



Synthesis of Ecofriendly Silver Nanoparticle from Plant Latex used as an Important Taxonomic Tool for Phylogenetic Inter-relationship

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ABSTRACT

Nano technology is the design, characterization, production & application of structures, devices and systems by controlling shape and size at the nanoscale. It involves the production, manipulation and use of materials ranging in size from less than a micron to that of individual atoms from not only chemical approaches but also biological materials. Silver nanoparticles were successfully synthesized from $AgNO_3$ through a simple green and natural route using the latex of 6 different plant taxa belonging to 6 different families. Nano particle synthesis is proved under UV-Vis absorption spectroscopy (Sigma-Aldrich Chemical Pvt.Ltd). By using different plant-latex, silver nano particles synthesized which are quite stable and no visible changes are observed even after a month or so, if the nanoparticle solutions are kept in light proof condition. As nano particles have great application in medical world like gene therapy, cancer therapy, drug delivery etc. So medical world also easily accept the plant world for nano particle synthesis and welcome the angiosperms for their potentiality of synthesis of non polluted, environmentally acceptable, safety for human health nano particles. In taxonomic view, this modern tool for synthesis of ecofriendly, non toxic, not expensive nano particles help us to open a new vista for the classification of angiosperms and detection of their phylogenetic relationships depend on plants- ability for the synthesis of nano particles which are variable in size & concentration in case of different family, different genus even also in the species level. So, the elucidation of exact mechanism of nano particles production using different plants needs much more experimentations.

KEY WORDS: Ecofriendly, Non toxic, Non polluted, Not expensive, safety

INTRODUCTION

Plant latex refers generically to a stable dispersion (emulsion) of polymer microparticles in an aqueous medium. It is found in nature as a milky sap which coagulates in nature. It may be yellow or orange and also may be watery in nature.

Nano is the ancient Greek word for dwarf. In scientific terms it has been used to identify length scales that are one billionth of a unit. Metal nanoparticles are intensely studied due to their unique optical, electrical and catalytic properties [1-3]. Various techniques, including chemical and physical means have been developed to prepare metal nanoparticles, such as chemical reduction [4-7], heat evaporation [8,9], electrochemical reduction [10,11], photochemical reduction [12,13] and so on. A great deal of effort has been put into the bio synthesis of inorganic materials, especially metal nanoparticles using microorganisms [14-19]. Both live and dead micro organisms are gaining importance by virtue of their facile assembly of nanoparticles. Among micro organisms, prokaryotic bacteria have primarily attracted the most attention [14]. An important demonstration was reported by klaus et al [20] who describe the formation of silver based particles at the cell poles of propagating *pseudomonas stutzeri* AG259. Sastry and coworkers [21,22] have opened the field to the synthesis of metal nano particles by eukaryotic organisms like *verticillium* sp.[23]. They demonstrated that the shift from bacteria to fungi had the added advantage that processing and handling of the biomass would be much simpler. Some well known examples of bio organisms synthesizing inorganic materials include magnetotactic bacteria which synthesize magnetic nanoparticles [24-26], S-layer bacteria [27,28]. While *Fusarium oxysporum* biomass resulted in the extracellular silver nano

particles [29]. The rate of intra cellular particle formation and therefore the size of the nanoparticles could, to an extent be manipulated by controlling parameters, such as pH, temperature, substrate concentration and exposure time to substrate [30].

Various techniques, including chemical and physical means have been developed to prepare metal nanoparticles. In most cases, the surface passivator reagents are needed to prevent nanoparticles from aggregation. Unfortunately many organic passivators such as thiophenol, thiourea, macro capto acetate, etc. are toxic enough to pollute the environment if large scale nanoparticle are produced [31-33].

Synthesis of nanoparticles using biological entities has great interest due to their unusual optical [34], chemical [35], photoelectro-chemical [36] and electronic properties [37]. The synthesis & assembly of nanoparticles would benefit from the development of clean, nontoxic and environmentally acceptable 'green chemistry' procedure, probably involving organisms ranging from bacteria to fungi and even plants [38],[39]. Hence, both unicellular and multicellular organisms are known to produce inorganic materials either intra or extracellularly [40].

Synthesis of nanoparticles could be advantageous over other environmentally benign biological processes by eliminating the elaborate process of maintaining cell cultures. Jose – Yacaman and co workers [41,42] first reported the formation gold and silver nano particles by living plants. Very recently green silver nanoparticles have been synthesized using various natural products like green tea *Camellia sinensis* [43], Neem *Azadirachta indica* [44], leaf broth natural rubber [45], starch [46], *Aloe vera* plant extract [47], lemongrass leaves extract [48,49], leguminous shrub (*Sesbania drummondii*) [50] etc.

Some specific plant parts or whole plant specially angiospermic plants are used for the great synthesis of nano particle. Alfaalfa roots [51,52] have capability for absorbing Ag (0) from agar medium and transferring them to shoot of the plant in the same oxidation state. In the shoot these Ag atoms arranged themselves to form nanoparticles by joining themselves to form larger arrangements. Transmission electron microscope (TEM) / Scanning electron microscope (SEM) analysis showed that the accumulated Ag atoms inside the plant tissue underwent nucleation and resulted in the formation of nanoparticle.

With the use of *Emblica officinalis* fruit extract [53] as reducing agent, the extracellular synthesis of highly stable Ag and Au nanoparticles has been achieved (Ankamwar et al. 2005a). Along with stability the control over the shape of nanoparticle production using plants and parts is also possible. Leaves of geranium plant *Pelargonium graveolens* [54,55] have also been synthesized nanoparticles of gold. Nano particle (Au) also obtained from *colletotrichum* sp. Related to geranium using of *Aloe vera* leaf extract as a reducing agent the synthesis of gold nanotriangles as well as silver nano particle was obtained in single crystalline triangular form. For this, aqueous chloroaurate ions were used with the *Aloe vera* plant extracts. Aqueous silver ions (AgNO_3) when incubated with *Aloe vera* extract produce only spherical Ag nanoparticles. The appearance of brownish red colour and faint yellow colour in the reactions indicate the formation of gold and silver nanoparticles respectively. *Cinnamomum camphora* [56] leaf extract has been identified very recently for the production of gold as well as silver nano particle.

The plant, *Jatropha curcas* is commercially importance one as biodiesel is extracted from its seeds on industrial scale[57,58]. Though the *Jatropha* latex has some ethnomedicinal uses like wound healing, coagulant activities of blood [59], it is acrid and irritable to the skin also [60].

From this latex, nano particle is synthesized using it reducing as well as capping agent. In this case, extensive literature survey revealed that curcacycline A (a cyclic octapeptide) & curcacycline B (a cyclic nonapeptide) has reducing property and enzyme curcain has stabilizing property [61].

MATERIAL AND METHODS

Source of AgNO_3

Silver nitrate (AgNO_3) analytical grade was purchased from sigma — Aldrich chemical Pvt. Ltd.

Source of latex

Crude latex was obtained by cutting the green stems and fruits of 6 plants of different families. Milky white latex and watery latex both were stored at -20°C until use. All the aqueous solutions were prepared using triply distilled de-ionized water.

Table: 1. Selected plant taxa with necessary informations (plant name, family, description, colour of latex & medicinal uses)

Name of plants	Family	Description	Colour Of latex	Medicinal uses of the latex
1. <i>Alstonia scholaris</i> (Linn.) R.Br.	Apocynaceae	A moderate to large sized evergreen tree grows upto 30 meters in height. Leaves 5 – 7 in a whorl, elliptic blong, obtuse, petiolate, flowers small greenish or white, found in umbellate cymes. Fruits falicles about 50 cm. long, contain papillose seeds with hairs.	Milky white	Warm latex applied to allay chest pain; to cure dental caries with kusum oil applied to treat scabies; given to cattle to cure dysentery.
2. <i>Calotropis gigantea</i> (Linn.) R.Br. ex Ait	Asclepiadaceae	Laticiferous shrub, leaves opposite, broad, decussate, sessile. Flowers in umbelliform cymes. Flowers purple in colour, Fruit of two thick follicles. White & milky latex.	Milky white	Purgative, used to treat wounds caused by poisonous insect & catfish bites, to cure pain in the gum of teeth, ringworm, to cure septic wounds of cattle.
3. <i>Ficus religiosa</i> Linn	Moraceae	Large , deciduous tree. Leaves are alternate, cordate, shiny, long petioled, with linear anceolate tail, the receptacles occuring in pairs, axillary, purplish when ripe. White milky latex.	Milky white	Wound healing property.
4. <i>Hevea brasiliensis</i> Mull. Arg.	Euphorbiaceae	Tree, 40 m. Large. The white latex occurs in latex vessels in the bark, mostly outside the phloem.	Milky white	Antimicrobial activity
5. <i>Musa Paradisiaca</i> L.	Musaceae	Tropical tree like herb, with large leaves of which the overlapping bases form the so-called false trunk from the center of the crown spring the flowers. Each plant bears fruit only once. Watery latex present in this plant.	Watery white	Use in medicine of diarrhoea, dysentery, jaundice.
6. <i>Achras sapota</i> Linn.	Sapotaceae	Tall, evergreen, edible fruit sappodillo having dull brown rind, the flesh being of a dirty, yellowish white colour, very soft, sweet called naseberry.	Milky white	Filling for tooth cavities. Antioxidant property.

Table 2: Physical properties of silver nano particles

Colour & form	Golden yellow or faint yellowish liquid.
Properties	Water soluble
UV – Vis (nm) peak up	401 nm. – 434 nm
Specification	Stable for more than 30 days except in case of <i>Musa</i> sp.

Table 3: Rank of plants depends on size of nano particle

Sl. No.	Plant	Peak up	Rank
1.	<i>Alstonia scholaris</i>	434	1st
2.	<i>Hevea brasiliensis</i>	426	2nd
3.	<i>Ficus religiosa</i>	422	3rd
4.	<i>Calotropis gigantea</i>	415	4th
5.	<i>Musa paradisiaca</i>	413	5th
6.	<i>Achras sapota</i>	401	6th

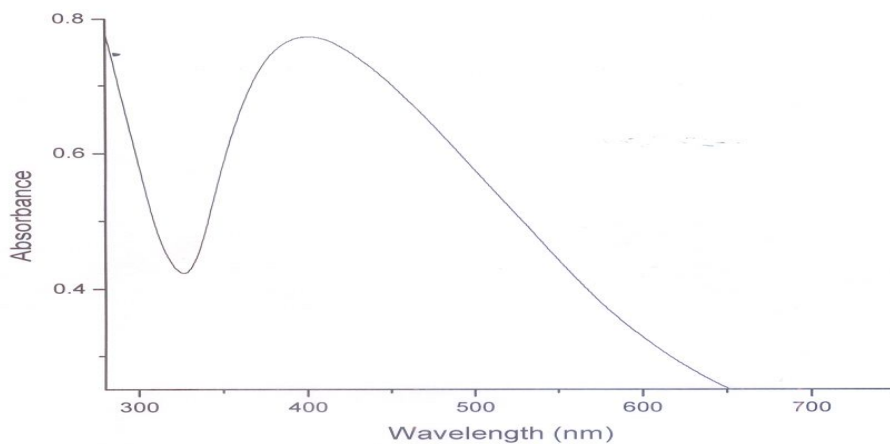
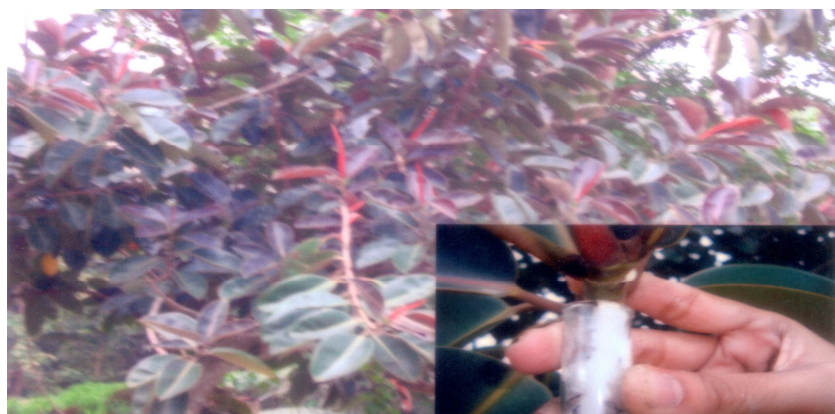


Fig:1 a.Latex collection from *Alstonia scholaris*. b.Absorption peak of silver nano particles using latex of *Alstonia scholaris* under UV- Vis absorption spectroscopy.



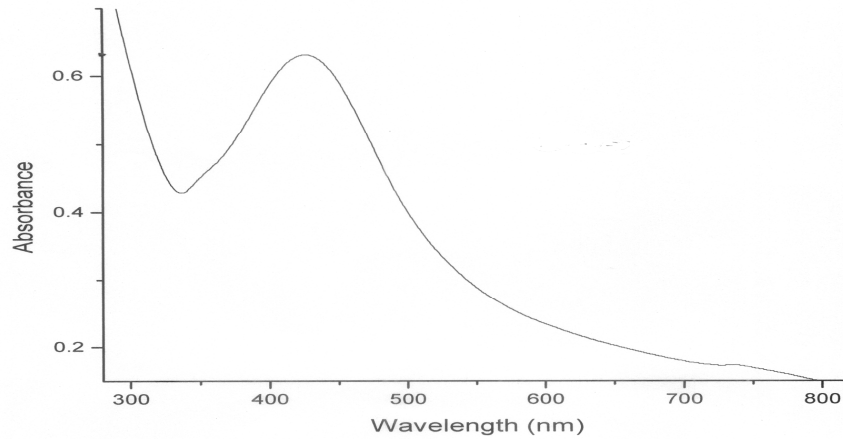


Fig:2 a. Latex collection from *Hevea brasiliensis*. **b.** Absorption peak of silver nano particles using latex of *Hevea brasiliensis* under UV- Vis absorption spectroscopy.

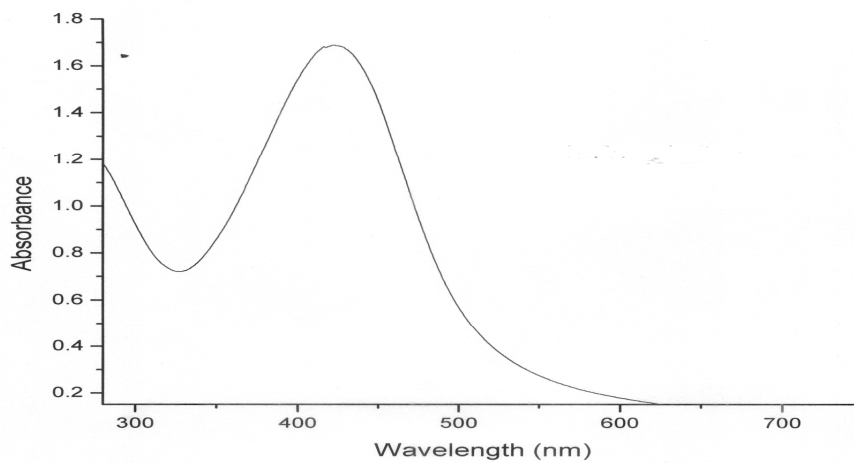


Fig:3 a. Latex collection from *Ficus religiosa*. **b.** Absorption peak of silver nano particles using latex of *Ficus religiosa* under UV- Vis absorption spectroscopy.

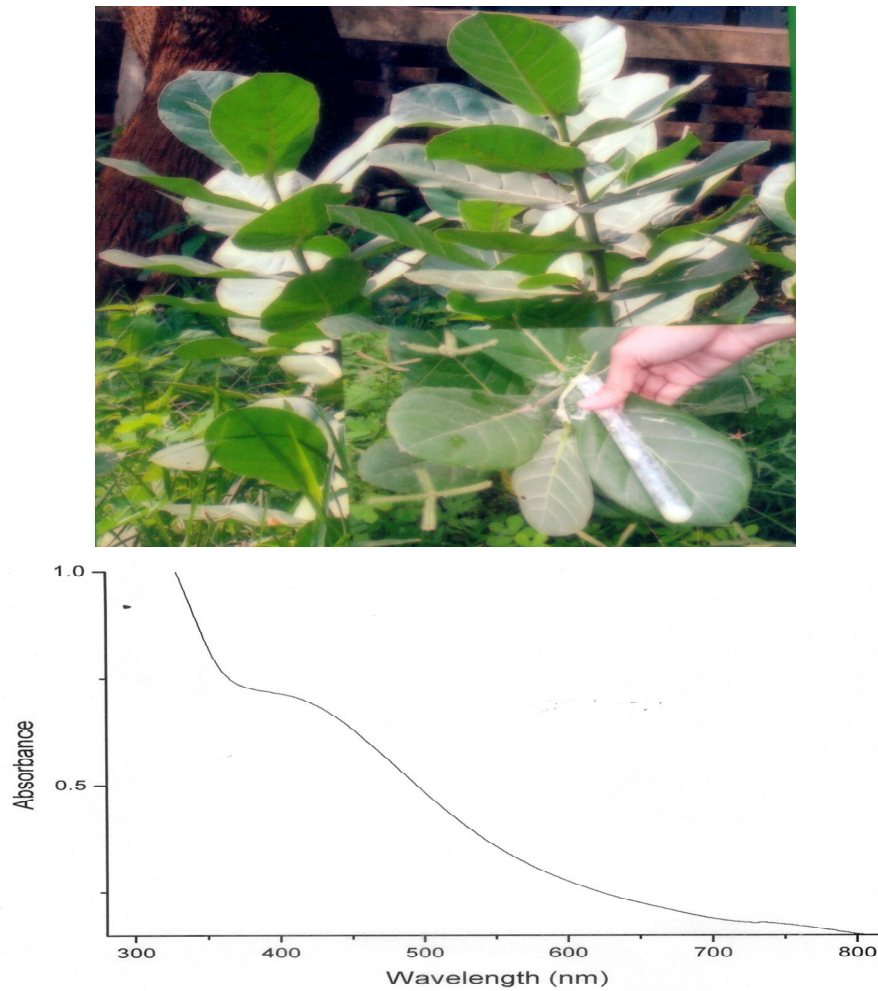


Fig:4 a. Latex collection from *Calotropis gigantea* **b.** Absorption peak of silver nano particles using latex of *Calotropis gigantea* under UV- Vis absorption spectroscopy.\



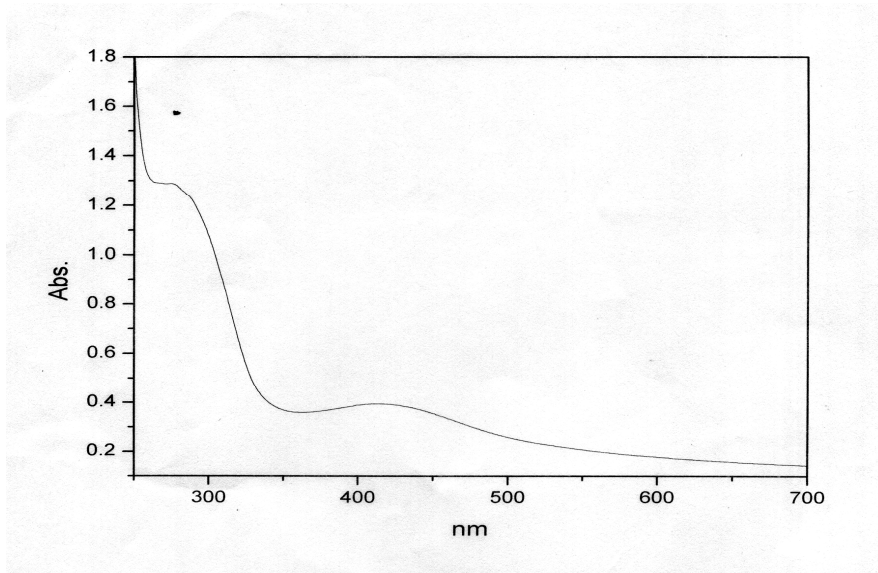


Fig:5- a.Latex collection from *Musa paradisiaca*. b. Absorption peak of silver nano particles using latex of *Musa paradisiaca* under UV- Vis absorption spectroscopy.

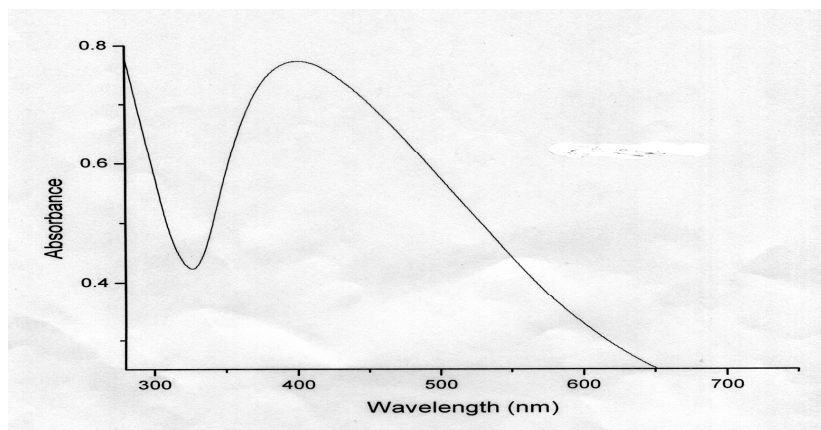


Fig:6- a. Latex collection from *Achras sapota*. b. Absorption peak of silver nano particles using latex of *Achras sapota* under UV- Vis absorption spectroscopy.

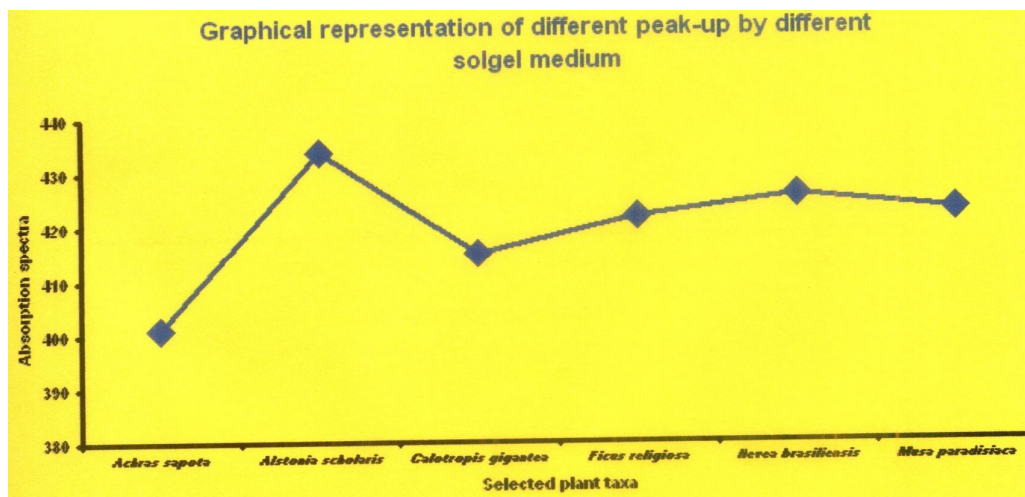


Fig:7- Graphical representation of different peak-up by different sol gel medium.

Synthesis of Ag nanoparticles

In a typical reaction procedure, 3 ml crude latex was diluted to 100 ml using triply distilled de-ionized water to make it 3% and 25 ml of this latex solution was taken in a R. B. Flask and heated at 60°C with constant stirring for 15 min in oil bath. Separately 25ml 2 millimolar aqueous silver nitrate solution was prepared and heated at 60°C with constant stirring for 15 min in oil bath. Then latex solution mixed with it and heated at 80°C for 30 to 45 mins and silver nano particles were obtained gradually. This naturally occurring nanoparticles are generated by the erosion and chemical degradation of plants.

Characterization techniques :

The characterization technique involves ultra-violet and visible spectroscopy UV-Vis absorption spectra were measured using shimadzu UV-1601 spectrometer at (range). We will show the nature of the particle through different absorption peaks of various samples.

RESULT AND DISCUSSION

Silver nanoparticles an effective germ fighter are widely recognized as being especially effective because of their enormously high surface area. Silver nanoparticles do not remain 'nanosize' when they come in contact with normal environmental samples such as water but they agglomerate to form much larger, much effective. There is no possibility that silver nanoparticles can ever form silver ions, except in the presence of strong oxidizing substances. With all the surface area and the energy that exists, the nanoparticles need to be held together 'somehow'. That is where the furry parts of tennis ball come into play as small molecules that hold on to the surface of the particle and stop it from breaking up under its own energy. It is like a tree whose roots can prevent soil erosion because the soil is bonded to the root in the ground. Surface plasmon resonance is a physical process that can occur when plane polarized light hits a metal film under total reflection conditions. This process gives different UV-Vis absorption peak up for different size of nano particle of different solgel medium. Which wavelength of light absorbed by maximum nano particles, that wavelength gives the peak up in UV-Vis absorption spectroscopy.

Surface means surface of metal nano particle, plasmons are electron density and its longitudinal motion, resonance occur when the momentum of incoming light is equal to the momentum of plasmon.

The plasmon resonances in silver nanoparticles depend on two components : absorption and a near field component that evolves into radiative far-field scattering. The relative contribution of these two components is related to the nanoparticle size. In the case of small nanoparticles (<30 nm), absorption generally dominates the extinction spectrum. For larger nanoparticles (>50 nm), the scattering component dominates the plasmon resonance extinction spectrum.

In this experiment which physical properties are shown by silver nanoparticles, are:

This metal with free electron (Ag) possess plasmon resonances in the visible spectrum which give rise to such intense colour i.e. golden yellowish colour.

One of the most interesting aspect of Ag nanoparticle is that their optical properties depend strongly upon the particle size & shape. This optical properties are dominated by the collective oscillation of conduction electrons resulting from the interaction with electro magnetic radiation. Controlling the spontaneous precipitation of silver nanoparticles occur in which medium, is called solgel medium. This controlling procedure is fully successful by different plant latex. When light absorbance capacity of solgel medium is increased, then size of nano particle is increased & when peak height for UV-Vis absorption(nm) is increased, then concentration of nano particles is increased. Nano particles have a large surface area compared with the total volume. The surface area to volume ration is interesting because chemical reactions typically occurs on surfaces, so nano particles that have a high surface to energy ration can be used in many interesting ways such as in catalysis.

CONCLUSION

Nano science is the study of phenomena and manipulation of materials at atomic molecular and macromolecular scales. Now in this science & technology, living organisms show huge potentiality. For detail study and deeply characterization of nano particles, in future X-ray diffraction measurements (X R D), Fourier transform infrared (FTIR) spectroscopy measurements, transmission electron microscopy measurements (TEM) & scanning electron microscopy measuremnts (S E M) may be practiced. So governing factors for the growth of nano structure can be known by those instruments or methods are:-

Kinetics of nucleation & growth,

Concentration of stabilizing agent.

Structure of the stabilizing agent.

Nature of the capping agent.

P^H of the medium employed & temperature.

Basically, nano particle has antimicrobial, antifungal applications, exteracellularly produced silver or gold nanoparticles using *Fusarium oxysporum* can be incorporated in several kinds of materials such as cloths. These cloths with silver nanoparticles are sterile and can be useful in hospitals to prevent or to minimize the infection with pathogenic bacteria such as *Staphylococcus aureus*. Nano particle is also used in biological detection, controlled drug delivery, optical filters, sensor design etc. Silver nanoparticles interact with HIV-1 Virus via preferential binding to the gp 120 glycoprotein knobs. Due to this interaction, silver nanoparticles inhibit the virus from binding to host cells. Bioremediation of radioactive wastes, resulted from nuclear power plants and nuclear weapon production such as Uranium have been achieved by using nano particles cells and S-layer proteins of *Bacillus sphaericus*. Gene expression could be enhanced by increasing the delivery of intact DNA to cell nuclei by producing hybrid polymer protein conjugate nanoparticles.

Last of all, in future, we can detect the phylogenetic relationship of angiosperms depends on the size, concentration or stability of nano particle synthesized by stabilizing agent like enzyme, reducing agent like protein which are most necessary for nano particle synthesis and all are present in plant body. Which plant is able to synthesis larger nano particle than the other plant, that plant is more advanced.

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