Synthesis of silver nanoparticles and their application in catalysis

Thesis submitted in partial fulfilment of the degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

Submitted By:

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Enrolment number- 207819

Under the guidance of:

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WAKNAGHAT-173234, HIMACHAL PRADESH, INDIA

(MAY-2022)

CERITIFICATE

This is to certify that the work titled **"Synthesis of silver nanoparticles and their application in catalysis"** submitted by **Girish Parmar (207819)** is partial fulfilment for the award of degree of Master of science in biotechnology from Jaypee University of Information Technology, Waknaghat has been carried out under my supervision. This work has not been submitted partially or wholly to any other university or institute for the award of this or any other degree or diploma.

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SELF – DECLERATION

I, **Girish Parmar**, student of MSC biotechnology, Jaypee University of Information Technology, Waknaghat, Solan, Himachal Pradesh do here by declare that the project entitled synthesis of **'Silver nanoparticles and their application in catalysis'** submitted towards the partial fulfilment for the award of degree **Masters of Science in Biotechnology** of Jaypee University of Information Technology is based on the result of the research work carried out by me written by me under the guidance and supervision of **Dr. Abhishek Choudhary**. This project or no part of this has been submitted elsewhere for the award of any degree or diploma.

_____

Girish Parmar

(207819)

Acknowledgement

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All may not be mentioned, but no one forgotten

Girish Parmar

Abstract

"Green synthesis" is an eco-friendly process which is used to decrease the toxicity of nanoparticles as compare to the other synthesis processes. In present study, metallic sliver nanoparticles (AgNPs) which holds an antibacterial and antifungal activity are synthesised using plant bark extract as a reducing agent. The optimization was done over several parameters like concentration of $AgNO_3$, volume/volume ratio of $AgNO_3$ and plant extract, pH and time. Then the Photocatalytic reduction was done using methylene blue dye which was observed at range of 300nm – 800nm wavelength on a UV-Vis. Spectrometer at different time intervals.

Contents

Chapter 1

Introduction

Introduction

When we talk about small things generally, millimetre and micrometre are the first thought comes in our mind but beyond that there are further small molecules which can't be seen from the naked eyes, we need to cover our eyes with the different characterisation techniques to see those particles, those minute particles are called as the "Nanoparticles". On which this whole study is based on, in the Nano world everything works on a different manner. All the parameters work differently and have a major role of "surface to volume ratio"[1]. Nanobiotechnology is a field which work on the world of minuscule particles that can't be seen normally and have unique qualities in their regular form. It is the area of science which combines nanotechnology with biology. Nanoscience and nanotechnology are not the same thing: nanoscience studies nanoscale structures and molecules, whereas nanotechnology exploits them in practical applications. If we try to calculate a nanometre in terms of meters it comes around one billion times of a metre, in nanotechnology we examine things on the basis of atoms and molecules of a substance, to see how small a nanometre is we can take an example of the diameter of human hair which is around 50.000 nanometre, it is ten times the diameter of the hydrogen atom, which is quite small to be seen[2]. The other rules of science are failed to show their impact on the nanometre level due to that it is more difficult to analyse and characterise the nanoparticles. They are nanoscale materials in which interference or surface properties have an impact on the bulk properties[3]. Some of its properties, such as colour, conductivity, and reactivity, differ significantly between the nano and macro scales. The durability and strength of a nanomaterial can be seen by the carbon nano-tubes which are 100x stronger than the steel but 6x lighter in weight[2], [4], [5]. Nanomaterials have a wide range of forms like rods, dots, small particles, nanotubes, nanowires, oxide particles, nanogels etc. they have specific size as compare to their type or form (table1.1). Several types of metallic nanoparticles are there for example, like silver nanoparticles, gold nanoparticles, copper nanoparticles, zinc nanoparticles etc other than metallic nanoparticles we can make nanoparticles from polymers, carbon source, lipids etc[6], [7]. Each and every nanoparticle have their different size, shape (generally spherical and round), colour, stability, and other physicochemical properties such as higher catalytic activity, greater stability, and altered optical behaviour, due to their enormous surface area[2], [4], [8]. In our daily life nanoparticles play a key role, they are used in variety of consumer products like textiles, paints, sensors, and other health-related items. We can manufacture the nanoparticles by keeping some points in mind like controlling the size, distribution, and shape of nanoparticles, also dispersion, aggregation stability, and nanocrystalline composition, also

we need to optimize our nanoparticles on the basis of different parameters like time, temperature, pH, concentration, ratio of metal ions and reducing agent, stability, environmental effects etc[2], [4], [7]. Nanotechnology gives us excess to study new opportunities in different application like, Novel diagnostic procedures and therapeutics, particularly in medicine and sensor biotechnology, promise a wide range of development potential, for example, novel drugs can be developed and supported by the progressive miniaturisation in the electronic industries, and interdisciplinary research on so-called Nanobots[2]. As well as, it is used in the food industry which is looking into using nanoparticles to raise or drop temperature and create a variety of flavours in a home oven to provide meals with a longer shelf life. Agriculture is using nanotechnology to advance the field of biological crop production. A natural continuation of minimally invasive surgery would be the development of long nanofiber containing devices that can be implanted into organisms. Furthermore, Nanotechnology is defined by the National Nanotechnology Initiative (NNI) as "a science, engineering, and technology done at the nanoscale"[2], [4], [8].

Table 1.1 Types of particles with their size.

Type of particle	**Size**	**References**
Atoms and small molecules	0.1 nm	[2], [4], [7]
Nanoparticles	1 to 100 nm	[2]
Fine particles (Particulate matter)	100 to 2300 nm	[4], [9]
Coarse particles (PM10 or dust)	2500 to 10000 nm	[8]
Thickness of paper	100,000 nm	[7]

There are two main ways through which nanoparticles get synthesised -

I. Top – down approach.

In the top-down approach a bulk material or substance get grounded or transformed into microscopic particles, by using different kinds of methods and altering their properties[10]. In

the result we can have specific kind of nanoparticles required[7]. Some processes of top-down approaches are listed below.

1. **Sputtering** - It is a vapour deposition method that deposits materials onto a particular surface by condensing atoms on to the surface[11].
2. **Chemical etching** - To remove material and create a permanent etched picture on metal, use a high-pressure and high-temperature spray[12].
3. **Thermal/laser ablation** - Fabrication of micropatterns through the removal of small fractions of a substrate under a laser beam[13].
4. **Mechanical/ ball milling** – Crushing and blending of materials used in mineral dressing process[10], [14].
5. **Explosion process** - Pulse current injected through the conducting wire to vaporise it and then condense it[15].

II. Bottom – up approach.

In this type of approach an angstrom size or very minute particles having less size than the nanoparticles like atom clusters are condensed together to achieve the size of nano particles, there are several processes used for the condensation of the minute particles to form nanoparticles like –

1. **Molecular condensation** - Two molecules of nanoparticles combined together to form a single molecule[16], [17].
2. **Vapour deposition** - Used to produce thin films. Substrate expose to one or more volatile precursor to produce thin film[18], [19].
3. **Sol-gel process** - The process of converting monomers into colloidal solution[3], [20].
4. **Spray pyrolysis** - When a precursor solution is sprayed upon a heated substrate, the precursor decomposes[21].
5. **Aerosol process** - Spaying a solution into a series of reactors where aerosol droplets get precipitated the nanoparticles[22].
6. **Bio reduction** - Bacterial mediated reduction mechanism[23], [24].

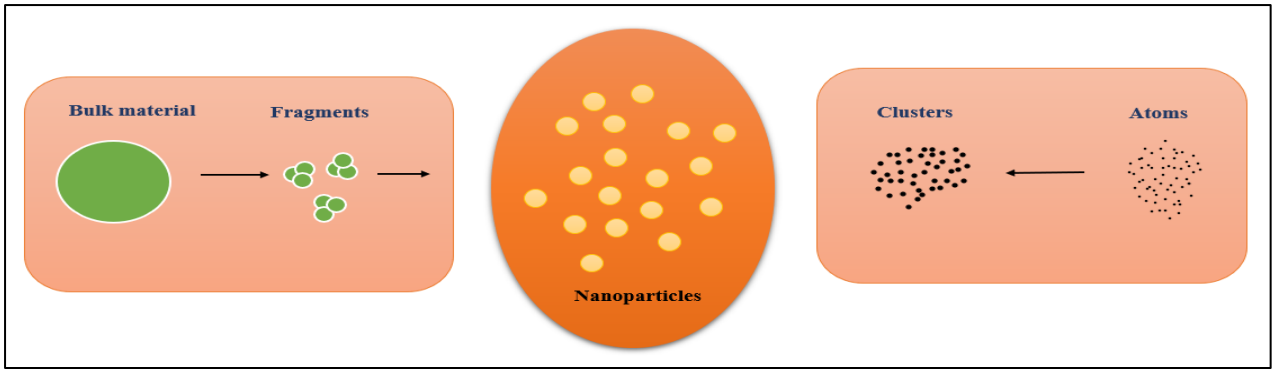


Figure 1.1 – Representing the top-down and bottom-up approach [8]

History and origin of nanotechnology

Every theory, material, or experiment have a beginning from where it discovered or coined for the public to enhance their knowledge and interest towards it. Nanotechnology had its influence from the time of Roman era 4th century AD. 'Lycurgus cup' is a present example of it, in which dichroic glass (made up of nanoparticles) is used which shows two different colours of glass in different lightning conditions. The foundation of nanotechnology can draw back to a lecture given by a legend himself "Dr. Richard Feynman" also known as the father of the modern nanotechnology, at the university of California (Caltech) in 1959, titled by "There is Plenty Room at the Bottom". By the title he was saying that even if you completely fill a glass with water or any other thing, there is still space remain in it because everything is made up of atoms and atoms have 99% empty space. He discussed the possible influence of molecules of the order of atoms. In 1974, a Japanese professor named 'Norio Taniguchi' originated the word "Nanotechnology" through which he described the contribution of semiconductor process and its potential applications. Prof. N. Taniguchi says "Nanotechnology is primarily concerned with separation, consolidation, and deformation of materials by one atom or molecule". The characterisation of nanoparticles also plays an important role in the history of nanotechnology due to their small they can't be seen with microscopes. Therefore, we need high resolution electron beam containing microscopes to identify them. Scientist named 'Ernest Ruska' and his team created the first ever Transmission Electron Microscope (TEM) at the university of berlin in 1931. 'Eric Drexier' has given the concepts for designing and manufacturing of complex mechanics and materials formed from single atoms. By using TEM, in 1991 'Iljimae and Mates' discovered hollow graphite tube, called as the carbon nanotubes. In year 2003 'George W. Bush' signed the "Nanotechnology Research and Development Act" for the enhancement and development of the nanotechnology. Carbon dot were discovered by 'Xu' in 2004. In present time nanotechnology is still growing with an upscale, the synthesis of

nanoparticles had taken a major turn towards the “Green Synthesis” in which the nanoparticles are synthesised from the organic materials like plants, animal waste and microorganisms. Due to which the toxicity is decreased and there is an improvement in the stability of the nanoparticles.

Classification

Nanoparticles are classified on the basis of their physical, chemical and biological methods. They different method for the synthesis using different material-based processes. Diagrammatically shown in the fig 1.3.

- **Physical methods are –**

i. Mechanical method

It is a top-down approach, this method includes mechanical milling through a ball and griding process. In a high energy mill, an appropriate powder charge (usually a combination of elements) is combined with a suitable milling medium. The purpose of milling is to lower down the particle size and mix particles in fresh phases. Various methods for ball milling, in which the balls contact the powder charge, can be utilised to synthesise nanomaterials [10].

ii. Vapour methods

This are those physical methods in which conditions are generated in vapor-phase nanoparticle synthesis when the vapour phase combination is thermodynamically unstable in comparison to the production of the solid substance to be synthesised in nanoparticulate form. It includes physical vapour deposition which is further divided into sputtering, laser deposition, and laser pyrolysis[19].

- **Chemical methods –**

Chemical are those methods in which different kinds of chemical reactions and processes are used for the synthesis of the nanomaterials. It includes chemical vapour deposition (CVD), spray pyrolysis, colloidal methods and sol-gel method. CVD is a process for depositing a solid material from a vapour using a chemical reaction that occurs in a typically heated medium [25]. Other methods have their specific reaction for the synthesis of nanomaterials like –

i. **Spray pyrolysis –**

It's an aerosol decomposition technique based on a liquid sample's thermal degradation. [26], It is a method of depositing a thin coating on a heated surface by spraying a solution on it. [21].

ii. **Colloidal method**

It's a wet chemistry method for making nanoparticles that involves first creating the particles in a solution, then dropping the wet particles onto a substrate and removing the solvents. Although it is time consuming and have chances of contamination[4].

iii. **Sol-gel method**

It is also a wet chemical method and exclusively for the oxide nanoparticles. The molecular progenitor are dissolved in water/alcohol, then heated and stirred to synthesise a gel via hydrolysis/alcoholysis[3].

- **Biological methods**

Biological methods mainly include the green synthesis of the nanoparticles by different parts of plants, animals and microorganisms. It is an organic process having less toxicity, environmentally friendly. Minimization of waste, reduced pollution, usage of organic solvents and renewable feedstock, due to which nanoparticle doesn't harm any living organism[5].

Synthesis of nanoparticles from biological approach also include the 'Green syntheses. Green synthesis is a eco-friendly method which use the plant. animal waste and microorganisms for the reduction and oxidation of the metallic nanoparticles. In this study we had used the extract of bark portion from plant named 'Myrica esculenta'[27]. This plant is mainly found in the north region of India (Himachal Pradesh. Sikkim etc). It has many antifungal and antibacterial properties, which combines with the properties of silver metal and work in form of prominent reducing agent for the producting the nanoparticles.

Myrica esculenta

It is a popular medicinal plant, commonly found in the Himalayan region of India. M. esculenta is used in the medicinal purposes to treat several diseases like anaemia, chorionic bronchitis, cough, asthma and several other disorders[27]. This pant is commonly known as *Kaphal* or *bay berry*. Other than the bark portion, every part of this plant has their own benefits which provide antibacterial qualities. Its classification is given in figure 1.2[27].

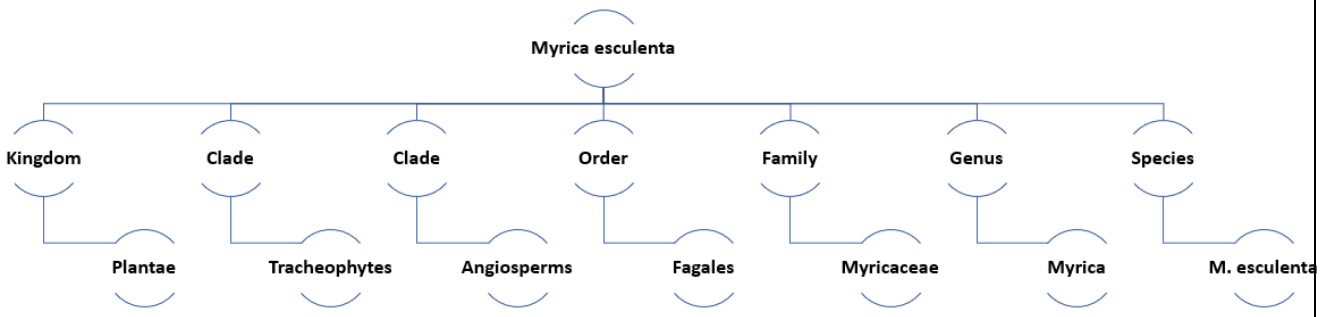


Fig. 1.2 shows – Classification of Myrica esculenta[27].

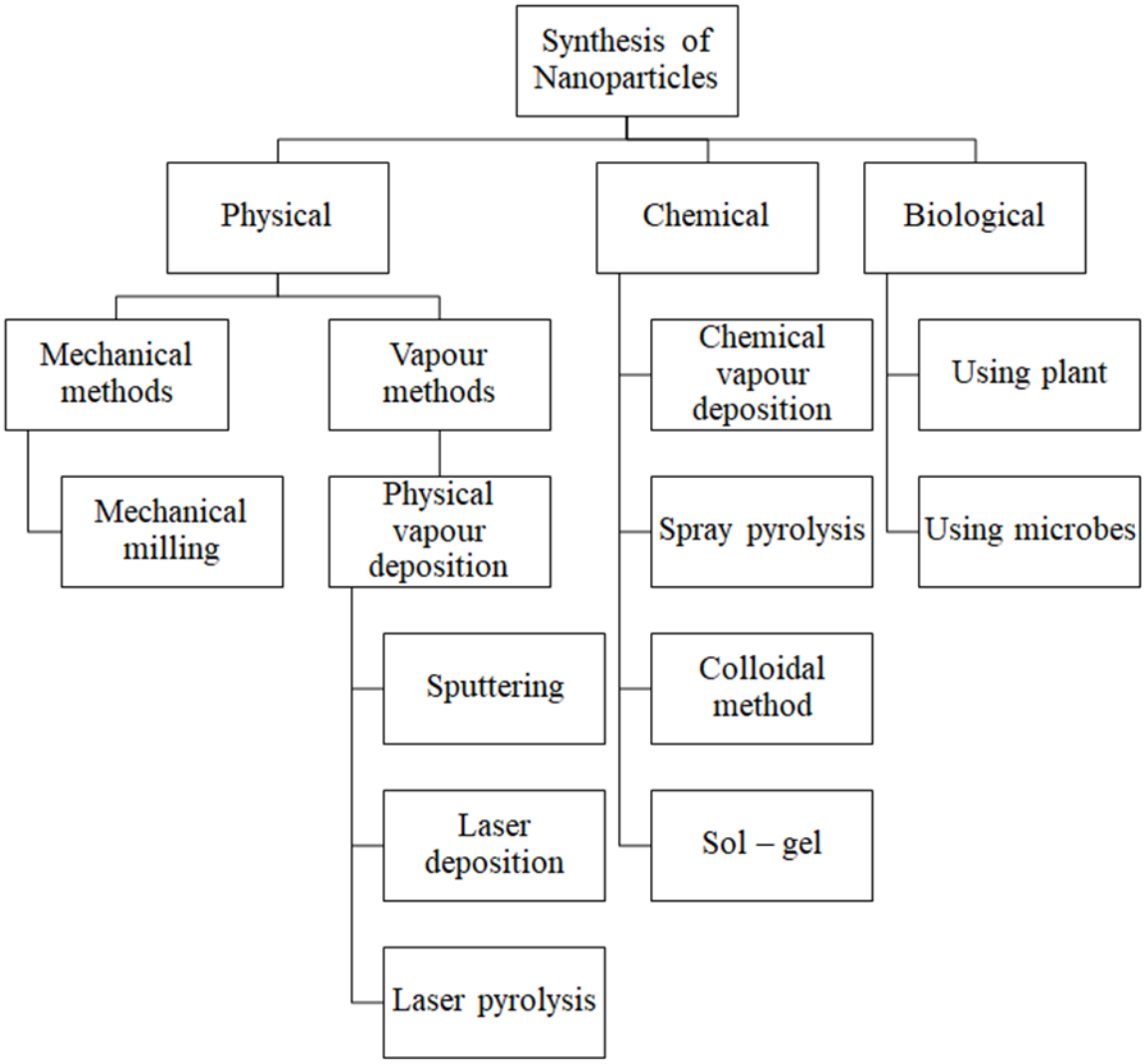


Fig 1.3 shows – The classification techniques for the nanomaterials[7].

Chapter 2

Review of literature

Review of literature (ROL)

Introduction

Nanobiotechnology is an area of science that deals with the world of minuscule particles that are invisible to the human eye and have unique qualities not found in their bulk form.[1] They can be recognised by their size, form, structure, and other characteristics.[1] Nanobiotechnology is an area of science that combines nanotechnology with biology.[1] A nano-meter is one billionth of a metre, and we examine how we may make things with general materials on the scale of atoms and molecules in Nanotechnology.[1] The diameter of a human hair is around 50,000 Nano-meters, 10x the diameter of a hydrogen atom.[28] Ordinary chemistry, physics, and other materialistic science rules are no longer applicable at this level.[2] Nanomaterials are nanoscale materials in which surface or interference features influence bulk properties. Some of its properties, such as colour, strength, conductivity, and reactivity, differ significantly between the nano and macro scales (for example, carbon nanotubes are 100 times stronger than steel but six times lighter). Gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), quantum dots, polymer-based NPs, carbon dots, metals and metal oxides, and other nanoparticles are examples.[2]

These nanomaterials can have superior physical and chemical properties, such as higher catalytic activity, greater stability, and altered optical behaviour, due to their enormous surface area.[1] Nanomaterials are used in a various of consumer products, involving textiles, paints, sensors, and other health-related items.[2] Controlling the size, distribution, and shape of synthetic nanoparticles, in addition to dispersion, aggregation stability, and nanocrystalline composition, is often critical for the manufacture of desired nanoparticles.[28]

Nanotechnology opens up a plethora of new study opportunities.[1] Novel diagnostic procedures and therapeutics, particularly in medicine and sensor biotechnology, promise a wide range of development potential, for example, novel drugs can be developed and supported by the progressive miniaturisation in the electronic industries, and interdisciplinary research on so-called Nanobots.[2] The development of lengthy fibrous devices that can be implanted into organisms would be a natural progression of minimally invasive surgery.

The food industry is investigating meals that have a longer shelf life by utilising nanoparticles to raise or lower their temperature and create diverse flavours in a home oven. Nanotechnology is being employed in agriculture to advance the field of biological crop production.[6]

The two major ways for making nanoparticles are 'Top-down' and 'Bottom-up.'
A bulk substance is ground or transformed into microscopic particles in a Top-Down manner using –

Name of method	Process
Sputtering	It is a vapour deposition method that deposits materials onto a particular surface by condensing atoms on to the surface
Chemical etching	To generate a permanent etched image on metal, high-pressure and temperature spray is used to remove material.
Thermal/laser ablation	Fabrication of micropatterns through the removal of small fractions of a substrate under a laser beam.
Mechanical/ ball milling	Grind and blend materials for use in mineral dressing process.
Explosion process	Pulse current injected through the conducting wire to vaporise it and then condense it.

Angstrom size or very minute particles are condensed together in the bottom-up process to achieve the size of nano particles.[1]

Name of methods	Process
Molecular condensation	Two molecules of nanoparticles combined together to form a single molecule.
Vapour deposition	Used to produce thin films. Substrate expose to one or more volatile precursor to produce thin film.
Sol-gel process	Monomers are converted into a colloidal solution.

Spray pyrolysis	A precursor solution is sprayed onto a hot substrate, leading to decomposition of the precursor.
Aerosol process	Spaying a solution into a series of reactors where aerosol droplets get precipitated the nanoparticles.
Bio reduction	Bacterial mediated reduction mechanism.

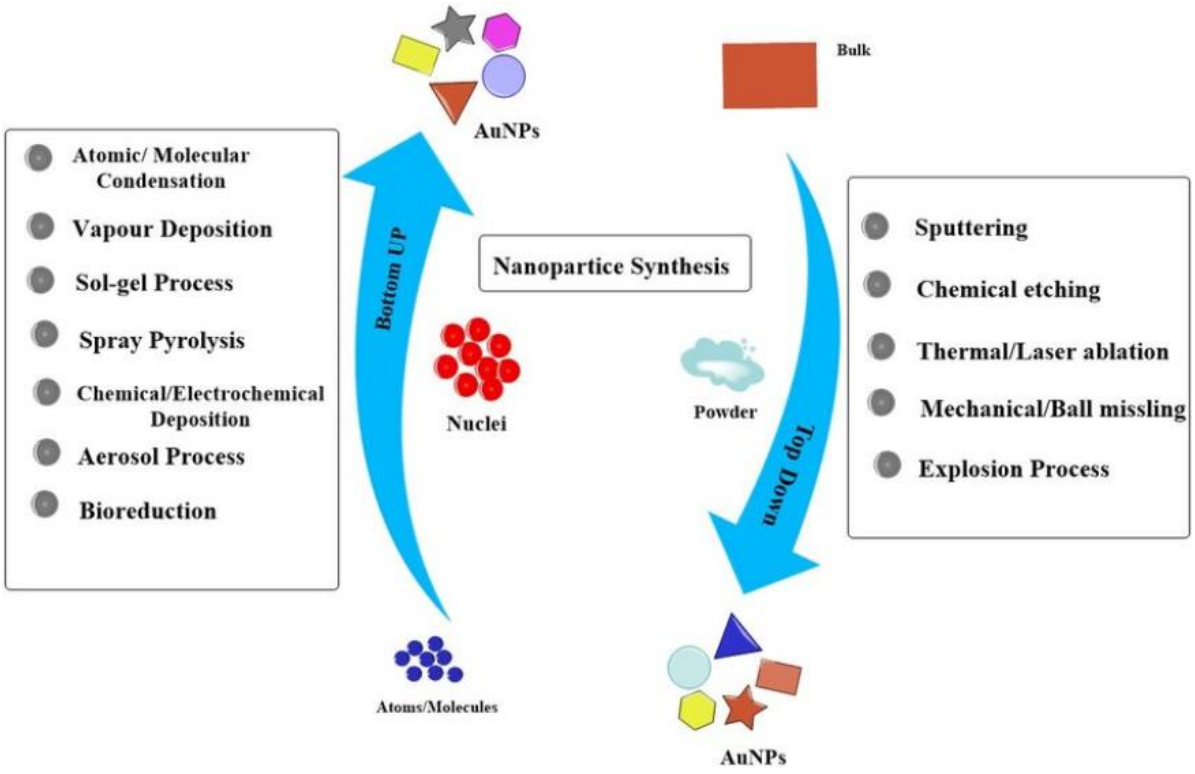


fig. 2.1 shows – The process of "Top-Down" and "Bottom-Up" approach. [29]

Organic and inorganic nanoparticles –

Organic nanoparticles and inorganic nanoparticles are the two main types of nanoparticles found in nature.[30] In terms of fabrication principles, organic nanoparticles differ from inorganic NPs.[9] In comparison to organic nanoparticles, inorganic nanoparticles are hydrophobic, very stable, non-toxic, and biocompatible since organic nanoparticles are manufactured from raw and natural materials, whereas inorganic nanoparticles are synthetic nanoparticles built for a specific procedure.[30] Because of the toxics included in the compounds, inorganic nanoparticle synthesis processes are harmful to health at first, but with

time, they switch to green synthesis, in which inorganic nanoparticles are turned into biocompatible products.[30] Sol-gel technique, mechanochemical processing, and physical vapour synthesis have all been successful in producing inorganic nanoparticles.[9] Acid hydrolysis, ultrasonication, ionic gelation, and the reverse micellar approach, on the other hand, are all effective ways for organic nanoparticles.[9]

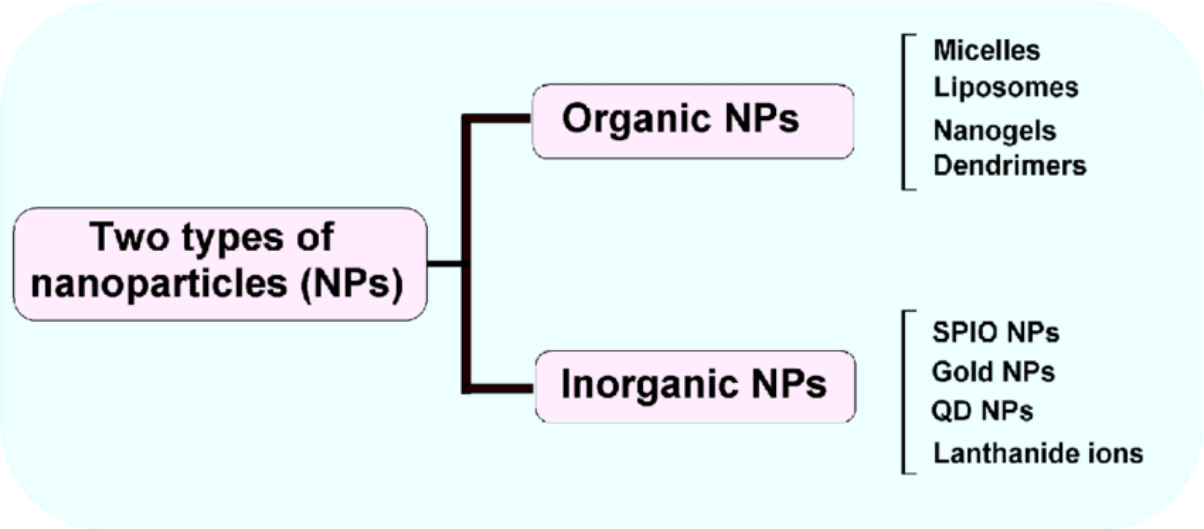


Fig. 2.2 shows – Types of Organic and Inorganic nanoparticles.[31]

Properties of nanoparticles –

Nanoparticles are different in size from other particles, their characteristics are likewise varied.[9] Physical qualities, chemical properties, magnetic properties, electrical properties, optical properties, and so on are all included.[2]

Size, shape, specific surface area, aspect ratio, surface morphology/ topography, structure; including crystallite and defect structure, solubility, and the process of assembling fine particles into cohesive units like pallets or clots, also known as agglomeration or aggregation state, are all examples of physical properties.[1] Structure formula, nanomaterial composition, phase identity, surface chemistry (composition, charge, tensions, reactive sites, physical structures, photo catalytic capabilities, zeta potential, and so on), and hydrophilicity or hydrophobicity are all chemical qualities.[1] Magnetic characteristics are a charge-based function; nanoparticles' magnetic properties are particularly useful due to their small size.[1] Drug delivery, therapeutic treatment, MRI imaging, bio separation, and in vitro diagnostics all involve magnetic

characteristics. Magnetite and maghemite are two types of magnetic nanoparticles that are produced by a co-precipitation technique.[1] Nanotechnology has made it possible to produce supra magnetic qualities in nanoparticles by shrinking the size of ferromagnetic and ferrimagnetic materials to a few nanometres or smaller than the supra-magnetic diameter[2]. When in a zero magnetic field, supra magnetic nanoparticles are not magnetic, but when exposed to an external magnetic field, they quickly become magnetised[1].

Nanoparticles are used to cover electrodes to enhance the electrical properties of materials with same chemical composition; the conductivity of electrodes rises because of the huge surface area of nanoparticles.[7] Conductivity of a bulk material is independent of its diameter or cross section area, but it has been discovered that the conductivity of carbon nanotubes changes with changes in cross section area and also when shear force is applied to the carbon nanotubes, indicating that they can act as a conductor or semiconductor.[7] Many experiments rely on the optical properties of nanoparticles, which include absorption, transmission, reflection, and light emission.[7] The inter electronic structure of the material employed determines these modifications.[7] Because of the presence of surface plasmon resonance (SPR), nanoparticles have different colours than their bulk counterparts. For example, gold metal in bulk is yellow-orange in colour, but gold metal in nanoparticle form is red in colour because the size decreases and it can only reflect high wavelengths.[32] Spectroscopic techniques can be used to determine optical characteristics.[32] The energy of both the highest and lowest molecular orbitals was altered by the reduction in the dimensions of nanomaterial's electronic effects.[32]

Characterisation techniques for nanoparticles –

Nanoparticles are so small that they can't be seen with the human eye, we need a special device to study them[32]. The primary techniques used to study nanoparticles include scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction, UV-radiation spectroscopy, FTIR (Fourier transform infrared radiation), AFM (atomic force microscopy), and so on[1].

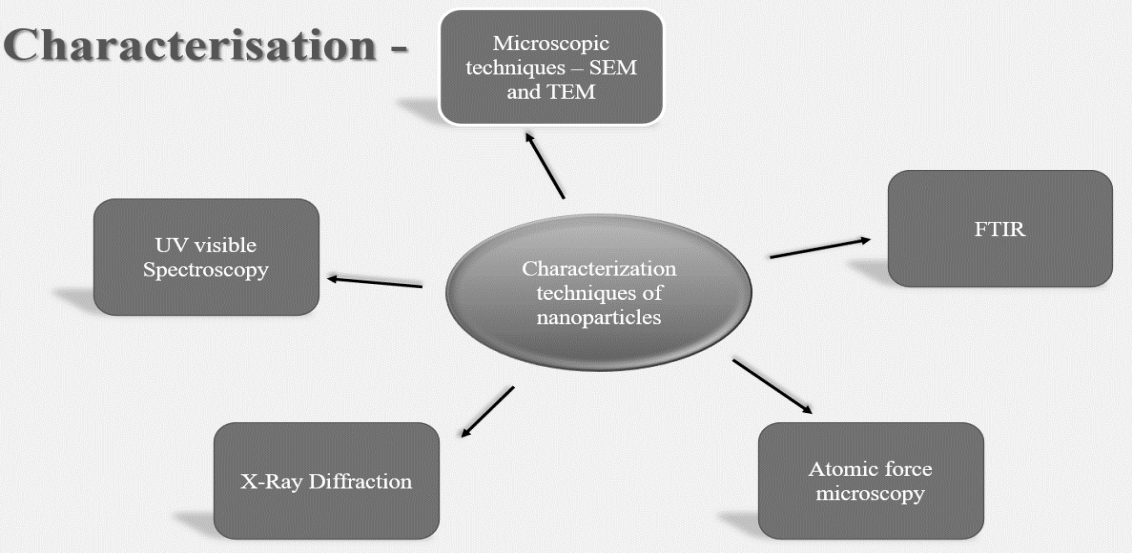


Fig 2.3 shows – Different characterisation techniques of nanoparticles

SEM (Scanning electron microscope)

Back scattered or diffracted electrons from the specimen are traced by the detector in scanning electron microscopy, and the image of the specimen is formed.[8] The electrons communicate with the atoms on the sample surface, generating signals which reveal the composition of sample, shape, and surface.[33] The electron beam is emitted using an electron cannon at the top of the SEM.[34] It features two condenser lenses to keep the electron beam narrow and preserve its path; below the condenser lenses is an objective lens with the specimen stage.[34] Then there's a detector, which aids in the detection of backscattered electrons and the formation of a picture from them.[34]

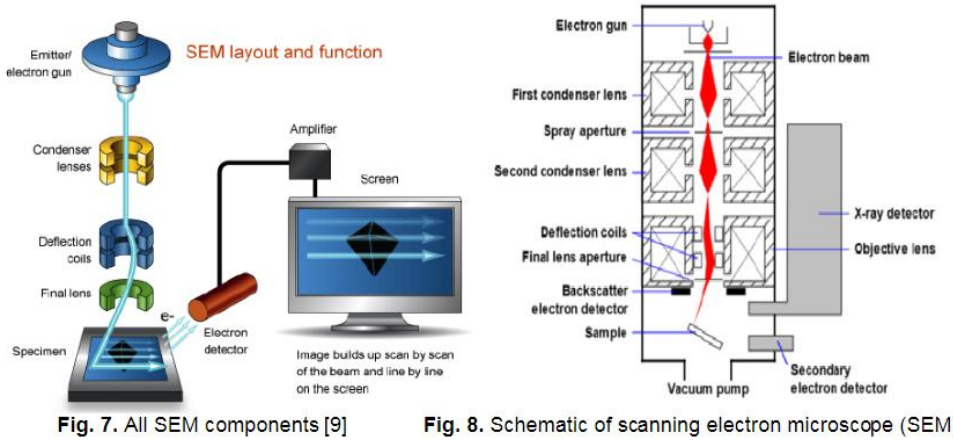


Fig. 7. All SEM components [9] **Fig. 8.** Schematic of scanning electron microscope (SEM) [9]

Fig 2.4 shows – Schematic diagram and components of SEM[34].

TEM (Transmission electron microscopy)

The transmitted light in a TEM picture shows the image of the specimen.[35] An electron emitting pistol, a condenser lens, a specimen stand, an objective aperture lens, an intermediate lens, a projector lens, and a fluorescent screen are among the features.[35] It necessitates the use of a vacuum chamber and relies on an electron particle beam to see specimens and generate highly magnified images.[36] The specimen is passed through a vacuum and condenser lens, where the electrons are dispersed or hit the fluorescent screen at the bottom of the microscope, resulting in a picture of the specimen with its asserted components appearing on the screen in various shades depending on its density.[36]

In this sort of microscopy, we need an extremely thin specimen, thus we use an ultramicrotome to prepare it so that the electron beam can easily travel through it.[36], [37]

The major difference between TEM and SEM is that for forming the images SEM uses reflected or knocked-off, whereas TEM uses transmitted electrons.[37]

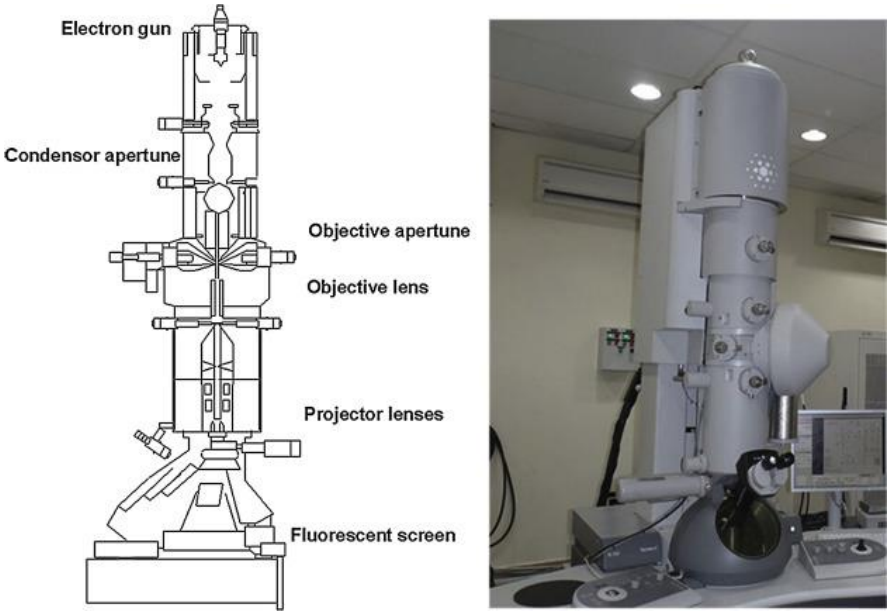


Fig 2.4 shows – Diagrammatic representation of TEM[38].

X-ray diffraction (XRD)

A crystalline substance is required for the XRD process.[39] It is based on monochromatic light and a crystalline material interacting constructively.[39] A cathode tube is used to produce x-rays.[39] Only when the incident rays meet the conditions of Bragg's law, which connects

the wavelength of electromagnetic radiation to the diffraction angle and lattice spacing of a crystalline solid, do they cause constructive interference.[39], [40]

This device offers data on the crystal's strain, grain size, crystallinity, and flaws.[40] The workhorse of XRD, a point detector with a Nal crystal scintillator, is the most frequent form of detector utilised in XRD.[39] This approach entails exposing the sample to incoming x-rays and then measures the intensities and scattering angles of the x-rays that get away.[40]

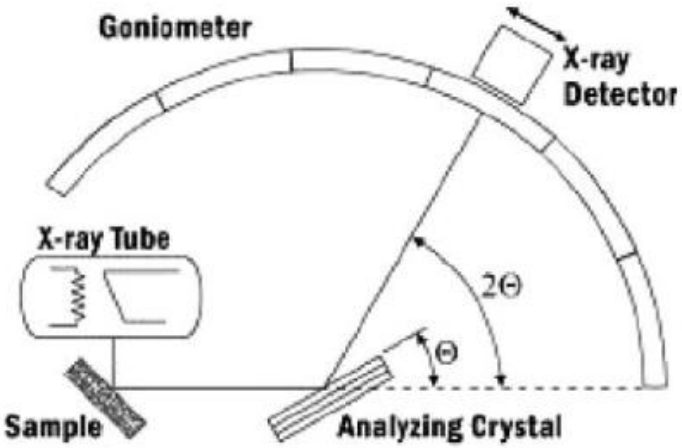


Fig 2.5 shows – Schematic representation of working of diffractometer[40].

UV-radiation spectroscopy

By measuring the amount of scattered light from the cuvette, this technique can be used to quantify the sample.[41] When a molecule or ion transitions from one energy state to another, it emits electromagnetic radiation, which is calculated and interpreted by spectroscopy.[42]

When a chemical material absorbs UV light, it produces a specific spectrum that can be used to identification of unknown compound.[15] A light source, monochromator, sample and reference cells, detector, amplifier, and recording devices are all part of the system.[42]

It is necessary to detect impurities in compounds, to clarify the structure of compounds, to quantify compounds, to check the functional group in the compound, and so on.[41]

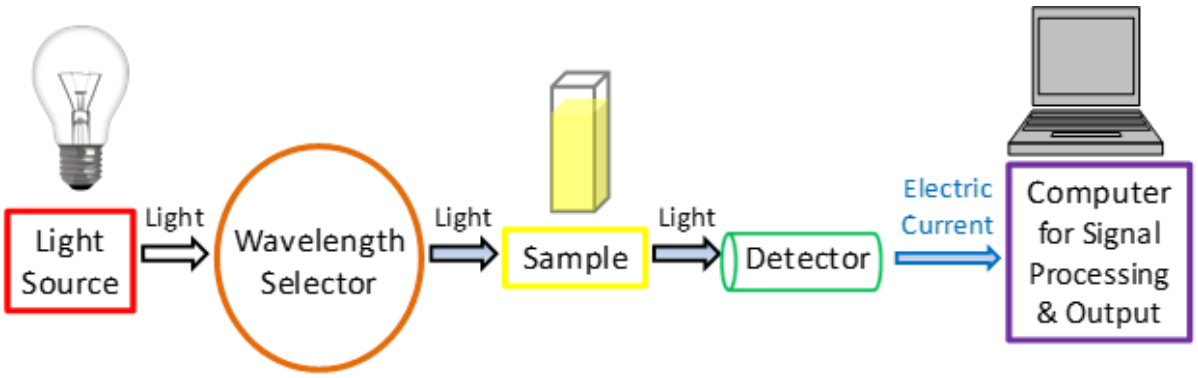


Fig 2.6 shows – Diagrammatic representation of UV Vis. Spectroscopy [42]

FTIR (Fourier transform infrared radiation spectroscopy)

The first generation of IR spectrometers was developed in the late 1950s, and the FTIR is the third generation of IR spectrometers, which signalled the end of monochromators and the rise of interferometers.[43] This approach analyses the vibrations and rotations of molecules impacted by infrared radiation at a certain wavelength (720nm – 1mm) and is used to determine the charges in functional groups such as alkane, thiol, alcohol, and others in biomolecules.[43] The sample absorbs IR radiation, causing a change in the strength of the radiation, which is recognised as an off-null signal.[44] The operations of synchronous motors are used to record the changes in signals.[44]

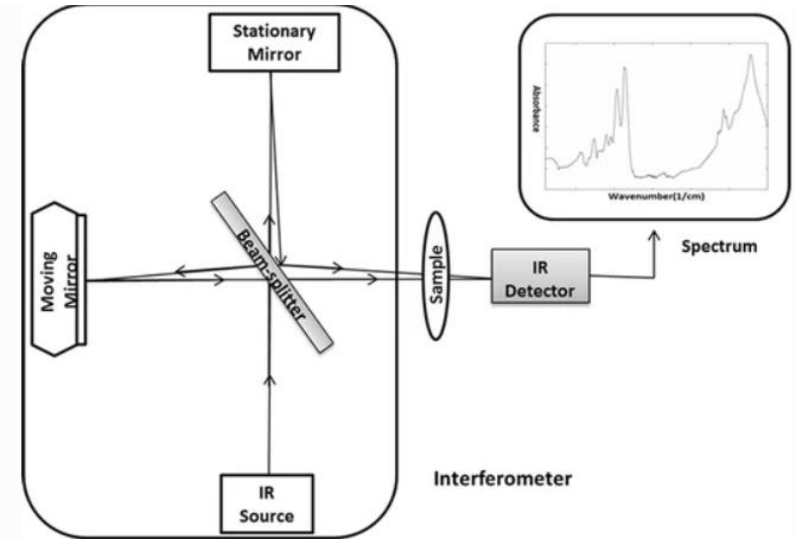


Fig.2.7 shows – Schematic representation of FTIR spectroscopy[44]**.**

AFM (Atomic force microscopy)

Binnig, Gerber, and Quate created it in 1985.[45] It is made up of a cantilever composed of diamond shards connected to gold foil and a laser beam that applies force to the Cantilever.[46] The sample interactions were provided by the interatomic Van der Waal's forces as the diamond tip made direct contact with the surface.[46] AFM control deflection and force management as well as angstrom-level positioning and feedback loop control.[46] From the back of the refractive AFM lever, a laser beam is reflected onto a position-service detector.[46] The cantilever's tips are made of Si or Si3N4 microfabrication.[46] In the AFM, Hokk's law is used, which states that force is equal to the product of the lever's stiffness and the distance the lever is bent.[45]

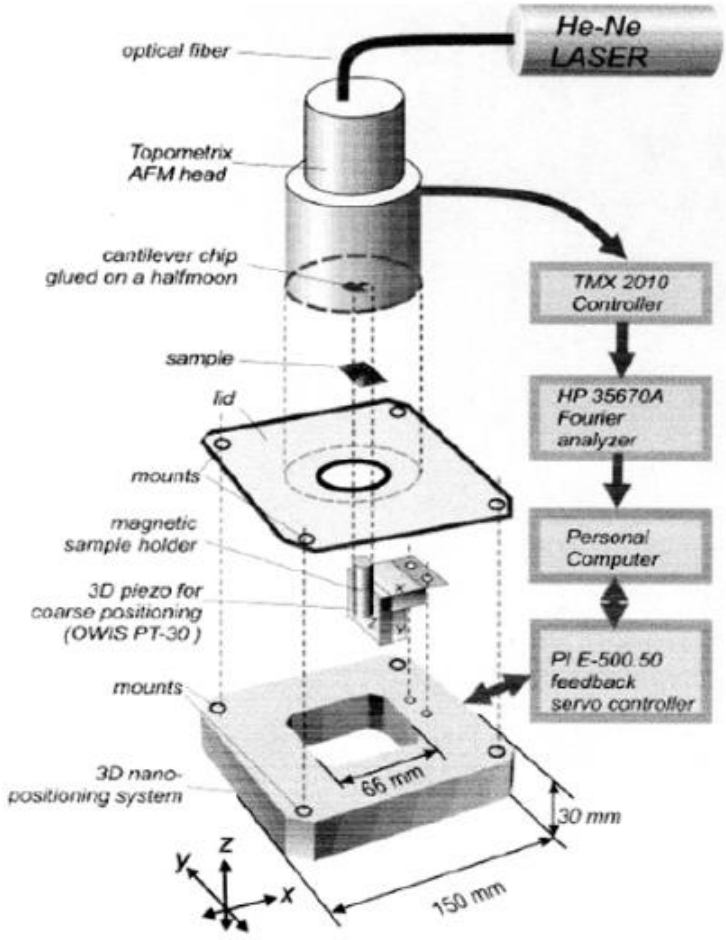


Fig. 2.8 shows – diagrammatic representation of AFM[47].

Applications of nanobiotechnology

Nanotechnology has uses in a wide range of fields, including electronics, renewable energy, biomedicine, health care, the environment, food, textiles, and military applications.[1], [2] Nanomaterials are expected to have a huge impact on biotechnology and medicine because of their similar size to biological constituents including enzymes, antibodies, proteins, and nucleotides, which makes them easier to utilise in medical applications.[1], [2] Nanoparticles offer unique features like as optical emission, a large surface-to-volume ratio, electrical and magnetic properties, and can be used in bioengineering applications ranging from drug delivery to biosensor manufacture. Metal NPs, semiconductors, and other carbon materials have expanded the applications for biosensor device development.[2]

Biosensors

Biosensors are analytical devices that generate electronic signals by interacting with a receptor and a target molecular analyte.[1], [2], [28] A bioreceptor, a transducer, an amplifier, electronics, and an interface are all components of a biosensor.[1] Because of its linearity, simplicity, stability, low cost, quick analysis, and downsizing, biosensors offer enormous potential.[1], [2] Clark and Lyons invented the first biosensor in 1962, which detected glucose content by immobilising glucose oxidase (GOD) enzyme molecules on the surface of an oxygen electrode.[1]

Biosensors are used to detect bio-receptor molecules such as cells, proteins, and enzymes, which are bonded to the biocompatible layer of nanomaterials.[2] We can examine the detected molecules using a microcontroller and display screen, which is attached to a nanomaterial and transducer via which it is amplified.[28]

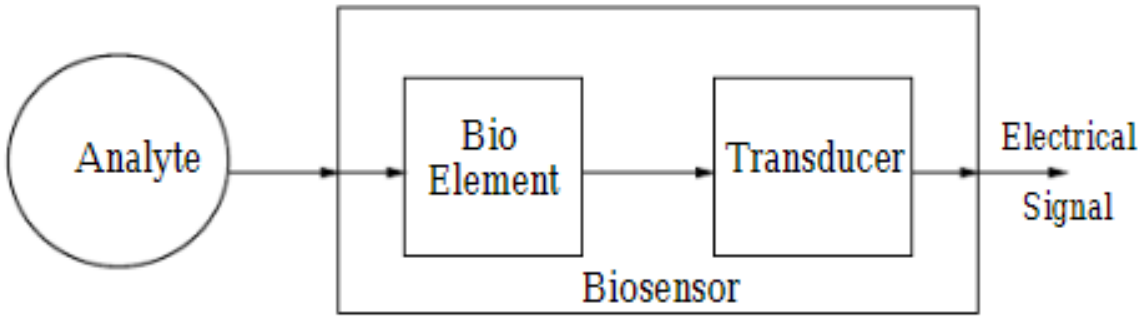


Fig. 2.9 shows – The schematic diagram of nanoparticle-based biosensor[48].

Types of biosensors -

They come in a variety of sorts depending on the application and transducer used in the building of a biosensor.[2] DNA or RNA-based biosensors, antibodies or antigen-based biosensors, microbial biosensors, and enzymatic biosensors are all examples of recognition elements.[28]

DNA based biosensor –

DNA biosensors are used to identify immunodeficiency and neurology-related disease.[49] Using nano diagnostic techniques, we can have more precise tools for early diagnosis and simplify the supply of surgical and modified medicine in healthcare.[50]

It is dependent on the immobilisation of a single-strand oligonucleotide probe onto the transducer surface, since the probe DNA is particular to recognise the complementary DNA target sequence via hybridization, and they apply a change in the detectable signal by generating a duplex structure.[49]–[51]

Antigen-Antibody based biosensor -

It works on the basis of affinity and are also known as immunosensors.[52] When antigen or antibodies are present in an immunochemical reaction, they are used as a biorecognition element immobilised on a transducer surface.[25] Because of their excellent selectivity and label-free detection, these sensors are useful for detecting malignant compounds, proteins, LDLs, and food pathogens, among other things.[53] It has the potential to enhance the loading of biorecognition molecules with large surface areas.[54]

Enzymatic biosensors -

In an enzymatic biosensor, an enzyme molecule is immobilised on a transducer surface and reacts with a chosen analyte to create a specified signal.[55] GOD can be used for quantitative monitoring of glucose concentration with or without mediator in the first glucose-based biosensor, which is also an enzymatic biosensor.[55] Another example is the detection of urea in a sample where urease is capable of converting hydrogen bicarbonate and ammonia.[56]

Optical biosensor -

It has a bioreceptor molecule and an optical transducer that make up an analytical gadget.[57] The transduction system causes changes in absorption, reflection, refraction, frequency, and amplitude when a physical or chemical change occurs in a typical reaction or process.[57] A

light source, a bioreceptor, and an optical detecting system are the essential components[57], [58].Absorption process, fluorescence, surface plasmon resonance, and fibre optic biosensors are some of the applications for optical biosensors[58].

Thermal biosensor -

They can detect temperature changes in immobilised bioreceptors and target molecules during a biological reaction.[59]

Drug delivery system –

Nanoparticles play an important role in medication delivery because they may conjugate with a variety of medicines and deliver them to the target location via diverse techniques. The NP surface is engineered with ligands to increase cell affinity and co-polymers to keep immune cells at bay. Because of its high selectivity towards the target region, nanotechnological applications are extremely essential in the field of medication administration, as it might lessen hazardous side effects of pharmaceuticals in normal cells.[60]

It can be defined as "Formulation of a device that allows medicinal chemicals to be introduced into the body and enhances efficiency and safety by controlling the rate, duration, and location of drug release in the body".[60]

The nanoparticles used in Drug Delivery Systems contain encapsulated, absorbed, dispersed, or conjugated drugs, which have lower toxic side effects, multifunctional targeting, delivery, and reporting capabilities, high saturation solubility, drug particle resistance to settling, and improved therapeutically index.[61]

Food

Consumer concerns about food quality and health benefits are driving academics to discover a technique to enhance the food quality while maintaining the nutritional value of the product to the greatest extent possible[62]. The food sector has broaden its desire for nanoparticle-based products because many of them include critical nutrients and have been proven to be non-toxic[63].

Nanostructured food components are being created with the hopes of improving flavour, texture, and consistency[64]. Nanotechnology extends the shelf life of many food ingredients and reduces food waste due to microbial infection[65].

Catalysis

Nanoscience and nanotechnology contributed to have this, going far away the traditional homogeneous and heterogeneous catalysts to create catalysts with unparalleled properties and performance[8],[66]. The mechanisms supporting the nano-effects remain vague, and there are still some places left for development of design for nano-catalysts. Ongoing design scheme rely on nanoscale production of highly active sites as well as micro-environment created through embedding the nano-catalyst in limited spaces in permeable nanomaterials[8], [66]. Modern characterization of nanoparticle is important for more logical nanoparticle design and production.

Nano-effects incorporate structural alterations and are bounded, and they have an effect on energy levels, that can be modify nanomaterials physical, electrical, and optical characteristics. The generation of bulky and fine chemicals in conventional petroleum-based refineries and biorefineries are based on biomass, carbon dioxide conversion, photocatalytic water splitting, reformation, and the enlargement in advanced sensor materials are all leading catalytic applications in sustainable chemistry[8], [66], [67].

Chapter 3

Materials and Methodology

Materials

- Materials for the preparation of the crude extract –

i. Myrica esculenta's bark portion
ii. Mortal and pastel
iii. Weighing balance
iv. 100 ml and 10 ml measuring cylinder
v. Milli-Q water
vi. Flasks of 100 ml. 250ml, 500ml.
vii. Beaker
viii. Hot plate
ix. Magnetic bead
x. 45 ml tubes (plastic vail/falcons)
xi. Centrifuge
xii. Cold storage
xiii. Parafilm

- Materials for the preparation of the $AgNO_3$ solution -

i. $AgNO_3$ (silver nitrate)
ii. Milli-Q water
iii. Weighing balance
iv. Flask
v. Measuring cylinder 100ml and 10 ml.

- Materials for the preparation of the silver nanoparticles –

i. Plant aqueous extract
ii. Sliver nitrate solution
iii. Flask/test tubes
iv. pH meter
v. NaOH (0.5 N)
vi. HCL (1 M)
vii. Micropipettes (1µl – 1ml)

viii. Microtips (1µl – 1ml)

Methodology

- **Preparation of the plant aqueous extract** -

We need 2gm of plant extract (name) and 200 ml of milli-Q water(name). Weight 2 gm of plant extract powder and put it into a flask. Then add 200 ml of the milli-Q water into it . So, it can make a 1% solution in respect of plant extract powder and the milli-Q water. Then put that flask on the hot plate(name) on 60˚ Celsius for 30 minutes. After the proper mixing of the plant extract powder with milli-Q water let it cool for some time. Then put equal amount of aqueous extract in plastic vails/ falcon tubes (name). Centrifuge them on 10000 rpm for 20 min. After the centrifugation process take out the supernatant in another plastic vail/ falcon tube (name) and discard the palate. Cover the mouth of the tubes with parafilm, otherwise it will get contaminated. Store the tube at 4˚ Celsius until the time of use.

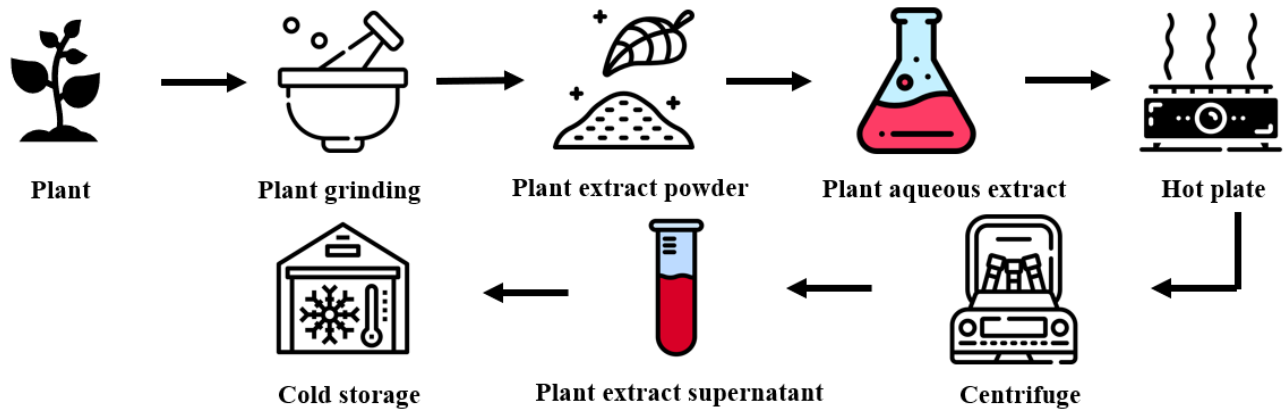


Fig.3.1 shows – Pictorial representation of the preparation of plant aqueous extract.

- **Preparation of the Silver nitrate ($AgNO_3$) solution** –

For the preparation of $AgNO_3$(name) solution, we need to make 7mM solution of $AgNO_3$ in 100 ml of milli-Q water(name). Weight the calculated amount of $AgNO_3$ and add it into a Flask. Then add 200 ml of water into the flask and shack them well until it mixes properly. Cover the flask and store it in the cold storage on 4˚ Celsius.

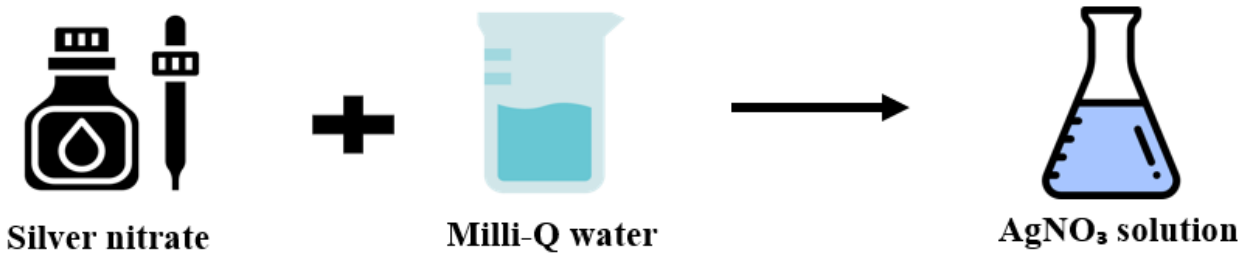


Fig. 3.2 shows – Preparation of the silver nitrate ($AgNO_3$) solution.

- **Preparation of the silver nanoparticles from plant extract** –

To prepare AgNPs, take 1.25 ml of plant extract (name) and pipette out it in 9 ml of 7mM $AgNO_3$ (name) in a glass test tube and mix it well. After properly mixing of solution, perform its pH. In this study we have taken 4,6,7,8,9,10,11 and 12 pH of AgNPs in different samples. From which we optimized pH 10 as the optimum pH for the stability, size and durability of the nanoparticles. After the pH, its spectra are done by multiskan (spectrometer)(name) on a range of 200 - 800 nm wavelength. 60 times diluted sample is taken for the scanning procedure.

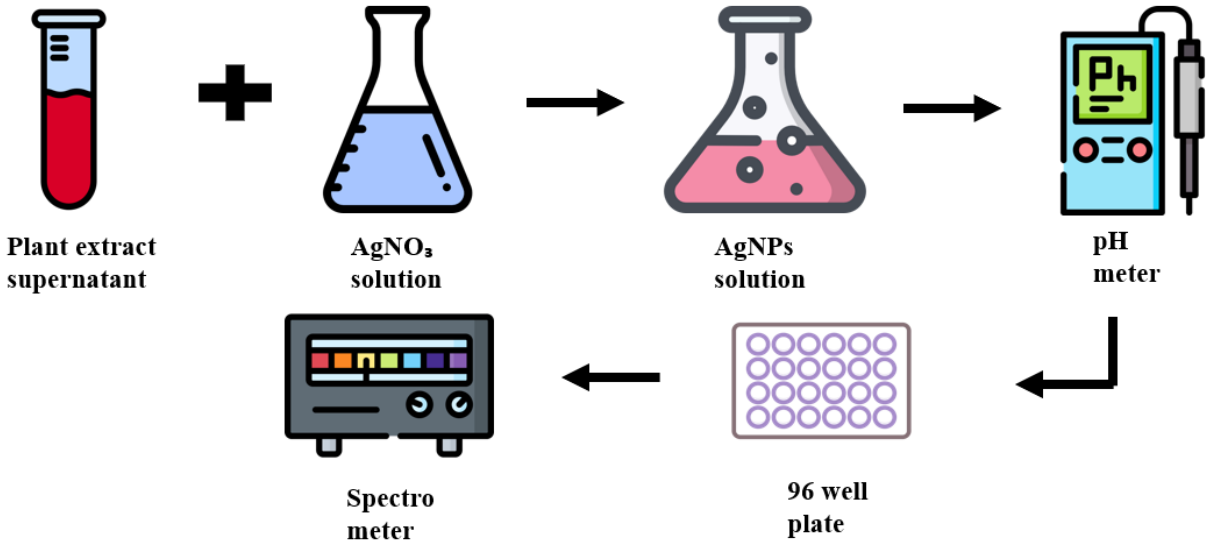


Fig. 3.3 shown – Procedure for the preparation of AgNPs.

Catalytic activity of AgNPs on reduction of methylene blue

Stock solution of methylene blue dye prepared by 200ml of milli-Q water and 2mg of dye[66]. About 2ml of biosynthesised AgNPs was added to 20ml of methylene blue dye solution using different pH level (6.0,7.0 and 8.0) (name)[66]. the suspension reaction mixed for 30 min with magnetic stirrer[66]. Then the solution was exposed to the light of the sun from morning until sunset[66]. At specific time intervals, 5µl of suspension aliquots were used to estimate the photocatalytic dye degradation at various wavelength using the UV-Vis spectrometer (multiskan)[66]. The dye degradation was measured at 600 nm – 700 nm using the absorbance value[66]. The proportion of dye degradation was calculated according to the following formula:

% Degradation = $(1 - A_t/A_0)$ {[66]}

A_t is the absorbance after time t min

A_0 is the absorbance at zero time

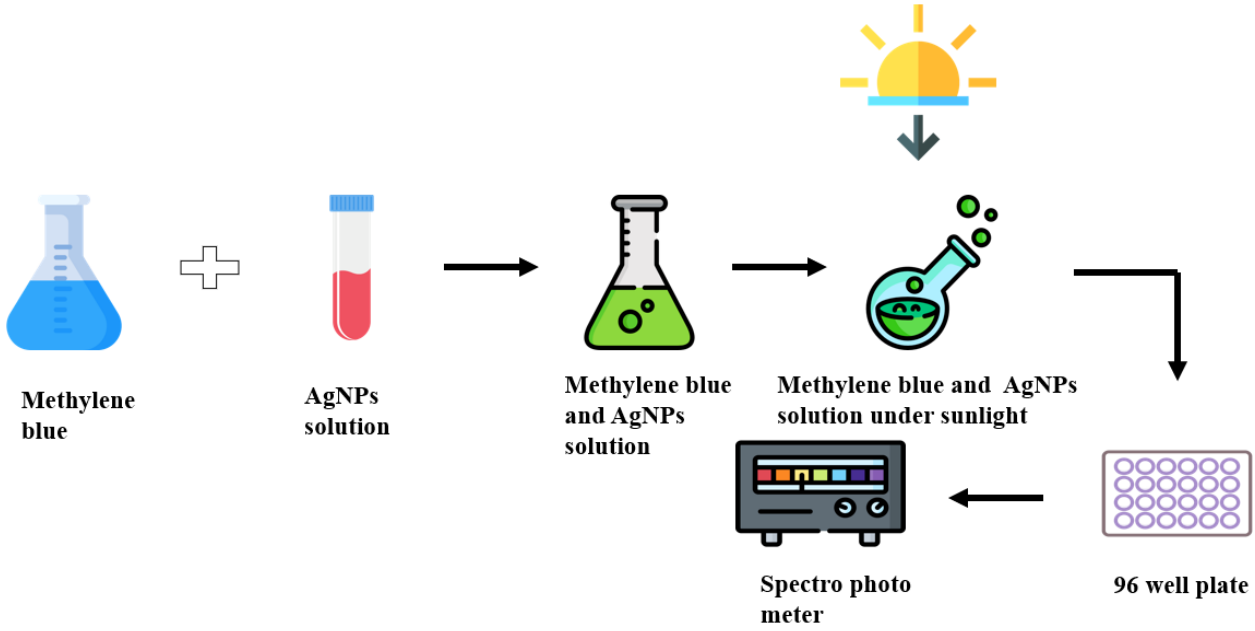


Fig. 3.4 shows – Photocatalytic reduction of AgNPs.

Chapter 4

Results

UV visible spectroscopy analysis:

Formation of biosynthesised silver nanoparticles (AgNPs).

Fig 4.1 shows the result of the formation of AgNPs by mixing the $AgNO_3$ with plant extract at the ratio of 9:1, respectively. By using the UV-Vis. Spectroscopy on the range of 300nm - 800 nm wavelength. The peak is shown over the 350nm - 450 nm wavelength which confirm the formation of nanoparticles.

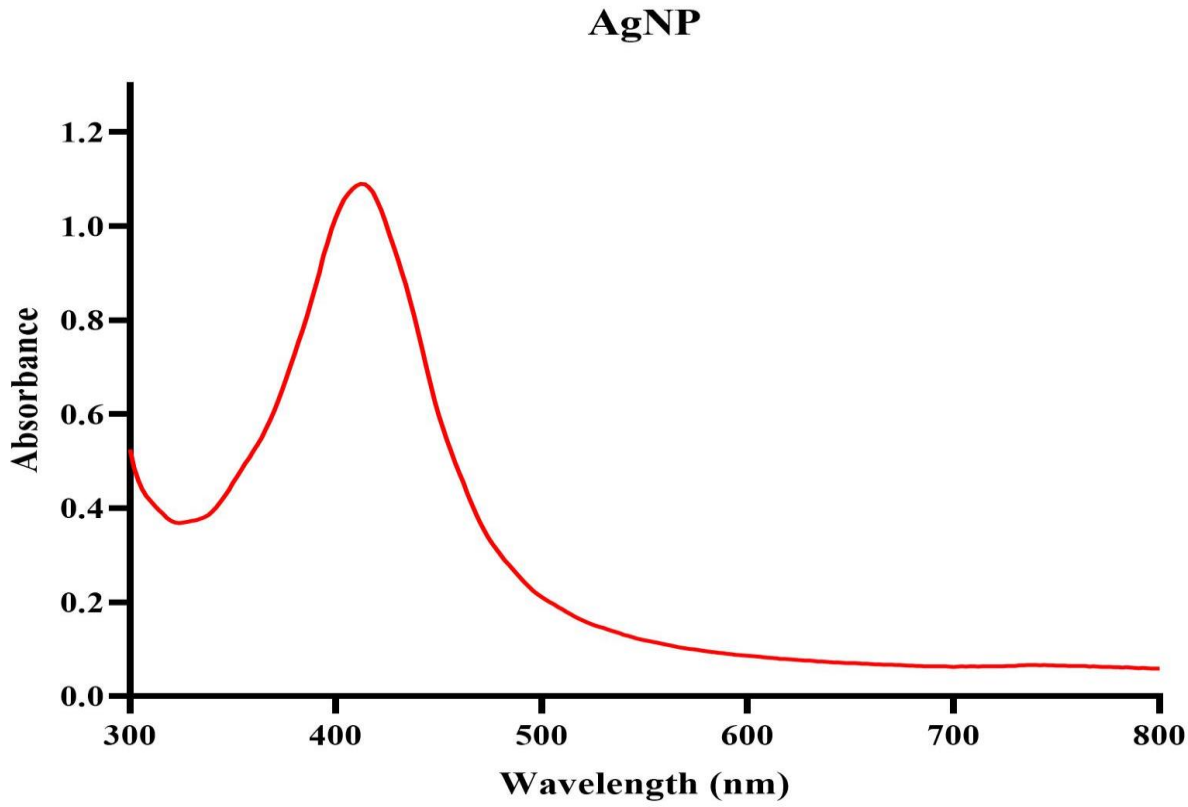


Fig. 4.1 shows the formation of the silver nanoparticles from the plant extract as the reducing agent. Result is done by UV-Vis. Spectroscopy.

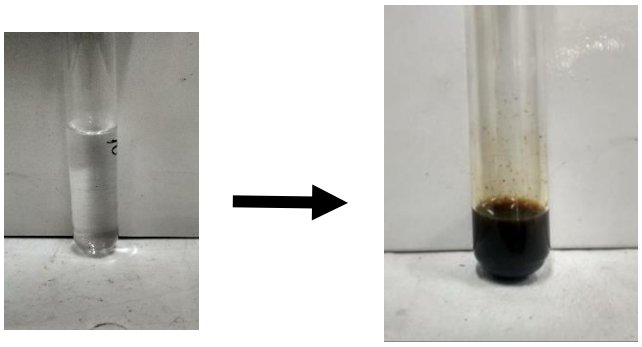


Fig 4.2 shows Colorimetric changes in silver nitrate and formation of AgNPs

Results for the optimization of concentration of the $AgNO_3$.

For the concentration optimization of $AgNO_3$, different amount of $AgNO_3$ is put into test tube with 10ml of milli-Q water and mixed well. After, mixing 1ml of plant extract (reducing agent) is pipette out into it and make the pH of the solution to 7. Then the spectra is preformed on UV-Vis spectrometer with 60 times diluted sample. The optimum concentration comes out to be 7mM shown in figure 4.1.

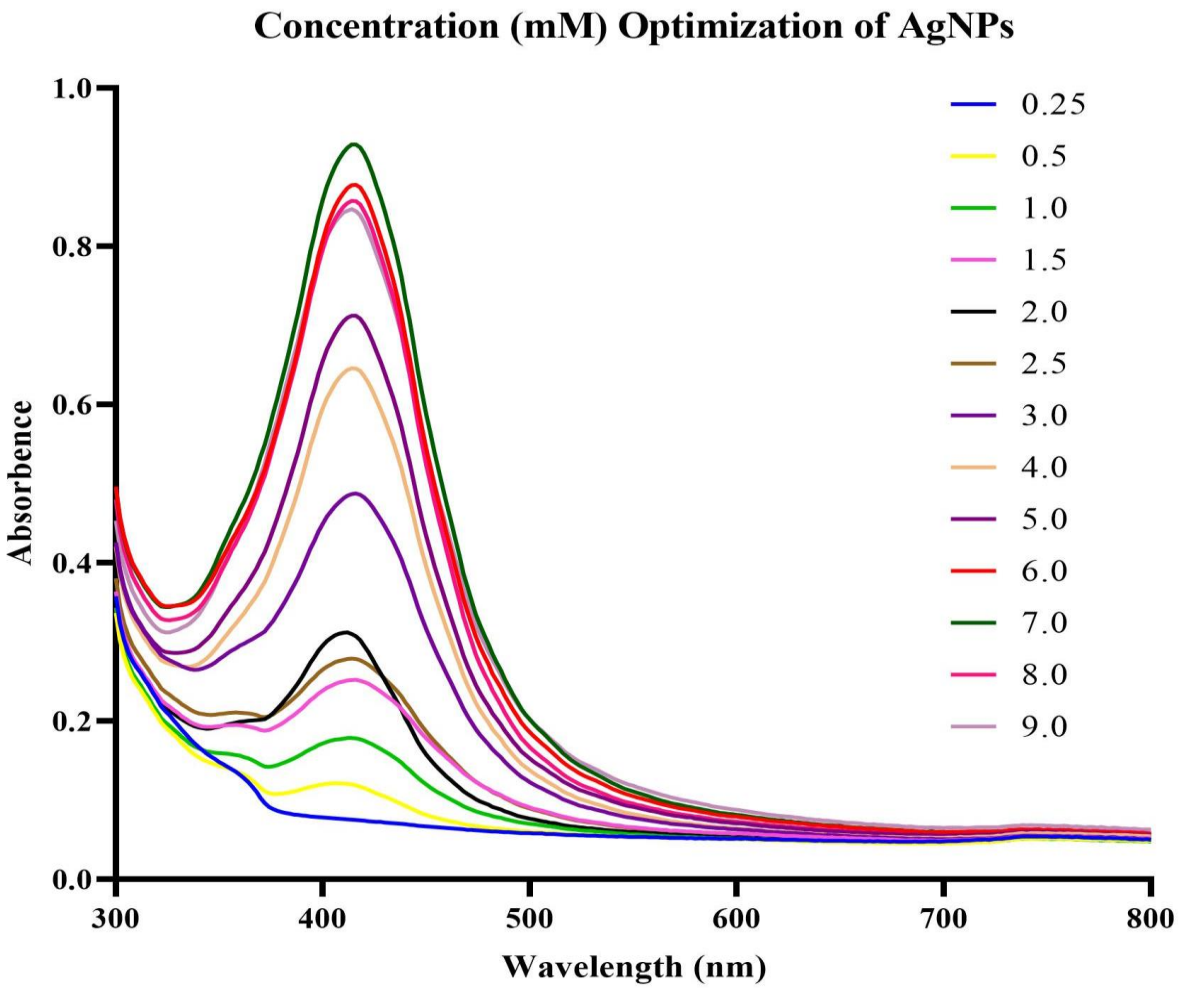


Fig. 4.3 shows- The optimization curves of $AgNO_3$ solution with plant extract.

Optimization of volume of the plant extract: -

For optimization of volume of plant extract, different volume (0.25ml, 0.5ml, 0.75ml, 1.0ml, 1.25ml,1.5ml,2.0ml,2.5ml,3.0ml) of plant extract is used and by using the UV-vis.

Spectroscopy, different absorbance is been calculated. 1.25ml is the optimized volume of the plant extract show in figure 4.2.

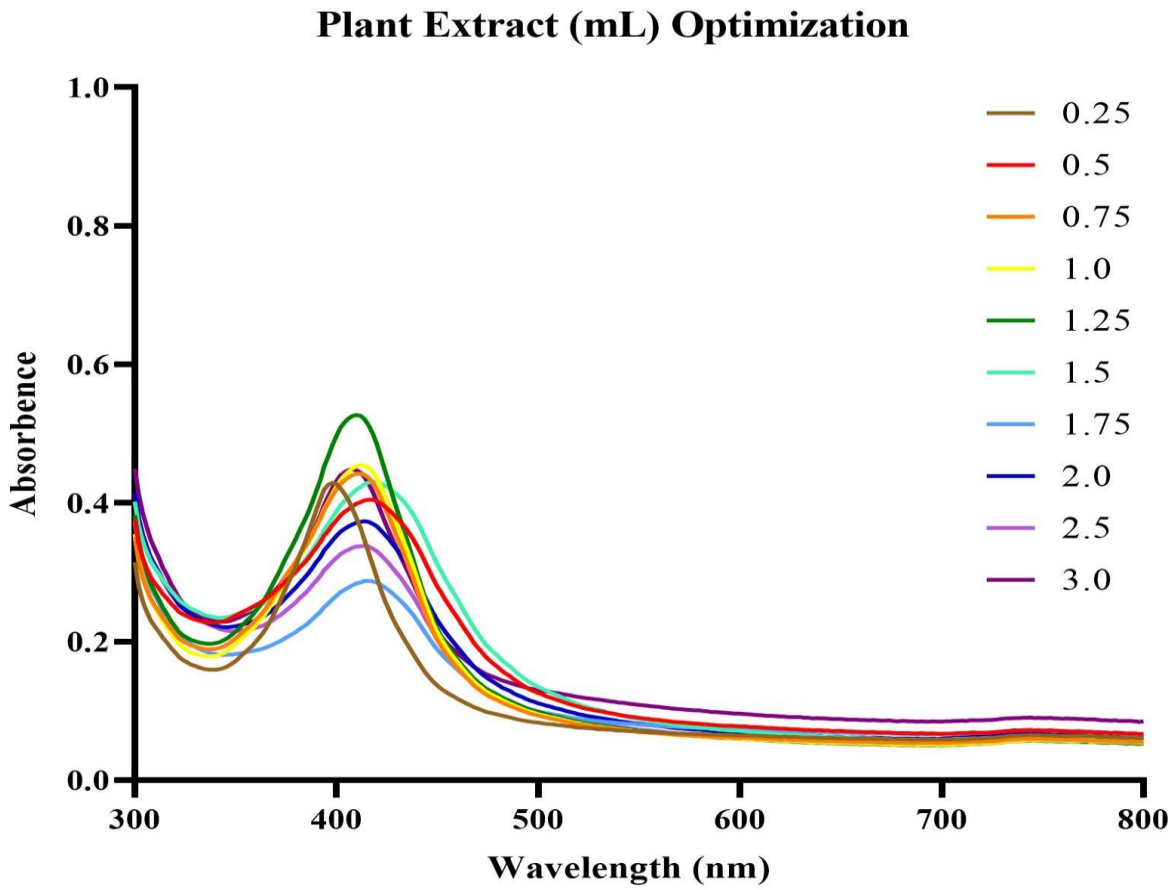


Fig. 4.4 shows the optimization volume of plant extract. 1.25ml is the optimum volume.

Optimization of pH of the silver nanoparticles: -

For the optimization pH of the nanoparticles different pH is done. By using the UV-Vis. Spectroscopy the results are shown in the fig 4.3. the peak is observed in between 350nm – 500 nm wavelength. Optimized pH is 10.

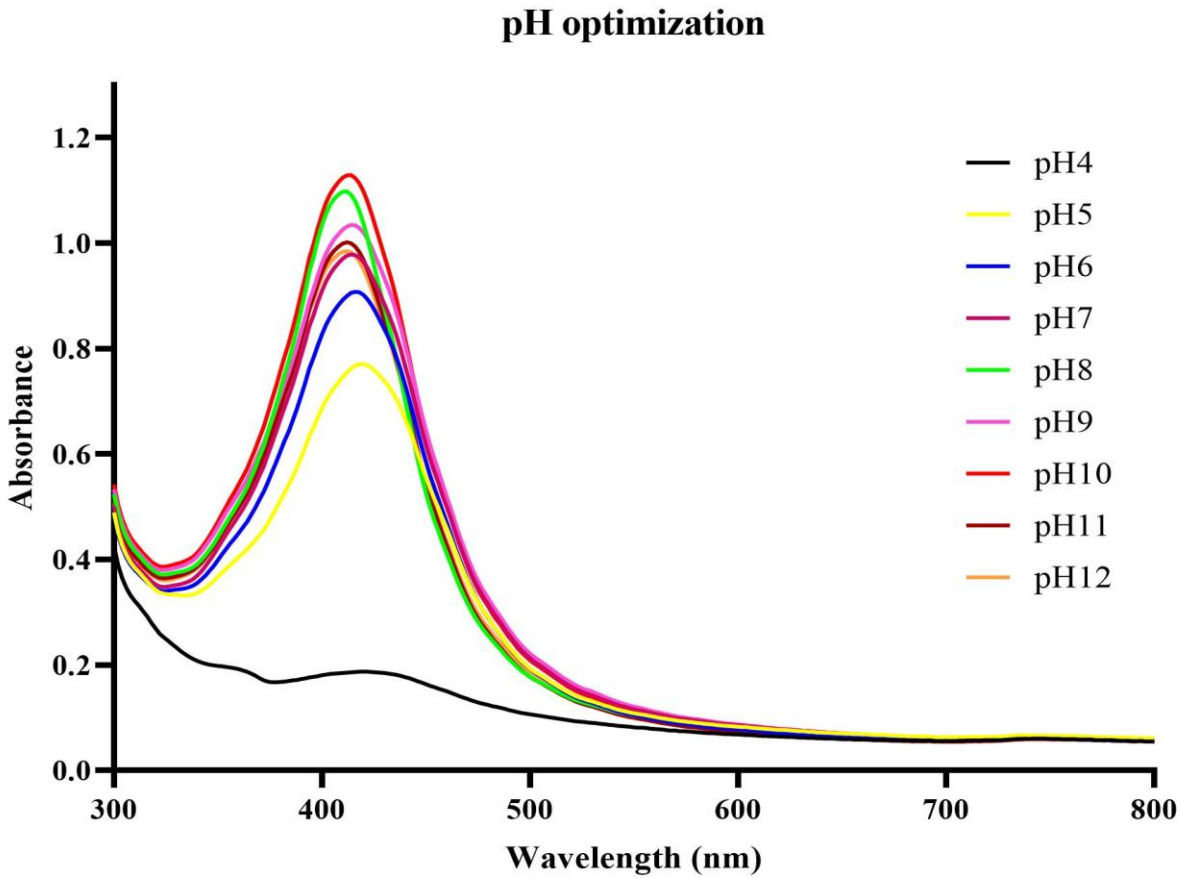


Fig. 4.5 shows the optimized results of pH optimization of AgNPs. UV-Vis. Spectral analysis is done on the range of 300nm - 800 nm wavelength.

Optimization of the silver nanoparticles: -

Time optimization is done by taking the spectra over a range from 300nm to 800 nm, on different time interval and observe the peak at maximum absorbance of the silver nanoparticle -s.

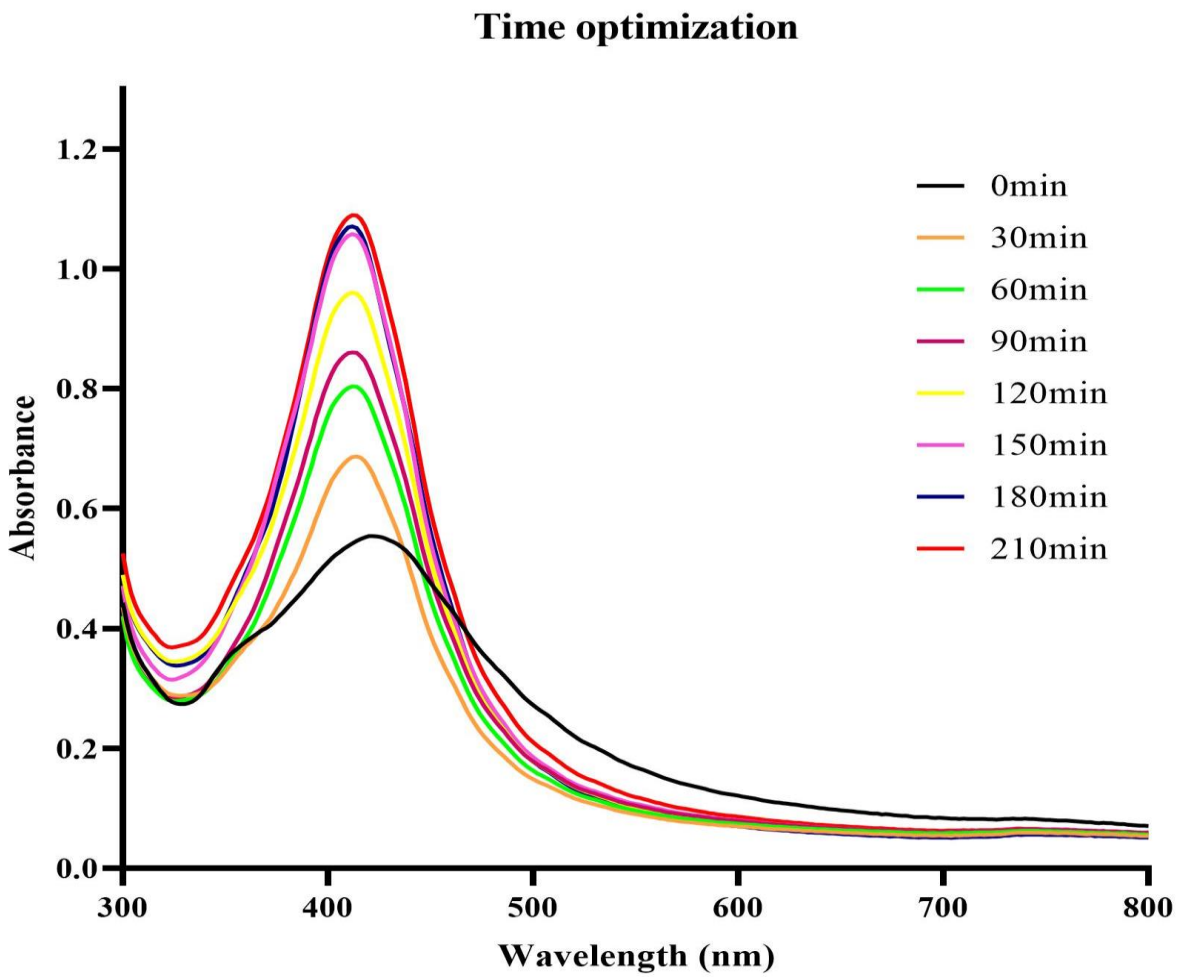
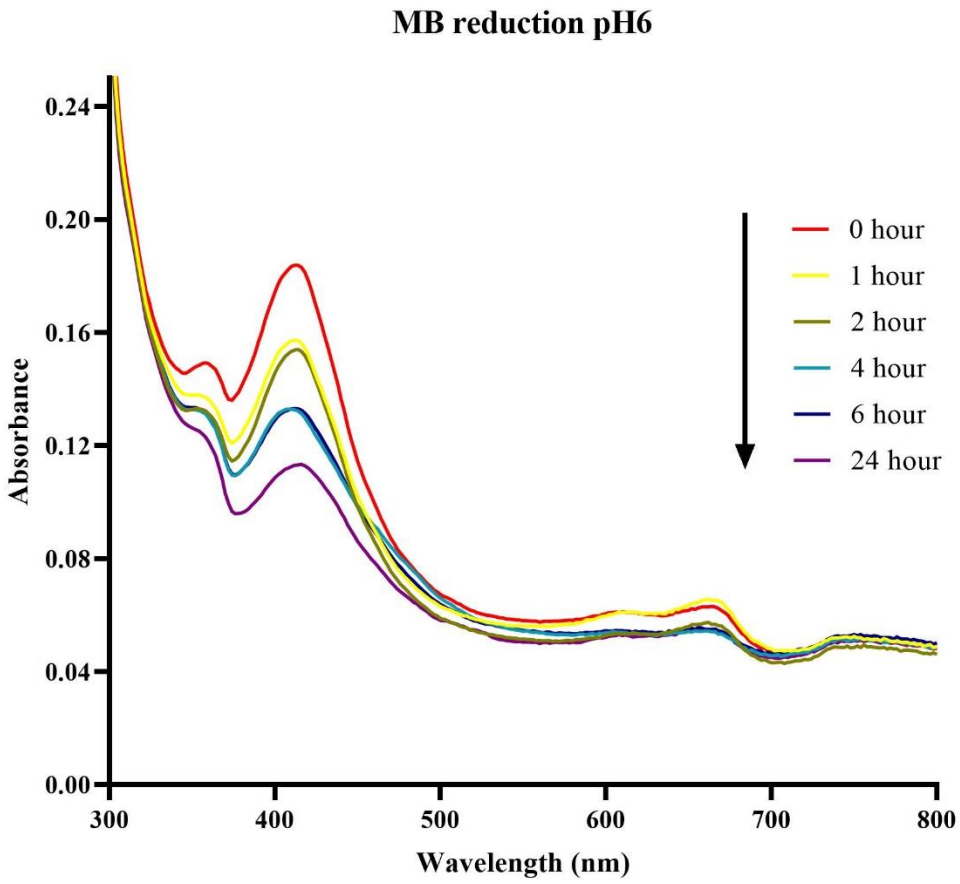


Fig. 4.6 shows the time optimization curve of AgNPs. By using UV-Vis. Spectroscopy over the range of 300nm - 800 nm wavelength.

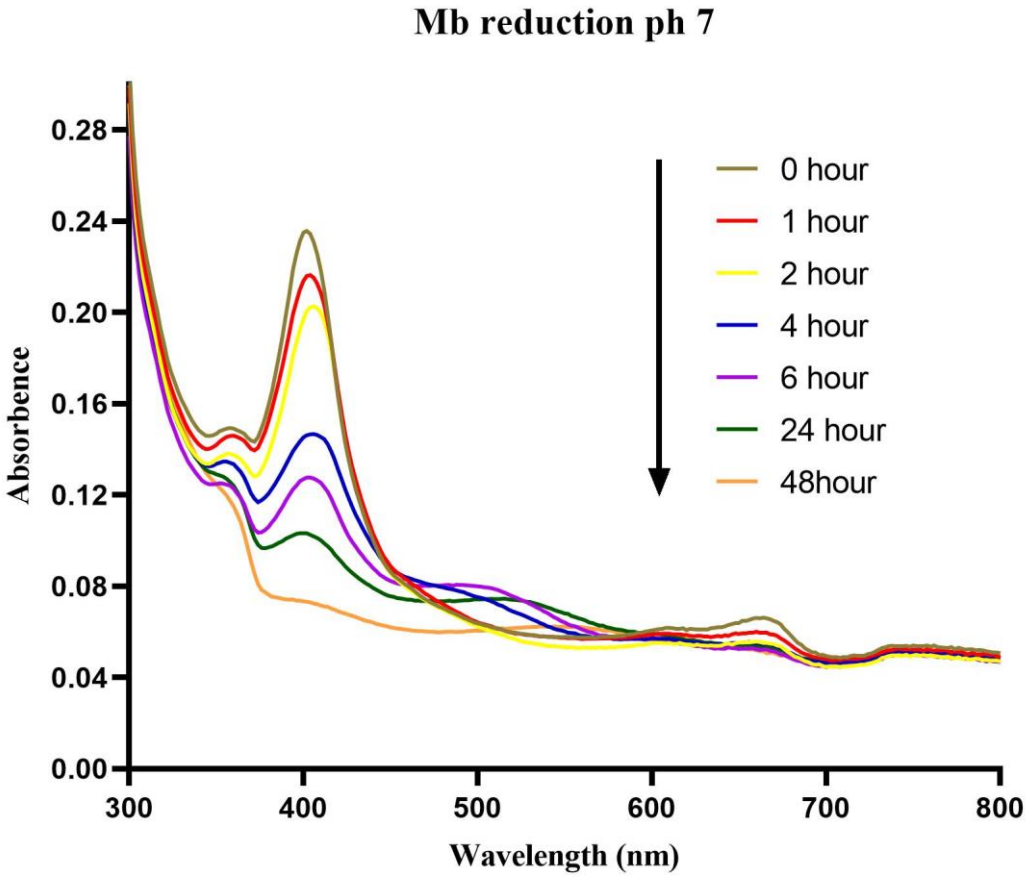
Degradation of AgNPs by methylene blue dye in the photocatalytic reaction.

Degradation of dye is initially observed by the colour change. Then by the absorption spectra over a range of 300nm - 800 nm wavelength. We have used different pH (6,7 and 8) for the degradation process, Shown in the figures.

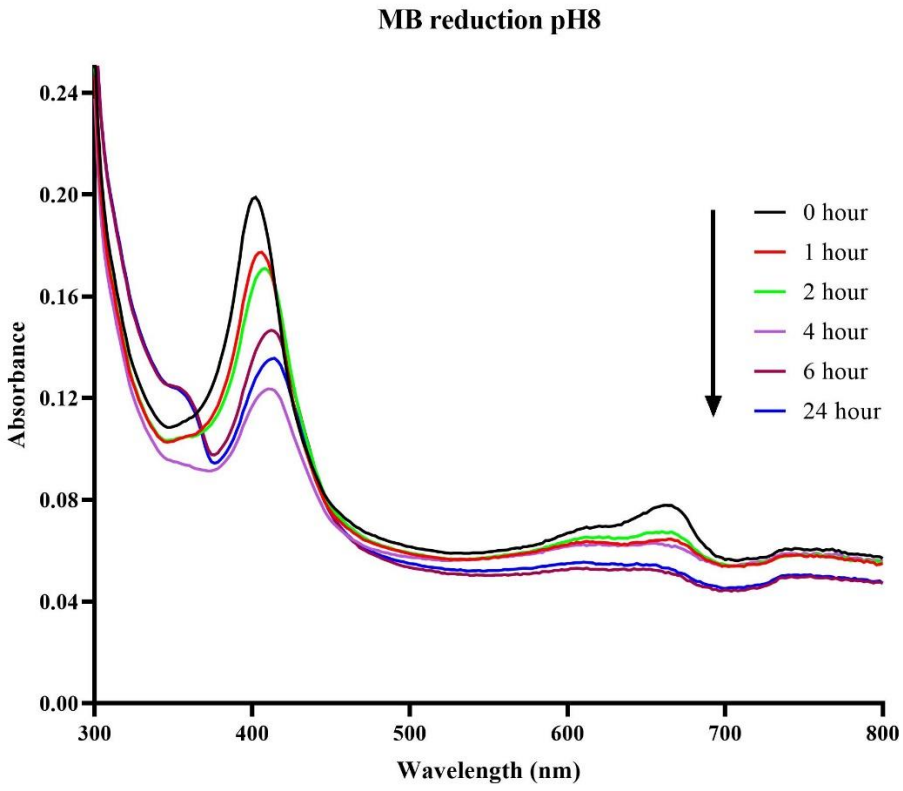
Fig 4.7 shows - The colour change in different pH after 24hr of catalytic reduction. pH 6, pH 7 and pH 8 respectively.



(a)


Mb reduction ph 7
Absorbence
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0.00
300
400
500
600
700
800
Wavelength (nm)
0 hour
1 hour
2 hour
4 hour
6 hour
24 hour
48hour

(b)


MB reduction pH8
Absorbance
0.24
0.20
0.16
0.12
0.08
0.04
0.00
300
400
500
600
700
800
Wavelength (nm)
0 hour
1 hour
2 hour
4 hour
6 hour
24 hour

(c)

Fig. 4.8 shows (a), (b) and (c) reduction process at the different pH 6, 7 and 8 respectively.

Table 4.1 – Degradation of methylene blue dye (%) by synthesised AgNPs (2ml) at pH 6

Exposure timing (h)	Degradation of dye (%)
1	14.0 ± 0.47
2	16.0 ± 0.21
4	27.0 ± 0.69
6	28.0 ± 0.78
24	38.0 ± 0.35

Table 4.2 – Degradation of methylene blue dye (%) by synthesised AgNPs (2ml) at pH 7.

Exposure timing (h)	Degradation of dye (%)
1	8.0 ± 0.18
2	14.0 ± 0.1
4	37.0 ± 0.75
6	45.0 ± 0.82
24	56.0 ± 0.21
48	66.0 ± 0.32

Table 4.3 – Degradation of methylene blue dye (%) by synthesised AgNPs (2ml) at pH 8.

Exposure timing (h)	Degradation of dye (%)
1	10.0 ± 0.85
2	14.0 ± 0.47
4	26.0 ± 0.24
6	37.0 ± 0.85
24	38.0 ± 0.31

By formula -

% Degradation = $(1 – A_t/A_0)$

XRD analysis data =

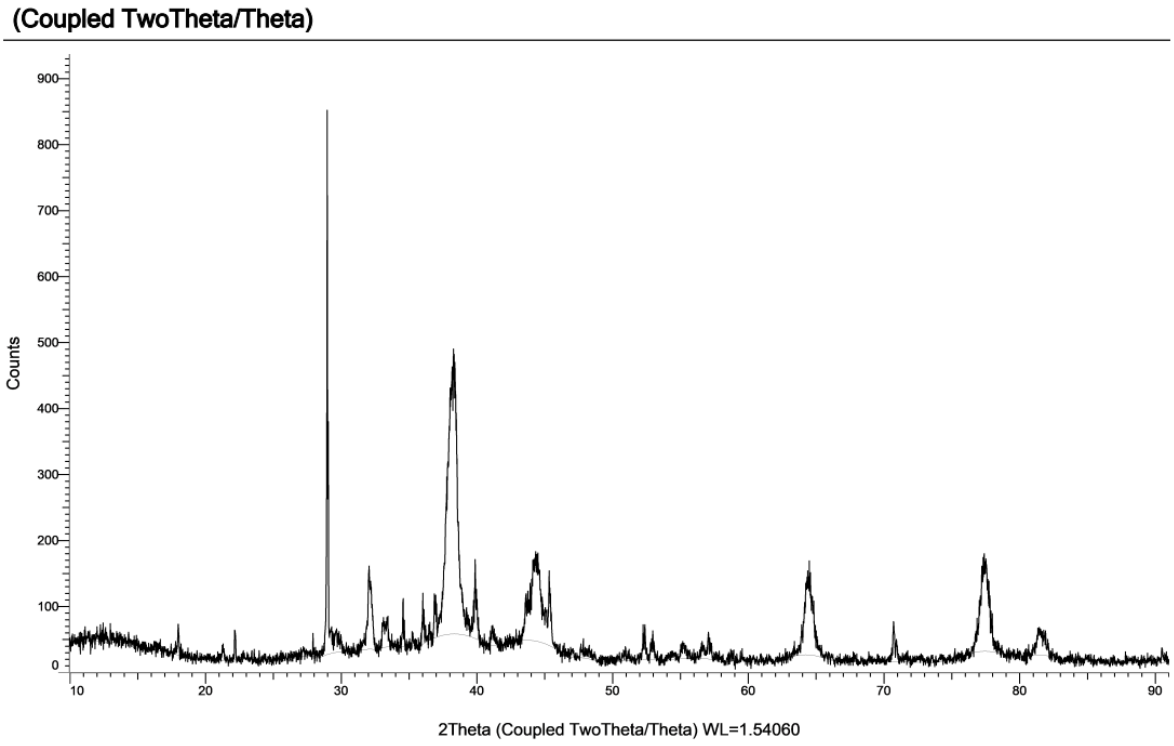


Fig 4.9 shows – The XRD analysis data of green synthesised silver nanoparticles. Sh

Chapter 5
Conclusion and Discussion

Conclusion

Using green synthesis metallic silver nanoparticles are synthesised and optimized on the basis of different parameters like concentration of $AgNO_3$ in synthesis solution, volume of plant extract, pH and time. For the confirmation of synthesis UV visible spectroscopy is used as the characterisation technique. After the formation of AgNPs photocatalytic reduction process is used in degradation of nanoparticles with methylene blue dye. Optimized concentration of $AgNO_3$ required for the synthesis of AgNPs was observed to be 7mM, optimum volume/volume ratio of $AgNO_3$ and plant extract used in the ratio of 9:1 was observed to be 1.25, optimum pH 10, and time was 210 minutes.

Degradation of nanoparticles by photocatalytic reduction is used over 2ml of silver nanoparticles synthesised using Myrica esculenta's bark extract achieves highest percentage of degradation of 66% at the 7 pH in 48 hours. The absorption peak of methylene blue dye was observed between 650-700 nm wavelength.

Discussion

There are many different methods present for the synthesis of nanoparticles but usually nanoparticles are synthesised from the hard metals which contains toxicity as well, green synthesis or green chemistry is a process incule reduction of those hard metals with organic substance and decrease the toxicity as compared to other processes. Synthesising silver nanoparticles by green synthesis is a one step process in which a solution of hard metal is used and it get reduced with an organic substance like plant extract, animal waste and through microorganisms.

In this study Myrica esculenta is used as our reducing agent for the reduction $AgNO_3$ and production of the silver nanoparticles (AgNPs). When the reducing agent react with the $AgNO_3$ in solution, the secondary metabolites which present in the plant extract will convert Ag+ ions into the Ag^0 ions by ionic reduction. When Ag^0 ions will combine together they will form a nanoparticle this process will continue until the end of the one of the reagents, pH plays an important role in the formation of nanoparticle because by the addition of NaOH and HCL the reduction process will increase or decrease, respectively.

The optimization process plays a key role in synthesis of nanoparticles since it provides information about the saturation points of the various parameters like concentration of $AgNO_3$ in synthesis solution, volume of plant extract, pH and time of saturation. It is necessary to optimize this parameter because we need them for our further application procedures.

Degradation of nanoparticles by photocatalytic reduction is used over 2ml of silver nanoparticles synthesised using Myrica esculenta's bark extract achieves highest percentage of degradation of 56% at the 7 pH in 24 hours. The absorption peak of methylene blue dye was displayed in between 650-700 nm wavelength.

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