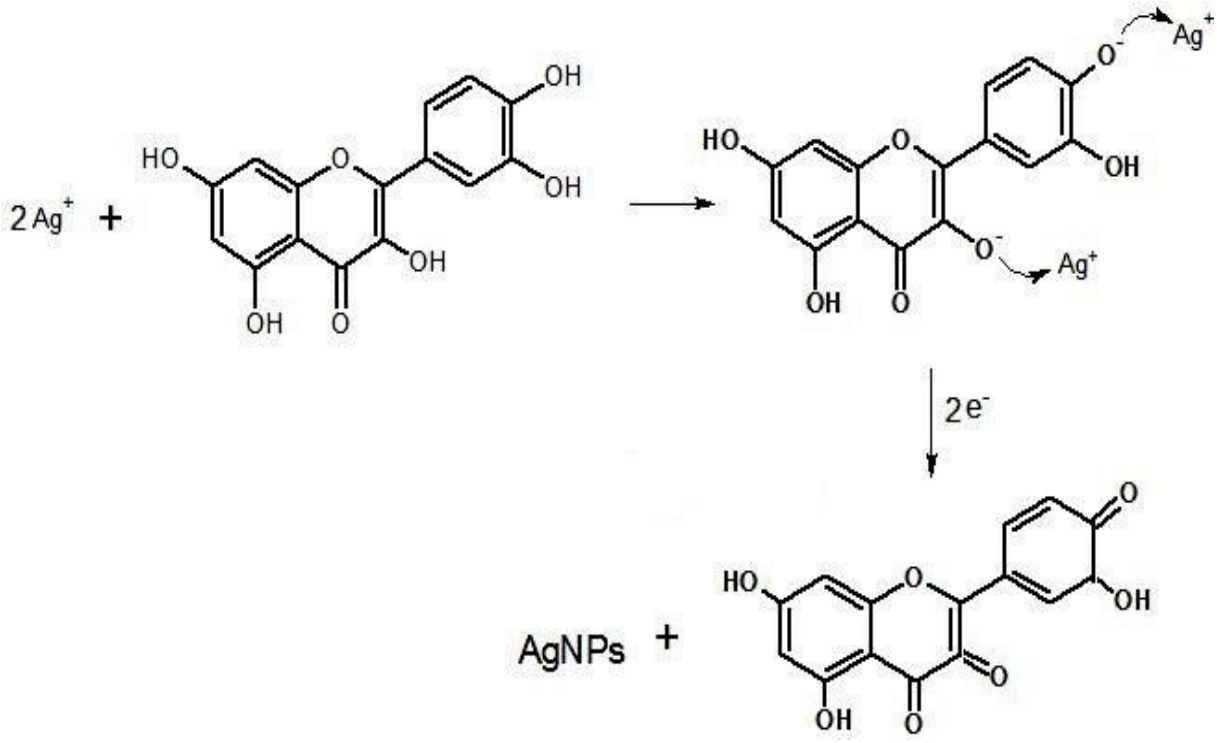



**Fig. 35. Mechanism of reduction of silver ion to AgNPs by Quercetin molecule.**

# Chapter-4

## Conclusion

We compared the effects of environmental conditions such as temperature, pH, reaction time and reactants concentration on the green synthesis of AgNPs using Tulsi leaf extract and quercetin separately as reducing agents. It was found that the results obtained using different characterization techniques showed prominent similarities. The synthesized AgNPs exhibit strong absorption between 400 and 450 nm and a strong PL at around 450 nm depending upon the size, shape and morphology of the resultant particles. The average particle size of synthesized AgNPs obtained using the green approach varied between 10-20 nm. The green synthesis technique was found easy, economic, fast and environment friendly as compared to other synthesis techniques. It is observed that the environmental conditions can significantly alter the size and shape of synthesized AgNPs, hence desirable size and shape of AgNPs for various applications can be synthesized easily using green method. Moreover, from the above experiments it can also be inferred that bio-molecules such as quercetin present in plant extracts of Tulsi, Neem etc. may be responsible for the reduction of silver ions to AgNPs. The synthesized AgNPs showed enhanced antibacterial property which is very useful for Bio-medical applications. It can be concluded that green approach is best technique not only for the synthesis of AgNPs but for a variety of metal nanoparticles having vast applications in field of electronics, medicine, cosmetics, nanotechnology etc.

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