

**pH MODULATED GREEN SYNTHESIS OF
SILVER NANO PARTICLES FOR ITS
GERMICIDAL AND ANTI-TOXICANT ACTIVITY**

*Thesis submitted in partial fulfillment of the requirement
For the award of the degree of*

**Master of Science
In**

CHEMISTRY

Submitted by:
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to the



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June 2016**

DECLARATION

I hereby declare that the work being presented in the dissertation entitled "*pH Modulated Green Synthesis of Silver Nanoparticles for its Germicidal and Anti-toxicant Activity*" in partial fulfillment of the requirements for the award of the degree of Masters of Chemistry, School of Chemistry and Biochemistry, Thapar University, Patiala is my own work during the period of January to June 2016, under the supervision of Dr. Diptiman Choudhury. My thesis has not previously formed the basis for award of any degree, or other similar title or recognition.

Patiala

Date: 15th July '16

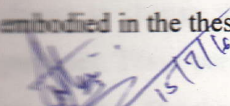


Vanshita Goel

ABSTRACT

CERTIFICATE

This is to certify that the thesis entitled "*pH Modulated Green Synthesis of Silver Nanoparticles for its Germicidal and Anti-toxicant Activity*" by Vanshita Goel (301402023) submitted to School of Chemistry and Biochemistry, Thapar University, Patiala for the Degree of Master of Science is a record of bonafide research work, carried out by her under my supervision. I believe that the thesis fulfils part of the requirements for the award of Master of Science. The results embodied in the thesis have not been submitted for the award of any other degree.


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ACKNOWLEDGMENT

Little achievements often require long, tortuous effort and bitter experiences including some sacrifices. And this is only possible when the almighty GOD keeps his handful of blessings on the head of anybody. I would like to submit everything beneath the feet of GOD.

I would like to acknowledge my approbation & humility to my esteemed faculties and my admired guide Dr. Diptiman Choudhury, Assistant Professor, School of Chemistry and Biochemistry, Thapar University, Patiala, for his constant counseling and proper guidance throughout my project work. I would like to extend all my gratitude to my all faculties Dr. Bonamali Pal, Associate Professor (HOD), School of Chemistry and Biochemistry, Thapar University, Patiala, Dr. Soumen Basu, Dr. Ranjana Prakash Assistant Professors, School of Chemistry and Biochemistry, Thapar University, Patiala, for their constant encouragement. I would like to thank Mr. Amit Mishra, Ms. Akansha Mehta, Ms. Manisha Sharma and Mr. Anirudh Sharma for constant help in this project.

I may be failing in my duties if I do not thank all my batch mates for their constant encouragement. I would more than ever like to thank Pawandeep Kaur for her constant help throughout my work.

Inadequate thought in terms of words and expression, this dissertation owes a lot to my beloved parents for their support, suggestions, inspiration, encouragement & good wishes for the success of my dissertation.



Vanshita Goel

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ABSTRACT

In recent science Nanotechnology is a burning field for the researchers. Nanotechnology deals with the Nanoparticles having a size of 1-100 nm in one dimension used significantly concerning medical chemistry, atomic physics, and all other known fields. Nanoparticles are used immensely due to its small size, orientation, physical properties, which are reportedly shown to change the performance of any other material which is in contact with these tiny particles. These particles can be prepared easily by different chemical, physical, and biological approaches. But the biological approach is the most emerging approach of preparation, because, this method is easier than the other methods, eco friendly and less time consuming.

The present work investigates the synthesis of silver nanoparticles (Ag NPs) by biological method using *Ocimum sanctum*. Silver nanoparticles were successfully synthesized from Tulsi by green synthesis method at different pH range (pH 4, 5, 7, 9, 11). The detailed characterization of the Ag NPs was carried out using UV-visible spectroscopy, Scanning Electron Microscopy (SEM), TEM (Transmission Electron Microscopy), Raman Spectroscopy, DLS and FTIR. From the UV-visible spectroscopy, the absorption peak was found at 435 nm. From the SEM images, it is confirmed that the sample contains spherical silver nanoparticles. The antibacterial activity of silver nanoparticles was determined by batch culture method, and found that silver nanoparticles have significant antibacterial activity against *E. coli* and *Bacillus sphaericus*. Finally, the adsorption of dyes and reduction of pesticides was done at all different pH range. The result came to be positive which showed inhibition of bacterial growth, adsorption of both cationic and neutral dyes and reduction of the pesticides.

OBJECTIVE

The principal objective of this research work is:

- Synthesis of AgNPs from silver salts using biological route
- Characterization of synthesized silver nanoparticles
- Determination of the size and shape of AgNPs
- Study of its antimicrobial activity against a few common bacteria
- Adsorption of cationic and neutral dyes
- Reduction of pesticides with temperature and time dependent parameters

INTRODUCTION

NANOPARTICLES: Building Blocks for Nanotechnology [1]

Nanoparticles serve as the fundamental building blocks for various nanotechnology applications. Nanotechnology, and alongside nanostructure materials, play an ever increasing role in science, research and development as well as also in every day's life, as more and more products based determined the physical and chemical properties of materials undergo extreme changes, which open up a wide range of future, but partly already realized applications. There are two more forces driving towards nanotechnology, mostly biomolecules and other bioentities are of nanometer size; thus the nanoscale provides the best opportunity to study such bio-entities and their interactions with other materials. Another impetus is semiconductor industry which, by its ever-lasting demand for miniaturization, has been driven deeply into the nano-realm. Nanotechnology deals with materials have dimensions in the nanometer range (<100 nm). They may consist of single, isolated nanoparticles, of assemblies of such nanoparticles, of thin films which are two-dimensional nanostructures, of composites in which the constituents are of nanosize dimensions (nanocomposites). Nanosized structures created by lithography and etching as for example semiconductor devices, or of structures created by self-organization of individual nanostructures. Nanoparticles are seen as solution to many technology and environmental challenges. By green synthesis methods of NPs reduced hazardous to the global efforts [2]. Implantation of these sustainable processes should adopt the fundamental principles of green chemistry. These principles emphasize on maximizing the efficiency of chemical processes without compromising safety concern of the products. The physical, chemical and biological properties of the nanoparticle change in fundamentally from the properties of both individual atoms/molecules and of the corresponding bulk material. Diverse chemical nature of nanoparticles, including: metals, metal oxides, non-oxide ceramics, carbon and biomolecules. Nanoparticles have several different morphology forms such as spheres, cylinders, platelets, tubes, etc. Consequently, they are designed with surface modifications tailored to meet the needs of specific applications they are going to be used for. The various diversity of the nanoparticles (Figure 1) arising from their wide chemical nature, shapes and morphologies, the medium in which the particles are present, the state of dispersion of the particles and most importantly, the several possible surface modifications the nanoparticles can be subjected to make this an important active field of science now a days.

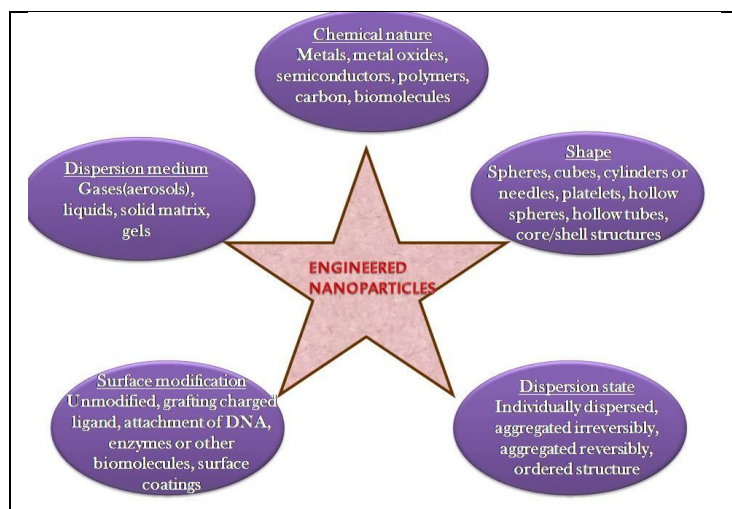


Fig1: Various features contributing to the diversity of engineered nanoparticles. The same chemical can generate a wide variety of nanoparticles.

Classification of nanoparticles

Nanoparticles fall into two categories: mainly organic and inorganic nanoparticles. Organic nanoparticles may include carbon nanoparticles (fullerenes) in other hand, inorganic nanoparticles may include magnetic nanoparticles, noble metal nanoparticles (like gold and silver) and semiconductor nanoparticles (like titanium dioxide and zinc oxide). There is a growing interest in inorganic nanoparticles as they provide superior material properties with functional versatility, have been examined as potential tools for medical imaging as well as for treating diseases due to their size features and advantages over available in chemical imaging drugs agents and drugs. When mesoporous silica combined with molecular machines prove to be excellent imaging and drug releasing systems. Gold nanoparticles have been used extensively in imaging, as drug carriers and in thermo therapy of biological targets [3]. Inorganic nanoparticles (metallic and semiconductor nanoparticles) exhibit intrinsic optical properties which may enhance the transparency of polymer- particle composites. For that reasons, inorganic nanoparticles have found special interest in studies devoted to optical properties in composites. Size dependent color of gold nanoparticles has been used to color glass for centuries [4]. Inorganic nonmaterial have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good compatibility, and capability of targeted drug delivery and controlled release of drugs [5].

Properties of Nanoparticles

The presence of a high fraction of atoms/molecules constituting the nanoparticle on the particle surface rather than in the particle interior and the immense surface area available per unit volume of the material is the most significant consequences for the nanoparticles. Both of these properties increase in magnitude with a decreasing particle size. As a result of the unique physical, chemical and biological properties of nanoparticles originate from these two features. Quantum effects of some nanoscale materials are exhibited allowing for a number of interesting applications. The large specific surface area of nanoparticles including: the origin of a number of their unique applications. Catalysis is enhanced by high surface area per unit volume and the homogenous distribution of nanoparticles. However, high surface areas give strong interactions between the nanoparticles and the solid matrix in which they may be incorporated. The platelet morphology and large specific surface areas of silicate nanoparticles particles enhance the barrier properties of polymer membranes by vastly increasing the pathway for molecular transport of permeating substances.

Physical and Chemical Properties of nanoparticles

The principal parameters of nanoparticles are their shape (including aspect ratios where appropriate), size, and the morphological sub-structure of the substance. Indirectly chemical agents can stabilize against coagulation or aggregation by conserving particle charge and by modifying the outermost layer of the particle. Depending on the growth characteristics and the lifetime of a nanoparticle very complex compositions will be possibly with complex mixtures of adsorbate. At the nanoparticle - liquid interface, polyelectrolytes have been utilized to modify surface properties and the interactions between particles and their environment. They have been used in a wide range of technologies, including adhesion, stabilization, and controlled flocculation of colloidal dispersions [6].

Nanoparticle - Nanoparticle Interaction

At the nanoscale level, particle-particle interactions are either dominated by weak Van der Waals forces, stronger polar and electrostatic interactions or covalent interactions. In suspended in air, nanoparticles charges can be accumulated by physical processes such as glow discharge or photoemission. In case of liquid, particle charge can be stabilized by electrochemical processes at surfaces. The nanoparticle - nanoparticle interaction forces and nanoparticle - fluid interactions are importance to describe physical and chemical processes, and the temporal evolution of free nanoparticles. The interaction forces, either attractive or repulsive, that determine the rate of individual and collective nanoparticles.

APPLICATIONS OF NANOPARTICLES

Medicine

Once materials are prepared in the form of very small particles, for that they change significantly their physical and chemical properties. Nano-dimension, percentage of surface molecule compare to bulk molecule is high and this enhances the activity of the particle in nano dimension and the normal properties of the particle like heat treatment, mass transfer, catalytic activity, etc are all increases. But as compare to non-metal nanoparticles, metal nanoparticles have more industrial application. Many new developments are offer by nanoparticles in the field of biosensors, biomedicine and bio nanotechnology-specifically in the areas:

- Drug delivery
- As medical diagnostic tools
- As a cancer treatment agent (Gold nanoparticles).

Nanoparticles and nanostructure are developing in human medical application, including imaging or the delivery of therapeutic drugs to cell, tissues and organs. Many drug loaded nanoparticles are interacts with organ and tissues and are taken up by cells. Several studies have shown that the tissue, cell and even cell organelle distribution [6-7] of drugs may be controlled and improved by their entrapment in colloidal nonmaterial's, like micelle structure, such as nanocontainer. Magnetic nanoparticles have wide range of applications, such as the immobilization of the proteins and enzymes, bioseparation, immunoassays, drug delivery, and biosensors [8]. Nanoparticles of ferromagnetic materials are of important, of their reduced sizes that can support only single magnetic domains. Due to their potential technological application as recording media in synthesis of arrays of 4 nm diameter FePt nanoparticles with an extremely narrow size distribution has promoted a significant research effort.

Environment

Researchers are using photo catalytic copper tungsten oxide nanoparticles to break down oil into biodegradable compounds. The nanoparticles are in a grid that provides high surface area for the reaction is activated by sunlight and can work in water, making them useful for cleaning up oil spills. Iron nanoparticles are being used to clean up carbon tetrachloride pollution in ground water. Iron oxide nanoparticles are being used to clean arsenic from water wells.

Commercial application of Nanoparticles

Existing and potential applications involving nanoparticles are almost endless. The amount of nanoparticles usage in different applications may vary significantly. The Project on Emerging Nanotechnologies lists more than 470 products in May 2007, that are verified to include some

form of nanotechnology, the number being double that was reported the same time in 2006. The positive view with which the nanoproducts are being greeted is reflected in the explicit choice of the term 'nano' in the names of many commercial products, for example, Nano & UV Artificial Teeth Cleaner, Nano Air Filter, Nano Anti-Aging Cream, Nano B-12 Vitamin Spray, Nano Bag, Nano Cold Catalyst Air Purifier, Nano Pacifier, Nano Trousers, Nanoceuticals Artichoke Nanoclusters.

Table 1: Present and Potential Applications of Nanoparticles

Biomedical	<ul style="list-style-type: none"> • Antibacterial creams and powders (Ag) • Biocompatible coatings for implants • Bio labeling and detection (Au, Ag, Quantum dots) • Cancer diagnostics and targeted drug delivery (magnetic nanoparticles) • Cell, receptor, antigen, enzyme imaging (Quantum dots) • Fungicides (ZnO, Cu₂O) • Gene delivery (CNT)
Consumer Goods and Personal Care Products	<ul style="list-style-type: none"> • Anti-bleaching, scratch resistance additives in paints • Anti-scratch coated tiles (alumina) • Barrier packaging (silicates) • Glass coatings for anti-glare, anti-misting mirrors (TiO₂) • Skin creams with antioxidant vitamins (nano capsules)
Electronics and Computers	<ul style="list-style-type: none"> • Chemical mechanical planarization (alumina, silica, ceria) • Coatings and joining materials for optical fibers (Si) • Conductive coatings/fabrics (rare-earth-doped ceramics) • Display technologies (conducting oxides) • Electronic circuits (Cu, Al)
Engineering Materials	<ul style="list-style-type: none"> • Cutting tool bits (Al₂O₃, ZrO₂, WC, TaC) • Thermal spray coating techniques (TiO₂, TiC-Co) • Flame retardant polymer formulations (nano clay) • Lubricants and sealants/hydraulic additives (Cu MoS)
Environmental	<ul style="list-style-type: none"> • Self-cleaning glass (TiO₂ based coatings) • Soil remediation (Fe) • Water treatment (photo-catalyst treatments, TiO₂)
Food	<ul style="list-style-type: none"> • Flavors and colors in food and beverages (nanocapsules) • Food packaging materials (SiO₂, TiO₂, Ag)
Power and Energy	<ul style="list-style-type: none"> • Dye-sensitized solar cells (TiO₂, ZnO, Au) • Environmental catalysts (TiO₂, CeO₂ as diesel additive)
Transportation	<ul style="list-style-type: none"> • Battery technology • High strength, light weight composites for • Increasing fuel • Efficiency

Silver Nanoparticles

Silver nanoparticles are of interest because of the unique properties (*e.g.*, size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles [9-10]. The most popular chemical approaches, including chemical reduction using a variety of organic and inorganic reducing agents, electrochemical techniques, physicochemical reduction, and radiolysis are widely used for the synthesis of silver nanoparticles. Recently, nanoparticle synthesis is among the most interesting scientific areas of inquiry, and there is growing attention to produce nanoparticles using environmentally friendly methods (green chemistry). Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens, biological, and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity. This chapter presents an overview of silver nanoparticle preparation by physical, chemical, and green synthesis approaches. The aim of this chapter is, therefore, to reflect on the current state and future prospects, especially the potentials and limitations of the above mentioned techniques for industries. Moreover, we discuss the applications of silver nanoparticles and their incorporation into other materials, the mechanistic aspects of the antimicrobial effects of silver nanoparticles.

The optical properties of silver nanoparticles were used by glass founders as far back as in the time of the Roman Empire. That evidenced, so-called Lycurgus cup (4th century AD) now exposed in the British Museum. A detailed study of the composition of its bronze-mounted insets of stained glass, carried out in the late 20th century, revealed the presence of metal nanoparticles (with the average diameter of 40 nm) that consists of silver (70 %) and gold (30 %) alloy [13]. It explained the remarkable feature in bowl to change its color from red in transmitted light to grayish green in reflected light. Before the 1980s, the scientific and practical interest in silver nanoparticles was exclusively caused by the possibility of their use as highly dispersed supports for enhancing the signals from organic molecules in the Raman spectroscopy [14-15]. Fundamental studies carried out in the last three decade shows that silver nanoparticles exhibit a rare combination of valuable properties including, unique optical properties associated with the surface Plasmon resonance (SPR), well-developed surfaces, catalytic activity, high electrical double layer capacitance, etc. [16]. For that reason silver nanoparticles serve as a material in the development of new-generation electronic, optical and sensor devices. In the past 20 years, the trend miniaturization and the necessity of modernization of technological processes led to the substantial increase in the number of scientific publication devoted to the synthesis and properties of silver nanoparticles. Silver is used as a catalyst for the oxidation of methanol to formaldehyde and ethylene to ethylene oxide [17]. This band is attributed to collective excitation

of the electron gas in the particles, with a periodic change in electron density at the surface [16, 18]. Some studies showed that use of a strong reductant such as borohydride, resulted in small particles that were somewhat monodisperse, but the generation of larger particles was difficult to control [14, 19]. Use of a weaker reductant such as citrate, resulted in a slower reduction rate, but the size distribution was far from narrow [20-21]. Synthesis of Ag NPs is based on a two steps reduction process [19]. In this technique a strong reducing agent is used to produce small Ag particles, which are enlarged in a secondary step by further reduction with a weaker reducing agent [15]. Different studies reported the enlargement of particles in the secondary step from about 20–45 nm to 120–170 nm [19, 20, 22].

NANOSILVER:

One of the substances used in nanoformulation is silver (nanosilver). Due to its antimicrobial properties, silver has also been incorporated in filters to purify drinking water and clean swimming pool water. To generate nanosilver, metallic silver has been engineered into ultrafine particles by several methods; include spark discharging, electrochemical reduction, solution irradiation and cryo- chemical synthesis [23]. Nano-silver particles are mostly smaller than 100 nm and consist of about 20-15,000 silver atoms [23]. In addition, nanostructures can be produced as tubes, wires, multifacts or films. At the nano-scale, the silver particles exhibit deviating physico-chemical properties (like pH dependent partitioning to solid and dissolved particulate matters) and biological activities compared with the regular metal [24]. This is due to the higher surface area per mass, allowing a larger amount of atoms to interact with their surroundings. Due to the properties of silver at the nanoscale, nanosilver is nowadays used in an increasing number of consumer and medical products. Because, silver is a soft white lustrous element, an important use of silver nanoparticles is to give a product's a silver finish. Still, the remarkably strong antimicrobial activity is the major direction for development of nano-silver products. Examples are food packaging materials and food supplements, odor-resistant textiles, electronics, household appliances, cosmetics and medical advices, water disinfectants and room sprays.

WHY SILVER?

Silver is one of the basic elements that make up our planet. It is a rare, but naturally occurring element, slightly harder than gold and very ductile and malleable. Pure silver has the highest electrical and thermal conductivity of all metals and has the lowest contact resistance. Silver can be present in four different oxidation states: Ag^0 , Ag^{2+} , Ag^{3+} . The former two are the most abundant ones, the latter are unstable in the aquatic environment [25]. Metallic silver itself is insoluble in water, but metallic salts such as AgNO_3 and Silver chloride are soluble in water. 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 gonorrhoea [25]. Although acute toxicity of silver in the environment is dependent on the availability of free silver

ions, investigations have shown that these concentrations of Ag⁺ ions are too low to lead toxicity. Metallic silver appears to pose minimal risk to health, whereas soluble silver compounds are more readily absorbed and have the potential to produce adverse effects [26]. 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 70-90µg/day. Since silver in any form is not thought to be toxic to the immune , cardiovascular, nervous or reproductive system it is not considered to be carcinogenic [27], therefore silver is relatively non-toxic [28]. Silver demand will likely to rise as silver find new uses, particularly in textiles, plastics and medical industries, changing the pattern of silver emission as these technologies and products diffuse through the global economy [25].

Synthesis of Ag nanoparticles

Material Scientists researcher is conducting to develop novel materials, with better properties, functionality and lower cost than the existing ones. Different physical, chemical and biological synthesis methods have been developed to enhance the performance of nanoparticles displaying improved properties with the aim to have a better control over the particle size, distribution and morphology [29, 30, and 31]. Synthesis of nanoparticles to have a better control over particles size, distribution, morphology, purity, quantity and quality, by employing as environment friendly economical processes has always been a challenge for the researchers.

METHODS FOR SILVER NANOPARTICLE SYNTHESIS:

Physical approaches:

Most important physical approaches include evaporation-condensation and laser ablation. Various metal nanoparticles such as silver, gold, lead sulfide, cadmium sulfide, and fullerene have previously been synthesized using the evaporation-condensation method. The absence of solvent contamination in the prepared thin films and the uniformity of nanoparticles distribution are the advantages of physical approaches in comparison with chemical processes. [33-34]. It was demonstrated that silver nanoparticles could be synthesized via a small ceramic heater with a local heating source [32]. The evaporated vapor can cool at a suitable rapid rate, because the temperature gradient in the vicinity of the heater surface is very steep in comparison with that of a tube furnace. This makes possible the formation of small nanoparticles in high concentration. This physical method can be useful as a nanoparticle generator for long-term experiments for inhalation toxicity studies, and as a calibration device for nanoparticle measurement equipment [32]. Silver nanoparticles could be synthesized by laser ablation of metallic bulk materials in solution [35-36]. The ablation efficiency and the characteristics of produced nanosilver particles

depend upon many factors such as the wavelength of the laser impinging the metallic target, the duration of the laser pulses (in the femto-, pico- and nanosecond regime), the laser fluence, the ablation time duration and the effective liquid medium, with or without the presence of surfactants [37-38]. One important advantage of laser ablation technique compared to other methods for production of metal colloids is the absence of chemical reagents in solutions. Therefore, pure and uncontaminated metal colloids for further applications can be prepared by this technique [39].

Chemical approaches:

The most common approach for synthesis of silver nanoparticles is chemical reduction by organic and inorganic reducing agents. In general, different reducing agents such as sodium citrate, sodium borohydride (NaBH_4), elemental hydrogen, Tollens reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers are used for reduction of silver ions (Ag^+) in aqueous or non-aqueous solutions. The aforementioned reducing agents reduce silver ions (Ag^+) and lead to the formation of metallic silver (Ag^0), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to formation of metallic colloidal silver particles [40-51]. It is important to use protective agents to stabilize dispersive nanoparticles during the course of metal nanoparticle preparation, and protect the nanoparticles that can be absorbed on or bind onto nanoparticle surfaces, avoiding their agglomeration [40]. The presence of surfactants comprising functionalities (*e.g.*, thiols, amines, acids, and alcohols) for interactions with particle surfaces can stabilize particle growth, and protect particles from sedimentation, agglomeration, or losing their surface properties. Recently, a simple one-step process, Tollens method, has been used for the synthesis of silver nanoparticles with a controlled size. In the modified Tollens procedure, silver ions are reduced by saccharides in the presence of ammonia, yielding silver nanoparticle films (50-200 nm), silver hydrosols (20-50 nm) and silver nanoparticles of different shapes [42].

Biological approaches:

In recent years, the development of efficient green chemistry methods employing natural reducing, capping, and stabilizing agents to prepare silver nanoparticles with desired morphology and size have become a major focus of researchers. Biological methods can be used to synthesize silver nanoparticles without the use of any harsh, toxic and expensive chemical substances [43-44]. The bioreduction of metal ions by combinations of biomolecules found in the extracts of certain organisms (*e.g.*, enzymes/proteins, amino acids, polysaccharides, and vitamins) is environmentally benign, yet chemically complex. Many studies have reported successful synthesis of silver nanoparticle using organisms (microorganisms and biological systems) [45-46].

Green Synthesis of Nanoparticles

The production of nanoparticles by living organisms or material of biological origin for example, nanoparticles may be synthesized using living bacteria or fungi, or using plant extracts. That techniques provided an advantages over more traditional methods of synthesizing nanoparticles because they are environmentally friendly, can take place around room temperature or lower, and require little intervention or input of energy. In these methods, organisms involved are generally easily cultured in simple organic media, are a renewable resource, and can usually simply be left to do their work. Various organisms could synthesize inorganic particles, including silica and calcium carbonate, or chalk, that has long been known. Many more microorganisms are able to reduce metal ions to metal. Several bacteria can produce magnetic material by the reduction of iron compounds, incorporating magnetic nanoparticles into bodies known as magnetosomes within their cells. Some types of bacteria have been successfully employed in the biosynthesis of nanoparticles. This can take place both intracellularly and extracellularly. It is thought that the bacteria use the nitrate anion (NO_3^-) as a source of nitrogen, leaving metallic silver. Fungi and flowering plants been used experimentally for synthesize nanoparticles.

NEED FOR GREEN SYNTHESIS:

Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. The need for biosynthesis of nanoparticles rose as the physical and chemical processes were costly. Often, chemical synthesis method leads to presence of some of the toxic chemical absorbed on the surface that may have adverse effect in the medical applications [47]. This is not an issue when it comes to biosynthesized nanoparticles via green synthesis route [48]. So, in the search of cheaper pathways for nanoparticles synthesis, scientist used microbial enzymes and plant extracts (phytochemicals). With their antioxidant or reducing properties they are usually responsible for the reduction of metal compounds into their respective nanoparticles. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals.

APPLICATION OF SILVER NANOPARTICLES

Human Health

The production of nanoparticles has many different effects on human health relative to bulk material [49]. Increase the biological activity of nanoparticles can be determined beneficial, detrimental or both. Nanoparticles are enough to access to skin, lungs, and brain [50-51]. Exposure of metal containing nanoparticles to human lung epithelial cells generated reactive oxygen species, which lead to oxidative stress and damage of the cells [52]. A study on toxic

effects of silver nanoparticles was done, that results show a deposition of particles on organs and severe developmental effects. The biocompatibility and toxicity of silver nanoparticles were exhibited at each development stage by observing single silver nanoparticle inside embryos.

Environment

Silver nanoparticles have a great concern to wastewater treatment utilities and to biological systems. The inhibitory effects of silver nanoparticles on microbial growth were evaluated at a treatment facility using an extant respirometry technique. The nitrifying bacteria were susceptible to inhibition by silver nanoparticles, which could have detrimental effects on the microorganisms in wastewater treatment. The environmental risk of silver nanoparticles was recently investigated by determining released silver from commercial clothing. The sock material and wash water contained silver nanoparticles of 10–500 nm diameter.

Catalytic Action

Due to high surface area and high surface energy predetermine metal nanoparticles for being effective catalytic medium. Growing small particles of silver have been observed to be more effective catalysts than stable colloidal particles. These growing particles catalyzed the borohydride reduction of several organic dyes. The reduction rate catalyzed by growing particles is distinctly faster compared to that of stable and larger silver particles, which are the final products of growing particles. Catalysis is due to efficient particle-mediated electron transfer from the BH₄⁻ ion to the dye. The catalytic activity of the particles depends on their size, $E^{1/2}$ of the dye, and the dye-particle interaction [53]. Catalytic activity of silver nanoparticles can be controlled by its size, as redox potential depends on the nanoparticle size [53].

Antimicrobial activity of silver nanoparticles

Due to the non-toxic, safe inorganic antibacterial agent of silver nanoparticles being used for centuries and incapable of killing about 650 microorganisms that cause diseases [54]. Silver has been described as being ‘oligodynamic’, that is, its ions are capable of causing a bacteriostatic (growth inhibition) or even a bactericidal (antibacterial) impact. Therefore, it has the ability to exert a bactericidal effect at minute concentration [55]. It has a significant potential for a wide range of biological application such as antibacterial agents for antibiotic resistant bacteria, preventing infections, healing wounds and anti-inflammatory [56]. Silver ions (Ag⁺) and its compounds are highly toxic to microorganism exhibiting strong biocidal effect on many species of bacteria but have a low toxicity towards animal cells. Bactericidal behavior of nanoparticles is attributed to the presence of electronic effects that are brought about as a result of change in local electronic structure of the surface due to smaller sizes. The effects are considered to be contributing towards enhancement of reactivity of silver nanoparticles surface. Silver in ionic form strongly interacts with thiol groups of vital enzymes and inactivates them. That lead DNA loses its replication ability once the bacteria are treated with silver ions [57]. Silver nanoparticles destabilize plasma membrane potential and depletion of levels of intracellular adenosine

triphosphate (ATP) by targeting bacterial membrane resulting in bacterial cell death. Compounds of silver such as silver nitrate and silver sulfadiazine are used to prevent bacterial growth in drinking water, sterilization and burn care.

Silver acts as odor controlling agent

Due to effective antimicrobial agent activity of silver nanoparticles provide terrific driving force for diffusion. The Silver nanoparticles are assembled with many different shapes, such as spheres, rods, cubes, wires, film, and coatings and can be integrated into a variety of materials like metals, ceramics, polymers, glass, and textiles via fine spraying of silver nanoparticle solution. Some Athletic clothing companies have incorporated silver nanoparticles into their products mainly for reduce the bad smell. Many textile industries insert silver nanoparticles into its products to allow the particles to attach to the filaments. Once the silver nanoparticles encounter sweat from the human body, they naturally release a low concentration of silver ions into the moist environment.

Silver as Bactericidal agent

Nanometers size of bacteria, use enzymes to metabolize nutrients and create energy in a similar fashion as living organisms. They are unicellular with only one compartment of protein and they store all the elements of the cell. Thus, in order to stop the exponential rate of bacterial replication and disrupt the bacterial enzymes and energy metabolism. Silver ions attack microbes in three different pathways including; respiration, replication, and cell wall synthesis. Silver nanoparticles penetrate the bacterial cell membrane and change their structural composition by interacting with the bacteria's sulfate groups, which are the active site of enzymes. Silver ions disrupt the bacterial enzymes responsible for energy metabolism and electrolyte transport. The lack of enzyme activity ultimately suffocates the bacteria. As an additional means of attack, these powerful silver ions also detached the bacterial replication process by disrupting their DNA backbone finally creating structural imperfections within the cell's protective layers and speeding the collapse or burst of the bacteria [58]. Therefore, by targeting these three areas, silver ions prevent bacteria proliferation by establishing a defense system, slowing bacterial growth, and eventually killing them.

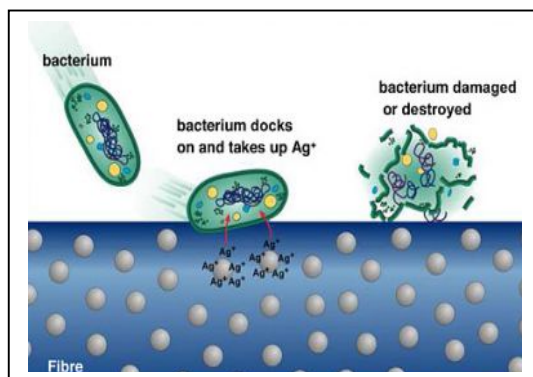


Fig2: When bacteria take up silver nanoparticles in its cell wall damaged the cell wall of those bacteria and the bacteria are killed.

TULSI SILVER NANOPARTICLES

Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals as well as provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganisms isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms. In this, we report rapid biosynthesis of silver nanoparticles using *Ocimum sanctum* leaf extract.

Herbal medicine also called botanical medicine or phytomedicine refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. As improvement in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing disease.

According to the ayurveda, plants have so many constituents which may be used for the treatment of so diverse ailments. Herbs had been used by all cultures throughout history but India has one of the oldest, richest and most diverse cultural living traditions associated with the use of medicinal plants.

Ocimum is a genus of about 35 species of aromatic annual and perennial herbs and shrubs in the family Lamiaceae, mostly native to the tropical and warm temperate regions of the Old World. Some medicinally important species includes.

Various Species of Tulsi

Ocimum americanum (syn. *O. canum*) is a native of tropical Africa. It is known as lime, hairy or hoary basil, is an annual herb with white or lavender flowers. It is used for medicinal purposes. Plant shows antimicrobial and antioxidant activity. *Ocimum americanum* contains volatile oil, flavanoids, carbohydrates, phytosterols, tannins and fixed oils.

Ocimum basilicum or sweet basil, a culinary herb. Most culinary and ornamental basil and there are many hybrids between species. *O. basilicum* var. *thyrsiflora*, or Thai basil, *O. basilicum* is a common ingredient in Thai cuisine, with a strong flavor similar to aniseed, used to flavour curries and stir-fries. It has been used as a folk remedy for an enormous number of ailments, including boredom, cancer, convulsion, deafness, diarrhoea, epilepsy, gout, hiccup, impotency, insanity, nausea, sore throat, toothaches, and whooping cough. Basil has been reported in herbal publications as an insect repellent.

Ocimum gratissimum, also known as African basil, or African Basil Wild basil in Hawaii, where it has naturalized. *Ocimum gratissimum*, also known as Clove Basil, *Ocimum gratissimum*

contains antibacterial, antidiabetic, Antitumor, anti-cancer, diarrhea, anti-fertility, hepatoprotective and analgesic activity and gastrointestinal disorders.

Ocimum micranthum, or Amazonian basil, is a South American variety often utilized in ayahuasca rituals for its smell which is said to help avoid bad visions. It possesses antibacterial, antiprotozoal and antioxidant activity.

Ocimum tenuiflorum (also known as tulsi, tulasī, or Holy Basil) is an aromatic plant in the family Lamiaceae which is native throughout the Old World tropics and widespread as a cultivated plant and an escaped weed. It is an erect, much branched sub shrub 30–60 cm tall with hairy stems and simple opposite green leaves that are strongly scented. Leaves have petioles, and are ovate, up to 5 cm long, usually slightly toothed. Flowers are purplish in elongate racemes in close whorls. There are two main morphotypes cultivated in India—green-leaved (Sri or Lakshmi tulsi) and purple-leaved (Krishna tulsi). It possess antioxidant and also used in memory improvement.

Ocimum sanctum, known as tulsi in Hindi and holy basil in English, is an erect softy hairy aromatic herb or undershurb found throughout India. Tulsi is commonly cultivated in gardens. Two type of *Ocimum sanctum* are met within cultivation i.e. tulsi plant with green leaves known as sri tulsi & tulsi plant with purple leaves known as Krishna tulsi. *Ocimum sanctum* is held sacred by Hindus and is used as medicinal plants in day to day practice in Indian homes for various ailments. It shows a number of medicinal activities. It used in anticancer, antifertility, antidiabetic and various other diseases.

Ocimum kilimandscharicum is an economically important medicinal perennial herb that is widely distributed in East Africa, India and Thailand. It is extensively grown in the Tropics.

In this study, biosynthesis of stable silver nanoparticles was done using Tulsi (*Osmium sanctum*) leaf extract. Tulsi is also known as “the elixir of life” since it promotes longevity. It has been established that the chemical constituents containing –OH, –SH, –COOH, and –NH₂ functional groups of various parts of natural plants, such as leaves, roots, flowers, fruits, bark and seeds are acted as a reducing and capping agents for the green synthesis of biogenic silver nanoparticles owing to its environmental friendly nature. Plant extract are less toxic and require minimum purification when compared to chemical methods. Silver nanoparticles have a high specific surface area and a high fraction of surface atoms. Because of the unique physicochemical characteristics of nanoparticles, including catalytic activity, antibacterial property, biosensors and antipesticial property. *Ocimum sanctum* (tulsi, belong to Lamiaceae family) is held blessed by Hindus in India and is used as medicinal plants in homes for various ailments. Recent study reveals that Tulsi is a powerful inhibitor because of its high content of eugenol and also shows protection against radiation poisoning. Some of the main chemical constituents of tulsi are—

oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, b-elemene (has anti-cancer properties), b-caryophyllene and germacrene D. urosolic acid which is one of the constituents of tulsi is involved in reducing the free radical induced damage by various drugs. Biogenic, biocompatible and inert nanomaterials have potential applications in the detection, diagnosis, therapy and monitoring the growth of cancer and tumor.

Chemical Composition of Tulsi (*Ocimum sanctum*)

Ocimum sanctum (tulsi, belong to Lamiaceae family) is held blessed by Hindus in India and is used as medicinal plants in homes for various ailments. Biogenic, biocompatible and inert nanomaterials have potential applications in the detection, diagnosis, therapy and monitoring the growth of cancer and tumor. Aqueous extract of leaves contains camphor, 1, 8-cineole, limonene, trans caryophyllene, camphene, myrtenol, a-terpineol, endo-borneol, linalool, flavonoids, tannins, saponins, sterols, carbohydrates, proteins and triterpenoids. Structures of some constituents are given. Out of these constituents, Linalool, and rosmarinic acid are water soluble, where as others are water insoluble, which were responsible for various biological activities. In this, we report the synthesis, characterization and mechanism of AgNPs formation using leaves extract derived from *Ocimum sanctum*. In addition, analysis of their antimicrobial activity against Gram-positive (*Bacillus sphaericus*) and Gram-negative (*Escherichia coli*) bacteria has been carried out and discussed.

LITERATUE REVIEW

There is an increasing demand of silver nanoparticles due to their unique properties and applications in various fields such as Medicine, catalysis, nanoelectronics, textile, field, pollution and water treatment. Nanosilver strong antimicrobial activity is the major reason for the development of nanosilver containing products. The silver nanoparticles are synthesis by various chemical and physical methods .The major drawbacks of chemical and physical methods are the synthesis process are expensive and many toxic chemicals are used. To overcome these problems Green Nanotechnology comes to play very crucial role for synthesis of silver nanoparticles. Use of various plants for synthesis of biogenic silver nanoparticles referred as Green Nanotechnology. In the present study, we reported the green synthesis of silver nanoparticles using by *Ocimum sanctum* (Tulsi) leaf extract in the exposure direct sunlight atmosphere without using any chemical additive. The *Ocimum sanctum* (Tulsi) leaf extract act as reducing agent as well as capping agent. Developed AgNPs were duly characterized and tested for their antibacterial activity. The stabilized Ag NPs were then characterized by the ultraviolet-visible (UV-Vis) spectroscopy; X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy. The Green synthesized Ag nanoparticles show highly antibacterial property against gram positive and gram negative bacteria when tested.

Silver nanoparticles (AgNPs) were synthesized using *Ocimum sanctum* (Tulsi) leaves aqueous extract as reducing as well as a capping agent in absence and presence of cetyltrimethylammonium bromide (CTAB). The resulting non material's were characterized by UV-visible spectrophotometer, and transmission electron microscope. The UV-Vis spectroscopy revealed the formation of AgNPs at 400–450 nm. TEM photographs indicate that the truncated triangular silver nanoplates and/or spherical morphology of the AgNPs with an average diameter of 25 nm have been distorted markedly in presence of CTAB. The AgNPs were almost mono disperse in nature. Antimicrobial activities of AgNPs were determined by using two bacteria (Gram positive *Staphylococcus aureus* MTCC-3160), Gram negative *Escherichia coli* MTCC-450) and one species of Candida (*Candida albicans* ATCC 90030) with Kirby-Bauer or disc diffusion method. The zone of inhibition seems extremely good showing a relatively large zone of inhibition in *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* strains. [59]

Development of green nanotechnology is generating interest of researchers toward ecofriendly biosynthesis of nanoparticles. In this study, biosynthesis of stable silver nanoparticles was done using Tulsi (*Ocimum sanctum*) leaf extract. These biosynthesized nanoparticles were characterized with the help of UV-vis spectrophotometer, Atomic Absorption Spectroscopy (AAS), Dynamic light scattering (DLS), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and Transmission electron microscopy (TEM). Stability of bioreduced silver nanoparticles was analyzed using UV-vis absorption spectra, and their antimicrobial activity was screened against both gram-negative and grampositive microorganisms. It was observed that *O. sanctum* leaf extract can reduce silver ions into silver nanoparticles within 8 min of reaction time. Thus, this method can be used for rapid and ecofriendly biosynthesis of stable silver nanoparticles of size range 4–30 nm possessing antimicrobial activity suggesting their possible application in medical industry. [60]

The present study was aimed to identify the antigen toxic effect of bio-synthesized silver nanoparticles (SNP) of *Ocimum sanctum* leaf extract against cyclophosphamide (CP). We tested the antigen toxic effect of bio-synthesized silver nanoparticles of *O. sanctum* leaf extract on human lymphocytes against CP by using chromosomal aberration assay (CAA). Silver nanoparticles was first synthesized from fresh leaf extract of *O. sanctum* and characterized. Their quality was checked by XRD technique and morphology by SEM. Three different doses of the bio-synthesized SNPs namely, 50, 100 and 200l/ml were selected and CP (100 lg/ml) was used as a positive control for CAA. CP administration to human lymphocytes culture caused reduction in mitotic index (MI) and increase in chromosomal damages. The three doses (50, 100 and 200 ll/ml) significantly ($P < 0.005$) reduced the chromosomal damages by CP and there was increase in MI. The biological way of synthesizing SNPs has advantages like cost effectiveness and ecofriendly. Also the bio-synthesized SNPs of *O. sanctum* leaf extract was found to be a powerful

genoprotectant. Furthermore works are to be carried out in future to find the extract mechanism of its genoprotective nature. [61]

In the present study the plant *Ocimum sanctum* was selected for its considerable Phenolic content and an attempt was made to evaluate antihelmintic potential against *Pheretema posthuma*. Aqueous extract is more potent than ethanolic extract. Aqueous extract at concentration 2, 4 and 10 mg showed paralysis and consequent death of the organism time that was comparable to piperazine at same concentrations. The aqueous extract of leaves of *Ocimum sanctum* showed good activity against *Pheretema posthuma* at the tested concentrations. [62]

Antihelminthic derived from plant source can be an answer to this world wide problem as they form secure and non-toxic with a modified site of action. *Ocimum sanctum* Linn known as Tulsi in India is a sacred plant for hindus known from centuries and being used in Ayurveda for its varied healing properties belonging to the Labiateae family. To this purpose we have studied in vitro antihelminthic activity of *Osmium* in comparison with albendazole. The leaves of *Ocimum sanctum* was taken from the Institute staff quarter's garden, which was endorsed by certified Taxonomist. The study was done using earth worms' adult type due to their anatomical resemblance with the intestinal roundworm parasites of humans. The suspension of aqueous extract of leaves of *Ocimum sanctum* Linn, concentration 100 mg/ml was prepared. Albendazole was used as standard reference drug and its 20 mg/ml concentration was prepared by as per the prescribed method. The antihelminthic activity was performed according to standard screening methods. Aqueous extract of *Ocimum sanctum* took 145±14 minutes to paralyze and 223±11 minutes to death of the worm, whereas Albendazole took 92±18 minutes to paralyse and 165±17 minutes to death of the worm with significant ($P<0.05$) value. Aqueous extract is more potent than control (NS) and lesser antihelminthic activity than albendazole. Time to paralysis and consequent death were significantly higher in aqueous extract of *Ocimum* that of Albendazole at same concentrations. Aqueous extract of *Ocimum sanctum* Linn is more potent than control (NS) and lesser antihelminthic activity than albendazole. Time to paralysis and consequent death were significantly higher in aqueous extract of *Ocimum* that of Albendazole at same concentrations.

According to the ayurveda, plants have been used for the treatment of so many diseases. Herbal drugs are easily available and have fewer side effects. So, many people are attracted towards the herbal drugs. *Osmium* is one of a few types of basil that is perennial. It is a well known plant in Indian traditional system of medicine. *Osmium* plant is widely used in the management of various ailments including colds, coughs, abdominal pains, measles, anti-ulcer, bronchitis, anorexia, memory disorders and diarrhea. This review delineates with all the phytochemical and pharmacological aspects of *Osmium* that will help the various practitioners and clinicians to get aware of the modern and traditional use of this plant. [63]

In several ancient systems of medicine including Ayurveda, Greek, Roman, Siddha and Unani, *Ocimum sanctum* has vast number of therapeutic applications such as in cardiopathy, haemopathy, leucoderma, asthma, bronchitis, catarrhal fever, otalgia, hepatopathy, vomiting,

lumbago, hiccups, ophthalmia, gastropathy, genitourinary disorders, ringworm, verminosis and skin diseases etc. The present review incorporates the description of *O. sanctum* plant, its chemical constituents, and various pharmacological activities.

Over the past few decades, nanoparticles of noble metals such as silver exhibited significantly distinct physical, chemical and biological properties from their bulk counterparts. Nano-size particles of less than 100 nm in diameter are currently attracting increasing attention for the wide range of new applications in various fields of industry. Such powders can exhibit properties that differ substantially from those of bulk materials, as a result of small particle dimension, high surface area, quantum confinement and other effects. Most of the unique properties of nanoparticles require not only the particles to be of nano-sized, but also the particles be dispersed without agglomeration. Discoveries in the past decade have clearly demonstrated that the electromagnetic, optical and catalytic properties of silver nanoparticles are strongly influenced by shape, size and size distribution, which are often varied by varying the synthetic methods, reducing agents and stabilizers. Accordingly, this review presents different methods of preparation silver nanoparticles and application of these nanoparticles in different fields. [64]

In present day scenario, pesticides are widely used for pest control in agriculture, because of which, the drinking water sources (both surface and ground water) gets contaminated. Majority of the pesticides are defiant to biodegradation and are found to be carcinogenic in nature even at trace levels. Various conventional methods employed for removal of pesticide are surface adsorption, photo-catalysis, membrane separation and biodegradation. However, these methods have disadvantages such as time consumption and expensive. Application of nanoparticles alleviates both of the above mentioned drawbacks. The nanoparticles need to be incorporated on a support to prevent the presence of it in the purified water after mineralization of pesticides. Previous researchers employed activated carbon and alumina as support for silver nanoparticles in pesticide mineralization. However, not many studies have been carried out on polyurethane foam (PUF) as support for silver nanoparticles in the mineralization of pesticides (Chlorpyrifos & Malathion) in water. Hence, a detailed study has been carried out to estimate the mineralization potential of silver nanoparticles supported on PUF. Silver nanoparticles synthesized using trisodium citrate as reducing agent was used for incorporating into polyurethane foam. Polyurethane foam was found to be having saturation for supporting AgNPs. The pesticide concentration found to be directly proportional to mineralization time. [65]

MATERIALS AND METHODS

Plant Material Collection

Ocimum sanctum leaves were collected from the local garden and washed thoroughly with distilled water to remove the dust particles. The thoroughly washed leaves were air dried for one hour. The dried leaves were cut into small pieces.

Preparation of Leaf Extract

About 3g of these finely sized leaves were weighed and transferred into 250mL beaker containing 100mL distilled water and boiled for about two hours. The extract was then filtered through Whatman No: 1 filter paper to remove particulate matter and to get clear solution and stored in refrigerator which to be used for further characterization and future works.

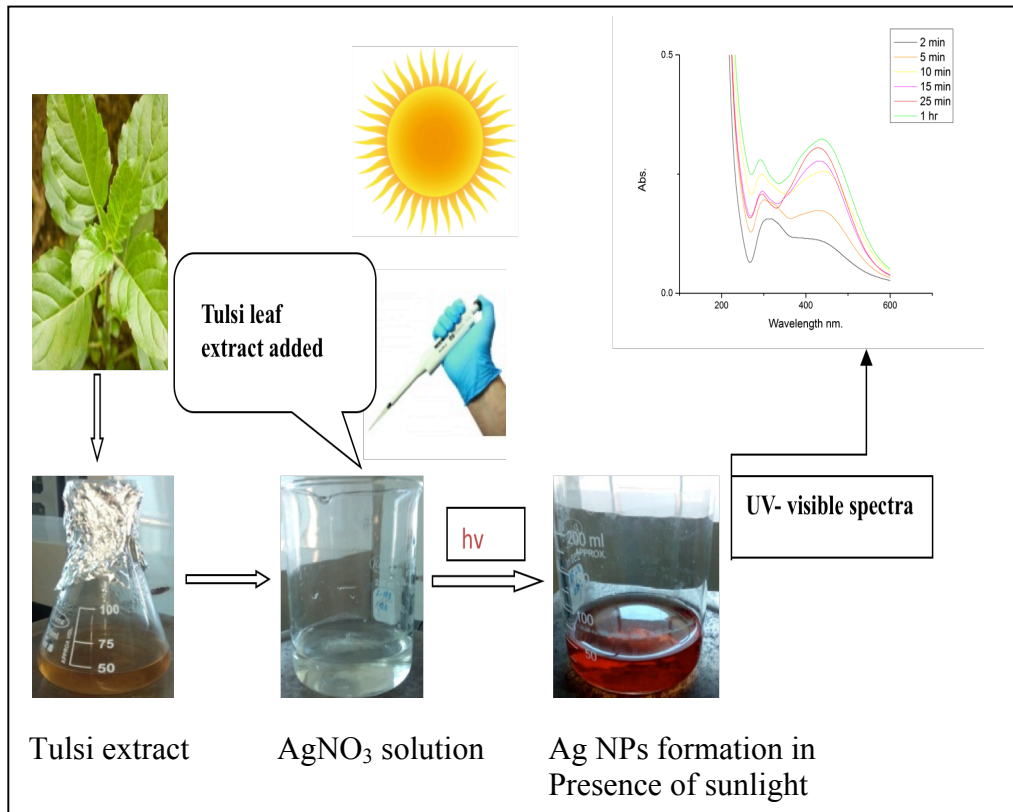
Preparation of Silver Nitrate Solution

Freshly solution of silver nitrate (stock solution) was prepared by adding 17mg of silver nitrate in 10ml of distilled water. This solution is kept covered and wrapped with aluminum foil in dark so as avoid its reduction.

Synthesis of Silver nanoparticles at different pH

Only Tulsi extract was taken then its pH was set at different pH i.e. 4, 5, 7, 9, 11. 4 ml of Tulsi extract each from above different pH was added into 0.0625ml of silver nitrate solution and kept in direct sunlight atmosphere for reduction of Ag^+ ions into Ag^0 . The complete reduction of AgNO_3 was confirmed by the change in color from colorless to pale yellow and finally reddish brown due to the surface Plasmon resonance phenomena.

GRAPHICAL ABSTRACT



DESCRIPTION OF CHARACTERIZATION METHODS

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

- **UV-vis Spectroscopy**
- **Transmission electron microscope (TEM)**
- **Fourier Transform Infrared Spectroscopy (FTIR)**
- **Dynamic Light Scattering (DLS)**
- **Secondary Electron Microscopy (SEM)**
- **Raman Spectroscopy**

UV–vis spectroscopy

The reduction of Ag^+ ions was confirmed by UV-vis spectrophotometer. The addition of *O. sanctum* leaf extract to silver nitrate solution resulted in color change of the solution from transparent to dark yellow due to the production of silver nanoparticles. These color changes arise because of the excitation of surface plasmon vibrations with the silver nanoparticles. To observe the optical property of biosynthesized silver nanoparticles, samples were periodically analyzed for UV–vis spectroscopic studies at room temperature operated at a resolution of 1 nm between 250 nm and 700 nm ranges. The peak of the Silver nanoparticles prepared at all pH by green synthesis lies between the range 420-480nm.

Transmission electron microscopy

Transmission electron microscopy (TEM) was performed for characterizing size and shape of biosynthesized silver nanoparticles. Fresh samples of all five pH were prepared. A drop of this solution was loaded on carbon-coated copper grids. 4-5 washings were given with distilled water. The grid were kept in incubator at 37°C for 30min. Inspection of TEM images also indicate the presence of thin black coating on the surface of AgNPs, which might be recognized to the adsorption of constituents of extract. Thus, we may state confidently that *Ocimum sanctum* leaves extract acts as a reducing as well as capping agents.

Fourier Transform Infrared Spectroscopy (FTIR)

For Fourier transform infrared spectroscopy (FTIR) measurements, the reaction mixture was centrifuged at 15,000 rpm for 15 min after complete reduction of AgNO_3 by the *O. sanctum* leaf extract to separate Ag nanoparticles from biomass or other bioorganic compounds. The Ag

nanoparticles pellet obtained after centrifugation were re-dispersed in water and washed (centrifugation and re-dispersion) with distilled water for three times. Finally, the samples were dried and grinded with KBr pellets. FTIR study imparts the biomolecules responsible for Ag^+ ions and stabilization of silver nanoparticles. The FTIR analysis in the range of $4000\text{-}400\text{cm}^{-1}$ was carry out to examine the presence of capping agent and role of molecules involved in the synthesis of silver nanoparticles.

Dynamic Light Scattering (DLS)

Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of silver nanoparticles. DLS study give us the information of size profile and stability of nanoparticles in solution .If the particles aggregate, there will be larger population of particles with larger radius.

Secondary Electron Microscopy (SEM)

The morphological study of synthesized silver nanoparticles using *Ocimum sanctum* (Tulsi) Extract was carried out by scanning electron microscope. After 24 hrs of the addition of AgNO_3 the SEM slides were prepared by making smear of the solutions on the slides. Then sample were characterized in the SEM at accelerating voltage of 20 KV.

Raman Spectroscopy

Raman Spectroscopy is a surface-sensitive technique that enhances Raman scattering by molecules adsorbed on rough metal surfaces. The increase in intensity of the Raman signal for adsorbates on particular surfaces occurs because of an enhancement in the electric field provided by the surface. When the incident light in the experiment strikes the surface, localized surface Plasmon's are excited. The field enhancement is greatest when the Plasmon frequency, ω_p , is in resonance with the radiation. The reaction mixture at different ph was centrifuged at 15,000 rpm for 15 min after complete reduction of AgNO_3 by the *O. sanctum* leaf extract. The Ag nanoparticles pellet obtained after centrifugation were re-dispersed in water and washed (centrifugation and re-dispersion) with distilled water for three times. The obtained pellet was dispersed on glass slides n kept for drying in incubator for 24hrs at 30°C . Then chemical composition and structure of material of AgNPs at different ph were observed with Raman spectroscopy under the range of $500\text{-}4000\text{nm}$.

APPLICATONS OF TULSI AgNPs

Antimicrobial activity

Biosynthesized silver nanoparticles were analyzed for their antimicrobial activity against gram-negative *E. coli* and gram-positive *Bacillus Sphaericus*. These silver nanoparticles were dispersed in autoclaved deionized water. Aqueous dispersions of silver nanoparticles of various concentrations (0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.50, 0.75, and 1.0) were made. Stock culture of *E. coli* and *Bacillus Sphaericus* were grown separately in liquid Luria broth medium were added to broth containing different concentrations of silver nanoparticles in each flask to observe the bacterial cell growth at 37 °C. Only bacterial cultures and AgNO₃ was taken as a control. Test tubes were kept in incubator cum shaker at 200 rpm/min. cultures was measured using UV-vis spectrophotometer at 600 nm *E. coli* after 14 hrs and *B. sphaericus* after 24 hrs.

Adsorption of dyes

Precise quantities of solid dye (Methylene Blue and Neutral Red each 5mg in 50ml) were dissolved in double distilled water to prepare the stock solutions of reactive dye. Solutions of various concentrations of dye were obtained by diluting this stock solution (5ml of each solution in 50ml of distilled water) making it as the working solution. The experiment was carried out using all the five ph (pH 4, 5, 7, 9, 11). In 5ml of Tulsi AgNP solution of each ph, 100µl of working solution of each dye at all ph was added then was kept for incubation in dark with stirring at 30⁰C. The reading was taken with UV-vis spectrophotometer at every 5min and 25min. The Methylene Blue showed its peak at 660nm and Neutral Red was observed at 550nm.

Adsorption of Pesticides

The pesticide used for adsorption here is Paradol (Methyl parathion). The experiment here was carried out by 2 ways.

1. Room temperature
2. At 80°C

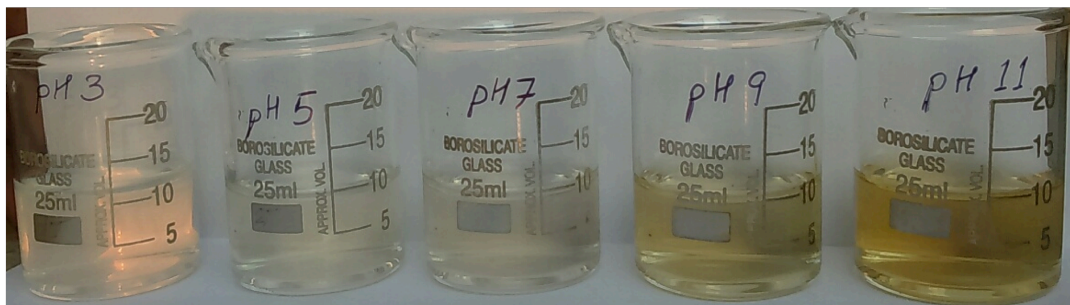
The stock solution was prepared by adding 40mg of pesticide in 100ml of double distilled water. Then for room temperature dependent batch 2.5ml freshly prepared solution of Tulsi AgNPs was taken and to it 100µl of pesticide solution was added and kept for incubation in dark for stirring at 30°C. The reading was taken at UV-vis spectrophotometer after every 0hr, 1hr, 3hr, 6hr and 24hr. The peak was observed at 400nm.

For 80°C temperature dependent batch the same stock solution prepared above was used with same concentration of Tulsi AgNPs and pesticide. It was kept in incubator at 80°C in dark with stirring. The reading was observed after every 0min, 10min, 30min, 45min, 60min, 90min and 120min. More than 90% of the reaction is complete within 15 minutes of the reaction time.

RESULTS AND DISCUSSION

Naked eye view: Change in color during the synthesis of Silver nano particles has been observed as in the Figure. The color of starting material Silver nitrate was initially yellow. When Tulsi leaf extract was added to Silver nitrate solution then the color immediately changes from yellow to color and to brownish. On further Sunlight exposure the color changes to dark brownish.

a) Figure showing AgNP solution after 0min of sun exposure



b) Figure showing AgNP after 5min



c) Figure showing AgNP after 15min



Fig3: (a) (b) (c) showing synthesis of AgNPs at different time intervals.

UV-vis spectrophotometer:

The formation of silver nano particles was initially confirmed visually and by using UV-visible spectroscopy technique which has been frequently used to characterize the synthesized metal and metal oxide nano particles. The change in color of the reaction mixture due to surface Plasmon resonance phenomenon provides a convenient signature to indicate the formation of silver nano particles in the reaction mixture. UV-visible analysis was performed to investigate the optical properties of silver nano particles. The UV-absorption spectroscopy of the sample shows an absorption peak is at about 435 nm, which confirm the synthesis of AgNPs.

This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range. Here is always an essential need for reducing entities in the reaction medium to bring such conversions. To ensure the presence of reducing entities in the reaction medium, reducing assay was carried out, and the results are shown. It shows the change in color of the reaction medium as an effect of presence of any type of reducing substance. As per this qualitative assessment of reducing potential of Tulsi extract, presence of significant amount of reducing entities was attested therein. In case of bacteria and fungi mediated synthesis of AgNPs, reduction of silver nitrate to elemental silver has been attributed to the presence of reductive enzymes but there is controversy regarding the plant extract components involved in reduction of silver nitrate to elemental silver. Shankar et al., (2005) have speculated the role of reducing sugars for the reduction of silver nitrate to silver nanoparticles. In another recent study, it has been suggested that different compounds such as caffeine and theophylline bring out the reduction processes and thus silver nanoparticles synthesis. Natural antioxidants have been reported to have strong reducing ability. As Tulsi possess a potent antioxidant activity, we attribute the reduction process to their presence of high quantity of antioxidants in the leaves extract. Graph shows UV-Vis absorption spectrum of silver nanoparticles. Through the plasmon band is broad due to the presence of components in Tulsi extract which are also being read in the spectrophotometric range, it is observed that the silver surface plasmon resonance (SPR) occurs at 430 nm and steadily increases in intensity as a function reaction time. There is no change in peak position, suggesting that nucleation of silver nanoparticles starts with initiation of reaction time only, and the size of the AgNPs formed changes with change in the pH range. As the pH increase the size of the nanoparticles formed decreases throughout the course of reaction. Inset of Figure represents the plot of absorbance at λ_{max} versus reaction time. The reduction

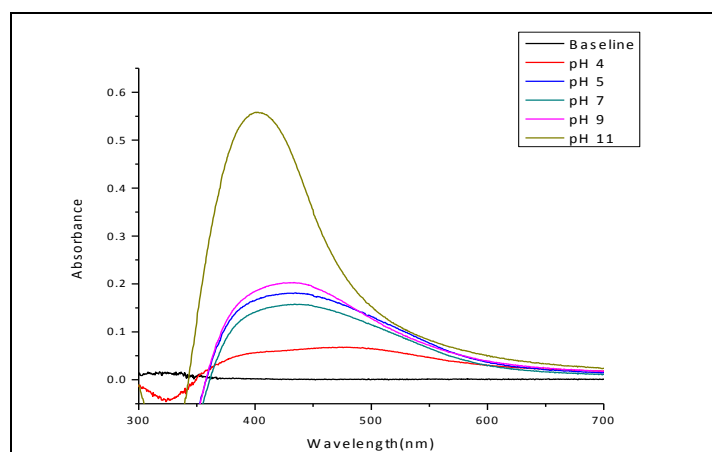


Fig4: shows the UV-Vis spectra of silver nanoparticles synthesized

TEM Analysis

Transmission Electron Microscopy offers the highest resolution we can achieve. This method helps us to observe the particle size of a material in nano-dimension and study the crystal structure meticulously. In a High Resolution Transmission Electron Microscope (TEM), a thin sample or specimen is irradiated with a sharp high-energy electron beam (usually in the range: 100-200 keV). The beam is strongly focused by magnetic lenses. The first few lenses before the specimen permit variation of the illumination aperture and the size of the illuminated area. The electron intensity distribution of the beam after interaction with the specimen is imaged onto a fluorescent screen by the objective lens and the post-objective lens system. Images are recorded by a digital CCD camera and can be reproduced or displayed on a computer monitor as well.

A TEM image of the prepared silver nano particles is shown. The Ag nano particles are roughly spherical in shape with a smooth surface morphology and irregular in distribution. The diameter of the nano particles is found to be approximately 14nm- 20nm in ph 4, 3nm-10nm in ph 7 and 15nm – 100nm in ph 11. TEM image also shows that the produced nano particles are more or less uniform in size and shape.

The diameter of the nanoparticles was determined using a millimetre scale.. These values indicate that the size of the particles increased with increasing ph. This may be because the higher ph, thus increasing the aggregation and particle size of the Ag ions.

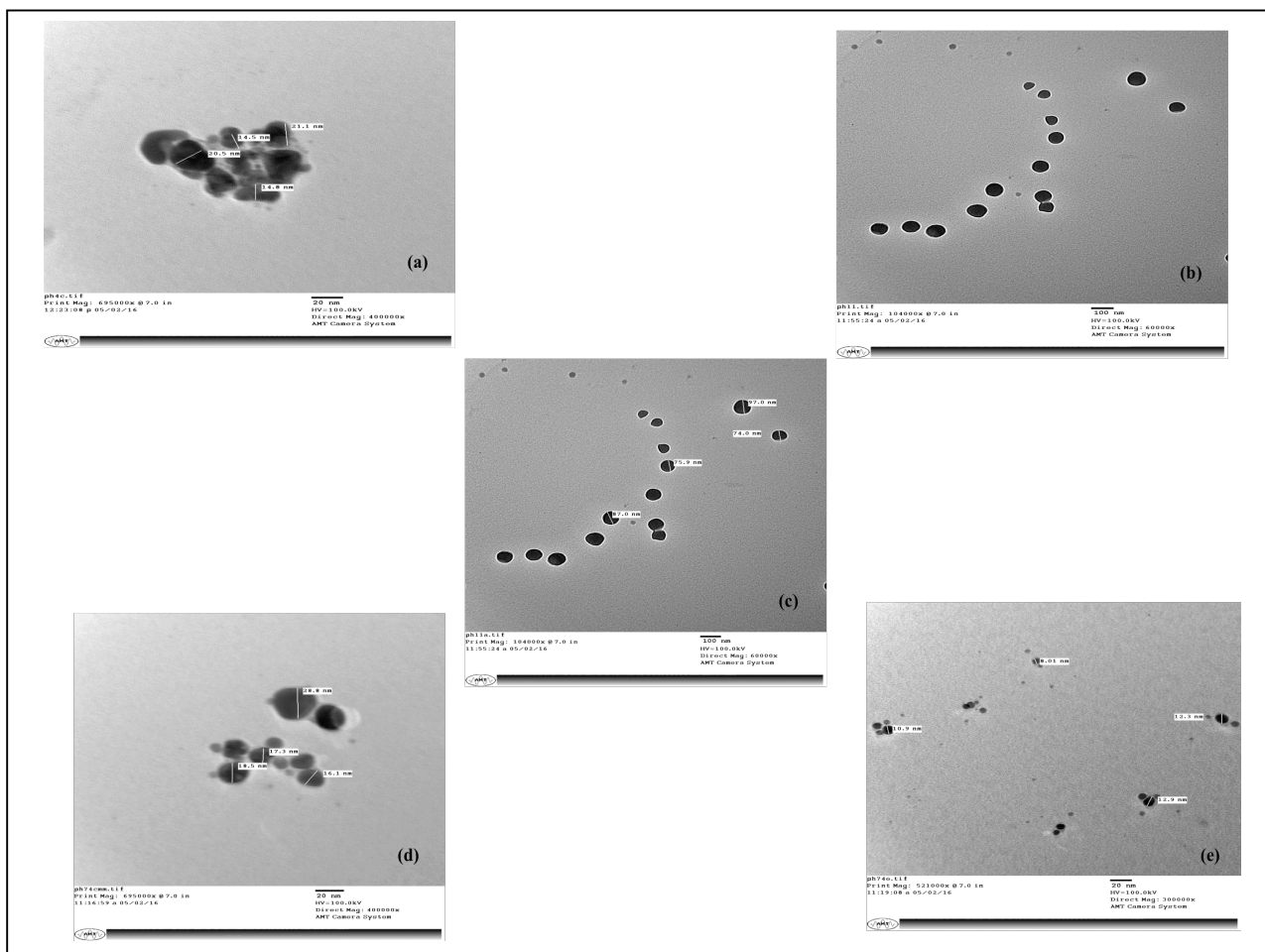


Fig5: (a) (b) showing TEM image of pH 4 (c) images of pH 7 (d) (e) images of pH 11

FTIR

FTIR (Fourier Transform Infra-red Spectroscopy) is a sensitive technique useful for identifying organic chemicals in a whole range of applications although it can also characterize some inorganic include paints, adhesives, resins, polymers, coatings and drugs. It is powerful tool for isolating and characterizing organic contamination.

FTIR is the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds to the bonds present in the molecule. The frequency range is measured as wave numbers typically over the range $4000 - 400 \text{ cm}^{-1}$. The background emission spectrum of the IR source is first recorded, followed by the emission spectrum of the IR source with the sample in place. The ratio of the sample spectrum to the background spectrum is directly related to the sample's absorption spectrum. The resultant absorption spectrum from the bond natural vibration frequencies indicates the presence of various chemical bonds and functional groups present in the sample. FTIR is particularly useful

for identification of organic molecular groups and compounds due to the range of functional groups, side chains and cross-links involved, all of which will have characteristic vibration frequencies in the infra-red range.

For Fourier transform infrared spectroscopy (FTIR) measurements, the reaction mixture was centrifuged at 15,000 rpm for 15 min after complete reduction of AgNO₃ by the *O. sanctum* leaf extract to separate Ag nanoparticles from biomass or other bioorganic compounds which may interfere in analyzing protein– AgNPs interaction. The Ag nanoparticles pellet obtained after centrifugation were re-dispersed in water and washed (centrifugation and re-dispersion) with distilled water for three times. Finally, the samples were dried and grinded with KBr pellets and analyzed.

The peak in FTIR was observed from 3500-1000cm⁻¹. The peak in 1035-1085cm⁻¹ species the presence of C-O bond, 1300-1400cm⁻¹ shows presence of C-H bond(stretch), 1595-1655cm⁻¹ shows N-H bend, 2850-3000cm⁻¹ shows C-H (bend) in alkanes and alkyls, range 3200-3300cm⁻¹ shows O-H (stretch) whereas 3400-3500cm⁻¹ shows N-H symmetric and asymmetric stretch. The data at different pH is observed with comparison to control (only Tulsi).

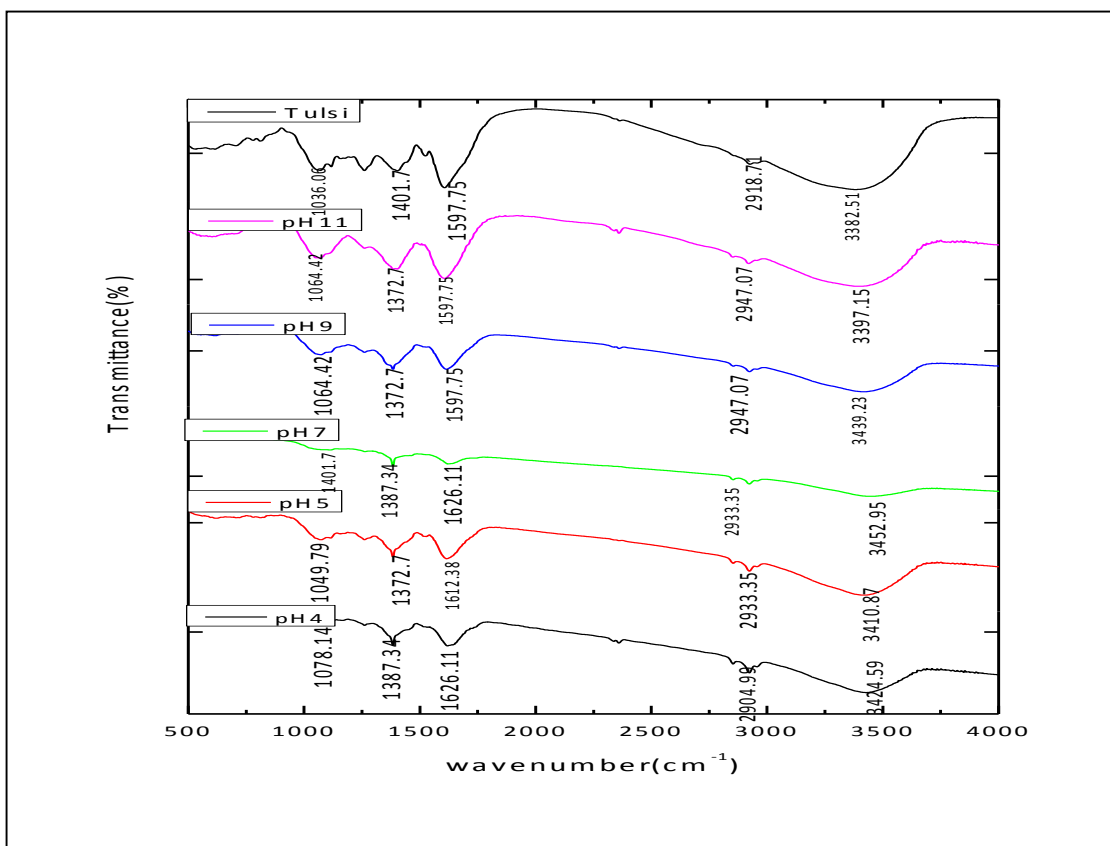


Fig6: showing analysis of different pH Tulsi AgNPs in FTIR

DLS

Dynamic light scattering or Photon Correlation Spectroscopy is a technique used in material physics for determining the size distribution profile of nanoparticles in suspension or polymers in solution. Light scattering technique is used here to determine the size distribution profile of nanoparticles present in the final solution after ultracentrifugation. From this measurement, the mean size of particles inside the sample is obtained along with the correlation between the number of particles of a particular size versus the size of the nanoparticles.

The particle size distribution (PSD) of synthesized silver nanoparticles of different pH (4, 5, 7, 9, 11) According to figure the colloidal solution of silver nanoparticles of ph 4 ranges at 800nm but in case of ph 11, the solution contains particles of uniform sizes ranging at 15nm. The particle size in case of ph 5 ranges at 23nm. Similarly the sizes of nanoparticles in case of ph 7 range at 17nm and ph 9 range at 10.5 nm. If we compare the below five results we can conclude that the ph 5, 7, 11 give uniform distribution of particles but ph 4 ranges at 800nm. Among them ph 9 is very appropriate since it gives lowest average size of nanoparticles.

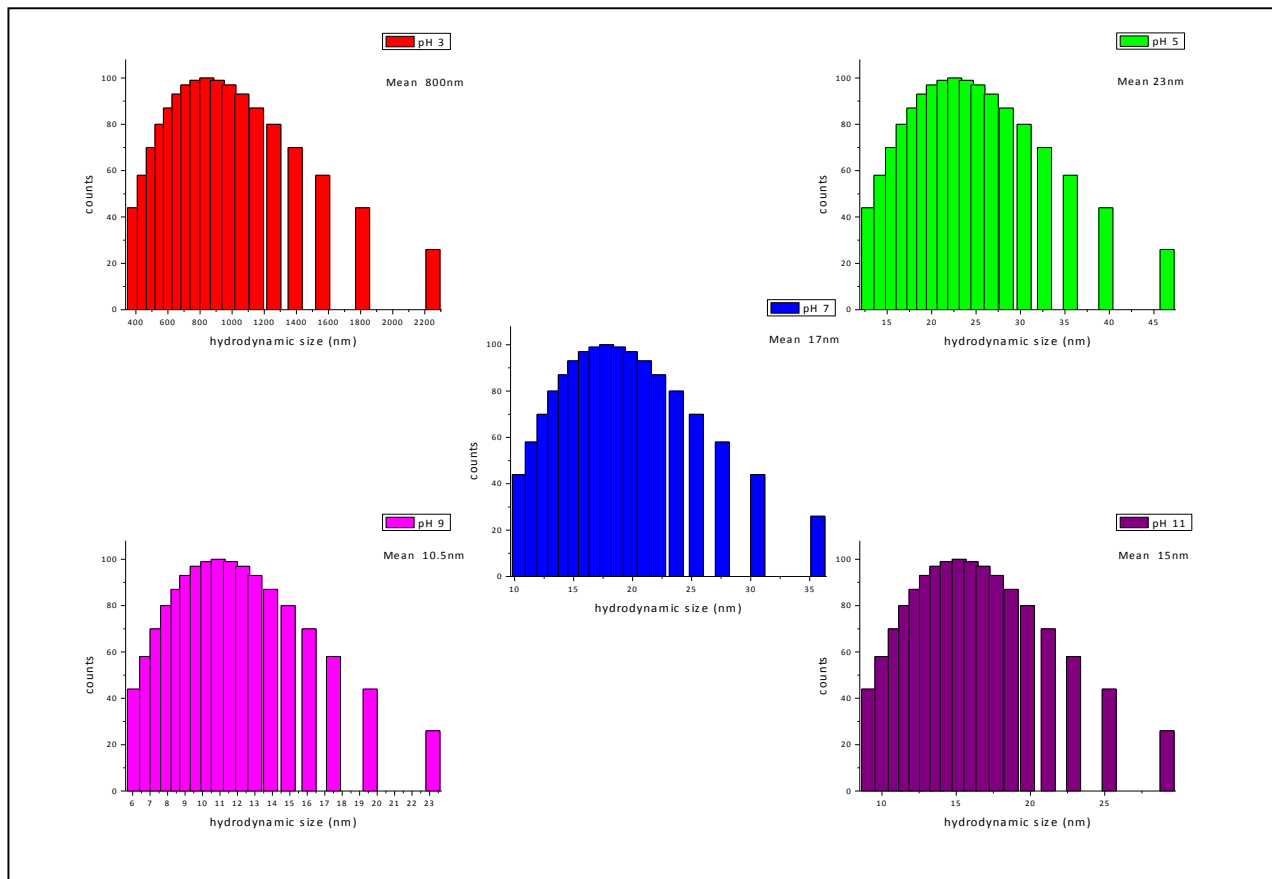


Fig7: showing the comparison between all ph range 4, 5, 7, 9, 11

SEM

Scanning Electron Microscopy is done for revealing the surface morphology of particles. Here, the bead for the SEM analysis was prepared by placing a drop of the silver nano- particle suspension on the carbon tape attached to the head of cylindrical bead and it was dried inside a vacuum dryer for a couple of hours. The particles on the top of the bead were scanned by Scanning Electron Microscope and the following image SEM images have greater depth of field yielding a characteristic 3D appearance useful for understanding the morphology material. Magnification is of order 10,000 X and resolution 10 nm.

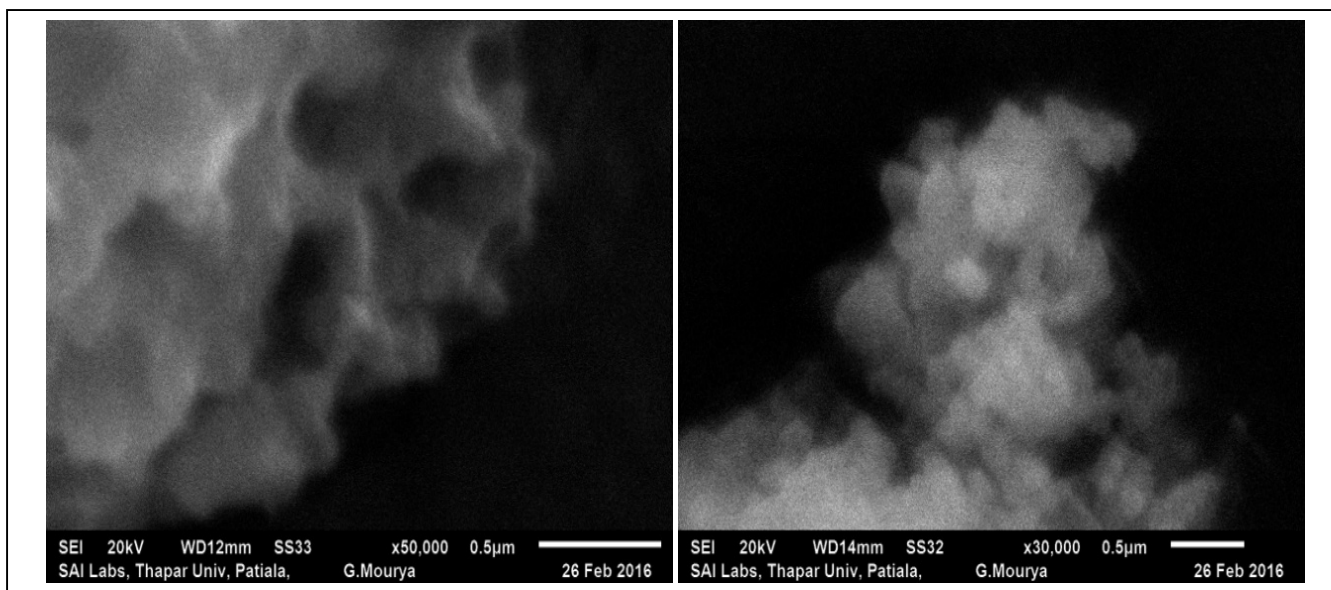


Fig8: SEM images of pH 7

Raman spectroscopy

In Raman spectroscopy the peak was ranges between 540-660nm in ph 4 and 5 whereas when we observe ph 7, 9, 11 we see the peak range from 1000- 3000nm. Range from 150-210nm shows x metal O, 300-500nm shows C-C aliphatic range. 500-1000nm specifies presence of C-H bond, C-N ranges between 1000-1500nm, 1500-2000nm shows range of C-C, 2000-3000nm shows presence of isonitrile. C-H is seen in range 3000-3500nm, 3500-4000nm shows presence of O-H bond.

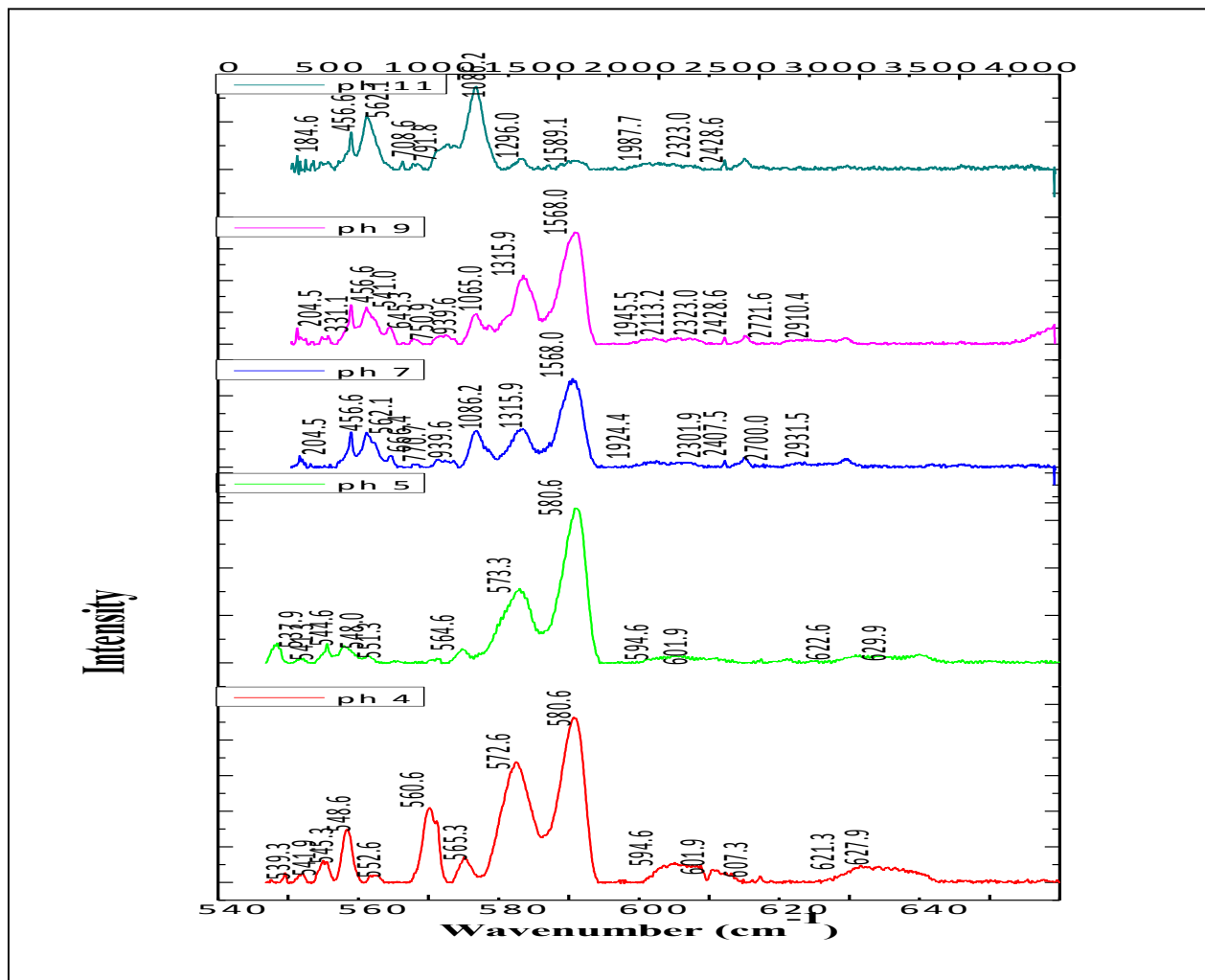
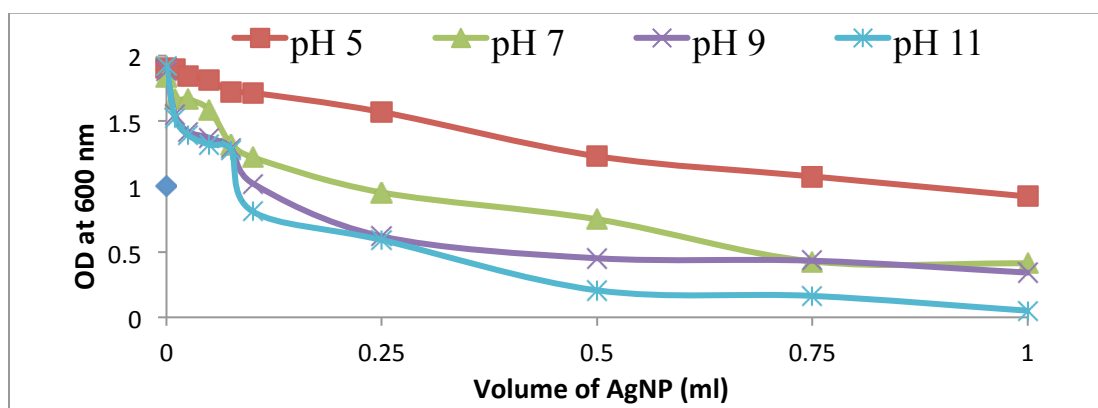


Fig9: Comparison of AgNP at different pH range

Antibacterial test

The antimicrobial activity of synthesized nanoparticles was studied by batch culture method. Aqueous dispersions of silver nanoparticles of various concentrations (0.01, 0.025, 0.05, 0.075, 0.1, 0.25, 0.50, 0.75, and 1.0) were made. Stock culture of *E. coli* and *Bacillus Sphaericus* were grown separately in liquid Luria broth medium were added to broth containing different concentrations of silver nanoparticles in each flask. Required amount of agar medium was prepared along with silver nanoparticles of concentration 100 μ g/ml. Another agar media without silver nanoparticles was also prepared which was used for culturing strains without nanoparticles. Both were autoclaved and poured into different test tubes. The above strains were cultured. The cultures without nanoparticles were kept as control for a comparison study.

(a)



(b)

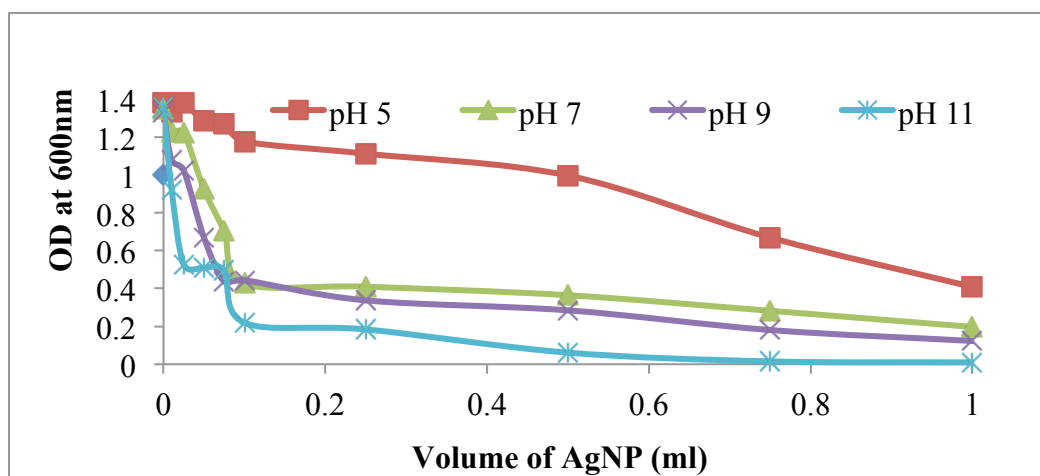


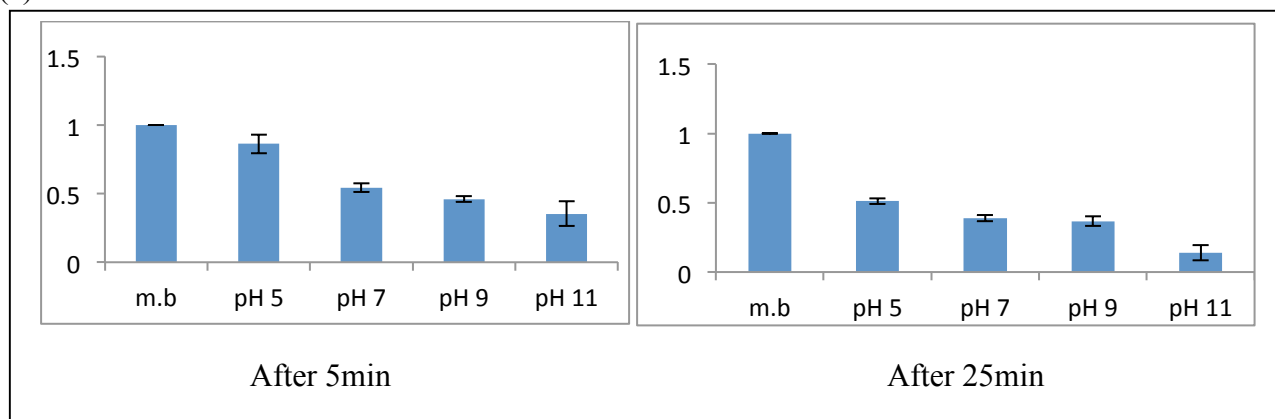
Fig10: (a) Graph showing growth inhibition of *E.coli* in different pH media

(b) Graph showing growth inhibition of *Bacillus sphaericus* in different pH medium

Adsorption of Dyes

The result proves that, after 25 minute of reaction between Methylene Blue and Neutral Red with strong reducing agent silver on the addition of silver nanoparticles, degradation of the dye MB is greatly enhanced. The disappearance of blue color of MB and red color disappearance of Neutral Red in solution was due to the reduction. The neutral dye n cationic dye solution turns into completely colorless solution within less than 25 minute in dark with vigorously stirred. Similar results show by using the biosynthesized AgNPs using *Ocimum sanctum* extract. The plot of relative absorption intensity with wavelength in a regular interval of time reveals that the complete reduction was accomplished in less than 30 min in the presence of Tulsi silver nanoparticles.

(a)



(b)

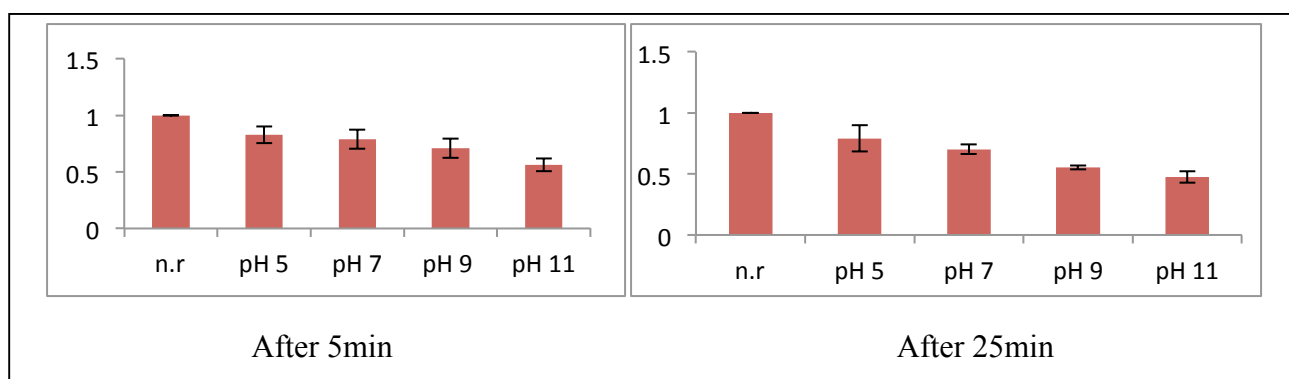


Fig11: (a) Graph showing reduction of *Methylene Blue* at 5min and 25min

(b) Graph showing reduction of *Neutral Red* at 5min and 25min

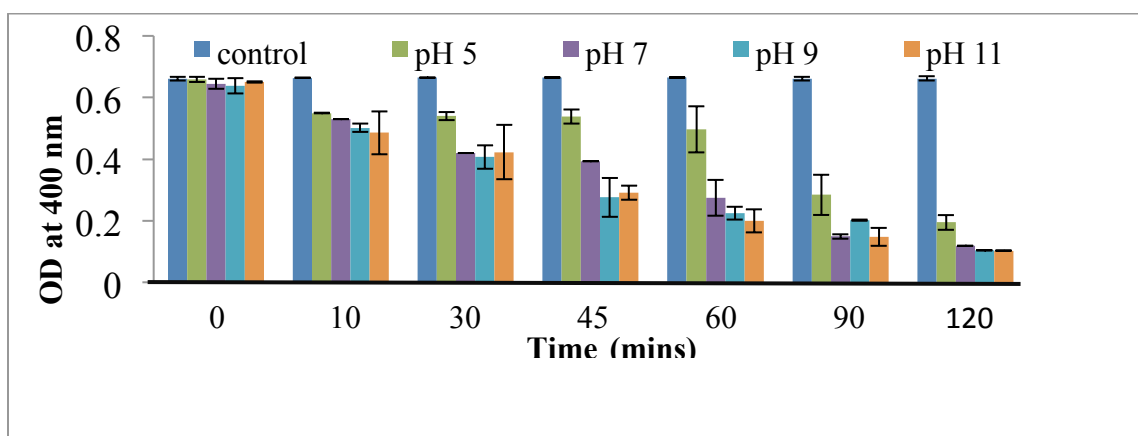
Adsorption of Pesticides

Nanoparticles exhibit a higher surface area-to-volume ratio with a decrease in the size of particles. It has been well established that the metallic nanoparticles such as zero-valent iron, copper, silver and gold have unique catalytic activity.

For room temperature dependent batch the same stock solution prepared above was used with same concentration of Tulsi AgNPs and pesticide. It was kept in incubator at 80°C in dark with stirring. The reading was observed after every 0min, 10min, 30min, 45min, 60min, 90min and 120min.

For 80°C time dependent batch solution of Tulsi AgNPs was taken and to it 100µl of pesticide solution was added and kept for incubation in dark for stirring at 30°C. The reading was taken at UV-vis spectrophotometer after every 0hr, 1hr, 3hr, 6hr and 24hr. The peak was observed at 400nm.

(a)



(b)

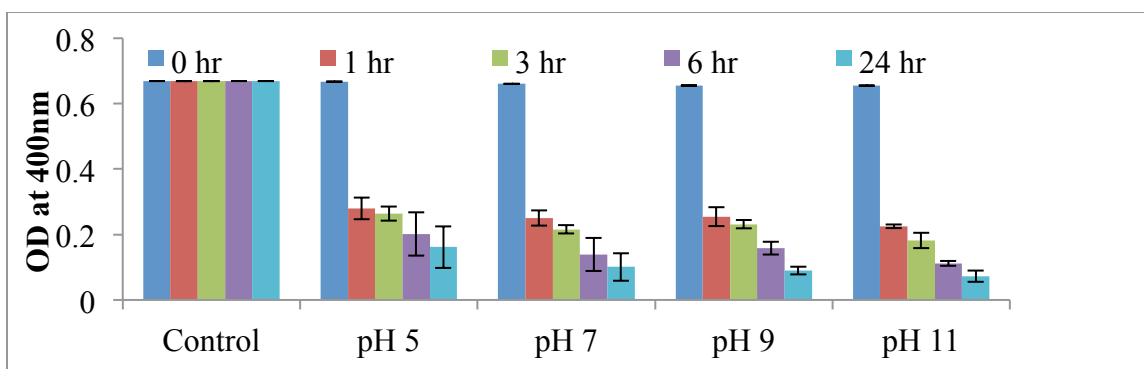


Fig12: (a) Graph showing *temperature dependent batch*

(b) Graph showing *time dependent batch at 80°C*

Conclusion

The rapid biological synthesis of silver nanoparticles using *Ocimum sanctum* leaves extract provides environmental friendly, simple and efficient route for synthesis of benign nanoparticles was done at different pH medium (4, 5, 7, 9, 11). The synthesized nanoparticles were of spherical and sheet shaped and the estimated sizes were 10-100 nm at different pH range. The nanoparticles were surrounded by a thin layer of tulsi that contains camphor, 1,8-cineole, limonene, trans caryophyllene, camphene, myrtenol, α -terpineol, endo- borneol, linalool, flavonoids, tannins, saponins, sterols, carbohydrates, proteins and triterpenoids having functional groups of amines, alcohols, ketones, aldehydes, etc., which were found from the characterization using UV-vis spectrophotometer, SEM, DLS, TEM, Raman spectroscopy, and FTIR techniques. All these techniques it was proved that the concentration of plant extract to metal ion ratio plays an important role in the shape determination of the nanoparticles. The higher concentrated nanoparticles had sheet shaped appearance where as the lower concentrations showed spherical shaped. The sizes of the nanoparticles in different pH range were also different which depend on the reduction of metal ions. From the data of DLS it was found that pH 11 solution had sharp nanoparticles of around 10nm and some has around 10-800 nm depending on different pH range. From the technological point of view these obtained silver nanoparticles have potential applications in the antibacterial activity, adsorption of dyes and reduction of pesticides and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

REFERENCES

1. R. Nagarajan *Synthesis, Stabilization, Passivation, and Functionalization*, ACS Symposium Series (Wash DC), 996: (2008), 1-14.
2. J.A. Dahl, B.L.S. Maddux, J.E. Hutchison *Toward greener nanosynthesis*, Chem. Rev. 107: (2007), 2228-2269
3. J. Cheon, G. Horace *Inorganic nanoparticles for biological sensing, imaging and therapeutics*, J. Mater. Chem. 19: (2009), 6249-6250.
4. W. Caseri *Inorganic nanoparticles as optically effective additives for polymers*, Chem. Eng. J. 196(5): (2009), 549-572.
5. Z.P. Xu, Z.Q. Pai, G.Q. Lu, A.B. Yu *Inorganic Nanoparticles As Carriers For Efficient Cellular Delivery*, Chem. Eng. Sci. 61: (2006), 1027-1040.
6. S. Liufu, H. Xiao, Y. Li *Investigation of PEG adsorption on the surface of zinc oxide nanoparticles*, Adv. Powder Technol. 145: (2004), 20-24.
7. C. Alexiou, W. Arnold, R.J. Klein, *Locoregional cancer treatment with magnetic drug targeting*, Cancer Res. 60: (2000), 6641-6648.
8. R. Savic, L. Luo, A. Eisenberg, et al. *Micellar nanocontainers distribute to defined cytoplasmic organelles*, Science (Wash DC) 300: (2003), 615- 618.
9. Y.H. Chen, C.S. Yeh *Colloids Surf. A*, Physicochem. Eng. Aspects. 197: (2002), 133-140.
10. A.R. Varlan, W. Sansen, A. VanLoey, et al. *Covalent enzyme immobilization on paramagnetic polyacrolein beads*, Biosens. Bioelectron. 11: (1996), 443-448.
11. T. Klaus, R. Joerger, E. Olsson, et al. *Silver-based crystalline nanoparticles, microbially fabricated*, Proc. Natl. Acad. Sci. 96: (1999), 13611-13614.
12. S. Senapati, *Biosynthesis and immobilization of nanoparticles and their applications*, University of pune, India, 188: (2005), 162-170.
13. D.J. Barber, I.C. Freestone *An investigation of the origin of the color of Lycurgus cup by analytical transmission electron-microscopy*, J. Archae. 32: (1990), 33-45.
14. J. Creighton, C. Blatchford, M. Albrecht, *Synthesis and Applications of Nanoparticles*, J. Chem. Soc. 75: (1979), 135-140.
15. P. Lee, D. Meisel *Adsorption and surface- enhanced Raman of dyes on silver and gold sols*, J. Phys. Chem. 86: (1982), 3391-3395.
16. A. Henglein, *Small-particle research: physicochemical properties of extremely small colloidal metal and semiconductor particles*, Chem. Rev. 89: (1989), 1861-1873.
17. A. Nagy, G. Mestl, *High temperature partial oxidation reactions over silver catalysts*. Appl. Catal. A. 188: (1999), 337-353.
18. B.G. Ershov, E. Janata, A. Henglein, A. Fojtik *Silver atoms and clusters in aqueous solution: Absorption spectra and the particle growth in the absence of stabilizing Ag ions*. J. Phys. Chem. 97: (1993), 4589-4594.

19. P. Schneider, H. Halbig, M. Grau **Nickel Photochemically prepared gold metal film in a carbohydrate-based polymer: A practical solid substrate for surface enhanced Raman scattering** J. Chem. Soc. 75: (1979), 790-795.
20. N. Shirtcliffe, U. Nickel, S. Schneider **Reproducible Preparation of Silver Sols with Small Particle Size Using Borohydride Reduction: For Use as Nuclei for Preparation of Larger Particles**, J. Colloid Interface Sci. 211: (1999), 122-127.
21. S. Emory, S. Nie **Synthesis and Applications of silver nanoparticles** Anal. Chem., 69: (1997), 2361-2368.
22. L. Rivas, S. Cortes, J.V.G. Ramos, G. Morcillo **Growth of silver colloidal particles obtained by citrate reduction to increase the Raman enhancement factor**. Langmuir 3: (2001), 574–577.
23. X. Chen, H.J. Schluesener **Nanosilver: A Nanoproduct in Medical Application**, Toxicol. Lett. 176: (2008), 1-12.
24. C.N. Lok , C.M. Ho, R. Chen , et al. **Proteomic analysis of the mode of antibacterial action of silver nanoparticles**, J. Proteome. Res. 5: (2006), 916-924.
25. M. Ramya **Green Synthesis of Silver Nanoparticles**, Int. J. Pharm. Med. & Bio. Sc. 1: (2012), 54-61.
26. L. Pamela, K.J. Hazelwood **Exposure-Related Health Effects of Silver and Silver Compounds**, Ann. Occup. Hyg. 49: (2005), 575-585.
27. A. Richard, A. Fuller **Inactivity of Two Noble Metals as Carcinogens**, J. Environ. Pathol. Toxicol. 1: (1978), 51-57.
28. X. Chen **Nanosilver: A Nanoproduct in Medical Applications**, Toxicol. Lett. 176: (2008), 1-12.
29. C. Granqvist, R.A. Buhrman, J. Wyns, et al. **Far-infrared absorption in ultrafine Al particles**. Phy. Rev. Lett. Vol. 37, 10: (1976), 625–629.
30. S. Shibata, K. Aoki, T. Yano, et al. **The Physics and Chemistry of Sol gel processing**, J. SOL-gel Sci. Tech. 11: (1998), 279-286.
31. S.S. Shankar, A. Ahmad, M. Sastry, **Geranium leaf assisted biosynthesis of silver nanoparticles**. Biotechnol. Prog. 19: (2003), 1627–1631.
32. J.O. Jung, H. Noh, S. Kim **Metal nanoparticle generation using a small ceramic heater with a local heating area**, J. Aerosol Sci. 37: (2006), 1662-1670.
33. F.F. Kruis, B. Rellinghaus **Sintering and evaporation characteristics of gas-phase synthesis of size-selected PbS nanoparticles**, Mater. Sci. Eng. B., 69: (2000), 329-324.
34. M.D. Magnusson, J. Malm, J. Bovin, et al. **Gold nanoparticles: production, reshaping, and thermal charging**, J. Nanopart. Res. 1: (1999), 243-251.
35. M.K. Mafune, Y. Takeda, T. Kondow, et al. **Formation of gold nanoparticles by laser ablation in aqueous solution of surfactant** J. Phys. Chem. B. 105: (2001), 5114-5120.
36. J.P.K. Sylvestre, E. Sacher, M. Meunier, et al. **Stabilization and size control of gold nanoparticles during laser ablation in aqueous cyclodextrins**, J. Am. Chem. Soc., 126: (2004), 7176-7177.

37. S.Y. Kim, K. Chun, W. Kang, et al. **Catalytic effect of laser ablated Ni nanoparticles in the oxidative addition reaction for a coupling reagent of benzylchloride and bromoacetonitril**, *J. Mol. Catal. A. Chem.* 226: (2005), 231-234.
38. N.B. Tarasenko, E. Nevar, N. Savastenko **Synthesis of nanosized particles during laser ablation of gold in water**, *Appl. Surf. Sci.* 252: (2006), 4439-4444.
39. K. Tsuji, N. Watanabe, M. Tsuji **Preparation of silver nanoparticles by laser ablation in solution: influence of laser wavelength on particle size**, *Appl. Surf. Sci.* 202: (2002), 80-85.
40. B.S. Wiley, B. Mayers **Shape-controlled synthesis of metal nanostructures: the case of silver**, *Chem. Eur. J.* 11: (2005), 454-463.
41. J.C. Evanoff **Size-controlled synthesis of nanoparticles and measurement of extinction, scattering, and absorption cross section**, *J. Phys. Chem. B.* 108: (2004), 13957-13962.
42. Y.L. Yin, Z. Zhong, B. Gates, S. Venkateswaran **Synthesis and characterization of stable aqueous dispersions of silver nanoparticles through the Tollens process**, *J. Mater. Chem.* 12: (2002), 522-527.
43. A.M. Ahmad, S. Senapati, D. Mandal, et al. **Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*** *Colloids Surf., B.* 28: (2003), 313-318.
44. B.D. Ankamwar, A. Ahmad, M. Sastry **Biosynthesis of gold and silver nanoparticles using *Emblia officinalis* fruit extract, their phase transfer and transmetallation in an organic solution**, *J. Nanosci. Nanotechnol.* 5: (2005), 1665-1671.
45. H.I. Korbekandi, S. Abbasi, **Production of nanoparticles using organisms**, *Crit. Rev. Biotechnol.* 29: (2009), 279-306.
46. S. Iravani **Green synthesis of metal nanoparticles using plants**, *Green. Chem.* 13: (2011), 2638-2650.
47. K. Parasharu **Bioinspired Synthesis of Silver Nanoparticles**, *Dig. J. Nanomater. Biostruct.* 4: (2009), 159-166.
48. N.A. Begum **Mondal Colloids and Surface** *Colloids Surf., B.* 71: (2009), 113-118.
49. M.A. Albrecht, C.W. Evans, C.L. Raston **Green chemistry and the health implications of nanoparticles**, *Green Chem.* 8: (2006), 417-420.
50. J.M. Koziara, P.R. Lockman, D.D. Allen, et al. **In situ blood-brain barrier transport of nanoparticles**, *Pharm. Res.* 20: (2003), 1772-1778.
51. G. Oberdorster, Z. Sharp, V. Atudorei, et al. **Translocation of inhaled ultrafine particles to the brain**, *Inhal. Toxicol.* 16: (2004), 437-440.
52. L.K. Limbach, P. Wick, P. Manser, et al. **Catalytic activity on oxidative stress**, *Environ. Sci. Tech.* 41: (2007), 4158-4164.
53. N.R. Jana, T.K. Sau, T. Pal. **Growing Small Silver Particle as Redox Catalyst**. *J. Phys. Chem.* 8: (1999), 103-115.
54. S.H. Jeong, S.Y. Yeo **The effect of filler particle size on the antibacterial properties of compounded polymer/silver fibers**, *J. Mater. Sci.* 40: (2005), 5407-5411.

55. S.L. Percival, P.G. Bowler, D. Russell. *Bacterial Resistance to Silver in Wound Care*, J. Hosp. Infect. 60: (2005), 1-7.
56. A.A. Taylor, I.M. Marcus, R.L. Guysi, et al. *Metal Oxide Nanoparticles Induce minimal phenotypic changes in a model colon Gut Microbia*, Environ. Eng. Sci. 10: (2015), 2014.0518.
57. J.R. Morones, J.I. Elechigerra *Interaction of silver nanoparticles with HIV-1*, J. Nanopart. Res. 16: (2005), 2346.
58. L. Geranio, M. Heuberger, B. Nowack *The Behavior of Silver Nanotextiles during Washing*, Environ. Sci. & Tech. (2009), 8113-8118.
59. E.S. Aazam, Z. Zaheer *Growth of Ag-nanoparticles in an aqueous solution and their antimicrobial activities against Gram positive, Gram negative bacterial strains and Candida fungus*, Bioprocess. Biosyst. Eng. 39: (2016), 575-584.
60. G. Singhal, R. Bhavesh, K. Kasariya *Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity*, J. Nanopart. Res. 13: (2011), 2981-2988.
61. P.P. Vijaya, B. Rekha, A.T. Mathew, et al. *Antigenotoxic effect of green-synthesised silver nanoparticles from Ocimum sanctum leaf extract against cyclophosphamide induced genotoxicity in human lymphocytes—in vitro* J. App. Nanosci. 4: (2014), 415–420.
62. K.C. Joshi, D. Nanda, P. Nainwal, et al. *In vitro anthelmintic activity of Ocimum sanctum* IJPSR 3: (2013), 287-288.
63. S. Narwal, A.C. Rana, V. Tiwari, et al. *Review on Chemical & Pharmacological Action of Ocimum kilimandscharicum*, IGJPS. 1: (2011), 287-293.
64. K. Nour, A. Eftaih, A. AlWarthan, R.A.A. Ammar, *Synthesis and applications of silver nanoparticles*, Arabian J. Chem 3: (2010), 135–140.
65. G. Manimegalai, S. Shanthakumar, C. Sharma *Pesticide Mineralization in Water Using Silver Nanoparticles Incorporated on Polyurethane Foam*, IJSR, 3: (2012), 2319-7064.
66. S.S. Shankar, A. Rai, A. Ahmad, et al. *controlling the optical properties of lemongrass extract synthesized gold nanotriangles and potential application in infrared-absorbing optical coatings*, Chem. Mater. 17: (2005), 566–572