Green Synthesis of Silver Nanoparticles using Tulasi Leaf at different Environmental Conditions

A dissertation submitted in partial fulfillment for the Degree of

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In

Nanoscience and Technology

by

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CERTIFICATE

This is to certify that the dissertation entitled "**Green Synthesis of Silver Nanoparticles using Tulsi Leaf at different Environmental conditions**" submitted to Delhi Technological University (Formerly Delhi College of Engineering) by Ms. Preeti Agnihotri (2K12/NST/15) in the partial fulfillment of the requirements for the award of the degree of **Master of Technology in Nanoscience and Technology (Applied Physics Department)** is a *bonafide* record of the candidate's own work carried out under my supervision. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honoring of any other degree.

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DECLARATION

I hereby declare that the Report of **"Green Synthesis of Silver Nanoparticles using Tulasi Leaf at different Environmental Conditions"** which is being submitted to the Delhi Technological University, in partial fulfilment of the requirements for the 4th semester **Major Project-II** course of the **Master of Technology** Degree in **Nano Science and Technology** in the **Applied Physics Department**, is report carried out by me.

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M.Tech. (NST)

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Abstract

This thesis is based on the use of Tulasi (ocimum sanctum) leaf broth in the synthesis of silver nanoparticles. On treating aqueous solution of silver nitrate with Tulasi leaf extract; a rapid formation of stable silver nanoparticles take place. In this process, aqueous leaf extract of Tulasi was used as capping as well as reducing agent. To characterize the synthesized nanoparticles we have used Scanning Electron Microscopy (SEM), X-ray diffractometry, Fourier Transform Infrared Spectroscopy, Emission and absorption spectroscopy. The synthesized nanoparticles which show a strong absorption and emission bands are crsytalline in nature. The rate of reduction of the metal ions by Tulasi leaf extract are much faster than those observed in earlier studies using micro-organisms such as fungi, demonstrating that synthesis of nanoparticles using biological methodologies can achieve rates of synthesis equivalent to the rates of synthesis of chemical methods. In addition, we have explored the effects of different parameters, such as pH, temperature, concentration on the synthesis of silver nanoparticle by biological methods and we have succeeded to control the size, quality and quantity of nanoparticles.

Chapter 1

Introduction and Literature Review

The field of nanotechnology is one of the most promising areas of research nowadays in modern material science and technology. Nanoparticles are the fundamental building blocks of nanotechnology [1]. In the recent past there have been impressive developments in the field of nanothechnology. Numerous methodologies have been formulated for the synthesis of nanoparticles of various shape and size depending on the particular requirements. The need to develop environmentally gentle synthesis processes i.e. which will not involve use of toxic chemicals in the synthesis process is picking up pace. As a result, researchers have started looking towards various biological systems and process for getting inspired. As many organisms (both unicellular and multi-cellular) have already been known to produce inorganic materials either intracellulary [2] or extracellularly [3], hence this change in stance of researchers doesn't seems surprising. A few well known examples of microorganisms synthesizing inorganic materials include; synthesis of magnetite nanoparticles by magnetotactic [4–6], synthesis of siliceous materials by diatoms [7–9] and production of gypsum and calcium carbonate layers by S-layer bacteria [10,11]. The secrets taken from nature have led to the growth of advanced nanomaterials using synthetic methods which mimic biochemical processes i.e. biomimetic approaches.

Even though there are many biotechnological applications such as remediation of toxic metals which employ microorganisms such as yeast [15] and bacteria [16] (the detoxification often occurring via reduction of the metal ions/formation of metal sulfides) it is only quite recently that materials scientists have started looking, with interest, towards such microorganisms as possible future ecofriendly nanofactories [17–23].Multiple species of bacteria have been shown to intracellularly synthesize metal nanoparticles [17–23] and their alloys [23]. Some of the researchers, in laboratory conditions, have shown that apart from bacteria (prokaryotic organisms), eukaryotic organisms (ex. Fungi) may also be used to grow nanoparticles of different chemical compositions and sizes [24–28] including quantum dots of the technologically important CdS by enzymatic processes [28]. Researchers have also been successful in the synthesis of fairly monodisperse gold nanoparticles of 8 nm average size using the alkalothermophilic (extremophilic) actinomycete Thermomonospora species [29] which is a fairly difficult to achieve through biological means currently. From the discussion so far it can be seen that the use of microorganisms in the deliberate and controlled synthesis of nanoparticles is a relatively new and exciting area of research with considerable potential for future development.

Although microorganisms such as fungi, bacteria and actinomycetes continue to be investigated in metal nanoparticle synthesis; in the meanwhile use of parts of whole plants in similar nanoparticle synthesis methodologies is an exciting possibility that is relatively underexploited and unexplored. Though the silver nanoparticles are considered biocompatible still the chemical synthesis methods may lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects while being used in medical/pharma applications. By synthesizing nanoparticles using microorganisms or plants can potentially eliminate this problem thereby making the nanoparticles more compatible for medical uses. Elimination of elaborate process of maintaining cell cultures can is an added advantage of using of plants for synthesis of nanoparticles over other environmentally benign biological processes. It can also be done on large scale to synthesize nanoparticles in bulk. Jose, Yacaman and co-workers demonstrated the synthesis of silver nanoparticles within live alfalfa plants by silver ion uptake medium [27, 28].

This thesis is on the use of Tulasi (ocimum sanctum) leaf broth in the synthesis of silver nanoparticles. On treating aqueous solution of silver nitrate with Tulasi leaf extract; occurrence of rapid formation of stable silver nanoparticles is observed. Addition of Silver salt to aqueous leaf extract of Tulasi was used as capping as well as reducing agent.

Nanoparticles

There is no accepted international definition of a nanoparticle, but usually “A nanoparticle (or nanopowder or nanocluster or nanocrystal) is a microscopic particle with at least one dimension less than 100 nm. There is a note associated with this definition: "Novel properties that differentiate nanoparticles from the bulk material typically develop at a critical length scale of under 100nm". A nanoparticle is the most important component in the production of a nanostructure, and is much smaller than the world of everyday objects that are described by Newton’ s laws of motion, but it is bigger than an atom or a simple molecule which are covered by quantum mechanics. Physical and chemical properties of metallic nanoparticles differ from that of bulk metals (e.g., higher specific surface areas, lower melting points, specific optical properties, specific magnetizations, and mechanical strengths), properties that might be attractive in various applications in industries. However, how a nanoparticle is seen and is defined depends very much on the very application.

Nanoparticles are in effect a bridge between bulk materials and atomic or molecular structures and hence are of great scientific significance.

Nanoparticle research is currently the most studied branch of science with the number of uses of nanoparticles in a variety of fields. The particles have wide assortment of potential applications in biomedical, optical and electronic.

Types of Nanoparticles

- **Quantum Dots: -** A quantum dot is a nanocrystal made of semiconductor materials that are small enough to exhibit quantum mechanical properties. The electronic properties of these materials are intermediate between those of bulk semiconductors and of discrete molecules [30][31][32]. Researchers have studied applications for quantum dots in diode lasers, LEDs, transistors and solar cells. They have in addition investigated quantum dots as agents for medical imaging and as potential quantum bit in quantum computing. Its electronic characteristics are closely related to its size and shape. For instance, the band gap energy in a quantum dot which determines the frequency range of emitted light is contrariwise associated to its size. In incandescent dye applications the frequency of emitted light increases with decrease in the size of the quantum dot. As a result, the colour of emitted light shifts from red to blue when the size of the quantum dot is made smaller, this allows the excitation and secretion of quantum dots to be highly controllable. Given that the size of a quantum dot may be set when it is being produced, its conductive properties may be carefully guided. Assemblies of quantum dots consisting of many different sizes can be made to exhibit a range of sought-after emission properties.

- **Carbon Nanotubes also known as Buckyballs and Buckytubes: -** Carbon nanotubes are allotropes of carbon with a cylindrical nano-structure. The nanotubes have been constructed with length-to-diameter ratio of up to 132,000,000:1, [30] significantly larger than for any other material. Such cylindrical carbon molecules have extraordinary properties, which are precious for nanotechnology, electronics, optics & other fields of material science & technology. Owing to their unusual thermal conductivity & mechanical & electrical properties, carbon nanotubes find applications as additives for various structural materials in the field.

- **Nanorods:** These are considered to be morphology of nano-scale objects. Each of their dimensions range from 1–100nm. These may be synthesized from metals or semi-conducting materials. Benchmark aspect ratios (length is to width ratio) are in the range of 3 to 5. Nanorods can be produced by direct chemical synthesis. A

mixture of ligands acts as shape control agents and bond to different facets of the nanorod with varying strengths. This allows diverse faces of the nanorod to grow at varying rates, producing a stretched out object.

- **Nanocrystals :-** A nanocrystal is a material particle having at least one dimension smaller than 100 nanometres[33] (a nanoparticle) and composed of atoms in either a single- or poly-crystalline arrangement.[34]

  The volume of nanocrystals distinguishes them from outsized crystals. For example, silicon nanocrystals can provide efficient light emission while bulk silicon does not[35] and can be used in various memory components.[36]

  When embedded in solids nanocrystals may exhibit much more complex melting behaviour than conventional solids[37] and may form the basis of a special class of solids.[38] They can behave as single-domain systems (a volume within the system having the same atomic or molecular arrangement throughout) that can help explain the behaviour of macroscopic samples of a similar material without the complicating presence of grain boundaries and other defects

- **Nanowires: -** A nanowire is a nanostructure, with the diameter in the range of a nanometer (10-9 meters). It can also be defined as the ratio of the length to width being greater than 1000. Alternatively, nanowires can be defined as structures that have a thickness or diameter constrained to tens of nanometers or less and an unhindered length. At these scales, quantum mechanical effects are important — which coined the term "quantum wires". Various types of nanowires exist, including metallic (e.g., Ni, Pt, Au), semiconducting (e.g., Si, InP, GaN, etc.), and insulating (e.g., SiO_2, TiO_2). Molecular nanowires are composed of repeating molecular units either organic (e.g. DNA) or inorganic (e.g. $Mo_6S_{9-x}I_x$).

Silver Nanoparticles: - Silver nanoparticles are nanoparticles of silver, i.e. silver particles whose size lies between 1 nm to 100 nm. While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface-to-bulk silver atoms.

Silver nanoparticles have unique thermal, optical, and electrical properties and are being built-in to products that range from photovoltaics to biological & chemical sensors. Examples include pastes, fillers and conductive inks which utilize silver nanoparticles for their sintering temperatures and high electrical conductivity & stability. Other applications include molecular diagnostics & photonic devices, which take advantage of the novel optical properties of nanomaterials. A widespread application of silver nanoparticles is for antimicrobial coatings; many keyboards, biomedical devices, wound dressings, & textiles now contain silver nanoparticles that continuously release a low level of silver ions to provide protection against germs (bacteria).

Applications of Silver Nanoparticle

Silver nanoparticles are being increasingly employed into numerous technologies and are used into a wide array of consumer products that take advantage of their antibacterial, optical and conductive properties.

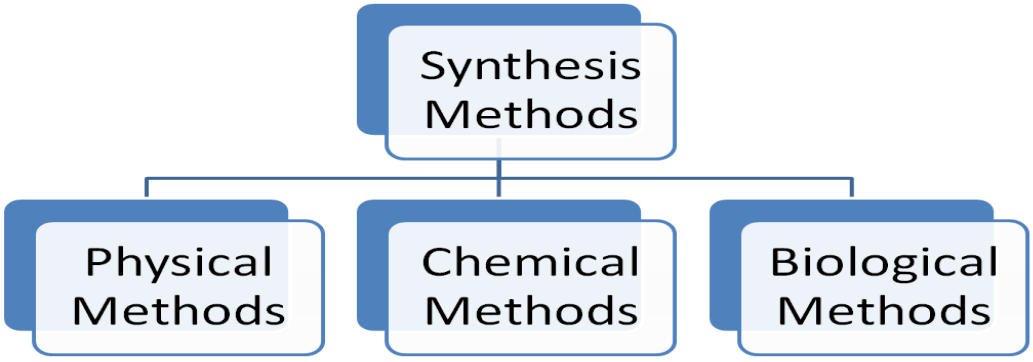
- ⇨ **Diagnostic Applications:** AgNPs are used in biosensors and numerous assays where the AgNP materials can be act as a biological tag for quantitative detection.

- ⇨ **Antibacterial Applications:** As anti-bacterial agents, silver nanoparticles are applied in a wide range of applications from water treatment to disinfecting medical devices and home appliances to [34]. Additionally, this encouraged the textile industry to use silver nanoparticles in different textile fabrics. Hence, silver nanocomposite fibres' were prepared which contained silver nanoparticles incorporated within the fabric. The cotton fibers containing silver nanoparticles exhibited high antibacterial activity against E-coli. [33-47]
- ⇨ **Conductive Applications:** AgNPs are being used in conductive inks and integrated into composites to enhance thermal & electrical conductivity.
- ⇨ **Optical Applications:** Silver nanoparticles are extensively being used to efficiently harvest light and for enhanced optical spectroscopies including metal-enhanced fluorescence (MEF) and surface-enhanced Raman scattering. Optical properties of a metallic silver nanoparticle depend mainly on its surface plasmon resonance, where the plasmon means to the collective oscillation of the free electrons inside the metallic nanoparticle. It is well recognized that the plasmon resonant peaks and line widths are sensitive to the size and shape of the nanoparticle, the surrounding medium and the metallic species. For example,

nanoclusters composed of 2 to 8 silver atoms could be the basis for a new type of optical data storage methods. Additionally, fluorescent emissions from the clusters could potentially also be used in electroluminescent displays and biological labels.

Synthesis of Nanoparticles

Nanoparticles can be synthesized by various methods which can be broadly classified under Physical, Chemical and Biological methods.



- **Physical Methods**: - In physical synthesis processes nanoparticles are created in a very controlled environment devoid of the direct use of chemicals. Metal nanoparticles are generally synthesized by evaporation/condensation; this could be carried out using a tube furnace at normal pressure. The source material inside a boat centred at the furnace is vaporized into a gas which acts as carrier. Nanoparticles of various materials, such as PbS, Ag, Au & fullerene, have already been produced in the past using the evaporation/condensation technique [39-42]. Physical methods can further be classified as
    - Mechanical:-
        - High energy ball milling
        - Melt mixing
    - Vapour :-
        - Physical Vapour deposition
        - Laser pyrolysis
        - Sputter deposition

        - Electric arc deposition
        - Ion implantation

- **Chemical Methods**: - Synthesis of nanoparticles can also be accomplished through chemical methods. Although chemical methods have their own disadvantages. But they still have many advantages over physical processes. Chemical methods are more simple and cheaper, and they permit the creation of particles with a unique shape that could otherwise not be achieved. In some cases, nanomaterials are obtained as colloidal particles in solutions, which can be filtered and dried to obtain powder form. In some other, we can obtain thin films or nanoporous materials by etching, electrodeposition, etc. In many other cases very well known chemical reaction route can be optimized to synthesize nanoparticles. Chemical methods can further be classified as:-
    - Colloids and colloids in solutions
        - Precipitation
        - Micro-emulsion synthesis
        - Sol gel synthesis
        - Spray Drying / spray pyrolysis
        - Thermal decomposition
- **Biological Methods**: - Biological methods using naturally occurring reducing agents such as polysaccharides, micro-organism such as bacteria and fungus or plants extract, i.e. green chemistry, are emerging as a simple and viable alternative to more complex chemical (synthetic) procedures to obtain nanoparticles. Chemical and physical methods of synthesis are costly, at times hazardous, un-ecofriendly burdensome and yields big particle size; on the other hand biological method of synthesis of nanoparticle is a green chemistry approach which is economic reliable eco-friendly technique of synthesis of nanoparticles however nanoparticles are not mono-dispersed and the rate of synthesis may be is slow. Although the silver and gold nanoparticles are considered biocompatible, chemical synthesis methods may still lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in case of medical applications. Whereas synthesis of nanoparticles using microorganisms or plants can potentially eliminate this problem by making the nanoparticles more biocompatible using plants for synthesis of nanoparticles.

Characterization Techniques of Nanoparticles

Characterization of nanoparticles is imperative to understand and control nanoparticles synthesis and their applications. Characterization can be performed using a variety of different techniques such as transmission and scanning electron microscopy (TEM, SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), powder X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), and UV–Vis spectroscopy [48-58].

These techniques are employed for determination of different parameters such as particle fractal dimensions, size, shape, crystallinity, surface area & pore size. Additionally, intercalation, orientation, & dispersion of nanoparticles and nanotubes in nanocomposite materials could be known by these techniques.

For example, the morphology and particle size could be determined by AFM, TEM & SEM. The benefit of AFM over traditional microscopes such as SEM and TEM is that AFM measures three-dimensional images so that particle height and volume can also be calculated. Additionally, dynamic light scattering is used for determination of particles size distribution in the sample. Besides, UV–Vis spectroscopy is used to confirm sample formation by showing the plasmon resonance, while X-ray diffraction is used for the determination of crystallinity.

Chapter 2

MATERIALS AND METHODS

Synthesis of Silver Nanoparticle using extract of Tulasi Leaf

Sample Collection: Fresh leaves of Tulasi were collected from nearby area of Delhi Technological University (DTU), Rithala. Leaves were washed thoroughly and allowed for air dry in room temperature.

A) Preparation of Leaf Extract

Materials

Beaker, distilled autoclaved water, leaves

Method

1) 25g of Tulasi leaves were weighed and washed thoroughly with distilled water.
2) Tulasi leaves were then cut into fine pieces and were boiled with 100 ml of distilled water for 20 minutes.
3) After cooling the sample was filtered through Whatman filter paper and filtrate was obtained.

B) Synthesis of silver nanoparticle

Material

Beaker, Silver Salt ($AgNO_3$), Tulasi leaf broth

Method

1) 5ml of Tulasi leaf broth was added to 45ml of 10-3M aqueous AgNO3 solution.
2) Colour change was observed after 15minutes.
3) A reduction of silver ions was monitored by measuring the UV–vis spectra of the solution at regular intervals after diluting a small aliquot (0.2 mL) of the sample 20 times
4) After 24 hours, the sample was centrifuged at 10,000 rpm for 15 minutes and the pellet was dissolved and heat dried to obtain silver nanoparticles.

Characterization of silver nanoparticles

A) Ultraviolet–visible spectroscopy

Ultraviolet–visible spectroscopy or ultraviolet-visible spectrophotometry (UV-Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. That is it uses light in the visible and adjacent (near-UV and near-infrared) ranges. The reflectance or absorption in the visible range directly affects the

perceived colour of the chemicals involved in the process. In this region of the electromagnetic spectrum molecules undergo electronic transitions. Which makes this technique is complementary to fluorescence spectroscopy, in sense that fluorescence deals with transitions from the excited state to the ground state, whereas absorption measures transitions from the ground state to the excited state.

Principle involved: - Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding orbitals of molecule. The more easily excited the electrons (that means lower energy gap between the HOMO and the LUMO) are, the longer the wavelength of light it can absorb.

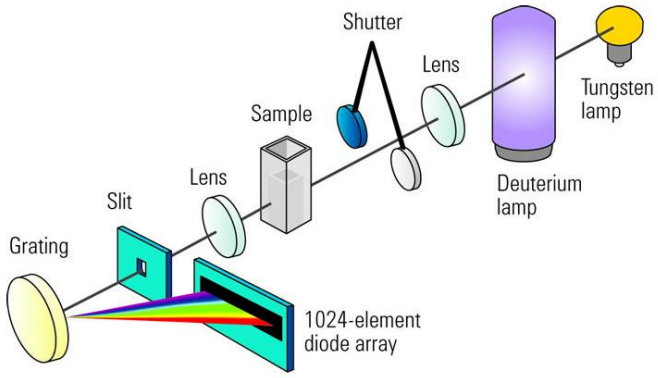


Figure 1: Schematic of UV-visible spectrometer

Applications of UV-Vis: - UV/Vis spectroscopy is regularly used in analytical chemistry for the quantitative determination of different components of interest, such as transition metal ions or highly conjugated organic compounds or biological macro-molecules. Typically spectroscopic analysis is carried out in solutions but solids and gases may also be studied.

- Solutions of transition metal ions can be coloured (i.e., absorb visible light) because d electrons within the metal atoms can be excited from one electronic state to another state. The colour of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands. For example, the colour of a dilute solution of copper sulfate is a very light blue; which on adding ammonia intensifies the colour and changes the wavelength of maximum absorption (λmax).

- Organic compounds, particularly those with a high degree of conjugation, can also absorb light in the UV or visible regions of the electromagnetic spectrum. Solvents for these determinations are often water for water-soluble compounds and ethanol for organic-soluble compounds. Solvent polarity and pH can affect the absorption spectrum of an organic compound.
- Whilst charge transfer complexes also give rise to colours, but these colours are often too intense to be used for quantitative measurement.

a) UV-Vis spectroscopy: The reduction of silver ions was monitored by measuring the optical absorption spectra of the solution at regular intervals after diluting a small aliquot (0.2 mL) of the sample 20 times. Absorption spectra were recorded by using UV/VIS/NIR Parkin Elmer （Lambda 750） spectrophotometer operated in the range of 250-700nm.

Material

Cuvette, UV-vis spectrometer (UV/VIS/NIR Parkin Elmer Lambda 750) , beaker,distilled water.

Method

1) A UV-vis spectrum of the solution at regular intervals was obatiend after diluting a small aliquot of the sample 20 times.

2) Distilled water was used to adjust the baseline.

b) Photoluminascene spectroscopy: Emission spectra of silver nanoparticle was obtained using by Jobin Yvon's PL Ka H photometer at excitation wave length 350nm in the range of 370-700nm.

Materials

Cuvette, beaker, distilled water, PL spectrophotometer

Methods

1) Emission spectra of the solution at regular intervals was obatiend after diluting a small aliquot of the sample 20 times

B) FTIR (Fourier transform infrared spectrum):

Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain an infrared spectrum of photoconductivity, Raman scattering, emission or absorption of a liquid, gas or solid. An FTIR spectrometer simultaneously collects spectral data in a

wide range of spectra. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time.

The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform (a mathematical process) is required to convert the raw data into the actual spectrum.

Fourier transform spectroscopy is a less intuitive way to obtain the same information. Instead of shining a monochromatic beam of light at the sample, in this technique a beam containing many frequencies of light at once is pointed at sample, then how much of that beam is absorbed by the sample is measured. Next, the beam is modified to contain a different combination of frequencies, to give a second data point. The same process is repeated many times. Thereafter, a computer takes all these data and works backwards to infer what the absorption is at each wavelength.

The beam described above is generated by starting with a broadband light source which contains the full spectrum of wavelengths to be measured. Light is pointed into a Michelson interferometer—a predetermined configuration of mirrors, one mirror is motor driven for auto adjustment. As this mirror shifts from its position, each wavelength of light in the beam is periodically blocked, then transmitted, then again blocked, again transmitted and so om, by the interferometer, which is due to wave interference. As different wavelengths are modulated at different rates, hence at each moment, beam coming out of the interferometer has a different spectrum.

As mentioned, computer processing is required to turn the raw data into the desired result. The processing required turns out to be a common algorithm called the Fourier transform. The raw data is sometimes called an "interferogram".

In a Michelson interferometer adapted for FTIR, the light from the polychromatic infrared source, roughly a black-body radiator is collimated and directed to a beam splitter. In an ideal condition 50% of the light is refracted towards the fixed mirror and 50% is transmitted towards the mirror which is moving. Light is reflected from the two mirrors back to the beam splitter and (ideally) 50% of the original light passes into the sample section. From there, the light is focused on the sample. Upon leaving the sample compartment the light is refocused on to the detector.

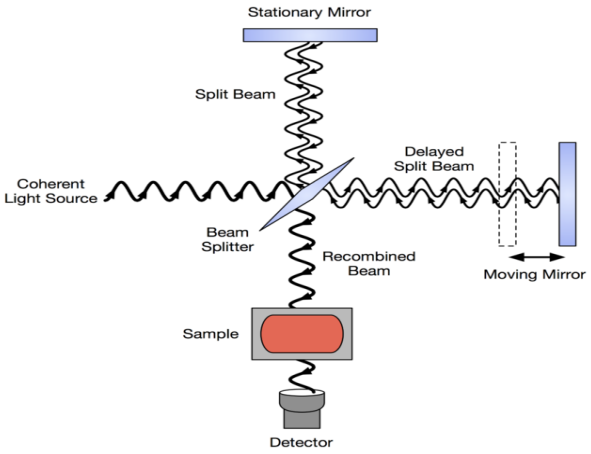


Figure 2: Schematic of an FTIR setup

The retardation is the difference in optical path length between the two arms to the interferometer. An interferogram is obtained by varying the retardation and recording the signal from the detector for various values of the retardation. Form of the interferogram when no sample is present depends on factors such as the variation of source intensity and splitter efficiency with varying wavelength. This should result in a maximum at zero retardation, i.e. when there is constructive interference at all a wavelength, which is followed by series of "wiggles". This position of zero retardation is determined accurately by finding the point of maximum intensity in the interferogram obtained. When a sample is present the background interferogram is modulated by the presence of absorption bands in the sample.

There are two primary advantages for an FT spectrometer compared to a scanning (dispersive) spectrometer.

1. The multiplex or Fellgett's advantage: - This arises from the fact that information from all wavelengths is collected at the same time. It results in a higher Signal-to-noise ratio for a given scan-time or a shorter scan-time for a given resolution.
2. The throughput or Jacquinot's advantage: - This result from the fact that, in a dispersive instrument such as XRD, the monochromator has entrance and exit slits which restrict the amount of light that passes through it. Whereas in interferometer throughput is determined only by the diameter of the collimated beam coming from the source.

Other insignificant advantages include less sensitivity to unwanted spectrum and "Connes' advantage" which results in better wavelength accuracy, whereas a disadvantage is that FTIR cannot use the advanced electronic filtering techniques that often make its signal-to-noise ratio inferior to that of dispersive measurements.

a) FTIR (Fourier transform infrared spectrum): Spectra were recorded on Thermoscientific Nicolet 380 in the range of 400-4000cm^{-1}.

Material

FTIR meter, liquid sample.

Method

1) Small amount of liquid sample has been taken and FTIR spectra were recorded in the range of 400-4000cm^{-1}.

C) Power X-ray Diffractometry (XRD)

When an X-ray is shined on a crystal, the diffraction happens in a pattern characteristic of the crystal structure. In powder XRD, the diffraction pattern is obtained from a powder of the material, not from a sole crystal. Powder diffraction is often easier and more convenient than single crystal diffraction since it does not require making individual crystals. Powder X-ray diffraction (XRD) also obtains a diffraction pattern for the bulk material of a crystalline solid, and not only of a single crystal which might not represent the overall material. The diffraction pattern plots intensity against the angle of the detector, 2θ.

Since most materials have unique diffraction patterns, so any compound can be identified by using a database of diffraction patterns. Also the purity of a sample can be determined from its diffraction pattern as well as the composition of any impurities present in it. A diffraction pattern can also be used to determine and refine the lattice parameters of a crystal structure.

A theoretical structure can also be refined using a method known as Rietveld refinement. Also, the particle size of the powder can be determined by using the Scherrer formula, it is used to relate the particle size to the peak width.

The Scherrer fomula is

$$t = \frac{0.9\lambda}{\sqrt{B_M^2 - B_S^2}\cos\theta}$$

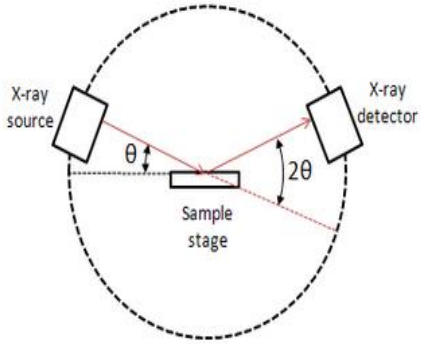


Figure 3: Wave diagram for XRD

Where λ is the x-ray wavelength, BM is the observed peak width, BS is the peak width of a crystalline standard and θ is the angle of diffraction.

a) X-Ray Diffraction:

The AgNPs dried powder was analyzed on XRD for their phase structure and exact material identifcation. X-ray diffraction (XRD) measurements of Tulasi leaf broth reduced Ag nanoparticles were carried on a Bruker D8 Advanced instrument operating at a voltage of 40kV and a current of 30 mA with Cu$K\alpha$ radiation.

Material

Dried sample, X-Ray difractometer

Method

1) The sample was dried at 600C, in an oven, and then XRD graph was obtained.

2) The Cu α radiation (k= 1.5418Å) was selected and the diffractogram was obtained in the 2θ range of 10–70 degree.

D) **Scanning electron microscopy (SEM)**

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. Electrons in the beam interact with atoms in the sample, thereby producing various signals that can be detected and that contain information about surface topography and composition of the sample. The electron beam is generally scanned in a raster scan pattern, with the beam's position combined to the detected signal to produce an image. SE microscopy can achieve resolution better than 1 nanometer. Specimen can be observed in high or low vacuum, in soaked conditions (in environmental SEM), and also at a wide range of cryogenic or elevated temperatures.

The most frequently used mode of detection is by secondary electrons emitted by atoms excited by the electron beam. Since on a flat surface, the plume of secondary electrons is mostly contained by the sample, whereas on a slanting surface, the plume is partially exposed and more electrons are emitted. Thus, by scanning the sample and detecting the secondary electrons an image displaying the topography of the surface is created.

In a usual SEM, an electron beam is thermionically emitted from an electron gun fitted with a tungsten filament cathode. IT is normally used in thermionic electron guns because it has the highest melting point and lowest vapour pressure of all metals, allowing it to be heated for electron emission, also because of its low cost. Other electron emitter materials include lanthanum hexaboride (LaB_6) cathodes, it can be used in a standard tungsten filament SEM if the vacuum system is upgraded and FEG, it may be of the cold-cathode type using tungsten single crystal emitters or the thermally assisted Schottky type, by using emitters of zirconium oxide.

The electron beam, which typically has an energy ranging from 0.2 k electron volt to 40 k electron volt, is focused by one or two condenser lenses to a spot about 0.4 nanometre to 5 nm in diameter. The electron beam passes through pairs of scanning coils or pairs of deflector plates in the electron column, usually in the final lens, which in turn deflect the beam in the x and y axes so that it scans in a raster fashion over a rectangular area of the sample surface.

When the primary electron beam interacts with the sample, these electrons lose energy by repeated random scattering and absorption within a teardrop-shaped volume of the specimen known as the interaction volume; this extends from less than 100 nm to approximately 5 µm into the surface. Size of the interaction volume depends on the specimen's density, the electron's landing energy and the atomic number of the specimen. The energy exchange between the electron beam and the sample results in the reflection of high-energy electrons by elastic scattering and emission of secondary electrons by inelastic scattering & the emission of electromagnetic radiation, all of which can be detected by specialized detectors. Beam current absorbed by the specimen can also be detected and used to create images of the distribution of current in specimen. Electronic amplifiers of various types are used to amplify the signals; these are displayed as variations in brightness on a computer monitor. Each pixel of

computer video memory is synchronized with the position of the beam on the specimen in the microscope; the resulting image is therefore a distribution map of the intensity of the signal being emitted from the scanned area of the specimen.

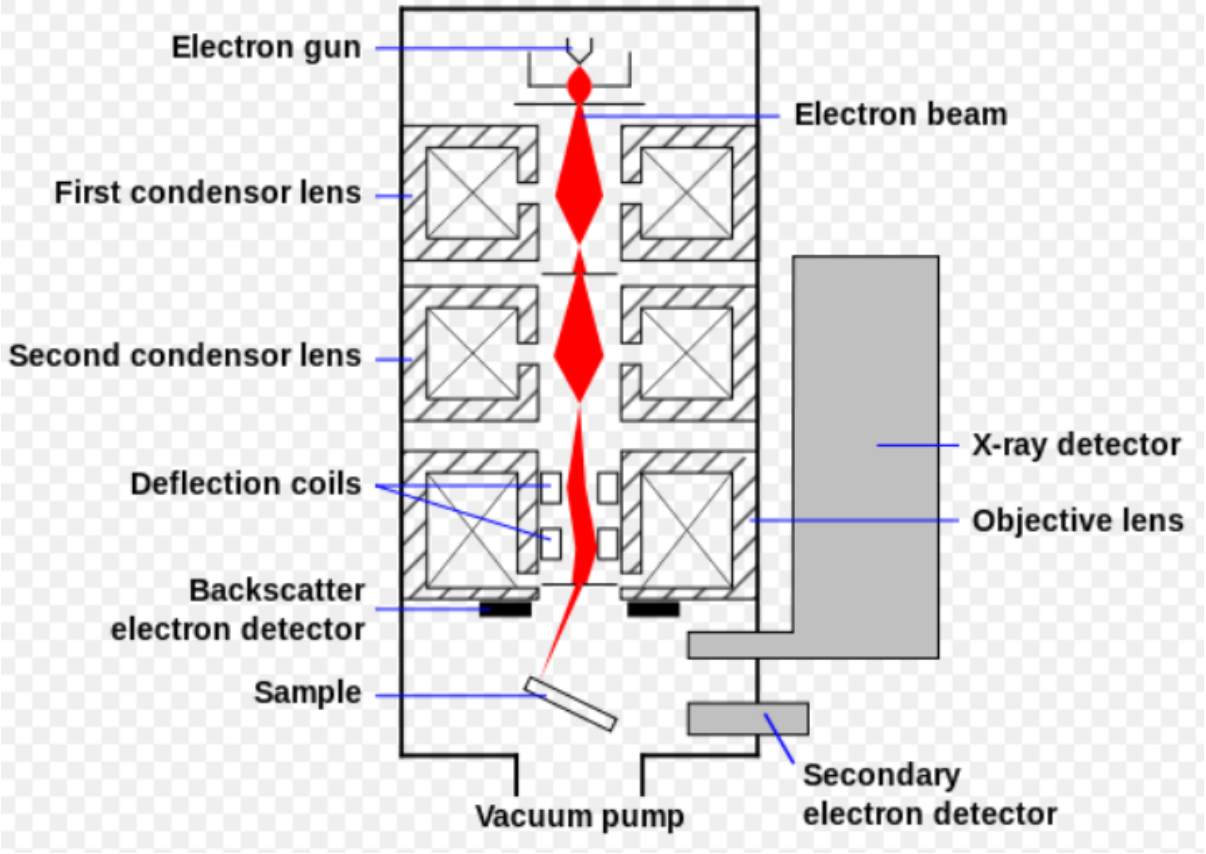


Figure 4: Schematic of an SEM

a) Scanning Electron Microscopy: SEM was done to study the surface morphology of the sample. The freeze-dried silver nanoparticles were mounted on specimen stubs with double-sided tapes and examined under a Hitachi S700N operated at a voltage of 15KV.

Material

Dried sample, SEM

Method

1) The dried sample was used for SEM analysis and the results were obtained.

E) **The Transmission Electron Microscope**

TEMs use electrons as "light source" and their much lower wavelength make it possible to get a resolution a thousand times better than what we can get with a light microscope.

You can see objects of the order of a few angstroms (10-10 m). For example, you can study small details in the cell or different materials down almost till atomic levels. The prospect for high magnifications has made the TEM a valuable tool in medical as well as materials & biological research.

A "light source" at the top of the microscope emits the electrons that travel through vacuum in the column of the microscope. In place of glass lenses focusing the light in the light microscope, TEM uses electromagnetic lenses to focus the electrons into a very fine beam.

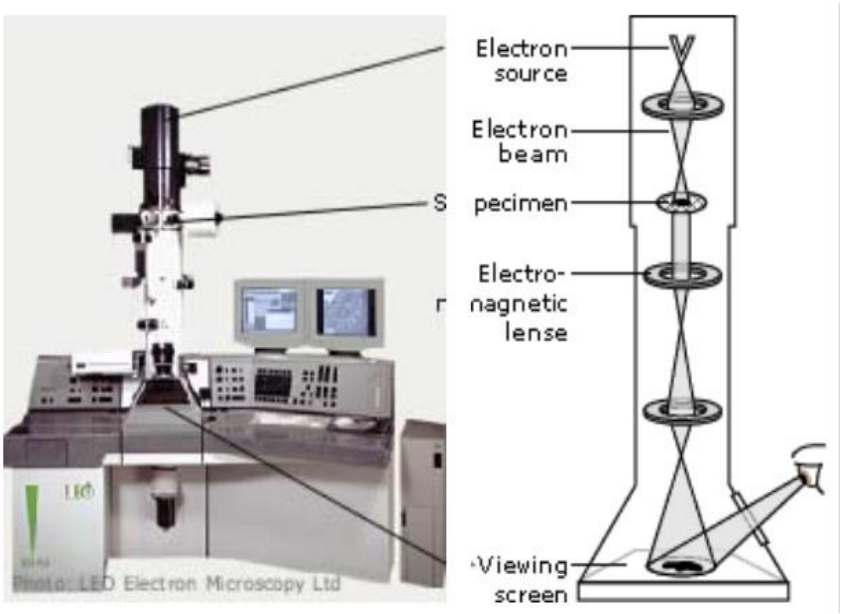


Figure 5: Schematic of a TEM

The electron beam then travels through the specimen we want to study. Some of the electrons are scattered and disappear from the beam, depending on the density of the material present. At the bottom of the microscope the unscattered electrons hit a fluorescent screen, which in turn gives rise to a "shadow image" of the specimen with its different parts displayed in varied darkness as per the density of the part. The image can be studied directly by the operator or photographed using a camera.

Resolution of the TEM is limited primarily due spherical aberration, but introduction of a new generation of aberration correctors have been able to partially overcome spherical aberration to increase resolution of TEM. Hardware correction of spherical aberration for the high-resolution transmission electron microscopy (HRTEM) has allowed the production of images with resolution below half angstrom (50 picometres) and magnifications above 50 million times. The capability to determine the positions of atoms within materials has made the HRTEM an important tool for nano-technologies research and development.

An important mode of TEM utilization is electron diffraction. The reward of electron diffraction over X-ray crystallography are that the specimen need not be a single crystal or even a polycrystalline powder, & also that the Fourier transform reconstruction of the object's magnified structure occurs physically and thus avoids the need for solving the phase problem faced by the X-ray crystallographers after obtaining their X-ray diffraction patterns of a polycrystalline powder or single crystal. The major disadvantage of the transmission electron microscope is the need for extremely thin sections of the specimens, usually about 100 nanometers. Biological specimens may be chemically fixed by dehydrating and embedding them in a polymer resin to stabilize them sufficiently to allow ultra thin sectioning to occur. Some sections of biological specimens (such organic polymers and similar materials) may require special treatment with heavy atom labels in order to achieve the required image contrast.

F) Atomic force microscopy

Atomic force microscopy (AFM) or scanning force microscopy (SFM) is a very high-resolution type of scanning probe microscopy with demonstrated resolution on the order of fractions of a nm's, more than 1000 times better than the optical diffraction limit.

The AFM is one of the foremost tools for measuring, imaging & manipulating matter at the nanoscale. In AFM the information is gathered by "feeling" the surface with a mechanical probe connected at the end of a cantilever. Typically, probe is made of piezoelectric elements that facilitate tiny but accurate and precise movements on (electronic) command enable the very precise scanning. Additionally, electric potentials can also be scanned using conducting cantilevers.

The cantilever is typically silicon or silicon nitride with a tip radius of curvature on the order of nm's. When the tip is brought into proximity of a sample surface, then the forces between the tip and the sample lead to a deflection of the cantilever according to Hooke's law [2]. Depending on the situation, forces that are measured in AFM include van der Waals forces, mechanical contact force, capillary forces, electrostatic forces, chemical bonding, magnetic forces, solvation forces, Casimir forces, etc. Along with force, other quantities may simultaneously be measured through the use of specialized types of probes.

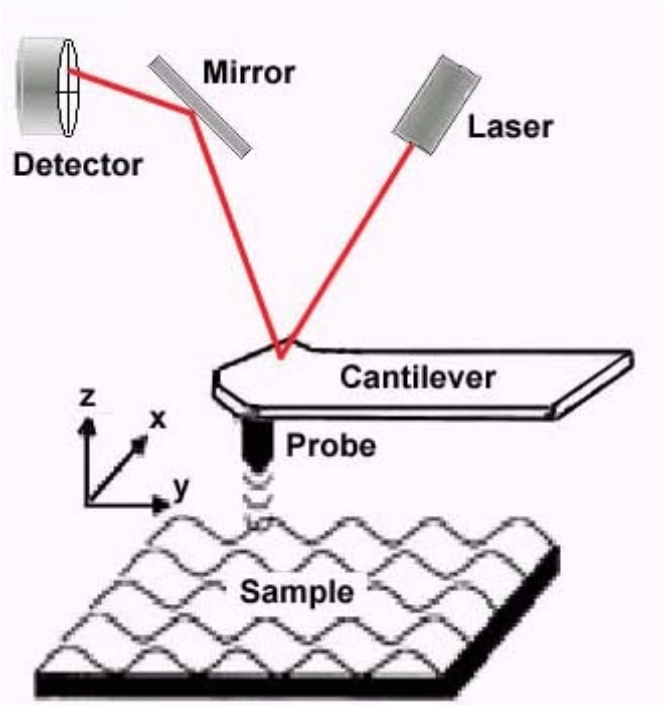


Figure 6: AFM Mechanism

Typically, the deflection is measured using a laser spot reflected from the top surface of the cantilever into an array of photodiodes. Some other methods that are used include optical interferometry, capacitive sensing (also known as piezoresistive) AFM cantilevers. The cantilevers are fabricated with piezoresistive elements that act as a strain gauge. By using a Wheatstone bridge strain in the AFM cantilever due to deflection can be measured, which may not be as sensitive as laser deflection or interferometry.

Depending on the application, the AFM can be operated in a number of modes. In general, possible imaging modes are divided into static (also called contact) modes and a variety of dynamic (non-contact or "tapping") modes where the cantilever is vibrated.

Imaging Modes

AFM operation is usually described as one of three modes, according to the nature of the tip motion:

- ⇨ contact mode, also called static mode (as opposed to the other two modes, which are called dynamic modes)
- ⇨ tapping mode, also called intermittent contact, ACmode, or vibrating mode, or, after the detection mechanism, Amplitude Modulation AFM

⇨ non-contact mode, or, again after the detection mechanism, Frequency Modulation AFM

AFM cantilever deflection measurement

Beam deflection measurement: - The most common method for cantilever deflection measurements is the beam deflection method. Laser light from a solid-state diode is reflected off the back of the cantilever and collected by a position-sensitive detector (PSD) consisting of two closely spaced photodiodes whose output signal is collected by a differential amplifier. In cantilever the angular displacement results in one photodiode collecting more light than the other, producing an output signal, which is proportional to the deflection of the cantilever. This detects cantilever deflections <10 nm. A long beam path (several centimeters) amplifies changes in beam angle.

Other deflection measurement methods

i. Piezoelectric detection
ii. Laser Doppler Vibrometry
iii. STM
iv. Optical Interferometry
v. Capacitive detection
vi. Piezoresistive detection

Chapter 3

Green Synthesis of Silver Nanoparticles using Tulasi Leaf at different Environmental Conditions: Effects of Concentration, Temperature and pH

Introduction

The field of nanotechnology is one of the most promising areas of research nowadays in modern material science and technology. Nanoparticles are the fundamental building blocks of nanotechnology [1]. In the recent past there have been impressive developments in the field of nanothechnology. Numerous methodologies have been formulated for the synthesis of nanoparticles of various shape and size depending on the particular requirements. The need to develop environmentally gentle synthesis processes i.e. which will not involve use of toxic chemicals in the synthesis process is picking up pace. As a result, researchers have started looking towards various biological systems and process for getting inspired. As many organisms (both unicellular and multi-cellular) have already been known to produce inorganic materials either intracellulary [2] or extracellularly [3], hence this change in stance of researchers doesn't seems surprising. A few well known examples of microorganisms synthesizing inorganic materials include; synthesis of magnetite nanoparticles by magnetotactic [4–6], synthesis of siliceous materials by diatoms [7–9] and production of gypsum and calcium carbonate layers by S-layer bacteria [10,11]. The secrets taken from nature have led to the growth of advanced nanomaterials using synthetic methods which mimic biochemical processes i.e. biomimetic approaches.

Although microorganisms such as fungi, bacteria and actinomycetes continue to be investigated in metal nanoparticle synthesis; in the meanwhile use of parts of whole plants in similar nanoparticle synthesis methodologies is an exciting possibility that is relatively underexploited and unexplored. Though the silver nanoparticles are considered biocompatible still the chemical synthesis methods may lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects while being used in medical/pharma applications. By synthesizing nanoparticles using microorganisms or plants can potentially eliminate this problem thereby making the nanoparticles more compatible for medical uses. Elimination of elaborate process of maintaining cell cultures can is an added advantage of using of plants for synthesis of nanoparticles over other environmentally benign biological processes. It can also be done on large scale to synthesize nanoparticles in bulk. Jose,

Yacaman and co-workers demonstrated the synthesis of silver nanoparticles within live alfalfa plants by silver ion uptake medium [12, 13].

Ocimum sanctum (Tulasi) is a medicinal herb which is generally found and cultured in India, Malaysia, Australia, West Africa, and some of the Arab countries [14]. It belongs to the family Lamiaceae. Tulasi is cultivated for religious and medicinal purpose and its leaves have been traditionally used for treatment of many infections.

This thesis is on the use of Tulasi (ocimum sanctum) leaf broth in the synthesis of silver nanoparticles. On treating aqueous solution of silver nitrate with Tulasi leaf extract; occurrence of rapid formation of stable silver nanoparticles is observed. Addition of Silver salt to aqueous leaf extract of Tulasi was used as capping as well as reducing agent.

MATERIALS AND METHODS: The detail of the materials and method is already discussed in Chapter 2.

Results & Discussion

Addition of silver salt to the Tulasi leaf broth resulted in colour change of the resultant solution. The colour changed from yellowish (Fig. 7b) to dark brown (Fig. 7d), which is due to the reduction of silver salt into silver nanoparticles. It took around one & half to two hours for the complete colour change to occur, there wasn't any further colour change after a period of two hours. This indicates that whole silver salt has been reduced to silver nanoparticles within this time frame and no further silver salt was available for reduction.

Figure 7: Colour change indicating synthesis of silver nanoparticles using Tulasi leaves. (a) Solution of 1mM AgNO3 without plant extracts, (b) 1mM AgNO3 with Tulasi leaves extract after 5 min and (c) after 15min (d) after 30 min.

While synthesizing nanoparticles with other biological methods such as the one using fungi, bacteria etc. takes longer duration of time, ranging from 1 to 5 days [1]. This also shows that biological methods of synthesis using plant extracts are more efficient as compared to other

biological synthesis methods. Formation of the silver nanoparticles by reduction of the silver salt during exposure to the broth of boiled O. sanctum leaves may be easily observed by optical absorption spectroscopy .

Fig. 8 shows the absorption spectra of synthesized Silver nanoparticles from Tulasi leaves. The UV absorption spectra was recorded at regular intervals of time by using spectrophotometer which was operated in the range of 250-700nm. From the observed absorption spectra we can conclude that with passage of time the optical density of the absorption peaks increased; which shows that more number of silver ions are getting converted in to silver nanoparticles, as the time passes. Note that the synthesized silver nanoparticles using Tulasi leaves absorbed maxima near 400nm. The increase in absorption intensity with time is given in Table 1 and shown in graph 1

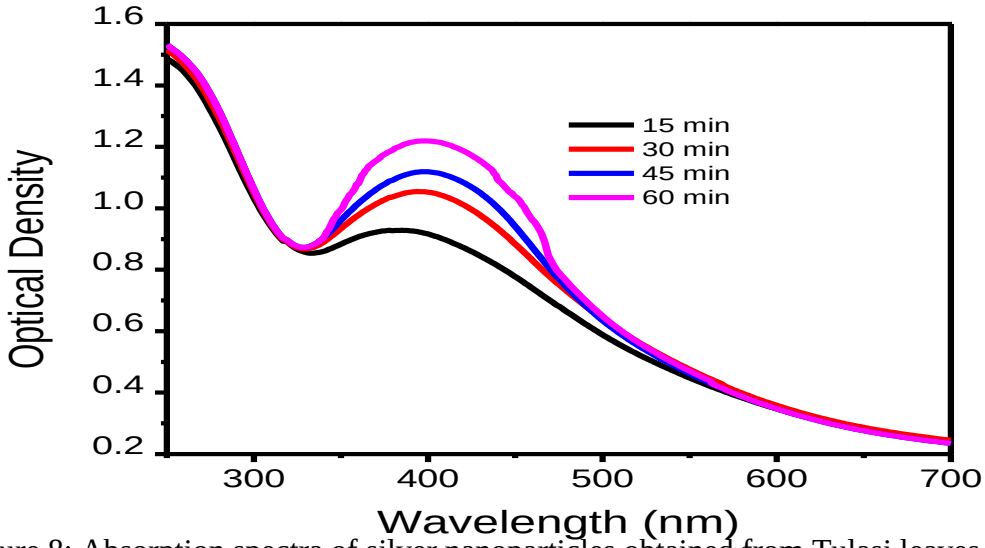
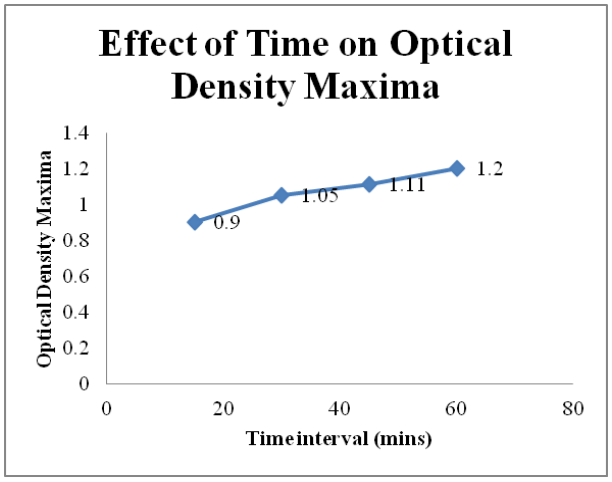


Figure 8: Absorption spectra of silver nanoparticles obtained from Tulasi leaves at three different times.

Table 1: Shows the maximum optical density with respect to time.

Optical Density Maxima	Time interval (mins)
0.9	15
1.05	30
1.11	45
1.2	60



Graph1: Plot of absorption intensity vs. reaction time

Fig. 9 shows emission spectra of the silver nanoparticle recorded at different time at excitation wavelength of 350nm. The band maxima was observed at around 450nm. As the time passes, an increase in intensity was observed due to the increase in amount of silver nanoparticle.

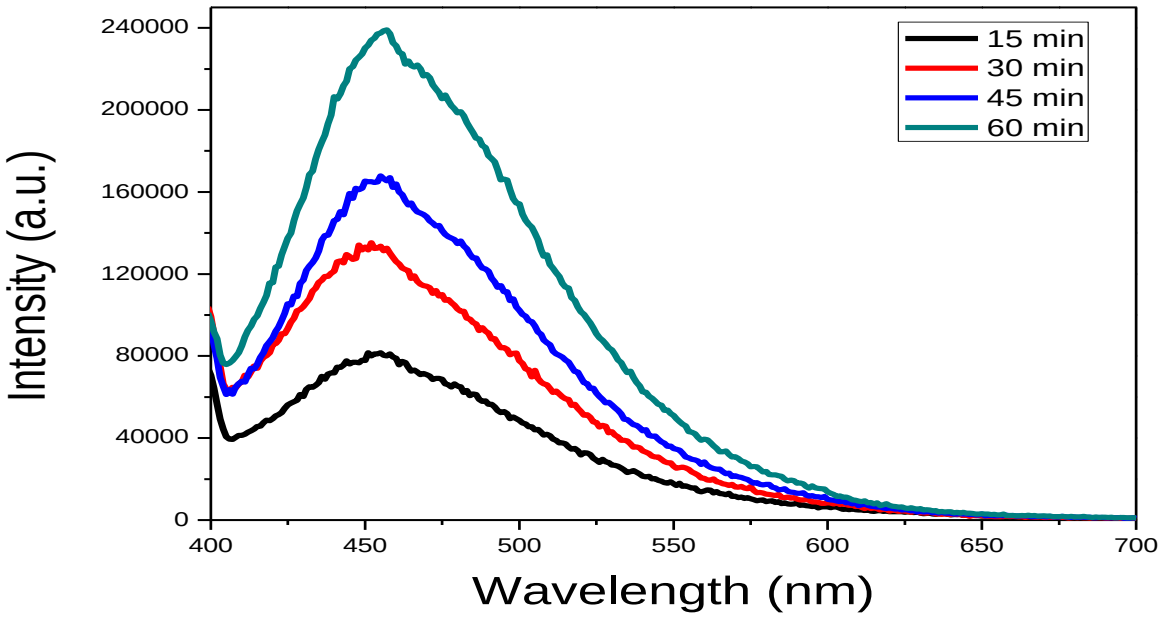


Figure 9: Emission spectra recorded as a function of time of reaction of 10^{-3}M aqueous solutions of silver nitrate with Tulasi leaf broth.

Effect of pH

pH is one of the important parameter to control the size and quality of nanoparticle synthesis [2-4]. To see the effect of pH, we prepare solution of silver salt and broth in presence of KOH for maintaining different pH levels. The colour change observed of the solution at different pH can be seen in figure q0.

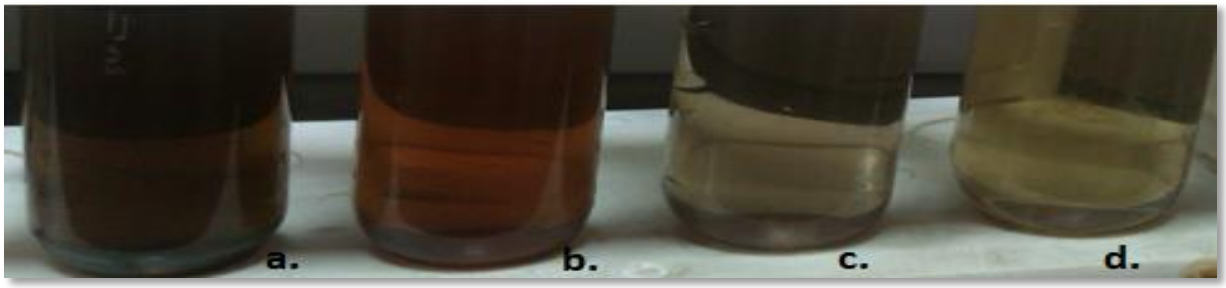


Figure 10: Change in colour solution of Tulasi broth and silver salt at different pH. (a) At pH=12, (b) at pH=11, (c) at pH=10 & (d) at pH=9.

Figure 11 shows the UV-Visible absorption spectra silver nanoparticles observed at different pH of the reaction mixture. Absorption spectra of the solutions at different pH levels were recorded. The growth of nanoparticles start at pH 9, however below pH 7, in acidic solutions no nanoparticles were synthesized. On increasing pH of the solution of Tulasi broth and silver salt towards more basic, formation of nanoparticle starts. However at higher pH, i.e. >12 the nanoparticles are less stable and agglomerated while keeping the solution for longer time (about 24 hrs). It was also noticed that on increasing pH of the reaction, the rate of reaction increases and at higher pH reduction of silver salt become more fast.

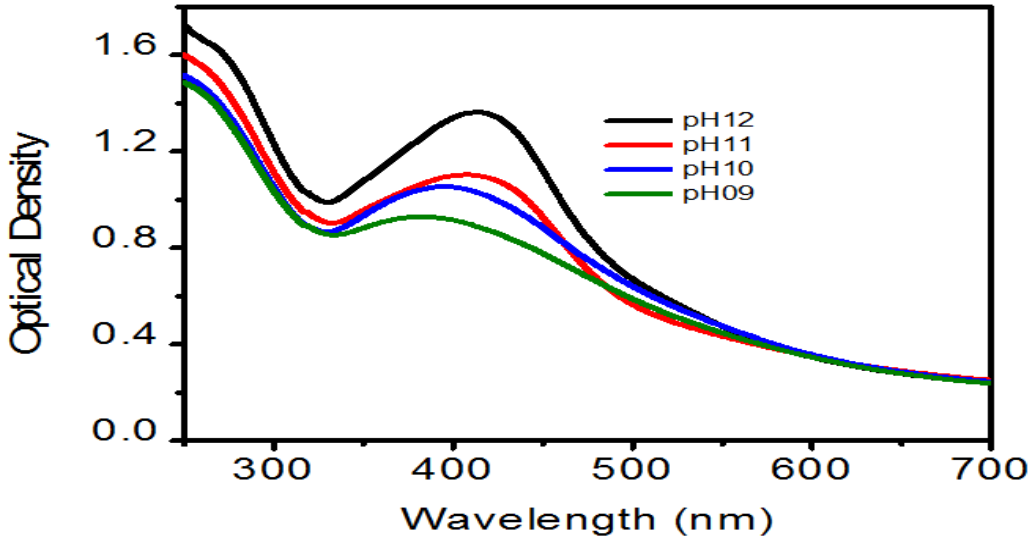


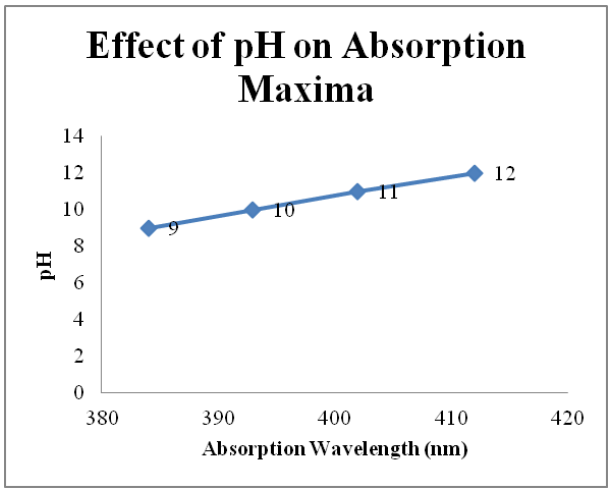
Figure 11: Absorption spectra of silver nanoparticles obtained at different pH of the reaction mixture.

As we change pH of the solution, absorption maximum of the nanoparticle shift towards red. Absorption maxima were observed at around 384nm at pH of 9 and 412nm at pH of 12. The absorption maximum shows a shift of about 28 nm while changing pH from 9-12 (Table 2).

Table 2: Shows absorption maxima corresponding the pH levels

Absorption Wavelength (nm)	pH
384	9
393	10
402	11
412	12

Emission spectra (figure 12) were recorded by photometer at different pH and at excitation wavelength (λex) of 350nm at different pH; for all the samples we take the same excitation wave length. Emission peak for all the samples was observed around 450nm. And as we increase the pH of the solution an increase in intensity was observed this is due to increase in the amount of synthesized colloidal silver nanoparticle.



Graph2: Plot of absorption maxima v/s pH of the Tulasi broth and silver salt solution

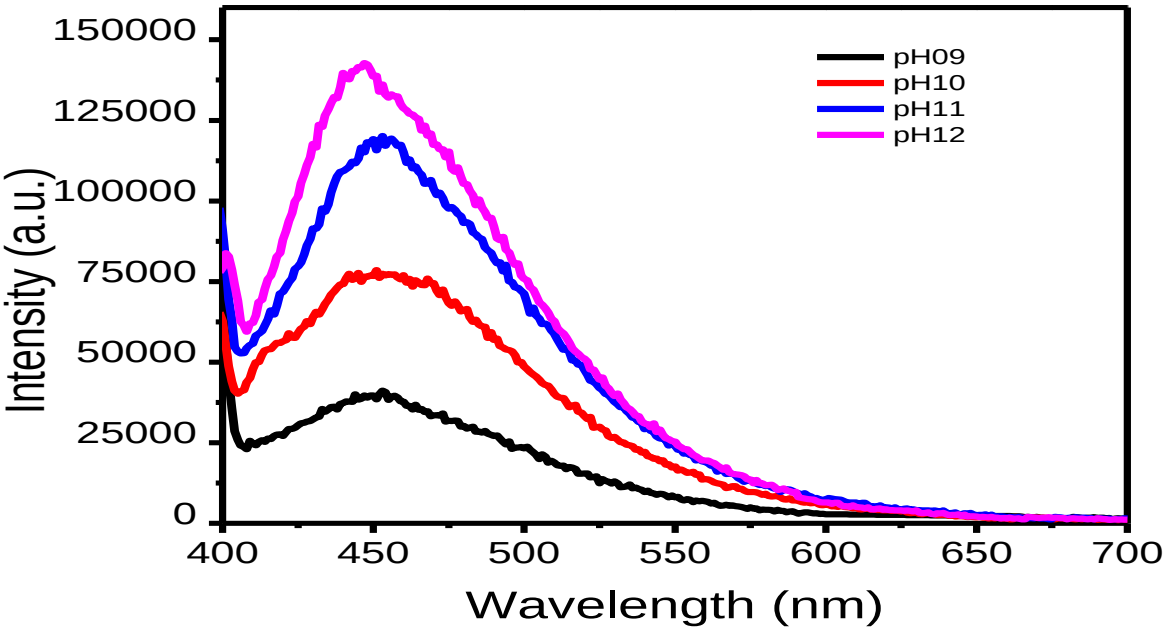


Figure12: Emission spectra of silver nanoparticles against pH values of the reaction mixture

Effect of Temperature

Temperature is one of the crucial factors which decides the shape and size of nanoparticles. By increasing temperature the reduction rate of silver salt to silver nanoparticle increases and the size of synthesized particles sharpens [5]. To analyze the effect of temperature we synthesized silver nanoparticles at different temperature, i.e., 15^{o}C, 25^{o}C, 35^{o}C and 45^{o}C and

recorded the absorption spectra in the range of 250-700 nm and illustrated in Fig. 7. It was noticed that at higher temperature, the rate of reduction of silver salt is faster as compared to the lower temperature. As an example, at higher temperature the reaction is completed in 1hour only.

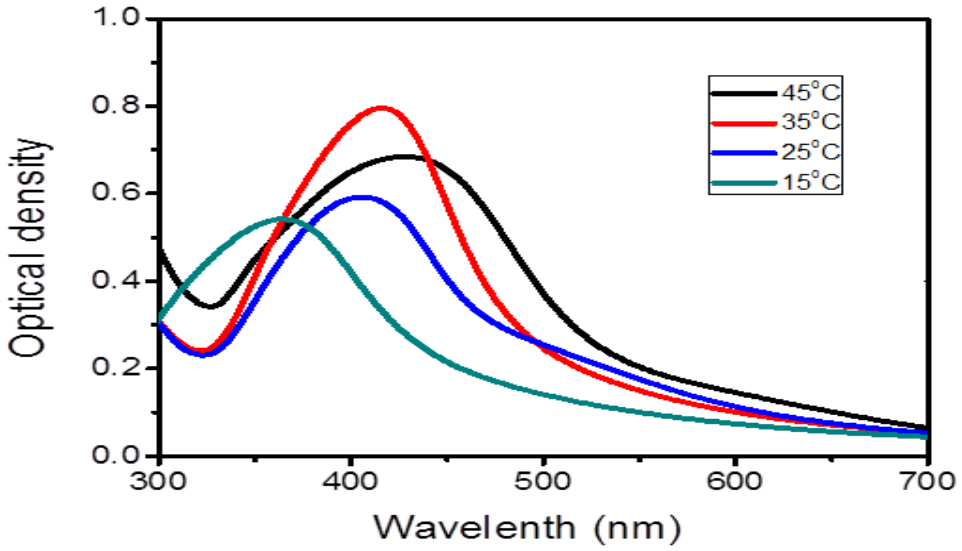
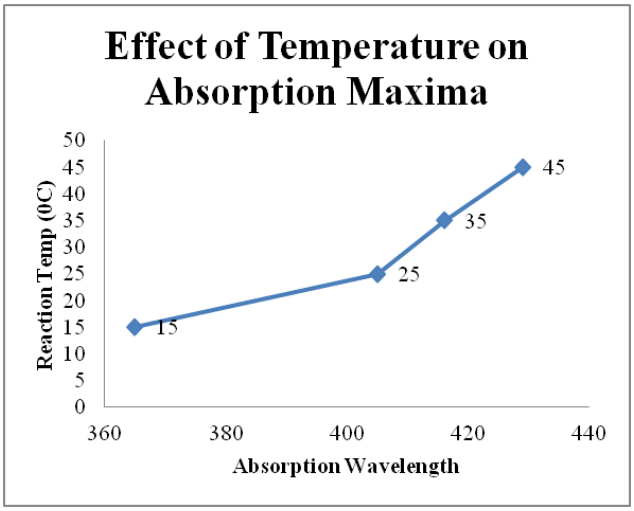


Figure 13: Absorption spectra of silver nanoparticles at different reaction temperature of 15℃, 25℃, 35℃ & 45℃.

It is interesting to note that on increasing the reaction temperature the absorption maximum is shifted towards longer wavelength, as shown given in Table 3 and graph. 3.

Table 3 shows the absorption maxima and the corresponding reaction temperature.

Absorption Wavelength	Reaction Temp (°C)
365	15
405	25
416	35
429	45



Graph3: Plot of absorption maxima v/s temperature of the Tulasi broth and silver salt solution

Fig. 14 shows photoluminescence spectra of silver nonoparticles at different temperature with 350nm excitation. The PL spectra lie in the range of 400-700nm.

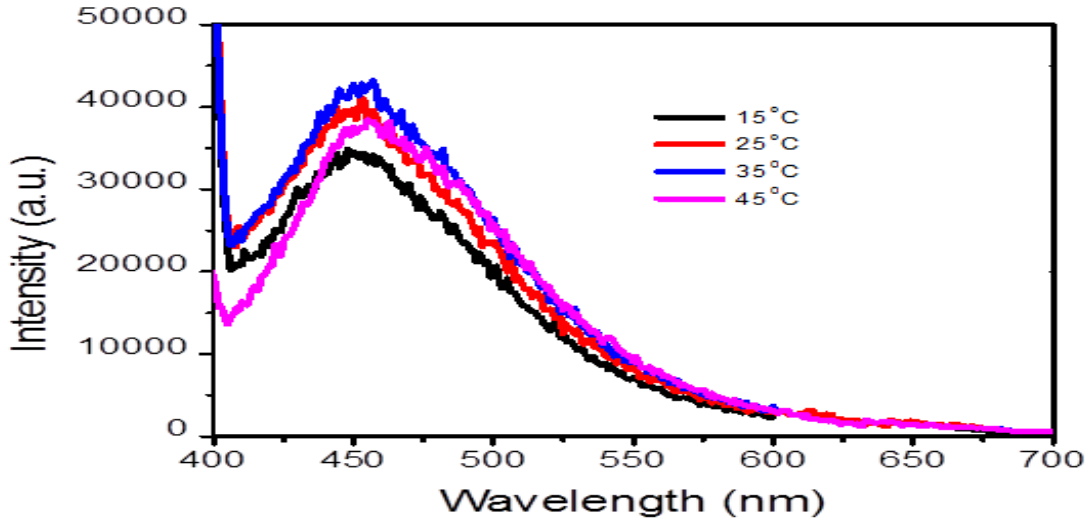
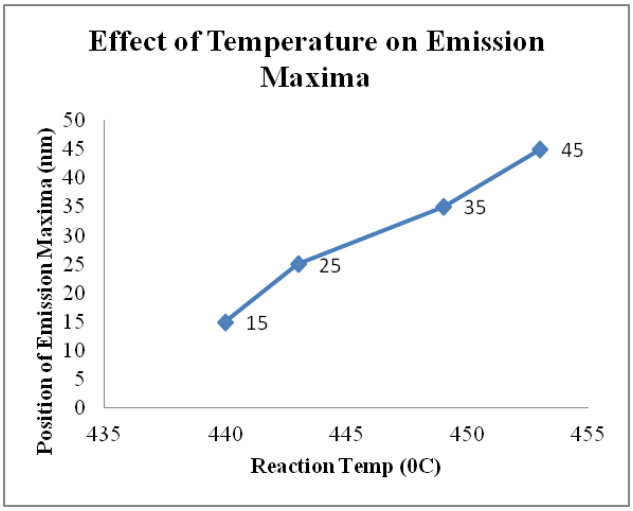


Figure 14: Emission spectra of silver nanoparticles at different reaction temperature 15^0C, 25^0C, 35^0C, 45^0C.

It was observed that with increase in temperature, the position of PL maximum shift towards longer wavelength region. A small change in PL intensity was also observed with changing the temperature.

Table 4 shows the position of emission maxima and the corresponding reaction temperature.

Position of Emission Maxima (nm)	Reaction Temp (^{0}C)
440	15
443	25
449	35
453	45



Graph4: Plot of emission maxima vs. temperature.

Effect of Concentration

Concentration of reaction solution is a crucial factor in deciding the quantity of silver nanoparticles. To see the effect of concentration, we mixed Tulasi broth and silver salt in different proportions. Upon mixing Tulasi broth in higher proportion to the reaction solution, the optical density of the silver nanoparticle increases. However, the absorption maximum remains at the same position.

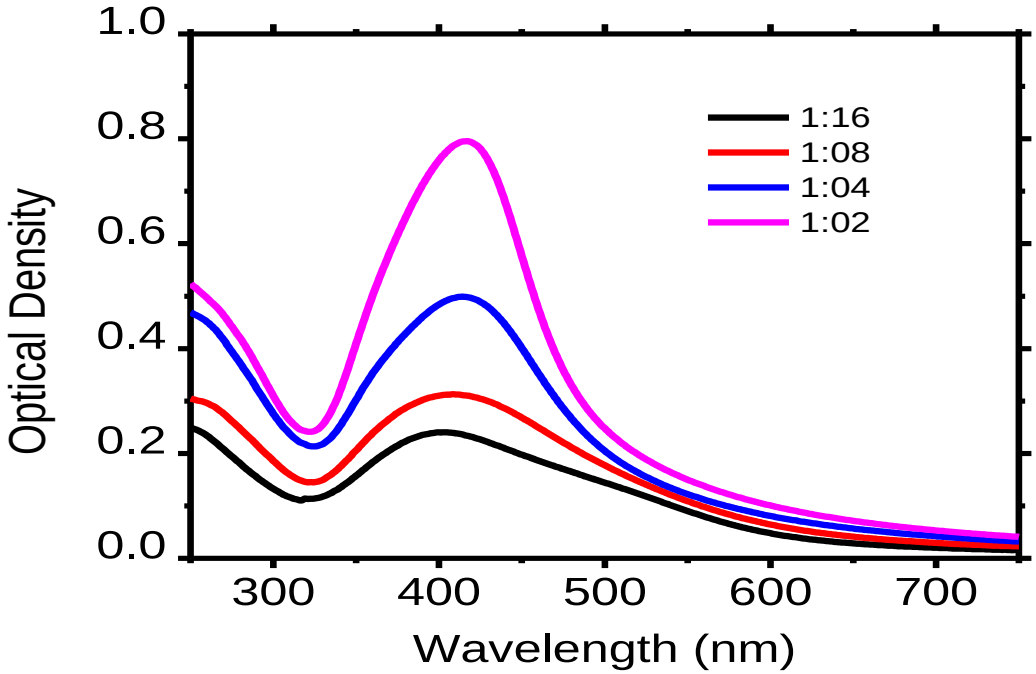
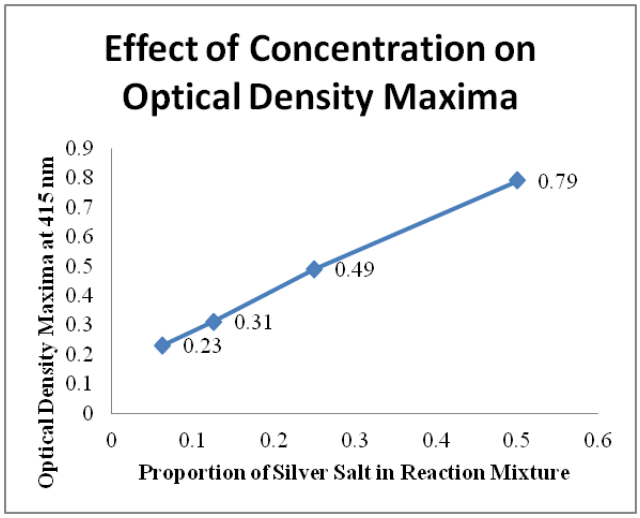


Figure 15: Absorption spectra of production of silver nanoparticles against various concentrations of Tulasi Broth (1:2, 1:4, 1:8, and 1:16)

Thus, we conclude that with increasing the concentration of Tulasi broth, the quantity of silver nanoparticles increases without changing the size of the particles (Table 5 and graph 5).

Table 5 shows the absorption intensity at 415 nm corresponding to the reaction mixture

Optical Density Maxima at 415 nm	Proportion of Silver Salt in Reaction Mixture
0.79	0.5
0.49	0.25
0.31	0.125
0.23	0.0625



Graph 3: Plot of proportion of silver salt in reaction mixture v/s observed optical density maxima

Fig. 16 shows the PL spectra of silver nanoparticles at different concentration in the range of 400-700 nm with 350nm excitation. The PL shows band maximum around 450nm. It was observed that as we increase the proportion of silver nitrate, the PL intensity increases. This is due to increase in amount of colloidal silver nanoparticle produced.

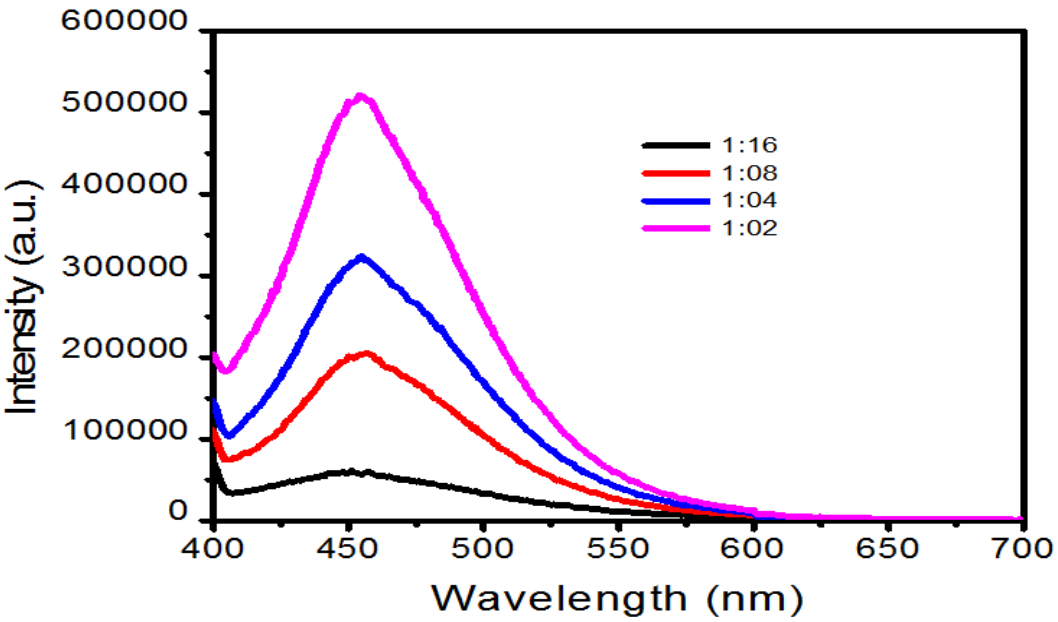


Figure 16: Photoluminescence spectra of production of silver nanoparticles at various concentrations of Tulasi broth (1:2, 1:4, 1:8, and 1:16)

Scanning Electron Microscopy

Scanning electron microscopy provided information about the morphology and size of the silver nanoparticles. To obtained features of the silver nanoparticles, analysis of the sample was performed using SEM analysis. Figures 17 and 18 show the SEM images of silver nanoparticles. From the SEM images, the calculated diameter of prepared nanoparticles in the

solution was approximately 150 nm. The particles are spherical in shape and are crystalline in nature. The size of synthesized nanoparticles is not the same, which is also inferred in absorption and PL. The average size of the particle is around 150 nm, which indicates that the particle synthesize by biological methods are not mono-disperse in nature they are poly disperse. The distribution of particles and the average particle size can also be seen in absorption and PL spectra

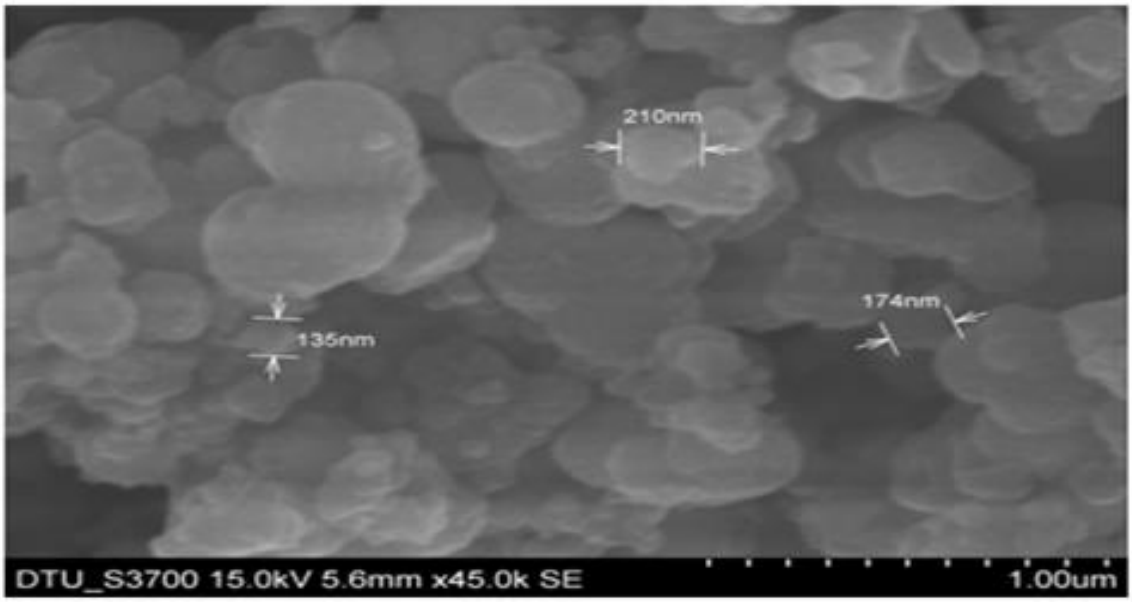


Figure 17: SEM image of synthesized silver nanoparticle

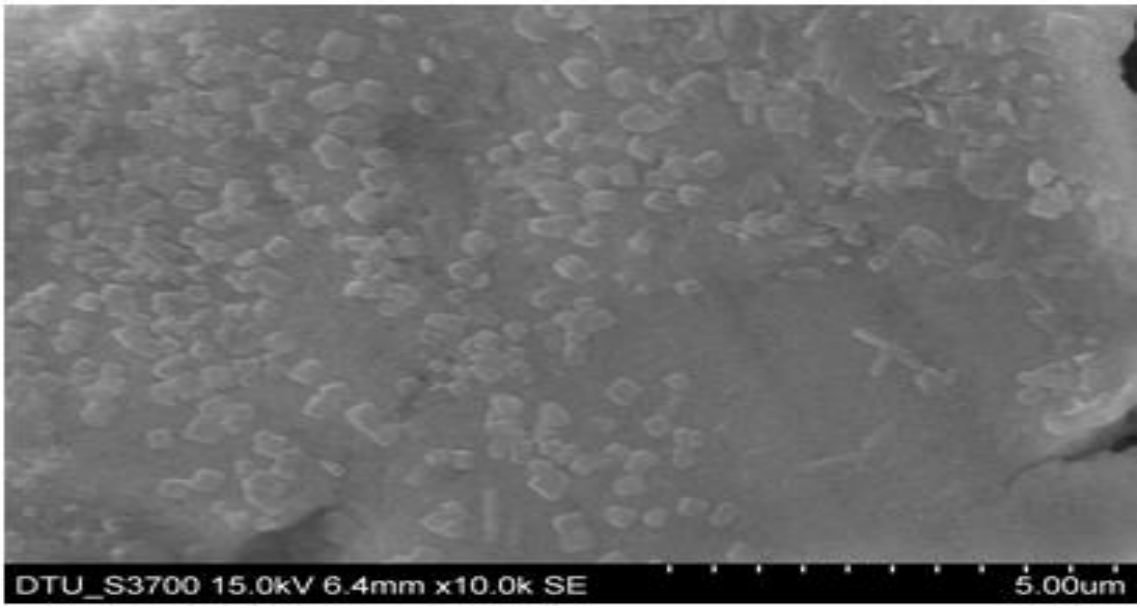


Figure 18: SEM image of synthesized silver nanoparticle

X-ray diffraction

Fig. 19 shows the XRD patterns obtained for silver nanoparticles synthesized using Tulasi leaf broth. The diffracted intensities were recorded from 0◦ to 80◦ at 2 theta angles and analyzed to confirm the nature and size of the silver nano particles. The XRD pattern thus clearly shows that the silver nanoparticles formed by the reduction of Ag+ ions by Tulasi leaf broth are crystalline in nature. The observed XRD pattern is compared with the standard, it is confirmed that the obtained silver particles are in the form of nanocrystals, as evident by the peaks at 2θ values at 38, which is at same position as in the standard XRD (Fig. 19).

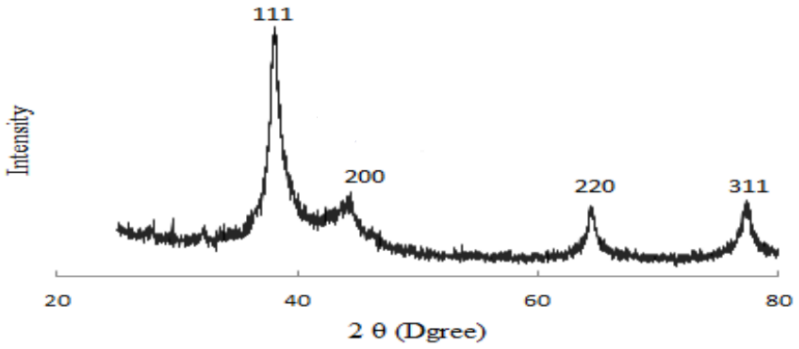


Figure 19: Standard X-ray diffraction pattern for silver nanoparticle

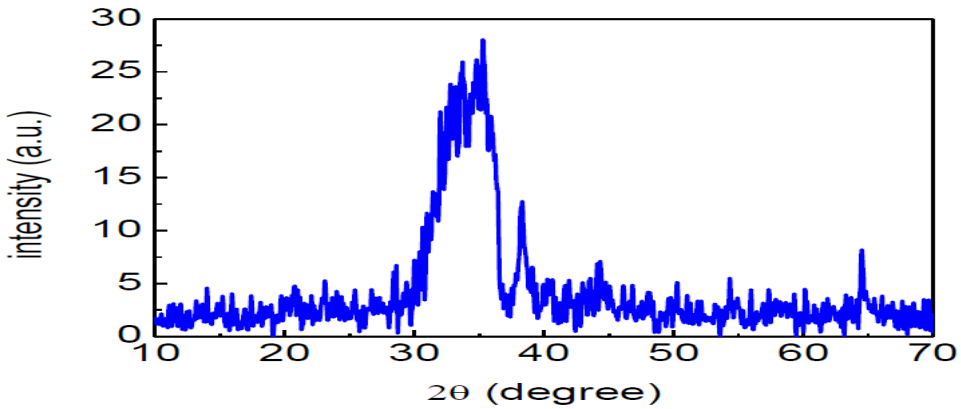


Figure 20: X-Ray Diffraction graph of Silver Nanoparticles synthesized by reduction of silver salt by Tulasi leaf broth

FTIR Results

To investigate the functional groups present in ocimum leave extracts, FTIR study was carried out. The spectrum is shown in figure 21. In the spectrum, a sharp peak is observed at

around 3389 cm^{-1}, which indicates the presence of OH group which in turn is due to presence of ascorbic acid ($C_6H_8O_6$) in oscimum leaves. And a peak at around 1635cm^{-1} indicates the presence of CO, C-O & OH groups bond stretching. This may be attributed to the presence of flavones ($C_5H_{10}O_2$) in the Tulasi leaves. Thus, FTIR study indicates that –OH and C-O groups are present in the Tulasi leaves which are involved in the reduction of $AgNO_3$ to Ag nanoparticles.

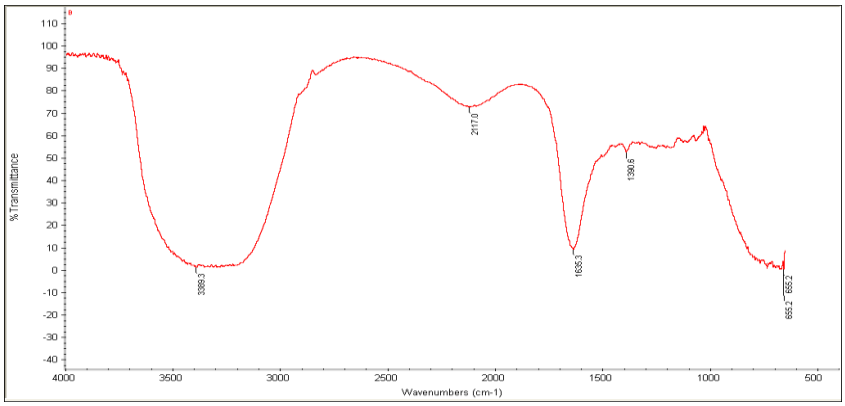


Figure21: FTIR spectra of Tulasi leaves broth

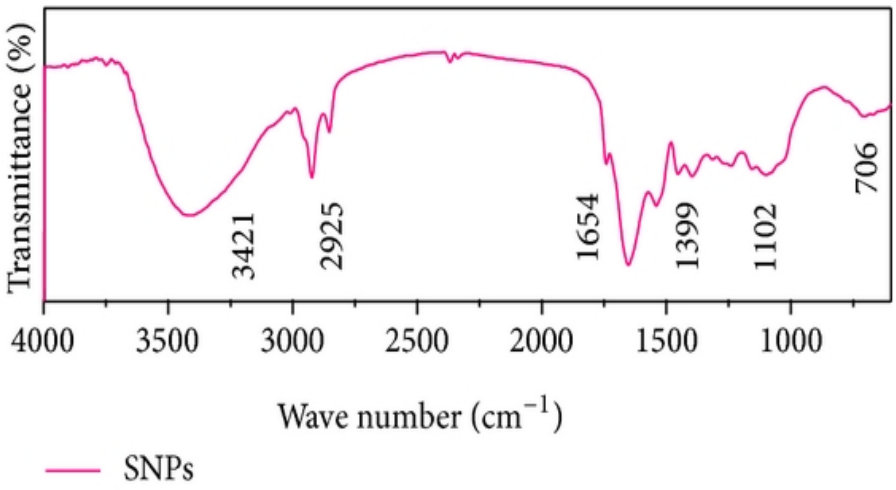


Figure 22: Standard FTIR for silver nanoparticles

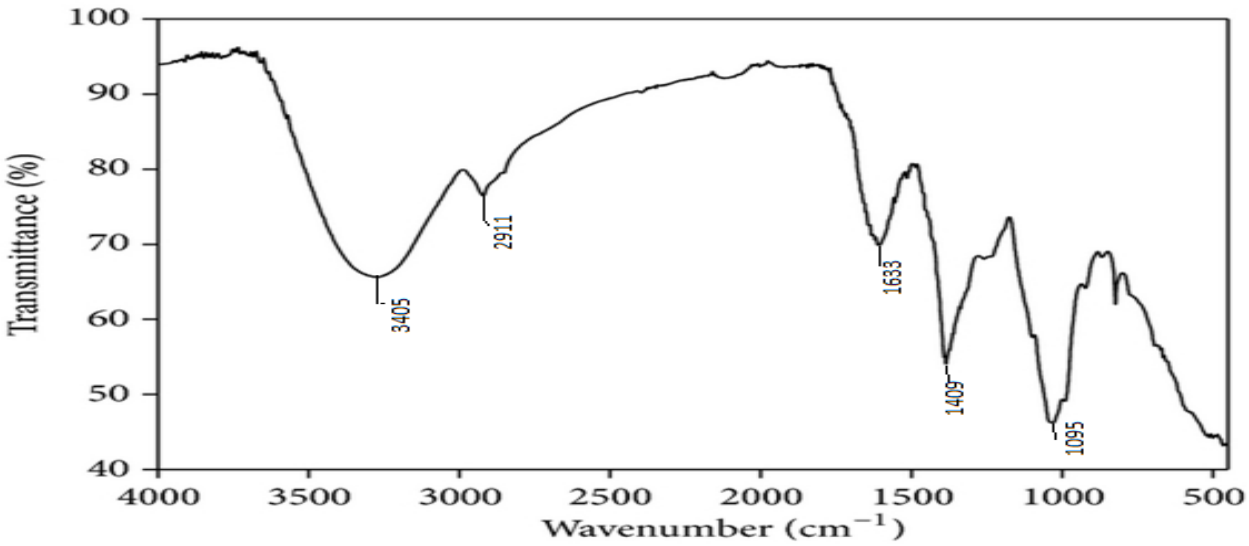


Figure 23: FTIR spectra of silver nanoparticles as observed for silver nanoparticles synthesized.

It is important to note that the FTIR peaks obtained at around 3405 $cm^{-1,}$ 2311 cm^{-1}, 1633cm^{-1}, 1409 cm^{-1}, 1095 cm^{-1} (Fig. 23)for the solution of $AgNO_3$ and Tulsi broth are quite resembled with the standard one (Fig. 22).

Conclusions

We have sucessfully synthesized silver nanoparticles by reduction of silver salt by Tulasi leaf broth. The synthesized silver nanoparticles are crsytalline in nature (XRD). The average size of the silver nanoparticles is about of 150 nm (SEM). We can also observed that the particles formed are poly-dispersed in nature all the synthesized particles are not of equal size.

By changesing the environmental parameters of reaction such as pH of the solution, temperature of solution, concentration of salt, we can control size, quality and quantity of the synthesized silver nanoparticles. Thus the biological synthesis of nanoparticles using plant extracts is scalable. The biological methods of synthesis using plant extracts are more efficient (completed in short time scale, nearly in 1hr) as compared to other biological synthesis methods, which may even take upto 2-3 days for synthesis. Thus the time taken for biological synthesis of nanoparticles using plant extracts is comparable to that of chemical methods.

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