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Thesis

SYNTHESIS OF METALLIC NANOPARTICLES USING PLANT LEAF EXTRACT

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Dedication

This thesis is dedicated;

First and foremost, to my family, **Ms. Benhakoum Ahlam** and loved ones who have supported me throughout my academic career with their support, encouragement, and cooperation.

I want to express my gratitude to all of my friends, especially those who never stopped encouraging, supporting and believing in me at all situations that were at hand.

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Dédicace.

Cette thèse est dédiée

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Abstract

Interest in the biosynthesis of silver nanoparticles (AgNPs) has been steadily increasing primarily due to their numerous applications in various fields, low cost, use of non-toxic environmentally-friendly materials and easy implementation. This study focused on the biosynthesis of AgNPs using unknown plant leaf extract, optimization of process conditions, and application in catalytic reduction of methylene blue dye to leucomethylene blue (colourless). AgNPs were synthesised by using silver nitrate as the precursor and plant leaf extract as a reducing and capping agent due to presence of phytochemicals (biomolecules) present like flavonoids, phenols, alkaloids, terpenoids, amides, aldehydes, carboxylic acid, and ketones and they were characterised using a uv visible spectrophotometer. A silver nanocomposite was synthesized using an unknown support, silver nitrate solution, ultra distilled water and the plant leaf extract. The silver nanocomposite was then applied in catalytic reduction of MB to LCMB with parameters; mass of the Ag-nano composite, concentration of sodium borohydride and concentration of MB were put into consideration. A time dependent uv vis spectrophotometer analysis for the catalytic degradation of MB dye by the biosynthesized Ag-nanocomposite for 5min indicated the maximum influence of nanocomposite. The MB dye was reduced rapidly with NaBH4 in the presence of Ag-nanocomposite due to their catalytic action. The findings of the study show the potential of unknown plant leaf extract for the biosynthesis of AgNPs with excellent catalytic activity of their nanocomposites for the reduction of MB dye to LCMB.

Keywords; silver nanoparticles, biomolecules, sodium borohydride, silver-nanocomposite, methylene blue, leucomethylene blue, catalyst.

Résume

L'intérêt pour la biosynthèse des nanoparticules argent (AgNPs) a progressivement augmenté principalement en raison de leurs nombreuses applications dans divers domaines, leur faible coût, l'utilisation de matériaux non toxiques respectueux de l'environnement et la facilité de mise en œuvre. Cette étude a porté sur la biosynthèse des AgNPs à l'aide d'extrait de feuilles de plantes inconnues, l'optimisation des conditions de processus et l'application dans la réduction catalytique de la teinture bleue de méthylène au bleu de leucométhylène. (Colourless). Les AgNPs ont été synthétisés en utilisant du nitrate d'argent comme précurseur et de l'extrait de feuilles de plantes comme agent de réduction et de captage en raison de la présence de phytochimiques (biomolécules) présents tels que les flavonoïdes, les phénols, les alcaloïds, les terpénoïde, les amides, les aldehydes, l'acide carboxylique et les cétones et ils ont été caractérisés à l'aide d'un spectrophotomètre UV visible. Un nanocomposite d'argent a été synthétisé à l'aide d'un support inconnu, d'une solution de nitrate d'argent, d'une eau ultra-distillée et de l'extrait de feuille de plante. Le nanocomposite d'argent a ensuite été appliqué dans la réduction catalytique de MB à LCMB avec des paramètres; la masse du composite Ag-nano, la concentration de borohydride de sodium et la concentrtion de MB ont été pris en compte. Une analyse du spectrophotomètre vis-UV dépendant du temps pour la dégradation catalytique de la teinture MB par le nanocomposite Ag biosynthétisé pendant 5min a indiqué l'influence maximale du nanocomposite. La teinture MB a été réduite rapidement avec NaBH4 en présence d'Ag-nanocomposite en raison de leur action catalytique. Les résultats de l'étude montrent le potentiel d'extrait de feuille de plante inconnue pour la biosynthèse des AgNPs avec une excellente activité catalytique de leurs nanocomposites pour la réduction de la teinture MB à LCMB.

Mots clés ; nanoparticules d'argent, biomolécules, borohydride de sodium, nanocomposite d'argent, bleu de méthylène, Bleu de leucométhylene, catalyseur

ملخّص

قد ارتفع بشكل مستمر نظرًا لتطبيقاتها (AgNPs) ازدياد الاهتمام بتخليق جسيمات الفضة النانوية العديدة في مجالات مختلفة، وتكلفتها المنخفضة، واستخدامها للمواد الصديقة للبيئة غير السامة باستخدام مستخلص غير معروف من أوراق AgNPs وسهولة تنفيذها. ركزت هذه الدراسة على تخليق النبات، وتحسين ظروف العملية، وتطبيقها في تخفيض لون الميثيلين الأزرق إلى لوكوميثيلين الأزرق باستخدام نترات الفضة كسابق ومستخلص أوراق النبات كعامل خفض AgNPs (عديم اللون). تم تخليق وتغليف بسبب وجود مواد نباتية كالفلافونويدات والفينولات والقلويدات والتربينويدات والأميدات والألدهيدات والحموض الكربوكسيلية والكيتونات وتم توصيفها باستخدام مطياف الأشعة فوق البنفسجية المرئية. تم تخليق نانومركب فضي باستخدام دعم غير معروف ومحلول نترات الفضة وماء MB مقطر بالتنقية الفائقة ومستخلص أوراق النبات. تم تطبيق النانومركب الفضي ثم في تخفيض حفاز MB. مع مراعاة المعلمات التالية: كتلة النانومركب الفضي، تركيز بوروهايد الصوديوم وتركيز LCMB إلى Ag بواسطة النانومركب MB أظهر تحليل مطيافية متوقتة للأشعة فوق البنفسجية المرئية لتحلل تلون MB المخلّق بطريقة الحياة الاصطناعية لمدة 5 دقائق تأثير الحد الأقصى للنانومركب. تم تخفيض صبغة بسرعة باستخد

بسبب تأثيرها الحفاز. تظهر نتائج الدراسة إمكانية استخدام مستخلص Ag في وجود النانومركب NaBH4 LCMB. إلى MB بنشاط حفاز ممتاز لتخفيض صبغة AgNPs غير معروف من أوراق النبات لتخليق

الكلمات الرئيسية: جسيمات الفضة النانوية، المركبات الحيوية، بوروهايد الصوديوم، نانومركب الفضة، الميثيلين الأزرق، لوكوميثيلين الأزرق، محفز.

Contents

Abbreviations

AgNPs: silver nanoparticles

SPR: surface plasmonic resistance

PVP: polyvinylpyrrolidone

CTAB: cetrimonium bromide

STP: standard temperature and pressure

NADPH: nicotinamide adenine dinucleotide phosphate

ROS: reactive oxygen species

ATP: Adenosine triphosphate

DNA: deoxyribonucleic acid

MB: methylene blue

LCMB: leucomethylene blue

RNA: Ribonucleic acid

General introduction

Nanoparticles have gained significant attention in various fields due to their unique properties and potential applications. These nanoscale materials, typically ranging from 1 to 100 nanometers in size, exhibit distinct physicochemical characteristics that differ from their bulk counterparts. The properties of nanoparticles, such as their high surface-to-volume ratio, size-dependent optical and catalytic properties, and enhanced reactivity, make them highly desirable for a wide range of technological advancements and environmental applications.

Nanoparticles possess several intriguing properties that make them suitable for diverse applications. Firstly, their high surface area allows for increased interactions with other substances, leading to improved catalytic activity and adsorption capabilities. Additionally, nanoparticles exhibit unique optical properties, such as plasmon resonance, which can be exploited for various sensing and imaging applications. Moreover, their small size enables nanoparticles to penetrate biological systems, making them potential candidates for drug delivery systems and biomedical applications.

Various methods are employed to synthesize nanoparticles, each with its advantages and limitations. These methods include chemical synthesis, physical methods, and biological approaches. Chemical synthesis involves the controlled reduction of precursor materials to form nanoparticles. Physical methods encompass techniques like vapor deposition, laser ablation, and attrition. Furthermore, biological methods utilize microorganisms or biological molecules to produce nanoparticles through biomineralization or enzymatic processes.

Methylene blue, a synthetic cationic dye widely used in the textile and printing industries, poses significant environmental concerns due to its persistence and potential toxicity. It is frequently discharged into wastewater streams, leading to water pollution and detrimental effects on ecosystems and human health. To address this issue, various methods have been explored for the efficient elimination of methylene blue from contaminated water sources.

One common method for methylene blue removal is adsorption, where adsorbent materials are employed to capture and bind the dye molecules, effectively removing them from the water. Other techniques include photocatalysis, chemical oxidation, and advanced oxidation processes, all of which utilize different mechanisms to degrade methylene blue molecules into less harmful byproducts.

Silver nanoparticles have emerged as promising agents for methylene blue elimination due to their unique properties and catalytic activity. Silver nanoparticles can be synthesized using various techniques, including chemical reduction, sol-gel synthesis, and green synthesis methods. Their small size and high surface area-to-volume ratio provide excellent adsorption and catalytic properties, making them efficient in capturing and degrading methylene blue molecules.

Furthermore, silver nanoparticles exhibit antimicrobial properties, which can potentially mitigate any microbial contamination present in wastewater. Their ability to generate reactive oxygen species under light irradiation also enhances their photocatalytic activity, enabling effective methylene blue degradation.

Considering the widespread application of silver nanoparticles and their potential in methylene blue elimination, further research and optimization of synthesis methods are crucial. Additionally, the assessment of their environmental impact and long-term stability is necessary to ensure their safe and sustainable utilization.

In summary, the properties of nanoparticles, along with the diverse methods of their synthesis, present numerous opportunities for their application in various fields. Methylene blue pollution poses significant challenges, but with the use of silver nanoparticles, a promising solution emerges. Harnessing the unique properties and catalytic activity of silver nanoparticles can lead to efficient and environmentally friendly approaches for eliminating methylene blue from contaminated water sources, contributing to a cleaner and healthier environment.

CHAPTER 1

BIBLIOGRAPHY STUDY

I. Chapter 1

I-1. INTRODUCTION

Nanotechnology is the science which deals in materials in the range of 1 to 100 nm. Nanomaterials have great importance as the physico-chemical properties of the metal is changed as it reaches the nano size, their properties are different as compared to the bulk metal.

These nanomaterials have multiple applications in various fields such as electronics, cosmetics, coatings, packaging, and biotechnology. Due to their optical properties the colloidal solution of metal nanomaterials is transparent, thus they are useful in cosmetics, coatings, and packaging.

Among metal nanoparticles, silver nanoparticle has wide application in industry and medicine due to its antibacterial, antifungal, antiviral and anti-parasitic characters. Because of their wide applications beneficial to humans there is a need to develop rapid and reliable experimental protocols for the synthesis of silver nanoparticles.

Different types of nanoparticles such as Ag, Au, Pt and Pd have been synthesized in the recent past by chemical, physical and biological methods. The chemical methods are the most popular but the use of toxic chemicals during synthesis produces toxic by-products [1].

The physical methods require large amount of energy to maintain high pressure and temperature required for the reaction [2]. Thus, the chemical and physical methods have their own limitations; these are considered expensive and unsuitable for sustainable ecosystem [3].

The synthesis of silver nanomaterials using biological entities is gaining momentum as; biological methods are providing, nontoxic and environmentally acceptable "green chemistry" procedures.
The physical and morphological features of metal nanoparticles are greatly affected by the solvents and reducing agents used.

The variation in size, shape and morphology influenced the applications of the nanoparticles. The morphology of silver nanoparticles is determined by reducing precursor. These reducing and/or stabilizing precursor can be induced by bacteria, fungi, yeasts, algae, or plants [4,5] as a whole or their products. The interaction of these biomolecules has been exploited earlier also in various applications such as recovery of metal/metals, bioremediation, bioleaching and bio-mineralization [6]. However, still the mechanism of synthesis of nanoparticles using biomolecules is yet to be explored and hence needs much more experimentations.

Synthesis of metal nanoparticles acquires special attention due to its specificity and environment friendly approach. Whether the microorganisms or the plants are being

employed, it is the biomolecules present in them which is responsible for the biosynthetic mechanism. These biomolecules may be carbohydrates, lipids, DNA and enzymes/ proteins or a combination of two or more.

I.2. SILVER.

I-2.1. DEFINITION

Silver is a shiny, malleable, and ductile metal that is naturally present in pepite. The name of the city Argentoratum, which is now the city of Strasbourg, was derived from the Latin Argentum, which gave the initials of its chemical symbol "Ag," and the ancient Greek argoV (argos), which means: white, or bright.

Silver has a very high malleability and ductility and, in its pure form, its electrical conductivity and thermal conductance are the highest of all metals. (7)

Silver (Ag) has two stable isotopes, 107Ag and 109Ag, with an abundance of 51.8% and 48.2% respectively. (8)

I-2.2. Physical And Chemical Properties of Silver
Silver is a noble metal that reacts more readily. The addition of copper increases the material's mechanical strength despite being quite malleable and ductile. It is extremely conductible, a quality used in electronics. Photography uses silver salts because they are photosensitive.

Table-I; 1; properties of silver

Properties	Ag
Electronic configuration	$[Kr]\ 4d^{10}5s^{1}$
Atomic number-mass	47-107.87
Crystalline structure	CFC
Density (g/cm$^{3)}$	10.5
Atomic radius (nm)	0.153
First ionisation energy (kJ mol^{-1})	758
Fermi speed(m/s)	$1.39*10^{6}$
Fermi energy(eV)	5.49
Electrical resistance (**µΩcm**)	1.59(20°c)
Fusion temperature(K)	1235.43
Boiling temperature (K)	2485
Thermal conductivity (W m^{-1}K$^{-1)}$)	429
Electrical conductivity (Sm^{-1})	$63*10^{6}$
Standard potential (V)	0.779(Ag^{+}/Ag)
Electronegativity	1.93

Silver is the second element of the first secondary group (IB) of the periodic table. The scientific name "silver" comes from the Latin "Argentum" which derives from the Greek word "arguros" which means "shiny white" or "bright white" [9].

The atomic configuration of silver (Z=47) is [Xe] 4d105s1. It is placed in group 11 (IB) after copper and between palladium (group 10) and cadmium (group 12) in the periodic table. (9)

I-3. SILVER NANOPARTICLES

Numerous other metal nanoparticles, including AgNPs, have also attracted interest due to their distinctive properties, which include chemical stability, electrical conductivity, antimicrobial, and catalytic activities. Due to its high surface-to-volume ratio, nano silver exhibits characteristics completely different from those of bulk silver.
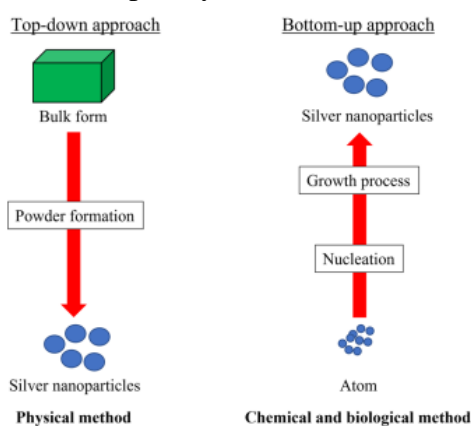
Figure -I; 1: Schematic representation of particle nucleation, growth, and formation of nanoparticles via physical, chemical, and biological methods. [100].

AgNPs are made using a variety of chemical, physical, and biological processes, resulting in a variety of sizes and shapes that are used in a wide range of applications. Thus, the top-down and bottom-up approaches are the two main categories into which the syntheses methods are divided**(fig1).**

The top-down method, uses complex techniques like laser ablation and lithography to mechanically reduce the bulk size of silver metal to the nanoscale.

When using the bottom-up approach, also known as the self-assembly technique, silver salt is dissolved in a solvent and Ag+ is reduced to its element by adding a reducing agent and stabilizing agents to stabilize the forming AgNPs and prevent agglomeration.

The bottom-up approach produces nanostructures with little or low defects, better long- and short-range ordering and chemical composition that are more homogenous [10]. AgNPs of desired morphology, size, and shape can be produced by the several physical and chemical methods (such as arc discharge, surface deposition, plasma

polymerization, emulsion polymerization, laser CVD (Chemical Vapour Deposition), and thermal decomposition in organic solvents, chemical reduction and photo reduction in reverse micelles [11].
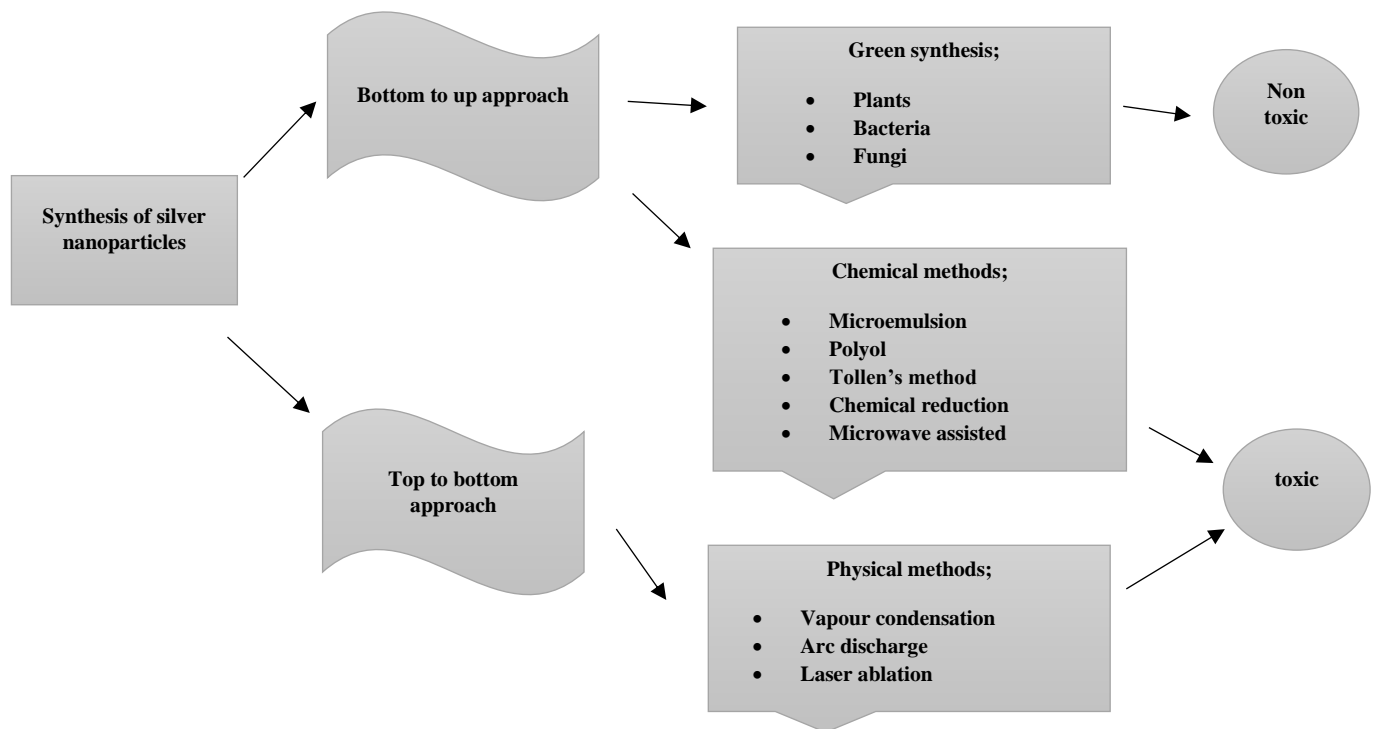
Figure -I; 2: Schematic representation of synthesizing silver nano particles

I-4. Properties Of Silver Nanoparticles

When an object transforms from a mass state to a nano-object state, new properties and traits appear. An overview of the impact of nanoparticle size on various physical characteristics, including optical, electrical, magnetic, mechanical, surface, catalytic, physico-chemical, antibacterial, and odorant properties, will be provided in the sections that follow
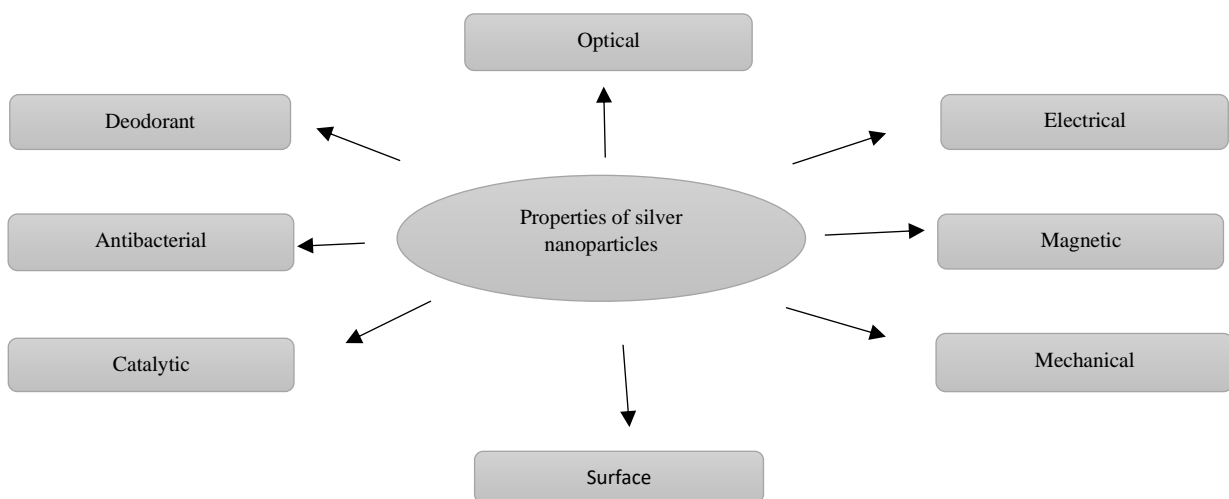
Figure -I; 3: properties of silver nanoparticles

I-4.1. Optical Properties

The use of the optical properties of silver nanoparticles as a functional component in various products and sensors is raising increasing interest. Silver nanoparticles are extremely effective in absorbing and diffusing light, unlike many colorants and pigments. They have a color that depends on the size and shape of the particle. The strong interaction of silver nanoparticles with light occurs because the conductive electrons on the metal surface undergo collective oscillation when excited by light at specific wavelengths, Figure. 4. Known as surface plasmonic resonance (SPR), this oscillation results in exceptionally strong diffusion and absorption properties [12].
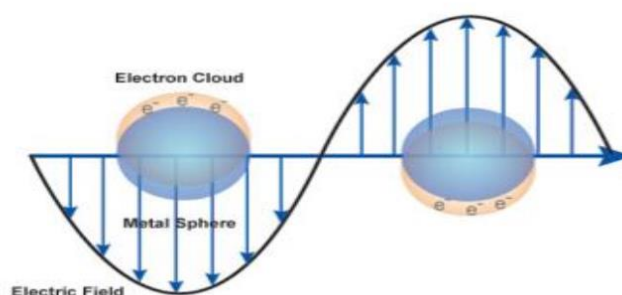
Figure -I; 4; Surface plasmonic resonance where the free electrons in the nanoparticles metal are put into oscillation due to a strong coupling with a wavelength specificity of the incident light. (12)

Surface plasmon resonance (SPR).

The phenomenon known as surface plasmon resonance (SPR) is the collective oscillation of conduction band electrons in resonance with the oscillating electric field of incident light, which will result in the non-radiative excitation of energetic plasmon electrons.

Silver nanoparticles' surface plasmon resonance (SPR) can be tuned across the visible and near-infrared spectrum depending on their size and shape. A simple and manageable method for creating silver nanocrystals with a specific shape and size is required for SPR applications.

I-4.2. Electrical Properties

This confinement at the nano-scale has changed the electrical properties because electrons cannot move freely at the nanometre level and are constrained in their movement. like

nanometre-scale superconductors or condensers in crane conductor/semiconductor materials. Silver nanoparticles smaller than 10 nm are also not regarded as conductors and cannot generate electricity

I-4.3. Magnetic Properties
For nanomagnetic materials, each spin behaves like a small magnet in nanomaterials. The interaction between neighbouring spins is dominated by the spin exchange interaction. Usually, most materials have j<0 and are nanomagnetic (paramagnetic or diamagnetically). In the case of para-magnetic materials, superparamagnetic nanoparticles return to zero magnetization after a small exchange between individual moments [13].

I-4.4. Mechanical Properties
At the nanometre level, changes in the mechanical properties of the material are observed. These properties include the young module, traction resistance (four times greater), weaker plastic deformation, greater hardness, more fragility, deformations of grain joints, decreased length, lower density of dislocation moments, increased short-distance dislocations [13].

I-4.5. Surface Properties
Surface-dominated properties, such as melting point, reaction rate, capillary action and adhesion, are controlled by their surface. Due to the high surface-to-volume ratio of nanomaterials, these properties present radical changes compared to their massive counterparts [13].

I-4.6. Catalytic Properties
From a chemical point of view, the reduction in nanoparticles size makes the material more reactive, making it a promising candidate for catalyst applications. It has been experimentally proven that metal nanoparticles have high catalyst activity for hydrogenation. [14 and 15]. The catalytic performance of NP-Ag supported by silicon spheres has been studied. The distribution of NP-Ag on silica spheres effectively protects aggregated metal particles, thus preventing deactivation and poisoning of the catalyst during the catalytic reaction [16].
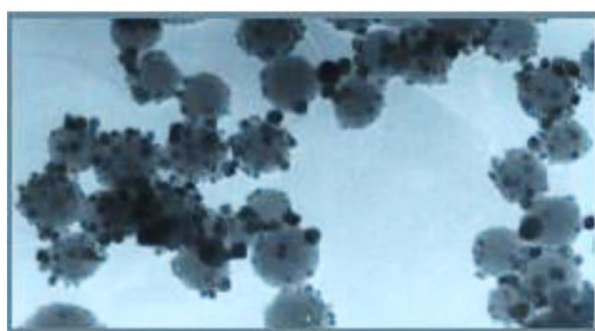
Figure -I; 5; TEM image of silver nanoparticles carried on silicon spheres (15).

I-4.7. Physical And Chemical Properties
Among the precious metal nanoparticles, Ag NP has received considerable attention due to its attractive physical and chemical properties [17]. Certain physico-chemical properties of Ag NP, including size (surface), surface coating load and shape, agglomeration and dissolving speed, are important in determining their interaction and biological effects. Smaller particles

have a larger surface area and therefore have a greater potential toxicity [18]. A small amount of silver is harmless to human cells, but it is fatal to microorganisms. (19)

I-4.8. Antibacterial Properties

There are different theories about the effect of NP-Ag on microbicides. NP-Ag has the ability to accumulate, anchor on the bacterial cell wall and subsequently infiltrate, causing changes in cell membrane structure and cell death [20]. The formation of free radicals by NP-Ag can be considered as another mechanism of cell death. Some electronic spin resonance spectra indicate that silver forms these free radicals when it comes into contact with bacteria. These free radicals can damage the cell membrane and make it porous, resulting in cell death [21].

I-4.9. Deodorant Properties

Nano-silver has odour-deodorizing properties used in refrigerators to eliminate bad odours [22].

I-5. METHODS OF SYNTHESIS OF SILVER NANO PARTICLES

AgNPs have many uses, making their synthesis an extremely important factor. The synthesis of nanoparticles can be accomplished in three ways: physically, chemically, and biologically, or "greenly." Based on the kind of chemical, physical, or biological component that is utilized in the process, each of these methods can be further categorized.

I-5.1. PHYSICAL METHODS

I-5.1.1. Vapour condensation method

Vaporization and Condensation are two different steps that are involved in this process and are done at atmospheric pressure using a tube furnace. A heat source is used for the process of vaporization and then these vapours are quickly condensed, resulting in nanoparticle synthesis [23]. There are some drawbacks, though, like the high energy and time requirements to raise the ambient temperature throughout the source material and achieve thermal stability.

I-5.1.2. Arc discharge method

It is an additional technique for the creation of nanoparticles physically. The direct-current arc voltage is applied across two graphite electrodes that are submerged in an inert gas, such as He, Ar, or Ne, without the use of any surfactants or stabilizers. In a study conducted by Tran et al., AgNPs were synthesized in deionized water with no surfactants using the arc discharge method. They used silver wires of 1-mm diameter as electrodes. The results showed that AgNPs synthesized using this method were 10 nm in diameter [24

I-5.1.3. Laser ablation method

The laser ablation technique allows for the solution phase synthesis of AgNPs on metallic bulk silver salt. It has been shown by Pyatenko et al. that 2–5 nm of spherical silver nanoparticles can be synthesized in pure water by irradiating the Ag target with the laser beam of wavelength 532 nm. The wavelength of the laser beam and the ablation time are the most important factors that determine the size of synthesized nanoparticles [25].
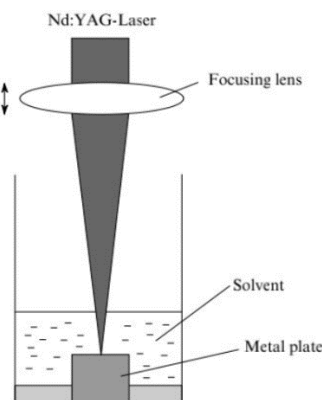
Figure -I; 6; Scheme of a unit for laser ablation in liquid media (101).

I-5.2. CHEMICAL METHODS

I-5.2.1. Chemical reduction method

This is the most commonly used chemical method that involves the use of a reducing agent and a stabilizing agent/capping.

$$Ag^+ + e^- \longrightarrow Ag^0$$

Some of the common reducing agents used for AgNP synthesis are sodium citrate, ascorbate, sodium borohydride (NaBH4), elemental hydrogen, polyol process, N, N-dimethylformamide (DMF), and poly (ethylene glycol) block copolymers. The reducing agent reduces Ag+ ions to Ag metal after which they agglomerate into clusters. Cluster formation can be prevented using stabilizing agents/capping agents [26, 27, 28]. Citrate, polyvinylpyrrolidone (PVP), cetyltrimethylammonium bromide (CTAB), polyvinyl alcohol (PVA), trisodium citrate, and polyethylene glycol (PEG) are some of the capping agents most frequently used for the chemical reduction method.

I-5.2. Microemulsion method

This approach includes the use of a mixture of water, surfactant, and oil. Sometimes a co-surfactant is also used along with the other components in this process. The commonly used surfactants are anionic surfactants like bis(2-ethylhexyl) sulfosuccinate, sodium dodecylbenzene sulfonate, and lauryl sodium sulphate and cationic surfactants like CTAB, PVP, and non-ionic surfactants like Triton X-100, etc. The surfactant molecules are surrounded by water droplets, which are like a silver nanoparticle synthesis micro reactor [29].
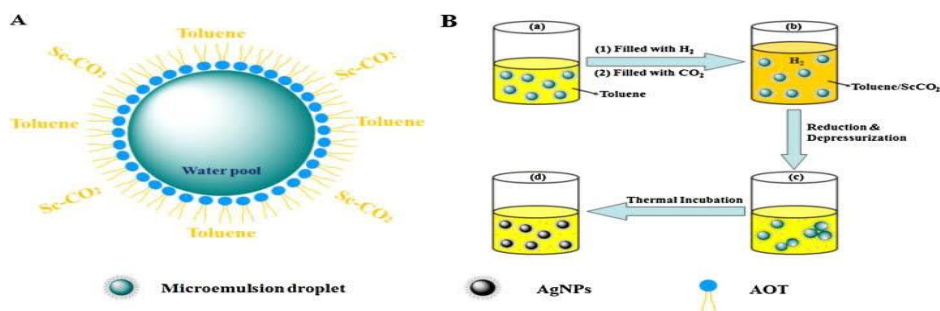
Figure -I; 7; Process of synthesizing silver nanoparticles using microemulsion process

I-5.2.3. Polyol process

This process includes the reduction of silver nitrate (AgNO3) at 160 °C with ethylene glycol. PVP acts as a capping agent. The reduction of AgNO3 with ethylene glycol and PVP produces monodisperse Ag nano cubes [30].

I-5.2.4. Tollen's method

AgNPs can also be created using reducing sugars and the Tollens reagent. These chemicals allow for room-temperature synthesis, and the reducing sugars act as a stabilizing agent over the AgNP surface as well as a reducing agent for the Ag+ ions, producing stable anti-bacterial Ag-NPs.
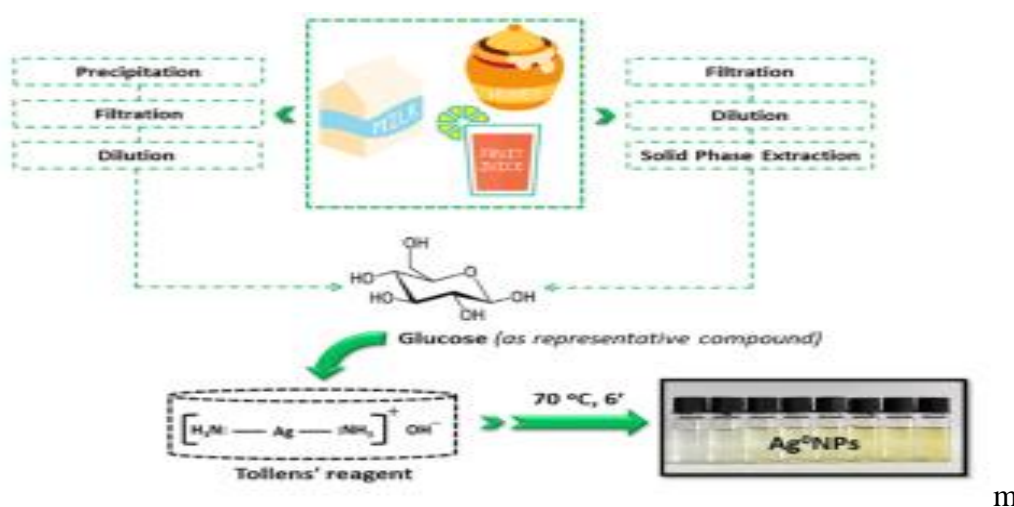
Figure -I; 8; Schematic presentation of the proposed method for the determination of reducing sugars in food extracts (inset photography was taken by one of the authors Selen Durmazel)

I-5.2.5. Microwave-assisted synthesis

Instead of using conventional heating during the synthesis process, this method uses microwave heating. Microwave use expedites the reaction and guarantees even heating. This process helps create silver nanoparticles without aggregation and with less energy. X. Zhao et al. synthesized AgNPs using microwave irradiation [31]. Alginate was used as a capping and stabilizing agent for the production. This method does not involve any chemical agent as alginate is a naturally occurring carbohydrate that is isolated from sea algae [31].

I-5.3. BIOLOGICAL METHODS

The biological approaches use a variety of natural products, such as phytochemicals, enzymes, and biodegradable polymerase reducing and capping agents, in place of potentially hazardous chemicals like sodium borohydride during the synthesis of Ag NPs. Water is also used as the solvent in these approaches, which adhere to the principles of green chemistry. Extracting microorganisms (extracellular and intracellular enzymes, metabolites), biodegradable polymers that will be discussed later, and various plant parts (roots, leaves, and flowers) can provide reducing agents for the synthesis.

I-5.3.1. Synthesis using plant extract

In contrast to fungi and microbes, green synthesis uses medicinal herbs of natural origin and their extracts, which contain a wide range of metabolites, particularly water-soluble flavones, quinones, which cause a rapid, and quick reduction of silver. The use of green chemistry is risk-free, economical, easily scalable to large-scale production, and easily accessible to raw materials at lower cost. When making AgNPs, phytochemicals directly contribute to the reduction of the silver ions.
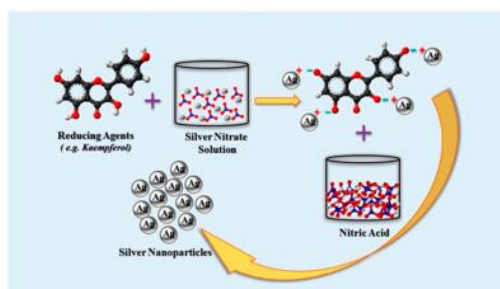
Figure -I; 9; Plant-based synthesis of Ag-NPs by reaction of AgNO3 with phytochemicals [102].

PHYTOCHEMICALS

A variety of plant parts, including leaves, roots, stems, bark, buds, fruits, and latex, have been used in previous studies to synthesize silver nanoparticles.

There has been a lot of work done up to this point on the green synthesis method for plant-mediated formulation of silver nanoparticles and the utilization of Phyto-chemicals for different plants.

By using spectroscopic analysis, the most significant phytochemicals involved in the reduction of silver were identified as flavonoids, phenols, alkaloids, terpenoids, amides, aldehydes, carboxylic acid, and ketones.

CAPPING AGENTS

Capping agents are frequently used in the bottom-up method of synthesis of silver nanoparticles in order to regulate the particle size, agglomeration, and morphology of the material.

Because capping agents have particular functional groups that can attach to the surface of synthesized silver nanoparticles, surface energy is decreased and grain growth and particle agglomeration are prevented.

In some cases, capping agents have the ability to stabilize the steric and electrostatic properties of silver nanoparticles in suspensions.
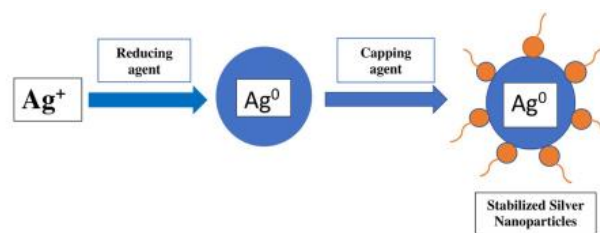
Figure -I; 10; The action of capping agent on the Ag NP (108)

Table-I; 2; List of different capping agents in chemical and biological synthesis (108)

METHOD	SILVER PRECURSOR	REDUCING AGENT	Stabilizing/ capping agents	SIZE (nm) AND SHAPE	REFERENCE
Chemical reduction	$AgNO_3$	Trisodium citrate	NaBH4	60 & 100 nm (0.75 ml and 1 ml of NaBH4 respectively) Spherical	[136]
Chemical reduction	$AgNO_3$	Sodium citrate	NaBH4	~23 Spherical	[137]
Chemical reduction	$AgNO_3$	MSA (mercaptosuccinic acid)	MSA	~65 Spherical	[137]
Plant	$AgNO_3$	Cleome viscosa	Alkaloids, phenolic compounds, tannins, proteins	5–30 Spherical	[140
Plant	$AgNO_3$	Callicarpa maingayi	Alkaloids, flavonoids, -OH, -NH, -C=O groups	13–15.6 Spherical	[141]
Fungi	$AgNO_3$	Fusarium oxysporum	Proteins	10–20 Spherical	[66]
Fungi	$AgNO_3$	Aspergillus oryzae	Proteins	< 105 (depending on pH, temp, and conc. of AgNO3) Spherica	[67]
Bacteria	$AgNO_3$	E. Coli	NH, -OH, Carbonyl,	118 Spherical	[144]

			CN triple bond in proteins		
Bacteria	AgNO₃	Bacillus cereus	Proteins	10–30 Spherical	[69]
Marine algae	AgNO₃	Spyridia fusiforms	Proteins, Secondary cyclic alcohols	5–50 Mostly spherical, some triangular and rounded rectangular	[146]

Table-I; 3; Green synthesis of AgNPs using plant extracts (106)

Plant extracts	Reducing/capping agents	precursor	Size(nm)	Morphology	Reference
Catharanthus roseus	Root extract	AgNO3	35-55	Spherical, FCC and crystalline	[90]
Aloe vera	Leaf extract	AgNO3	70	Spherical, cubical and triangular	[92
Lens culinary	Seed exudate	AgNO3	13	Crystalline and spherical	[93]
Banana	Peel	AgNO3	23.7	Crystalline and spherical	[94
Macrotyloma uniflorum	Seed extract	AgNO3	12	FCC and nearly spherical	[95]

I-5.3.2. Synthesis using bacteria

Pseudomonas stutzeri which is the first strain of bacteria form which AgNPs were synthesized and isolated form Ag amine [32]. Many of the bacterial strains and microorganism developing resistance to metal at lower concentration. Resistance mainly produced due to efflux, change in solubility, toxicity via oxidation/reduction and precipitation of metals [33]. There is evidence that while microorganisms are alive at lower concentrations of metal ions, exposure to these ions at higher concentrations causes microbial death. Nitrate reductase converts nitrate to nitrite during the biosynthesis of silver.
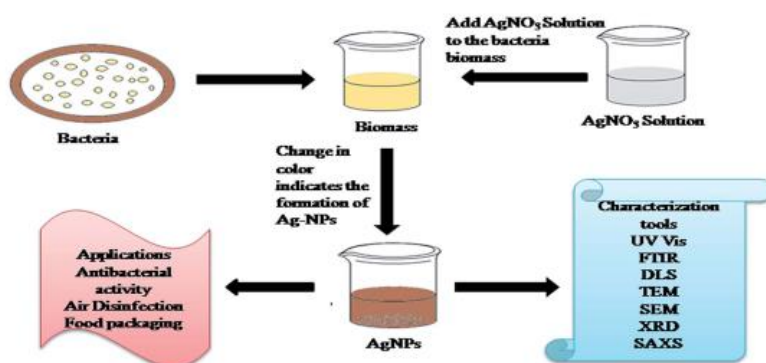
Figure -I; 11; Mechanism for the green synthesis of AgNPs from bacteria

Table-I; 4; Green synthesis of AgNPs using bacteria (106)

Bacteria	Intracellular/ Extracellular	Precursor	size (nm)	Morphology	Reference
Marine Ochrobactrum spp	Intracellular	AgNO3	38–85	Spherical	[46]
Exiguobacteriumm exicanum	Extracellular	AgNO3	5–40	Spherical and cubic	[47]
Shewanella oneidensis	Extracellular	AgNO3	4 1.5	Spherical	[48]
Pseudomonas putida NCIM 2650	Extracellular	AgNO3	70	Spherical	[52
Rhodococcus spp.	Intracellular	AgNO3	5–50	Spherical	[54

I-5.3.3. Synthesis using fungi

High production yield AgNPs synthesized by fungi obtained when compared to bacteria due to fungi secret higher number of proteins that directly responsible for increased production [34]. Higher production rate is mainly due to silver ions entered in to fungal cell wall which leads to reduction of silver ions by fungal enzymes such as naphthoquinones and anthraquinones [35]. The only drawback of fungal synthesis of AgNPs is its slower rate and process, so green synthesis is preferred over other methods.
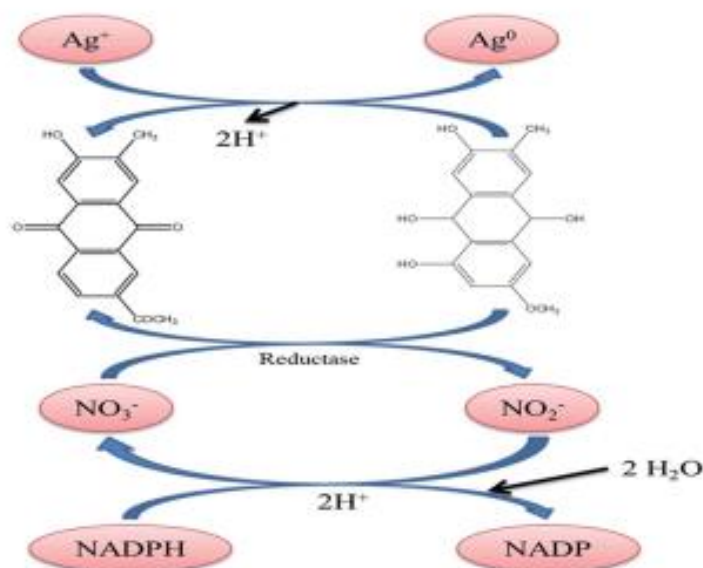
Figure -I; 12; Mechanism for the synthesis of AgNPs using fungi

Table-I; 5; Green synthesis of AgNPs using Fungi (106)

Fungi	Intracellular/ Extracellular	Precursor	Size (nm)	Morphology	References
Aspergillus niger	Extracellular	AgNO3	1-20	Spherical and Polydispersed	[67]
Fusarium acuminatum	Extracellular	AgNO3	13	Spherical	[69
Penicillium fellutanum	Extracellular	AgNO3	5-25	Spherical	[70]
Aspergillus clavatus	Extracellular and intracellular	AgNO3	10-25	Polydispersed and Spherical	[71]
Aspergillus flavus	Extracellular	AgNO3	8.92 ±1.61	Monodispersed	[73]

I-5.3.4. Synthesis using biodegradable polymers

Chitosan, a linear polysaccharide (sugar) derived from the tough exoskeleton of various marine organisms, serves as both a reductant and a stabilizer, making it the most beneficial for the biosynthesis of Ag NPs. Similarly, when preparing Ag NPs using starch, a paste of starch with NaOH may be used as it dissolves starch without degradation and the unmodified starch with high molecular weight prevents the agglomeration of particles for a longer time (six months) [36].
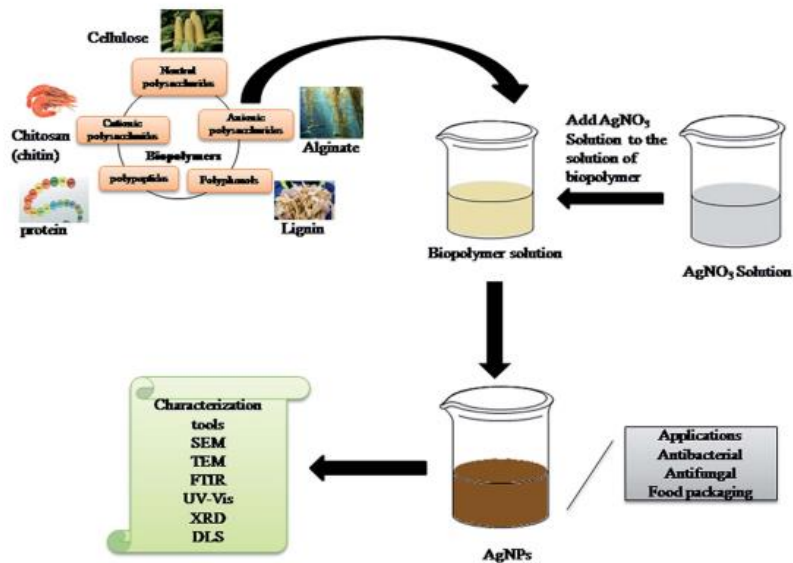
Figure -I; 13; Procedure for the synthesis of AgNPs using biopolymers

Advantages and Disadvantages of Biological Methods

i.   Advantages
• These methods are inexpensive, environmentally friendly and non-toxic.
• No complex configuration is required to drive the synthesis process.
• It is not necessary to use stabilizing agents to prevent NPs from agglomerating.
• Because these processes are performed in environmental conditions, they are not energy-efficient.
• These methods provide more precise control of the size and shape of NPs compared to chemicals and physical methods.

ii.   Disadvantages
• Synthesis by biological methods is not as fast as chemical synthesis.
• Due to the presence of many biomolecules present in biological sources, it is difficult to identify the exact biomolecules responsible for the synthesis of NPs.
• If biological synthesis is carried out on a large scale, an ecological imbalance can result from the overuse of different biological species.
• Microbial toxins can be collected by biological synthesis [37].

I-6. Stabilization of silver nanoparticles

A large number of capping agents are used for stabilizing AgNPs. The type of capping agent determines the stabilization mechanism of AgNPs. The aggregation kinetics of stabilized AgNPs is carried out in three mechanisms (electrostatic, sterile and electro steric) (38).

I-7. Mechanism of formation of silver nanoparticles [39 and 40]:

Metal nanoparticle formation is frequently viewed as a multi-stage sequence. Temperature, concentration, and the type of reagents and reducer can all play a role in the formation of nanoparticles.

Nucleation:

Zero valence metal atoms are created by dissolving and then reducing metal precursors. Collisions between atoms with high saturation in insoluble atoms produce small aggregates. The localized formation of a new, thermodynamically stable solid phase within a liquid phase is referred to as nucleation in this second stage. After some time, nuclear formation causes the saturation to decline. The result is a decrease in the rate of nucleation and a constant number of particles in the system.

Growth:

When a nucleus reaches a certain radius, it can continue to grow by ingesting the dissolved monomers. Thermodynamically speaking, this stage of growth is preferred. The rate of particle growth increases with atom concentration.

Murder:

The over-saturation rate continues to decline throughout the reaction. The Oswald death phenomenon refers to the point at which growth can no longer proceed and a final stage can occur. From the smallest to the largest particles, atoms are inter diffusing.

(a)

(b)
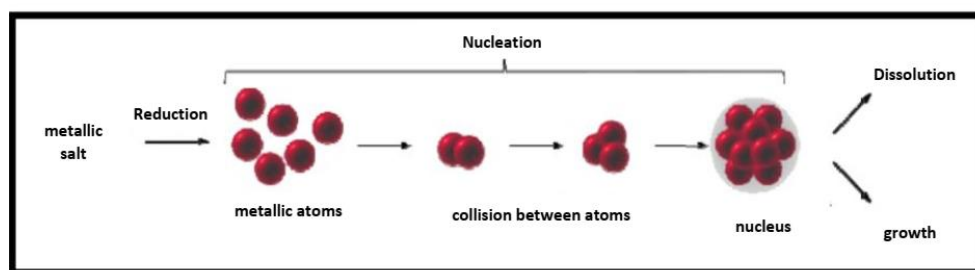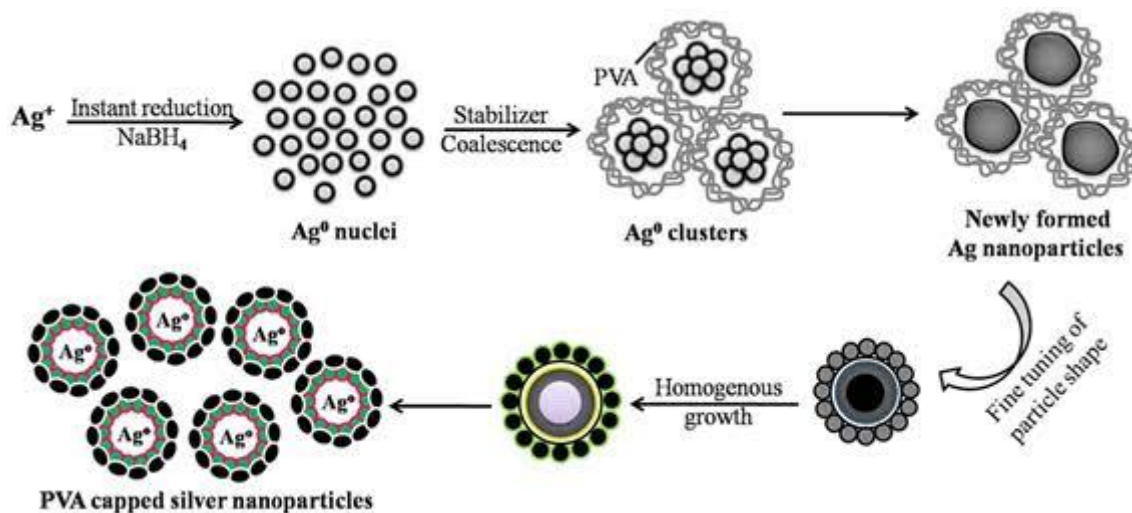
Figure -I; 14; Representation of stages that take place under nucleation (a) and mechanism of synthesis of AgNPs.

I-8. MECHANISMS OF ACTION(MOA) OF AGNPS

I-8.1. AgNP's antimicrobial MOA

When AgNP reaches toward cell they release Ag+ ions. These released ions then interact with sulphur and phosphorus containing compound present in cell wall. This led to disarranged cell wall formation and small pits forms in the cell wall.

Formed pit gives access to entry of ions and other foreign material to entry into cell. This increases intracellular osmotic pressure. As pressure built up in the cell, it begins to swell.

Finally, all these events led to bursting of cell wall and cell lysis take place. This type of antimicrobial activity is more in gram −ve cell than gram +ve cell. As gram +ve cell have more cross-linked peptidoglycan layer and teichoic acid in their cell wall.

The gram −ve cell have less or no peptidoglycan layer and have more lipopolysaccharide in their cell wall. So, the AgNP's easily interact with gram −ve cell due less barrier [41].
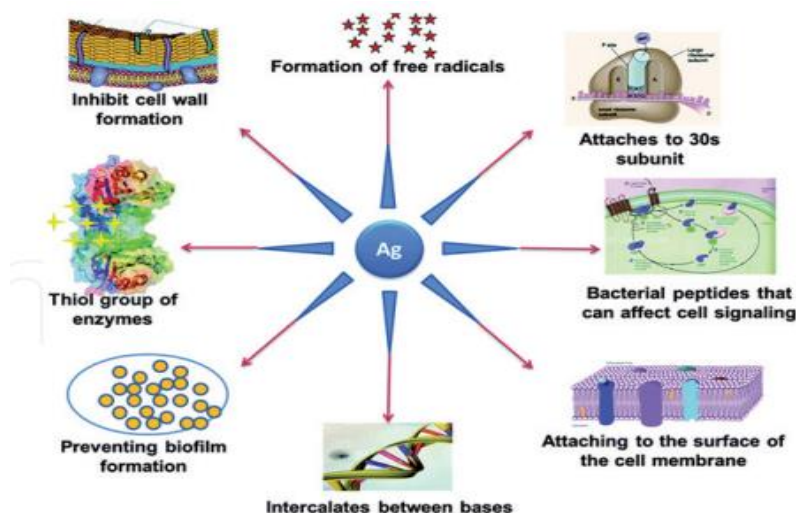
Figure -I; 15; Factors affecting to the bactericidal effect of silver nanoparticles. (109)

I-8.2. AgNP's anticancer MOA

As described in above when pit formation takes place in the cell wall, the Ag+ ions released by AgNP's get entered into cell. Then they reach to mitochondria where they interact with thiol groups and bind to NADPH dehydrogenase enzyme and liberates ROS. These formed ROS in mitochondria interacted with respiratory enzymes damage ATP formation and respiratory cycle of cell.

Formed ROS also interact with protein, sulphur and phosphorus containing cell constituent. Also, these formed ROS also bind to phosphorus elements of DNA and RNA which lead to inhibit cell replication and protein synthesis. Due to binding with DNA aggregation of damage protein synthesis which lead to cell death.

Another possible action is by autophagy. AgNP's have ability to induce autophagy by accumulation of autophagolysosomes in human ovarian cancer cell. This autophagy work by mainly 2 ways; at lower level they increase cell life i.e., surviving rate, but when its level increases it led to cell death [42].
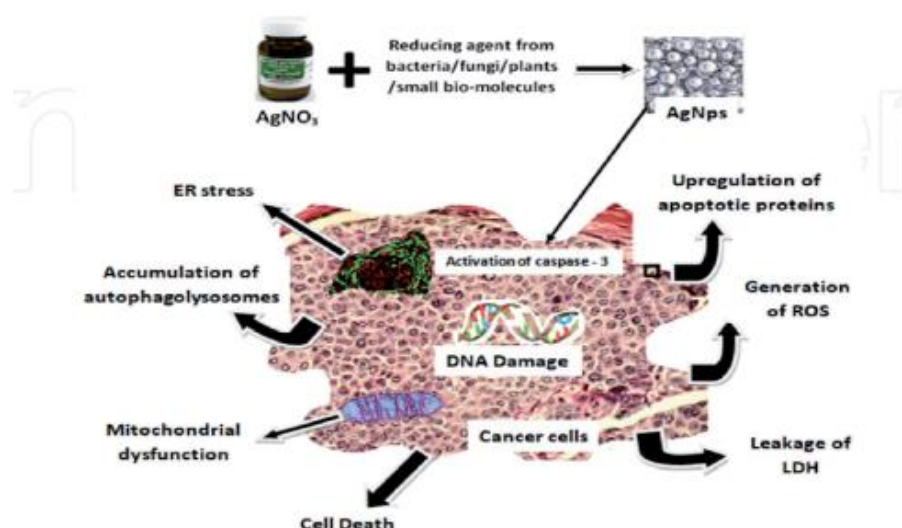
Figure -I; 16; Anticancer mechanism of action of silver nano particles. (109)

I-9. METHODS OF CHARACTERISATION OF SILVER NANO PARTICLES

The nature, size, shape, distribution, state of stability or aggregation, morphology, elemental composition, and dispersity (monodisperse or polydisperse) of nanoparticles are all characterized using various analytical and spectroscopic techniques. Following is a description of some of the key methods frequently used to characterize silver nanoparticles, along with a discussion of their benefits and drawbacks.
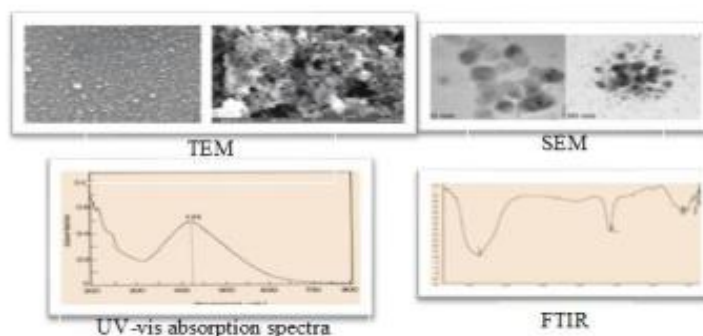
Sample analysis of silver nanoparticles by TEM, SEM, UV-vis spectroscopy, FTIR. (105)

I-9.1. Uv-vis spectroscopy

UV-vis spectroscopy is the primary but crucial tool that explains silver nanoparticles' formation at the initial synthesis stage. It is very characteristic depending upon the shape, size, and distribution of Ag NPs due to surface plasmon resonance. The smaller and spherical silver nano particles absorb at near 400nm and have narrow peaks, while larger Ag NPs have redshift (absorb at longer wavelength) with broad peaks.

It is also indicative of the stability of Ag NPs since the peaks start to decrease in intensity and broaden with the appearance of secondary peaks at higher wavelengths, as particles aggregate [43]. Similarly, the broadening of peaks also provides information about the distribution of Ag NPs. Generally, broad peaks indicate the formation of broader size range distribution (wide dispersity) of Ag NPs in solution [44, 45].

The colloidal Ag NPs exhibit size- and shape-dependent optical properties, i.e., the color of the solution during synthesis is also a function of size and shape, and the peculiar color of the solution makes it possible to observe and monitor the development of the synthesis reaction. However, UV-vis is insufficient on its own to provide a complete picture of synthesized Ag NPs.
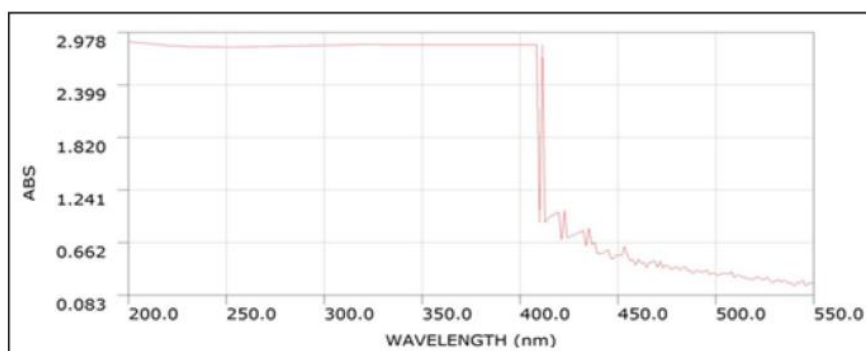
Figure -I; 18; shows a decrease in intensity of the characteristic surface plasmon band in the ultraviolet–visible (UV–Vis) spectroscopy for the spherical particles at k max 416 nm appeared as shown in figure above(112)

I-9.2. FOURIER TRANFORM INFRARED SPECTROSCOPY (FTIR)

In a similar way, Fourier transform infrared spectroscopy (FTIR), which involves the interaction of infrared (electromagnetic radiation) with bonding in molecules in the form of stretching and bending vibrations (in the 4000-400cm-1 region), characterizes the role of chemicals, metabolites involved in reducing and capping of Ag NPs, and the presence of chemical residue on the surface of Ag NPs.

It is fast, inexpensive, non-destructive (when used with the attenuated total reflectance accessory), and highly reproducible, but it is less sensitive to nanoscale analysis and gives only qualitative information [46]. The FTIR spectral analysis shows the involvement of amide (-CO-NH2), carbonyl (-CO), and hydroxyl (-OH) functional groups responsible for the reduction, capping, and stability of Ag NPs [47, 48, 49, 50].
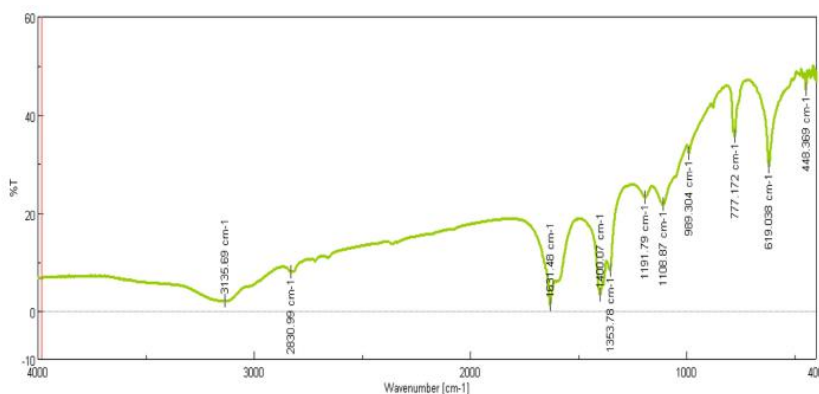
Fig. 3. FTIR measurements of AgNPs.

Figure -I; 19; shows the IR transmission is measured out the absorptions observed at lower wave numbers. The characteristic bands corresponding to the Ago stretching mode are observed at 448 cm1. The other peaks in the FTIR measurements responsible for capping, reducing and stabilizing the AgNPs present in the leaf extract. The peaks at 3135 cm1 correspond to the O–H stretching of hydroxyl groups. The relatively strong absorption peak around 1631 cm1 indicated the characteristics IR absorption of polysaccharides. Peak at 2830 cm1, it represents C–H stretching. (112).

I-9.3. X RAY DIFFRACTION (XRD)

Since every crystalline material has a unique and characteristic X-ray diffraction (XRD) pattern, it is used as a fingerprint to identify the material and determine the crystallinity. It is a non-destructive technique. The Joint Committee on Powder Diffraction Standards (JCPDS) has the databases of card number standards, which are used to identify the crystalline material.

For example, the characteristic powder diffraction pattern for face-centred cubic (FCC) silver lines is identified by **JCPDS no. 04-0783**, whereas the silver oxide is identified by **JCPDS no. 01-076-1489**. It also gives the information of phase impurity in the material.

For example, the presence of silver oxide in the synthesized Ag NPs could be formed due to the oxidation of Ag NPs on hot air oven drying of synthesized AgNPs that are mainly performed by vacuum freeze-drying [51].
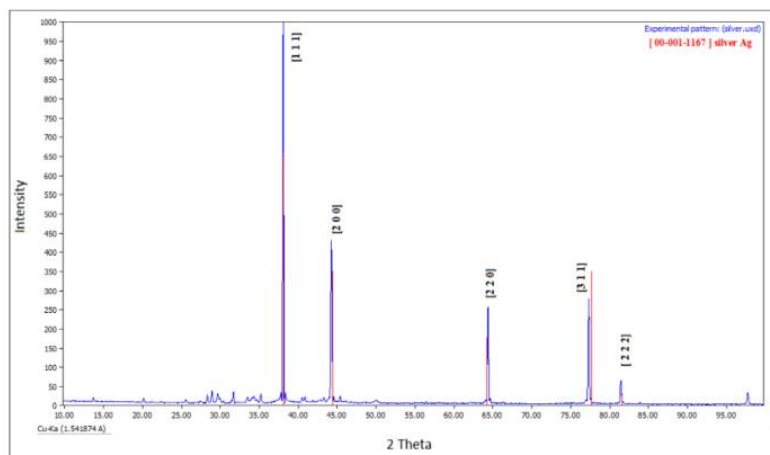
Figure -I; 20; shows the XRD pattern of the Cubic structure of AgNPs.
The peaks are extremely sharp. Characteristic peaks at 38.08, 44.26, and 64.37, 77.30, 81.44, 97.76 for cubic (JCPDS No.00-004-0783) marked by their indices [1 1 1], [2 0 0], [2 2 0], [3 1 1] [2 2 2] were observed. The crystallite sizes of the as synthesized AgNPs, Dp, was calculated from the major diffraction peaks of the base of (1 1 1) using the Scherrer formula;

$$D_P = \frac{k\lambda}{\beta_{\frac{1}{2}} \cos\theta}$$ (112).

I-9.4. Energy dispersive x ray spectroscopy (EDX)
Likewise, energy-dispersive X-ray spectroscopy (EDX) is used to characterize the elemental composition, relative abundance, and impurities of nanoparticles, which involves X-ray interaction with the sample.

The presence of emission peaks at 3keV reveals the presence of silver crystallites, and the absence of any other peaks confirms the impurity-free Ag NPs [49, 52], while the presence of other peaks like carbon and oxygen could be the interaction of metabolites with Ag NPs on the surface or due to oxidation of Ag NPs.

I-9.5. Dynamic light scattering (DLS)
Another quick, simple, and non-destructive method to measure particle size at the micrometre and nanometre scales is dynamic light scattering (DLS). In this method, a monochromatic light source, such as a laser, is passed through a suspension of nanoparticles, which scatter light at various intensities due to Brownian motion, and size is calculated using the Stokes-Einstein relationship.

It functions best with monodisperse nanoparticles and typically measures the hydrodynamic diameter of nanoparticles influenced by the surfactant, stabilizing, and capping agents as well as the presence of an electrical double layer adsorbed on the surface of nanoparticles.

Thus, the nanoparticle's measured size is somewhat larger than the actual size measured from XRD and TEM [53, 45, 54,55]. The DLS also measures the zetapotential of AgNPs where the large magnitude value indicates the electrostatically stabilized nanoparticles (i.e., no aggregation) [56, 57, 58].

I-9.6. Scanning electron microscopy

Additionally, scanning electron microscopy (SEM), which employs electron beams as imaging probes, is used to characterize the surface morphology, size, aggregation, and distribution of nanoparticles.

It offers high-resolution images at the nanometer scale, the function of the biomatrix, or the encapsulation of Ag NPs, and is used to calculate the size of nanoparticles. However, it requires a dry and conductive sample, and sometimes the size measurements from SEM are not as accurate as TEM due to Van der Waals clusters of small entities [59].
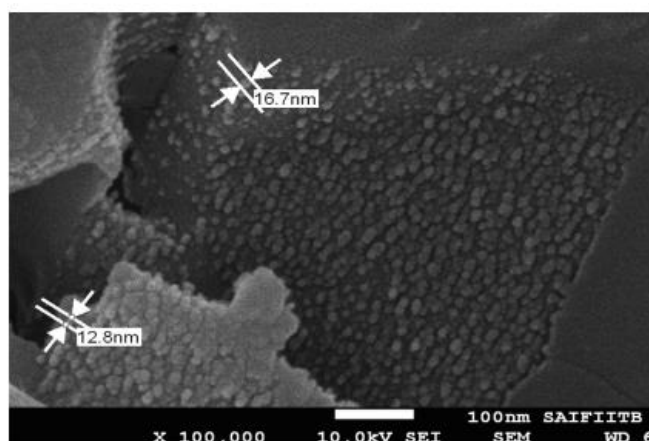
Figure -I; 21; shows the SEM image of AgNPs image was taken at 25,000 magnification shows AgNPs are spherical in shape with smooth surface and the size of the particles around 15–30 nm. (112)

I-9.7. Atomic force microscopy

In addition, atomic force microscopy (AFM), a cutting-edge 3D technique, employs a cantilever with a mechanical probe to characterize the topology of nanoparticles at a nanometer scale.

In comparison to methods like XRD, DLS, SEM, and TEM, it has a special advantage in that it can calculate the height and volume of a sample, analyse the 3D topology of the particles, and imagine hydrated nanoparticles as well as distribution (dispersity) and variation in nanoparticle size and shape. However, it is a time-consuming process and gives the exterior structural information only [46].
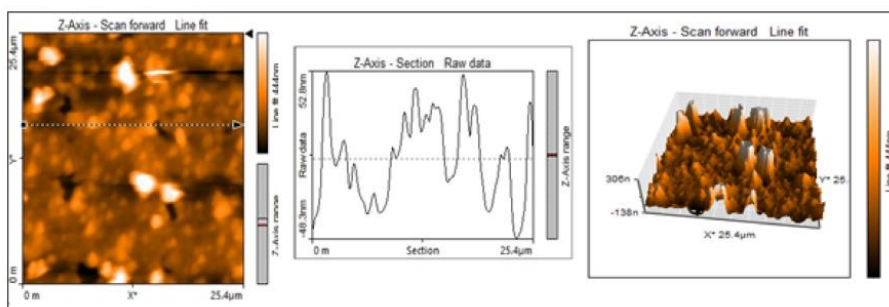
Figure -I; 22; shows size and shape of the nanoparticles which obtained directly from tip-corrected AFM measurements, and the shape of the nanoparticles is estimated on the basis of

AFM images and line scans. The tip-corrected measured the size of 20–40 nm, have average Surface roughness is 52.8 nm and spherical in shape. (112)

I-9.8. Transmission electron microscopy

Accordingly, transmission electron microscopy (TEM), which also employs an electron beam for interaction with the ultrathin sample, provides the most precise and high-resolution imaging information about the size, shape, morphology, state of aggregation, and distribution of nanoparticles at nanometer resolution.

It also makes it easier to understand how metabolite encapsulation of Ag NPs and capping agents work. The preparation of the ultrathin sample requires high vacuum, expertise, and time, and results are more reliable for homogeneous samples.
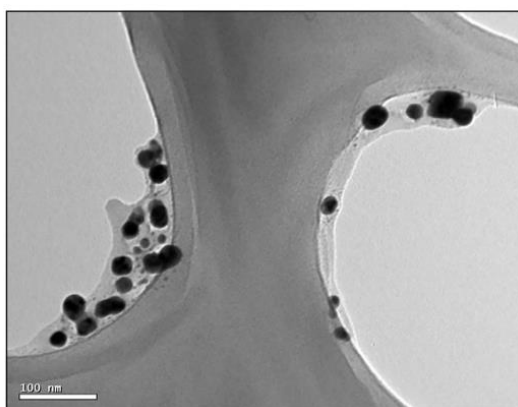
Figure -I; 23; Transmission electron micrograph of biogenic silver nanoparticles (111)

I-10. APPLICATION OF SILVER NANOPARTICLES
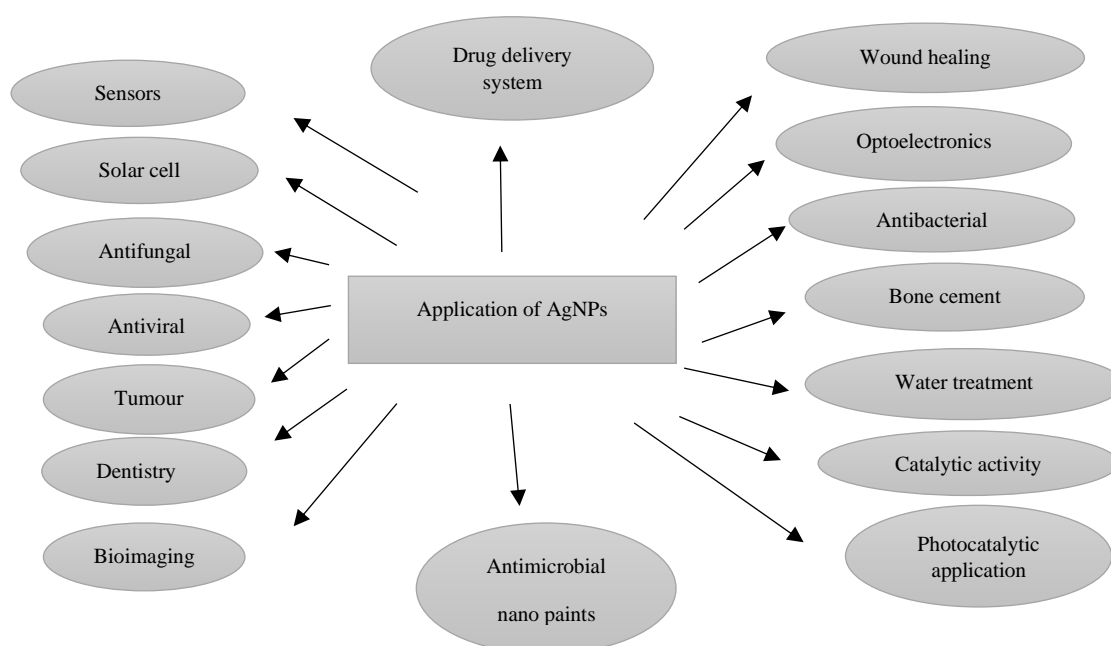
Figure -I; 24; *Application of silver nanoparticles*

I-10.1. Drug delivery system;

AgNPs are also being used for targeted drug delivery now a day. Its effectiveness in delivering the anti-cancer drugs to the tumour tissue has already been observed. The size of the nanoparticles makes it easy for them to penetrate the tissue and ensure effective drug delivery [62].

The use of AgNPs for delivering anti-cancer drugs is of great interest to researchers because of the cytotoxic effect shown by the convention methods of drug delivery [62, 63]. Both healthy or normal cells and tumour cells die as a result of conventional treatment.

Figure -I; 25; Drug delivery in nanoparticles

I-10.2. Wound healing

Due to the involvement of numerous different cell lineages, wound healing is a difficult and drawn-out process. The age of the patient, the size and depth of the wound, the use of medication, nutritional status, etc. are some extrinsic and intrinsic factors that influence the rate of healing.

In order to reduce the risk of infection at the site of the injury and prevent further complications, wound management is crucial. A perfect wound dressing removes extra fluid or dead skin cells and keeps the area around the wound moist. It also provides protection from microorganisms.

Conventionally, 6 different types of drugs are used for wound management and healing, which include antiseptics, tropical antibiotics, granulation tissue suppressing agents, herbal therapeutics, enzyme treatment, and some other tropical agents (vitamin E, scarlet oil).

But there are several limitations or drawbacks related to conventional medication therapy, like narrow antimicrobial spectrum, skin irritation, allergies and cytotoxic effect on body cells, etc. AgNPs can be a perfect alternative for conventional medicines as they have low toxicity to the system, effective against pathogens that are multi drug resistant, and they are not responsible for causing drug resistance due to their multilevel antimicrobial effect [64].

Figure -I; 26; AgNP wound-healing mechanism. Exposure to AgNPs promotes wound closure by preventing bacterial colonisation and inflammation in the wound site. Open wound image adapted from [103].

I-10.3. Sensors

Environmental monitoring is the process of assessing the state of the environment to determine the number of pollutants present. Environmental contaminants like mercury, cadmium, lead, copper, nickel, chromium, and others can be toxic metals. Silver nanoparticles can be effectively used in optical sensing for the detection of heavy and toxic metals, according to recent advances in nanotechnology.

I-10.4. Solar cell

One of the recent applications of AgNPs is in the dye sensitized solar cell as they induce surface plasmon resonance which thereby increases the absorption coefficient of dye [65].

 Ihara et al. discovered that the efficiency of the dye-sensitized solar cell increased from 1.5 to 2.5% in the presence of polymer-modified AgNPs. Here, the nanoparticles improved the photoelectric conversion efficiency of the solar cell [66].

I-10.5. Optoelectronics

Xie et al. [60] worked on substrates of homogeneous silver-coated nanoparticles for enhancement of fluorescence detection. In their study, a monolayer of Fluorescein isothiocyanate (FITC)-conjugated Human Serum Albumin (FITC-HSA) was used to investigate the degree of the fluorescence enhancement and it was tested by the help of laser scanning microscopy at excitation wavelength of 488 nm.

When it comes to fluorescence enhancement, Au Core-Ag shell nanostructures on the surface of the glass are good substrates with outstanding macroscopic homogeneity, according to a report based on spectrofluorometer spectra.

I-10.6. Photocatalytic application

The amount of surface energy and surface area play a role in how well silver nanoparticles work in catalytic media. Smaller silver particles are more effective catalysts than stable colloidal particles, according to several studies.

In comparison to stable and bulk AgNPs, the reduction rate of organic dyes catalysed by growing AgNPs is faster. In a study, the results of the photocatalytic investigation of ZnO: Ag

composite revealed that the AgNPs and their composites exhibit high catalytic performance in degrading and removing dye [61].
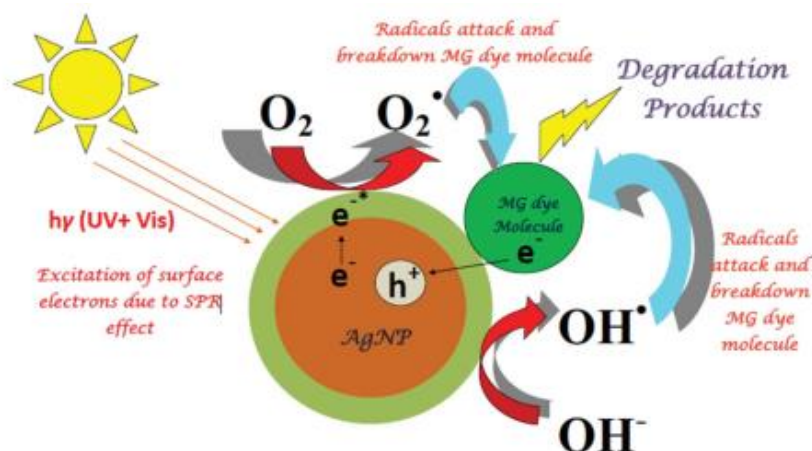
; Mechanism of MG dye degradation using solar light active photocatalytic AgNPs synthesised using CN extract. (110)

I-10.7. Antibacterial.

Due to the bacterial development of antibiotic resistance, there is a demand for an alternative antibacterial treatment. Against both Gram-positive and Gram-negative bacteria, the AgNPs exhibit excellent bactericidal action.

The antimicrobial activity of AgNPs is may be due to either;

- (i)   formation of pores in the cell wall, which ultimately leads to leakage of cellular content
- (ii)   the silver ion penetrate through ion channels does not damage the cell membranes; rather denatures the ribosome and inhibits the expression of enzymes and thiol containing proteins essential for the production of ATP and DNA thus resulting in cell death [67,68].

I-10.8. Antifungal

Extreme fungal infections have recently played a significant role in the rising prevalence of a specific disease and the mortality of patients with compromised immune systems. One of the most common pathogens responsible for fungal infections is Candida species. It often causes nosocomial infection with an associated mortality rate of up to 40% [69].

Kim et al. demonstrated the antifungal activity of silver nano formulation on a total of 44 antifungal strains of six fungal species [70]. The literature revealed that AgNPs are effective against C. glabrata, C. albicans, C. krusei, C. parapsilosis and T. mentagrophytes effectively. Recently studies showed that the Tulsi (Ocimum sanctum L.) mediated AgNPs exhibited antifungal activity against an opportunistic human fungal pathogen [71].

I-10.9. Antiviral

The cytoprotective properties of silver is well known and has been employed for the prevention of HIV interaction to the host cells [72]. AgNPs can also be used to prevent infection after surgery and acting as anti-HIV-1 agents [73].

Therefore, AgNPs interaction with microorganisms and viruses is another flourishing field of research.

The studies reported that AgNPs interact with HIV-1 by binding preferentially to gp120 glycoprotein knobs [74]. This sort of interaction of AgNPs specifically inhibits the binding of virus to host cells [38].

I-10.10. Medical devices.

AgNPs are extensively used in medical devices and implants. Additionally, they are also added to consumer products such as colloidal silver gel and silver-embedded fabrics which are now used in sporting equipment [75].

Silver coated biomedical devices [76], implants [77], textile fibres [78] are employed for the treatment of wounds or burns and glass windows and other surfaces to maintain sanitization and hygienically conditions.

I-10.10.1. Catheters

The plastic catheters are coated by bioactive AgNPs. The researchers have developed a coating method which yielded a thin (~100 nm) layer of nanoparticles of silver on the surface of the catheters. The nano particles coated catheters are biocompatible as they are nontoxic and have tendency to release specific and sustained release of silver at the implantation site [76].

The infection risk is highly reduced in these catheters due to significant in vitro antimicrobial activity by the inhibition of biofilm formation using Escherichia coli, Enterococcus, Staphylococcus aureus, coagulase-negative staphylococci, Pseudomonas aeruginosa and Candida albicans [79].

I-10.10.2. Bone cement

AgNPs with additive poly (methyl methacrylate) (PMMA) has been used as a bone cement [80]. Such bone cements are antibacterial due to presence of AgNPs. Bone cement is the material which are employed by orthopaedician for annexing prostheses like hip and knee replacement surgery. The joint replacement surgery has high infection rates i.e., approximately 1.0–4.0% [81].

Therefore, clinicians are switching over another type of bone cements which greatly reduce the rate of infection to 0.4% and 1.8%. The use of antibiotics is limited due to development of bacterial resistance. It has been demonstrated that nano silver -PMMA bone cement decreases the incidence of resistance by the versatile mode of action, and has also been known for its antibacterial activity and low cytotoxicity [82].

I-10.11. Tumour

Reactive oxygen species (ROS) can cause damage to cellular components such as proteins lipids and DNA and eventually lead to death of the cell. It has been found that AgNPs can induce cell death and oxidative stress in skin carcinoma cells and in human fibrosarcoma [82].

AgNPs is also known to induce a p53-mediated apoptotic pathway through which majority of the chemotherapeutic drugs triggers apoptosis [82].

I-10.12. Water treatment

The AgNPs can be employed for purification in water filtering apparatus which may be due to its enhanced antimicrobial nature [83]. An overview for treatment of waste water using AgNPs is shown in Figure 22. Silver nanotechnology can be used in water purification systems as world health organization (WHO) approved [84].

Antimicrobial agents have received a lot of consideration for use in biomedical devices, health, as well as in the food and hygiene industries, to prevent the growth of harmful microorganisms.

Antimicrobial coatings ought to be effective against bacteria, simple to make, and low in toxicity. Antimicrobial coatings made of silver or containing silver have been used extensively.
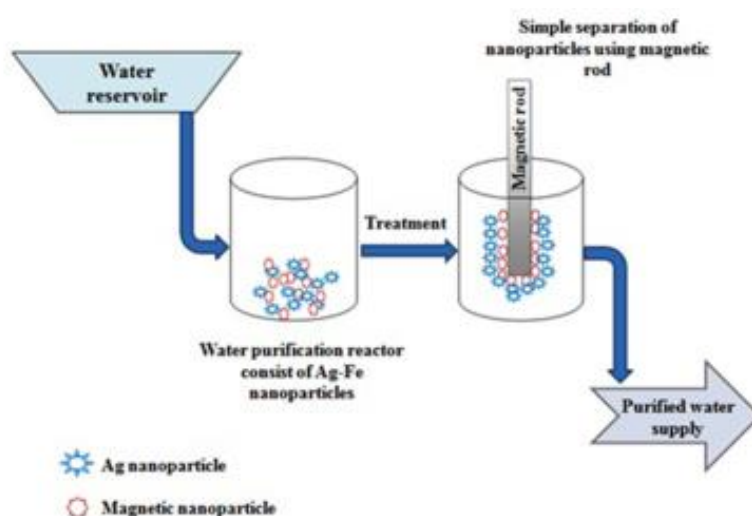
Figure -I; 28; Silver–magnetic nanocomposite involved in water purification. Wastewater is treated with synthesized silver–magnetic nanocomposite which after treatment is removed using a magnetic rod to obtain nanoparticle-free purified drinking water (107)

I-10.13. Catalytic activity

The catalytic activity of AgNPs is size dependent. The researchers have investigated the reduction of Methylene Blue (MB) by NaBH4 using catalytic activity of the synthesized AgNPs from the plant Guggulutiktham Kashayam [85].
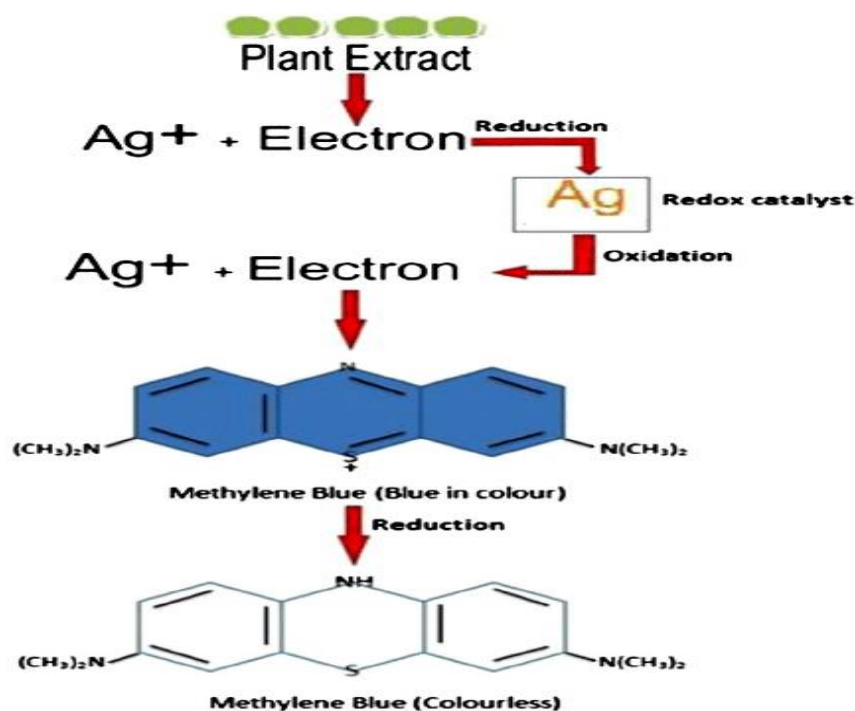
Figure -I; 29; Representation showing catalytic action of silver nanoparticles against methylene blue as it turns colourless

I-10.14. Antimicrobial nano paints

The production of nano fibre embedded with AgNPs are gaining interest. These polymeric nanocomposites are highly stable. They can withstand high temperature i.e., up to 200°C by resisting oxidation and aggregation, which enhances their application in production of silver nanoparticle embedded homogeneous paints [86].

I-10.15. Bioimaging

The AgNPs due to its plasmonic properties can be detected by numerous optical microscopy techniques and are advantageous over commonly used fluorescent organic dyes that decompose during imaging (photobleaching). As AgNPs are photostable thus they can be used as biological probes to monitor continuously dynamic events for an extended period of time [87].

AgNPs are used in bioimaging in one of two ways: by functionalizing a biomolecule on the surface of AgNPs, which increases specificity for cell membrane, or by incubating AgNPs with cells to observe physical interactions and uptake. The first is simpler because the second requires a particular biofunctionalization molecule.

I-10.16. Dentistry

AgNPs have been used to increase bacterial resistance while maintaining the cutting bonding strength of orthodontic adhesives in bandages, dental composites, and instruments. by lessening microbial colonization of the coating materials and enhancing antifungal properties.
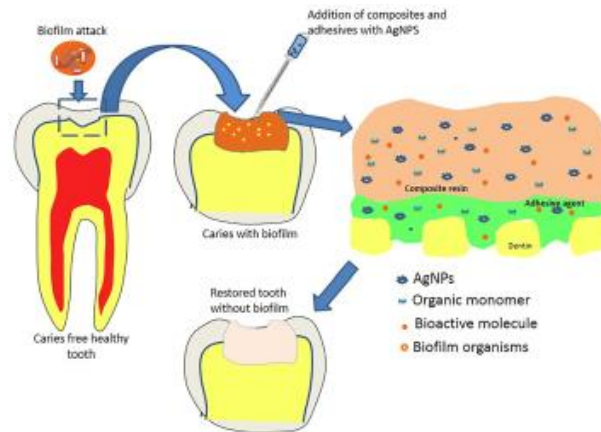
Figure -I; 30; Incorporation of AgNPs to Composite resins and Adhesive system eradicates the biofilm organisms and prevents loss of tooth structure. It prevents the microleakage increasing the longevity of restoration (104)

I-11. FACTORS AFFECTING SYNTHESIS OF SILVER NANO PARTICLES
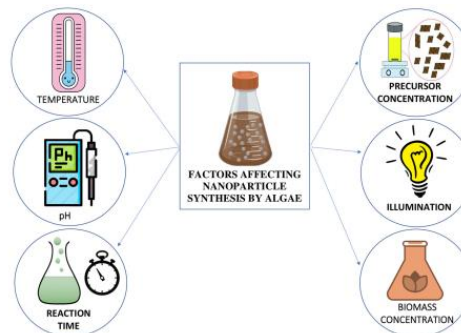
Figure -I; 31; Factors affecting the synthesis of silver nanoparticles (108)

I-11.1. Particular Method

Silver nanoparticles can be created using a variety of techniques, from physical ones involving mechanical processes to chemical or biological ones involving various organic or inorganic chemicals and living things.

Each procedure has unique advantages and disadvantages. However, compared to conventional methods, biological methods for the synthesis of nanoparticles use non-toxic and environmentally safe materials along with green technology, making them more acceptable and eco-friendlier.

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I-11.2. PH

The size and morphology of the biologically synthesized nanoparticles can be influenced greatly by the change in pH [92, 93]. It is because the electrical charge of biomass and capping agents are altered strongly at different pH conditions which causes alteration in their ability to bind and reduce metal ions. Hamouda et al. showed that the optimum pH for AgNP synthesis by O. limnetica was 6.7, but as soon as the pH was altered to 4.7 or 5.7, NP synthesis was completely inhibited [109]. Several reports exhibit that an acidic medium promotes the

production of large-size AgNPs whereas small-size AgNPs were formed in the alkaline medium [94]

I-11.3. Temperature.

Temperature is another important parameter that affects the synthesis of nanoparticles using all three methods. The physical method requires the highest temperature (>350∘C), whereas chemical methods require a temperature less than 350∘C. In most cases, the synthesis of nanoparticles using green technology requires temperatures less than100∘C or ambient temperature. The temperature of the reaction medium determines the nature of the nano particle formed [88].

Alteration in temperature can also affect the shape of nanoparticles. Lengke et al. reported that during the synthesis of AgNP with cyanobacterium Plectonema boryanum, the shape of the AgNp changed with a temperature change. Spherical silver nanoparticles were served at all temperatures ranging from 25 to 100 °C but a octahedral-shaped NP was produced only at 100°C [95].

I-11.4. Illumination

Light can act as a catalyst for many reactions. Illumination is a critical physical factor that can affect the synthesis of AgNP. There is multiple experimental evidence that establish the effect of illumination during nanoparticle synthesis. Patel et al. in an experiment showed that extracellular polysaccharides isolated from the Scenedesmus sp. were able to synthesize silver nanoparticles in the presence of light but failed to do so in the dark. This indicates that how important light is for the synthesis of silver nanoparticles in this condition [96].

I-11.5. Time.

The quality and type of nanoparticle synthesized using green technology are greatly influenced by length of time for which the reaction medium is incubated [89]. Similarly, the characteristics of the synthesized nano particles were also altered with time and greatly influenced by the synthesis process, exposure to light, and storage conditions, and so forth [90,91].

Particles may aggregate as a result of long-term storage, they may contract or expand during that time, they may have a shelf life, and so forth, all of which have an impact on their potential.

The quantity of nanoparticles typically increases with a longer reaction time, but only temporarily. The instability of the silver nanoparticles after that may cause them to aggregate. The effects of increasing time on synthesized nanoparticles, however, won't be felt if they are stable.

I-11.6. Precursor concentration

It is well known that precursor concentration not only influences the yield but also affects the morphology of synthesized nanoparticles. In a study conducted by Rahman et al., the researchers used two different precursor concentrations (0.650 mM and 1.250 mM) for the synthesis of silver nanoparticles using C. reinhardtii. They reported that the formation of AgNPs was faster at 0.650 mM of AgNO3 than at 1.250 mM. This depicts that the rate of conversion of Ag^+ to AgNps depends on the concentration of precursors [97].

I-12. Toxicity of AgNPs
The morphological structure, type of coating material, and particle size are just a few of the variables that affect this toxicity. AgNPs' cytotoxicity is size-dependent, as evidenced by numerous reports.

I-12.1. The negative effects of silver nanoparticles on humans
Different pathways exist for silver nanoparticles to enter the human body. Metal nanoparticles can enter the blood and/or lymphatic circulation after ingestion or skin contact in the presence of a skin lesion, accumulate in organs, and then cause changes in the structure of organs and cell nuclei as well as loss of cellular identity and reduced proliferative capacity.

Nanoparticles are dangerous because they can easily be inhaled and interact with the respiratory and then cardiovascular systems due to their size and shape.

One of the harmful effects of ionic silver for people is argyria (or argyrose, from the Greek "argyros" which means silver), which is characterized by the blue-gray colouring of the skin, including the face, arms, hands, the base of hair follicles, the nails, and mucous membranes.

I-12.2. Effects of silver nanoparticles in the Environment
Increased use of products containing nanoparticles may result in significant environmental discharge, which may affect soils, aquatic environments (fish, invertebrates, Phyto- and zooplankton, and algae), and terrestrial environments (potentially harmful effects on bacteria and plants).

Additionally, some nanoparticles are toxic to fish because they suppress branchial activity and interfere with their ability to grow and reproduce. A very insoluble precipitation is created when Ag+ ions combine with Cl, SO4–2, and S2 in freshwater or a marine environment. The cellular viability of soil bacteria, such as denitrifiers, which are crucial for nitrogen fixation, nitrate removal, the breakdown of organic compounds, and the development of symbiosis with plants, is decreased by nanoparticles.

I-12.3. MECHANISM OF TOXICITY
There are several proposed mechanisms for AgNP toxicity; four of them are summarised below. AgNPs are suspected to induce cellular toxicity by:

(i) adherence of AgNPs to the cell membrane, resulting in physical destruction and cell membrane impairment;

(ii) internalisation of AgNPs, which can cause malfunction of the intracellular organelle (mitochondria, vacuoles and ribosomes), and biomolecules (proteins, enzymes, lipids and DNA);

- (iii) induction of cytotoxicity and oxidative stress by generation of ROS and free radicals, which also disrupts the activity of biomolecules and intracellular micro-organelles;
- (iv) modulation of intracellular signal pathways [98,99].
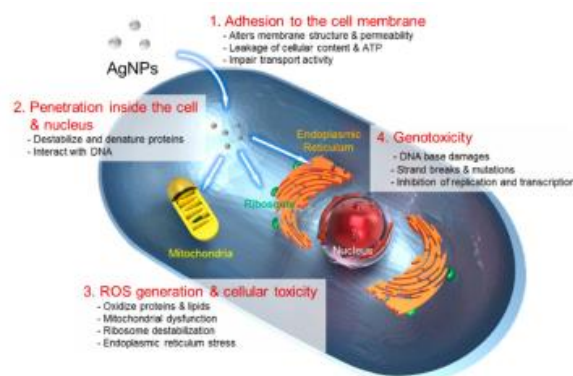
Figure -I; 32; Routes of cytotoxicity action for AgNPs. (**1**) Adhesion to cell wall; (**2**) Cellular internalisation; (**3**) ROS generation; (**4**) Genotoxicity [99].

I-13. Conclusion

One of the most alluring nanomaterials for commercial use is Ag-NPs. Antimicrobial, electrical, and medicinal goods have all made extensive use of them. In this study, we present a thorough overview of the Ag-NPs, including their methods of synthesis, antibacterial properties, and potential toxicological implications for both humans and the environment. Regarding environmental treatments, the disinfecting properties of Ag-NPs nanoparticles are also highlighted. A summary of a few recent uses of Ag-NPs in disinfection applications was presented and addressed. A few promising prospective applications of Ag-NPs-based nanomaterials for the treatment of infectious illnesses were also discussed. It will be possible to advance environmental treatments such as air, water, surface, and personal hygiene using a new class of nanosilver-containing disinfectant nanoproducts, which will help stop the spread of disease. However, further research is required to ascertain how to securely design, use, and discard items containing silver without posing a fresh risk to people or the environment before silver-based NPs can be employed in the field of treating infectious diseases.

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CHAPTER 2

SYNTHESIS OF SILVER NANOPARTICLES

II. CHAPTER 2

II-1. INTRODUCTION
This chapter describes the stages of manipulation performed as part of silver nano particles. The experimental part is divided into three stages: the first is a description of the plant used as a bio-reductor due to the presence of phytochemicals in the leaf extract that act as reductors and stabilizers in the formation of silver nanoparticles; the second is the synthesis of silver nanoparticles from the leaf extract; and the third is the formation of silver nano composites and their application in the reduction of methylene blue.

II-2. MATERIALS
II-2.1. Plant
In this study, we used an agro plant as a source of phytochemicals (biomolecules) which act as reducing and stabilization agents during the formation of AgNPs. This plant was obtained from the southern part of Algeria and we only used its leaves during our experiment. The suspected biomolecules present in the study include; flavonoids, phenols, alkaloids, terpenoids, amides, aldehydes, carboxylic acid, and ketones.

Figure-II; 1; showing the plant from which the plant extract was extracted
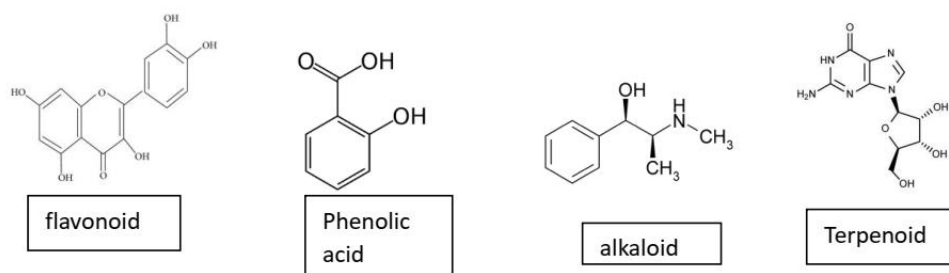
Figure-II; 2; structure of some of the biomolecules expected to be present in the plant leaves.

II-2.2. NaBH₄
Sodium borohydride is an inorganic compound whose chemical formula is $NaBH_4$. Under normal conditions, this compound exists as a white, powdery solid. $NaBH_4$ is a reducing agent which is widely used both industrially and on the laboratory scale. It can be noted that sodium borohydride is also known as sodium tetra-hydroborate and sodium tetra-hydridoborate.
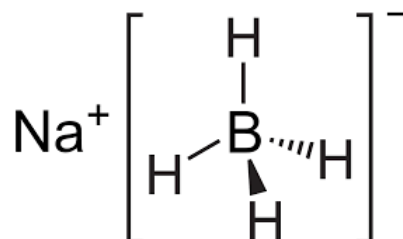
Figure-II; 3; structure of sodium borohydride

This salt consists of the Na^+ cation and the BH_4^- anion. It can be noted that the BH_4^- ion has a tetrahedral structure. $NaBH_4$ has three stable polymorphs, namely the α, β and γ polymorphs.

PROPERTIES OF SODIUM BOROHYDRIDE

Physical properties of NaBH₄

- The molar mass of $NaBH_4$ is 37.83 grams per mole.

- This compound does not have any characteristic odor.

- The density of sodium borohydride at STP corresponds to 1.07 grams per cubic centimeter.

- It has a melting point of 637K. However, it tends to undergo decomposition at this temperature.

Chemical properties of NaBH₄

- Despite being soluble in most protic solvents like water, this compound slowly reacts with the protic solvent, resulting in the formation of dihydrogen.

- Sodium borohydride generally undergoes decomposition in acidic and aqueous media but not in basic media.

- This compound is a reducing agent and can reduce a wide spectrum of organic carbonyls.

- $NaBH_4$ also releases hydrogen when exposed to many metal catalysts

II-2.3. Methylene blue
Methylene blue is a colorful organic chloride salt compound that is used as a dye in medicine and by biologists to help them see under a microscope. It is also known as methylthioninium chloride or Swiss blue. It is represented by the formula C16H18CIN3S. Its molecular weight is 319.85 g/mol.

Structure
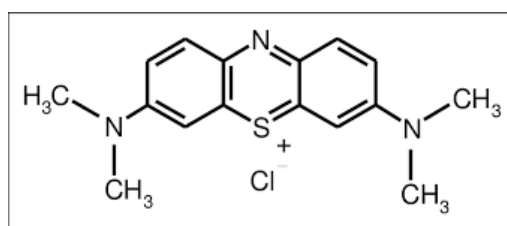
Figure-II; 4; Structure of Methylene Blue $C_{16}H_{18}CIN_3S$.

PROPERTIES OF METHYLENE BLUE

- Methylene blue is deep blue in color
- Methylene blue exhibits antioxidant properties
- It is highly soluble in water, chloroform, ethanol, and glacial acetic acid
- It is insoluble in ethyl ether, xylene, and oleic acid
- It is also having the property of being antimalarial
- It gets decomposed while heating and emits toxic fumes of nitrogen oxides, Sulphur oxides and chlorides.
- It is basic in nature.
- It acts as the enzyme inhibitor

Applications of Methylene Blue.

- Methylene blue has been used successfully to treat septic shock. It works by inhibiting the nitric oxide-cyclic guanosine monophosphate pathway, which is involved in septic shock.
- Animal studies have revealed that resistance to methylene blue is quite low. As a result, it is used as an antimalarial medication.

- Methylene blue is used in non-medical fields as a biological stain, redox indicator, peroxide generator, and so on.
- Methylene blue is used in sulphide analysis because the intensity of the colour corresponds to the amount of sulphur in the sample.
- Methylene blue is used as a disinfectant due to its antibacterial and antiparasitic effects on bacteria and other parasites. This is most likely due to its interactions with cytoplasmic cell structures and involvement in redox processes.

- Methylene blue has also been shown to be useful in aquaculture for the prevention of bacterial and fungal infections on freshwater fish eggs.

II-2.4. Silver nitrate (AgNO₃)

Silver nitrate is an $AgNO_3$ chemical compound. The silver cation $(Ag+)$ and the nitrate anion (NO_3^-) form an ionic bond. Because of this compound's ionic nature, it readily dissolves in water and dissociates into its constituent ions.

Silver nitrate is a precursor to a variety of silver compounds, including those used in photography. When exposed to light, $AgNO_3$ is quite stable when compared to silver halides, which are used in photography due to their sensitivity to light

An illustration describing the structure of the silver nitrate molecule is provided below. It can be observed that silver has an oxidation number of +1 in this compound.
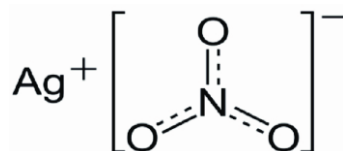
Figure-II; 5; structure of silver nitrate

The nitrate ion described above is made up of one nitrogen atom surrounded by three oxygen atoms. This ion's nitrogen-oxygen bonds are similar to one another. The nitrogen atom has a formal charge of -1, while each oxygen atom has a charge of -2/3. The nitrate ion has a net charge of -1, which is quenched by the +1-charge held by the Ag+ ion via an ionic bond in $AgNO_3$. It is worth noting that the nitrate ion's structure is stabilized by resonance.

PROPERTIES OF SILVER NITRATE

Physical properties

- The molar mass of silver nitrate is 169.872 grams per mole.

- $AgNO_3$ has a colorless appearance in its solid state and is odorless.

- In its solid state, it has a density of 4.35 grams per cubic centimeter. It's density in the liquid state at a temperature of 210°C corresponds to 3.97 g/cm^3.

- The melting and boiling points of silver nitrate are 482.8K and 713K respectively. However, this compound tends to decompose at temperatures approaching its boiling point.

- Silver nitrate, like most ionic compounds, dissolves readily in water. Its solubility in water corresponds to 22 g/100mL at 0°C and 256 g/100mL at a temperature of 25°C.

- The crystal structure of $AgNO_3$ is orthorhombic.

Chemical properties

- The hazards of $AgNO_3$ include its toxic and corrosive nature.

- The reaction between silver nitrate and ethanol is explosive.

- When heated to 440°C, this compound completely decomposes to give oxygen, nitrogen dioxide and silver.

- Silver nitrate on decomposition gives silver, oxygen gas and nitrite. The chemical equation is;

$$2AgNO_3(l) \longrightarrow 2Ag(s) + O_2(g) + 2NO(g).$$

II-2.5. Bi-distilled water and ultrapure water
Bi-distilled water: is the water that results from two distillations. The water has a very low concentration of inorganic salts, organic matter, microorganisms, soluble gases, and volatile impurities.

Ultrapure water: In ultrapure water, not only is the conductive medium almost completely removed, but the non-dissociated colloidal substances, gases, and organic matter in the water are kept to a very low level.

Figure-II; 6; setup of the bi-distilled and ultrapure water

II.2.6. Uv-visible spectrophotometers
The operation of an ultraviolet-visible spectrophotometer is based on the idea that chemical compounds absorb ultraviolet or visible light, creating unique spectra in the process. The Uv-vis spectroscopy analysis was conducted in the chemistry laboratory of Ain Temouchent University.
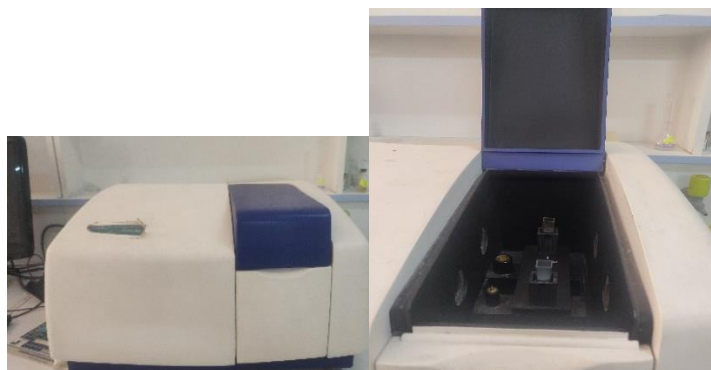
Figure-II; 7; structure of uv visible spectrophotometer

II-2.7. Support.

A support of fish's beak was used in the synthesis of silver nanocomposite. The support was provided by the chemistry laboratory of Ain Temouchent University.

Figure-II; 8; sample of the support used in synthesizing silver nanocomposite.

II-3. PREPARATION OF THE LEAF EXTRACT

In a round-bottomed flask, 1g of plant leaves were mixed with 100g of distilled water. The round-bottomed flask was then placed on a hot-bath plate at 70°C for 20-30 minutes, with continuous stirring during that time. After allowing the mixture to cool, it was filtered.
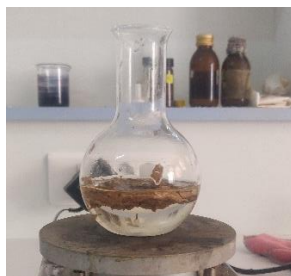
Figure-II; 9; solution containing plant leaves before heating at 70°C for about 25-30minutes

II-4. SYNTHESIS OF SILVER NANO PARTICLES

In different test tubes, different amounts of leaf extract (0.1-0.9) ml were reacted with (9.9-9.1) ml of silver nitrate (AgNO3) of 10^{-3} M. The mixtures in respective test tubes were observed for color changes with time variation, which took approximately 3 days.

Table-II; 1; showing different test tubes with varying volumes of plant leaf extract and silver nitrate

Experiments	1	2	3	4	5	6	7	8	9
Vol. of leaf extract (ml)	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
Vol. of AgNO$_3$ (ml)	9.9	9.8	9.7	9.6	9.5	9.4	9.3	9.2	9.1

- Characterization by uv-visible spectrophotometer.

To obtain the SPR band for the silver nano particles, 3ml of the brown solution of 9.5ml of silver nitrate (AgNO3) and 0.5ml of plant leaf extract was placed in a cuvette and inserted into a uv-visible spectrophotometer.

II-5. Synthesis of a nano composite AgNPs/Support

1g of the support was crushed, 0.5g of it was put inside 95ml of ultra-distilled water and then let for 5min while stirring continuously. Then 0.030g of silver nitrate (AgNO$_3$) of 10^{-3}M was added to the mixture and let for 10minutes with continuous stirring still. After the time had finished, 5ml of leaf extract were added to the mixture and there was a continuous stirring for 3days.

After three days, when the solution had completely turned brown, it was filtered and dried in an oven at 35.5°C for about 24 hours. The dried sample was then removed, and the color of the support and the sample obtained was compared.

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Figure-II; 10; a setup of solution containing support, ultrapure water, silver nitrate and plant leaf extract that has been left for 3days with continuous stirring

II-6. Reduction of methylene blue using the silver nano-composite
To convert methylene blue from blue to colorless, we used the following protocol: 0.0185g of NaBH4 in 25ml of distilled water, 10^{-3} M Methylene blue in 100ml of distilled water, and a UV-visible spectrophotometer.

2ml of methylene(10^{-4}M) blue was added to the cuvette, followed by 1ml of $NaBH_4$(0.1M), and the solution was left for a few seconds to confirm that NaBH4 has no effect on methylene blue. The absorbance peak was obtained by adding 0.005g of the nanocomposite sample to the mixture in the cuvette, which was then placed in the uv-visible spectrophotometer.

After obtaining absorbance peak, the cuvette was removed and tapped three times with a finger before being replaced for different times. Different absorbance peaks with varying intensities were obtained until the mixture became colorless, indicating that the silver nano-composite had reduced the methylene blue.

II-7. Parameters affecting the reduction of methylene blue using the nano-composite
II-7.1. Effect of the mass of the nano-composite sample
To determine the effect of nano-composite mass on methylene blue reduction, different masses of the nano-composite sample were used while timing the time it took for the methylene blue to turn from blue to colorless.

We also used 0.0185g of NaBH4 in 25ml of distilled water, 10^{-3}M Methylene blue in 100ml of distilled water, a UV-visible spectrophotometer, and different masses of the nano-composite sample (3, 5, 7) mg.

2ml of methylene blue was added to the cuvette, followed by 1ml of NaBH4, and the absorbance peak was obtained by adding 0.003g of the nanocomposite sample to the mixture in the cuvette, which was then placed in the uv-visible spectrophotometer.

After obtaining an absorbance peak, the cuvette was removed and tapped on three times with a finger before being replaced for different times. Absorbance peak of MB decreased until the mixture became colorless, indicating that the silver nano-composite had reduced methylene blue(blue) to leucomethylene blue(colorless)

This procedure was repeated for the nano-composite sample masses of 0.005g and 0.007g. For each case, we measured and recorded the time it took for the methylene blue to change color from blue to colorless at masses of 0.003g, 0.005g, and 0.007g of nano-composite sample.

Table-II; 2; showing experiments carried out on reduction of methylene blue using different masses of the Ag-nanocomposites

Experiments	1	2	3
Concentration of methylene blue (M)	10^{-4}	10^{-4}	10^{-4}
Concentration of $NaBH_4$ (M)	0.1	0.1	0.1

Mass of the silver nanocomposite (g)	0.003	0.005	0.007

II-7.2. Effect of the concentration of NaBH$_4$.

We used different concentrations of 0.1M, 0.15M and 0.2M of NaBH4 to determine the effect of NaBH4 concentration on the reduction of methylene blue from blue to colorless and kept the other factors constant because their variation with time was also noted.

10^{-3}M Methylene blue in 100ml of distilled water, 0.005g of the nano-composite sample, different concentrations of 0.1M, 0.15M and 0.2M NaBH4, and a uv-visible spectrophotometer were used.

2ml of methylene blue was added to the cuvette, followed by 1ml of 0.1M NaBH4, and the absorbance peak was obtained by adding 0.005g of the nanocomposite sample to the mixture in the cuvette, which was then placed in the uv-visible spectrophotometer.

After obtaining an absorbance peak, the cuvette was removed and tapped on three times with a finger before being replaced for different times. Absorbance peak of MB decreased until the mixture became colorless, indicating that the silver nano-composite had reduced methylene blue(blue) to leucomethylene blue(colorless)

This procedure was repeated with NaBH4 concentrations of 0.15M and 0.2M. For each case, we measured and recorded the time it took for the methylene blue to change from blue to colorless at NaBH4 concentrations of 0.1M, 0.15M, and 0.2M

Table-II; 3; showing experiments carried out on reduction of methylene blue using different concentrations of NaBH$_4$

Experiments	1	2	3
Concentration of methylene blue (M)	10^{-4}	10^{-4}	10^{-4}
Concentration of NaBH$_4$ (M)	0.1	0.15	0.2
Mass of the silver nanocomposite (g)	0.005	0.005	0.005

II-7.3. Effect of the concentration of methylene blue

To determine the effect of concentration of methylene blue on reduction of methylene blue from blue to colorless suing silver nano-composite, different concentrations (2, 5, 8) $*10^{-5}$ M of methylene blue were used and their variation with time were noted as the factors were kept constant.

0.0185g of NaBH$_4$ in 25ml of distilled water, $2*10^{-5}$M Methylene blue, a UV-visible spectrophotometer and 0.005g of the nano-composite sample were used.

In the cuvette, 2ml of $2*10^{-5}$ M methylene blue was added, followed by 1ml of 0.1M of $NaBH_4$ and then, 0.005g of the nanocomposite sample was added to the mixture in the cuvette; which was after put in the uv-visible spectrophotometer so as to obtain the absorbance peak.

Then after obtaining an absorbance peak, the cuvette was removed and tapped on using a finger for about three times and then put back for different times. Absorbance peak of MB decreased until the mixture became colorless, indicating that the silver nano-composite had reduced methylene blue(blue) to leucomethylene blue(colorless)

This procedure was repeated with a different concentration of $(5, 8) *10^{-5}$ M of methylene blue. For each case we determined and noted the time it took the methylene blue to turn from blue to colorless with respective to the concentrations $(5, 8) * 10^{-5}$ M of methylene blue.

Table-II; 4; showing experiments carried out on reduction of methylene blue using different concentrations of methylene blue

Experiments	1	2	3
Concentration of methylene blue (M)	$2*10^{-5}$	$5*10^{-5}$	$8*10^{-5}$
Concentration of $NaBH_4$ (M)	0.1	0.1	0.1
Mass of the silver nanocomposite (g)	0.005	0.005	0.005

STRUCTURE

II-8. CONCLUSION

The biosynthesis of silver nanoparticles using a plant extract is an intriguing process because it is both environmentally friendly and non-toxic. The phytochemicals in the plant extract served as both reducing and stabilizing agents. The formed nano particles were combined with the support to form silver nano composites, which were used to reduce methylene blue from blue to colourless.

CHAPTER 3

RESULTS AND DISCUSSION

III. CHAPITER 3
RESULTS AND DISCUSSION

III-1. Preparation of the leaf extract
The solution containing plant leaves and distilled water turned to pale yellow solution after 30 minutes at a temperature of 70°C. On filtration, a pale-yellow filtrate of the plant leaf extract was obtained.
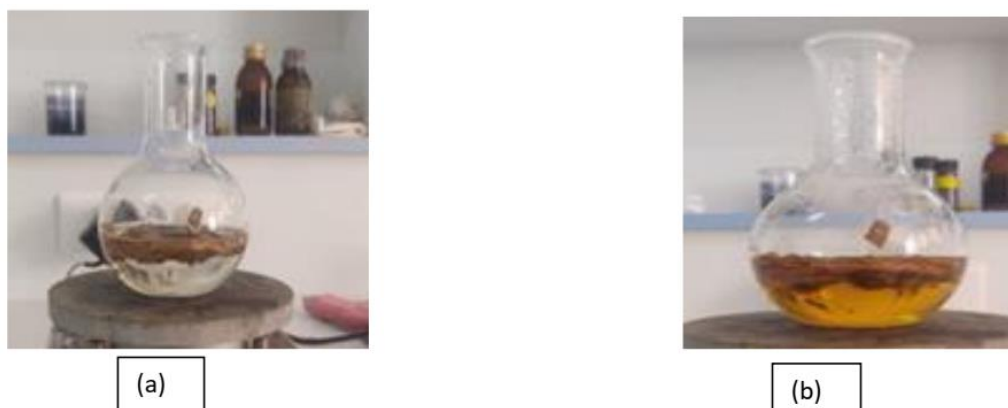

Figure-III; 1; solution containing plant leaves before heating at 70°C for about 25-30minutes fig(1a) and after the heating under same conditions fig(1b)

During the extraction process, the biomolecules (phytochemicals) present in the plant leaves are extracted out in aqueous form; which makes them perform their role as reducing and stabilizing agents. The presence of biomolecules can be confirmed by the FTIR by detecting the functional groups of different biomolecules present in the plant leaf extract. Though for our case, we didn't prove it but several reports have confirmed it.

III-2. Synthesis of silver nanoparticles
On reacting silver nitrate solution in different proportions (9.9-9.1) ml of 10^{-3}M with the plant leaf extract (0.9-0.1) ml in different test tubes at room temperature. There was a colour change to dark brown indicating the formation of AgNPs. The strongest dark brown colour was formed in the test tube containing 9.5ml of silver nitrate solution and 0.5ml of plant leaf extract.
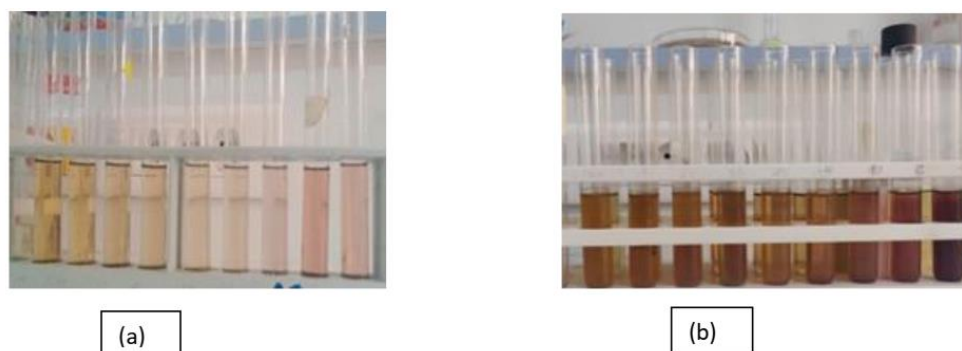
Figure-III; 2; shows solution of plant leaf extract and silver nitrate at 1minute fig 2(a) and 3days after fig 2(b)

When AgNO$_3$ solution was reacted with the plant extract, the biomolecules present in the extract reduced the Ag$^+$ ions to Ag^0, thus forming silver nanoparticles. The formation of silver nanoparticles was confirmed with the colour change to dark brown as shown in the figure 2 above.

$$Ag^+ + e^- \longrightarrow Ag^0$$

III-2.1. Characterisation

The solution containing 0.5ml of plant extract and 9.5ml of AgNO$_3$ was characterised by the uv spectrophotometer to confirm the initial presence of silver nanoparticles. The uv visible spectrum represents the result of Surface Plasmon Resonance (SPR). The SPR of AgNPs is influenced by the size, shape interparticle interactions, free electron density and surrounding medium, which indicates that is an efficient tool for monitoring the electron ejection and aggregation of NPS. It is the strong interaction of metal nanoparticles with light resulting in the collective oscillation of conduction electrons on the metal surface. (1)

The formation of AgNPs is confirmed from the change in colour from yellow to dark brown. The concentration of AgNPs exhibited a maximum absorption peak at 420nm.

According to Mie's theory, only a single SPR peak is expected for spherical metal nps whereas an isotopic particle can give rise to two or more peaks depending upon their morphology (2)

In the present study, a single peak is observed for AgNPs thus suggesting that our silver nanoparticles are spherical in shape.

Also, according to Godel et al. a decrease in absorbance is due to aggregation among nanoparticles over a period of time. (2)

III-3. Synthesis of silver nanocomposite (AgNPs/support)

The solution mixture of support (0.5g), ultra distilled water (95ml), AgNO$_3$ of 10^{-3}M (0.030g) and then 5ml of leaf extract turned brown after 3 days under continuous stirring. The solution was filtered and then the residue formed was dried in an oven at 35.5°C for about 24hours. On drying the sample, there was a colour change, a dark brown colour of the sample was obtained. The colour of the sample was compared to that of the support; that is, the sample was dark brown and the support was of brown colour. The colour change between the sample and support indicated formation of Ag nano-composite.
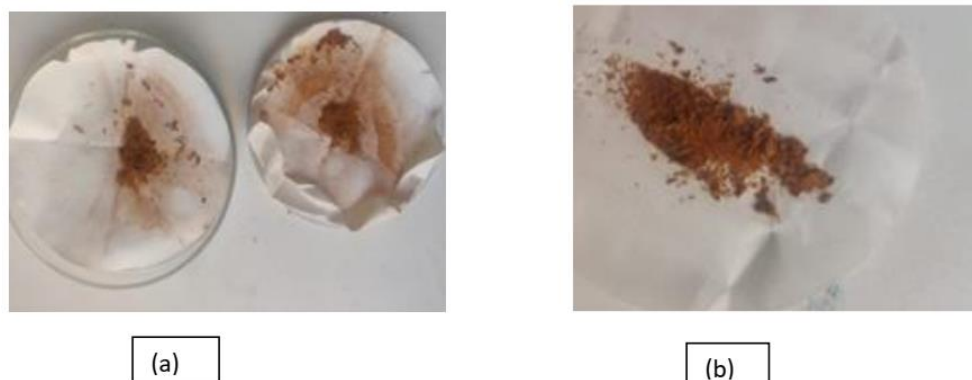
Figure-III; 3; shows samples of dried Ag-nanocomposite fig 3(a) and that of support fig 3(b)

III-4. Reduction of MB using the silver nanocomposite

A solution of MB (2ml) 10^{-4}M and NaBH$_4$ (1ml)0.1M was put into a cuvette, and the mixture was left for about 10minutes to see if the NaBH$_4$ had an effect on MB. There was no observable change in the colour of MB when observed by our naked eyes as shown in figure 5. 0.005g of silver nanocomposite was added to the mixture of MB and NaBH$_4$. Then the uv visible spectrophotometer was used to determine the absorbance intensity, as time for the decolorizing of methylene blue (MB) to leucomethylene blue (LCMB) was noted. An initial absorbance maximum was observed at 663, then the cuvette was removed and tapped on sides using a finger so as to remove the hydrogen gas bubbles formed, the same procedure was repeated until the absorbance maxima of MB was not seen anymore. MB almost or completely decolorised under 5minutes, thus indicating the catalytic activity of AgNPs.

Figure-III; 4; shows a solution containing Ag-nanocomposite, methylene blue and sodium borohydride before fig 4(a) and after fig 4(b) reduction of methylene blue(blue) to leucomethylene blue (colourless)

MB is a basic aniline dye also known as methylthionine chloride, which has many uses in diverse fields including biological, chemistry as a stain and as medicine. (3) MB is a water-soluble tricycle phenothiazine drug/ dye (4). MB has a blue colour in oxidized state and loses its colour upon reduction forming colourless leucomethylene blue. (3)

$NaBH_4$ is a weaking reducing agent used in many industrial processes as well as in waste water treatment that generates hydrogen gas via a hydrolysis reaction in aqueous solution. (5) After the addition of $NaBH_4$ to MB, no reaction took place up to 10minutes
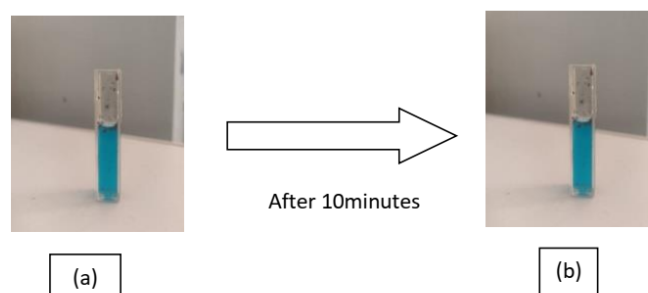

Figure-III; 5; a solution of methylene blue and only sodium borohydride observed for colour change using naked eyes before fig 5(a) and after fig 5(b) after 10minutes

Figure 5 shows that reduction of MB with $NaBH_4$ in absence of silver nanocomposite at room temperature occurs at extremely slow rate without any differences in colour by observing using naked eyes.

This result proves that even after a long time of about 80minutes between MB and $NaBH_4$, there is no reduction of MB that can be seen (6). Therefore, $NaBH_4$ is a weak reducing agent that requires a catalyst for its efficient action. (2)

Time-dependent readings were monitored using a uv vis spectrophotometer. MB usually has its maximum absorbance at 663nm due to π-π* and n-π*transitions. (3)
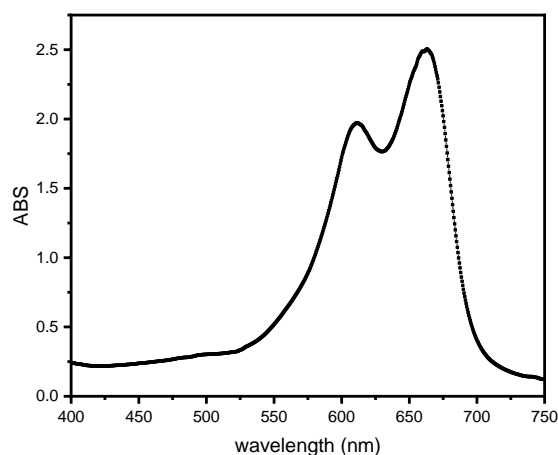
Figure-III; 6; variation of absorbance of methylene blue solution with wavelength.

After recording the uv spectra of MB, NaBH$_4$ and Ag-nanocomposite were added to the MB solution and the readings were monitored by the uv visible spectrum at regular time intervals.

After certain time intervals, the peak's absorbance tends to decrease as soon as the characteristic colour of MB solution faded, there was no more evidence of the presence of MB's distinctive peak. This took 5-6 minutes for MB to be reduced by NaBH$_4$ and Ag-nanocomposite.

During the time intervals, the cuvette was removed and tapped on using a finger, so as to prevent the bubbles released during the action of the reduction of MB from affecting our results as they tend to increase the time of reduction.

In terms of mechanism, since both catalyst and reducing agent are present in solution, that resulted into a successful decolourisation, this can be potentially explained by the Lagmuir-Hinshe (wood model). According to the model, the reducing agent (BH$_4^-$) and the dye (MB) can be adsorbed at the surface of the catalyst through affinity, physical sorption or hydrogen bonding.

Then, the electron from reducing agent (BH$_4^-$) is transferred to the dye (MB) which reduces it to its colourless LMB form (7). The catalyst Ag-nanocomposite consists in the process as the transfer medium.
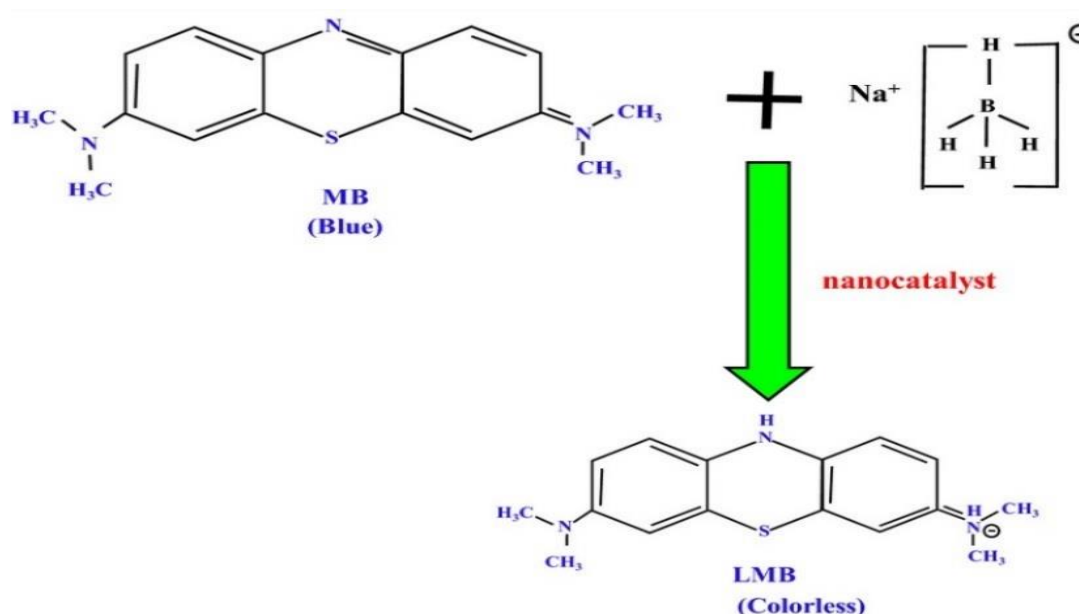
Figure-III; 7; reaction of methylene blue and sodium borohydride in presence of a Nano catalyst to form leucomethylene blue which is colourless in nature

BH_4^- ions are nucleophilic while MB are electrophilic in nature with respect to (8) Ag-nanocomposite, where the nano catalyst accepts electrons from BH_4^- ions and convey them to MB.

Here Ag-nanocomposite acted as an electron relay point that behaved alternatively as acceptor and donor of electrons.

Therefore, the Ag-nanocomposite speed up the reduction of the dyes by facilitating the electron exchange between $NaBH_4$ and MB dye as they proved as an electron-rich surface for interaction between reactants. The reduction is confirmed by colour change from blue to colourless.

The results of the study were compared to several researches that used AgNPs for MB decolourisation. Table (1) shows the comparison in terms of the used particle size, the initial MB concentration, reducing agent for decolourisation, exposure time and percent of decolourisation. (7)

Table-III; 1; comparison of result of this work with studies using AgNP for MB decolourisation (7) in respect of time for reduction

Nanoparticle	Size (nm)	Prepared MB concentration (ppm)	Reducing agent (decolorization)	Time (min)	Decolorization (%)	Reference
Bark extract-AgNP	17.5-66.5	0.32	NaBH4	30	~100	Sreekanth et al. 2016
polyvinyl alcohol-AgNP	10-13	*	NaBH4	35	< 100	Sagitha et al.
Kernel shell-AgNP	< 20	10	NaBH4	13	~100	Khodadadi et
Graphene oxide-AgNP	10-49.5	0.32	NaBH4	15	~100	Sreekanth et
Seed extract AgNP	22-32	320	NaBH4	< 19	~100	Vidhu §Philip 2014

The results in the table show that the Ag-nanocomposite is a better catalyst compared to some the reported methods in terms of time of reduction of methylene blue(blue) to leucomethylene blue(colourless).

To act as an effective catalyst, the redox potential of Ag-nanocomposite needs to be found between the redox potential of donor ($NaBH_4$) and acceptor (MB). These results prove that Ag-nanocomposites can act as an effective catalyst in the reduction of MB by $NaBH_4$ into phytochemically inactive LCMB. In absence of a catalyst, there is no change in the colour of MB after 10minutes with naked eyes, indication that reduction does not occur without catalyst.

The solution of MB and only Ag-nanocomposite also showed very minimal decolouration. In literature, MB can be adsorbed by the Ag-nanocomposite due to its high surface area to volume ratio thus attraction of MB dye on the surface of the Ag-nanocomposite.

This result suggest that both the reducing agent and catalyst are necessary to achieve high rate of decolourisation which is achieved potentially through AgNPs acting as electron relay centre for the NaBH$_4$ and the MB dye.

One can also notice that studies that incorporated NaBH$_4$ as a reducing agent have relatively achieved decolourisation at a quicker period.
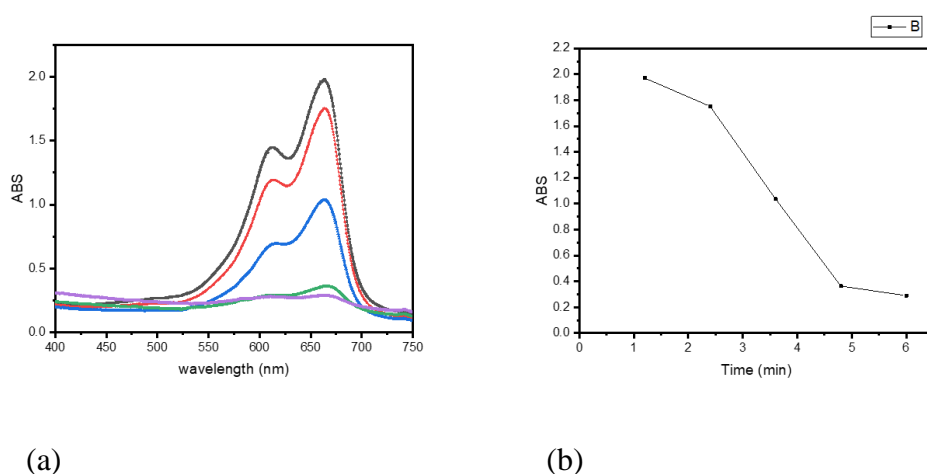

(a)   (b)

Figure-III; 8; time dependent uv vis spectroscopy analysis fig 8a and variation of maximum absorbance with time for each peak fig 8b.

III-5. MECHANISM OF REDUCTION OF MB

The bond dissociation energy (BDE) plays an important role during chemical reaction in breaking and formation of new bond. Electron transfer takes place during the reaction between MB dye and NaBH$_4$ in which NaBH$_4$ acted as a donor and the dye as acceptor. The addition of Ag nano catalyst in reaction mixture performed as a potential intermediate between MB dye and BH$_4^-$ ions. At first, it lowered the BDE and make the electron transfer between them more efficient. Thus, the rate of reduction of MB by NaBH$_4$ was increased in the presence Ag nanocomposite. (3)

Clearly, the Ag nanocomposite showed promising results in catalytic reduction of methylene blue(blue) to leucomethylene blue(colourless).
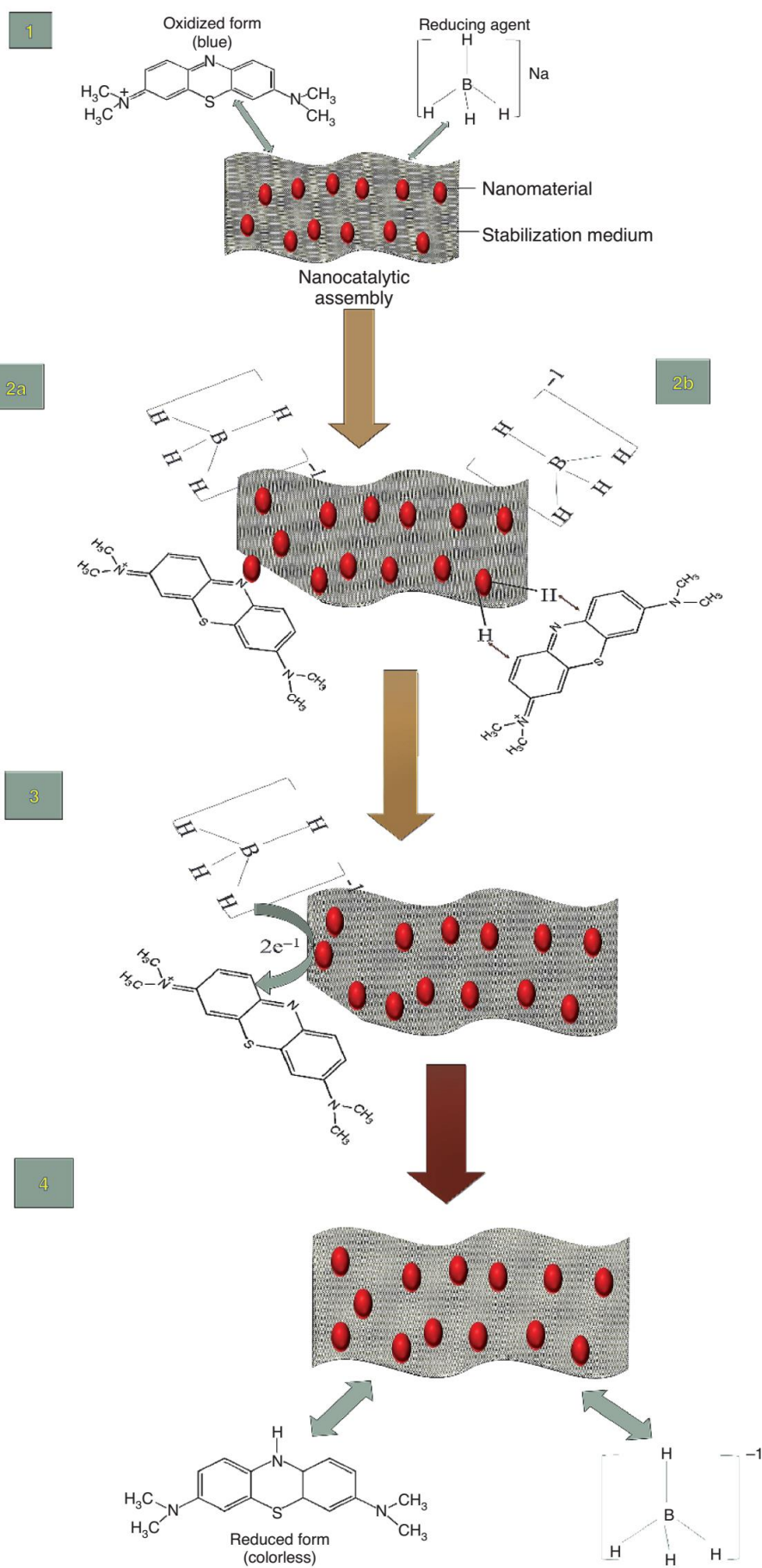
1

Oxidized form
(blue)

Reducing agent

H_3C
H_3C —N$^+$ ... N—CH$_3$
CH$_3$

B
H   H   H   Na

Nanomaterial

Stabilization medium

Nanocatalytic
assembly

2a

2b

II

3

$2e^{-1}$

4

Reduced form
(colorless)

Figure-III; 9: Langmuir–Hinshelwood model for chemical reduction of MB. (10)

(1) Reaction medium containing both dye and reductant molecules;

(2) adsorption of reductant and dye molecules over the surface of nanocatalysts via (a) direct absorption and (b) hydrogen bonding-assisted absorption of dye molecules;

 (3) transfer of electron from reductant to the dye molecule and

 (4) formed product and reductant become desorbed from the surface of the nanocatalytic assembly and are released into the reaction medium (10)

III-6. Parameters affecting reduction of MB.
III-6.1. Effect of the mass of the Ag-nanocomposite

The effect mass of the silver nanocomposite on reduction of MB was investigated. The amount of the Ag-nanocomposite is the main parameter for the reduction studies. Here, we used different masses of the Ag-nanocomposite. At mass of Ag-nanocomposite 3mg, the reduction occurred in a duration of 16.5minutes; 5mg, the reduction of MB occurred in a duration of 6mnutes; 7mg, the reduction of MB occurred in a duration of 13.9minutes.

The increase in mass of the Ag-nanocomposite while concentrations of $NaBH_4$ and MB were kept constant, lead to an increase in the concentration of the catalyst, thus an increase in the reduction efficiency of MB was observed. The catalytic reduction reduces slowly as the catalyst dosage increased as shown when the mass changed from 5mg to 7mg. this is due to increase in the number of active sites on the catalyst by increasing its amount thus increasing the rate of reduction of MB to LCMB by $NaBH_4$ (BH_4- ions present).

Table-III; 2; showing experiments carried out on reduction of methylene blue using different masses of the Ag-nanocomposites

| Experiments	1	2	3
Concentration of methylene blue (M)	10^{-4}	10^{-4}	10^{-4}
Concentration of $NaBH_4$ (M)	0.1	0.1	0.1
Mass of the silver nanocomposite (g)	0.003	0.005	0.007

Time (min)	16.5	6	13.9

When the catalyst amount was more than 5mg, the catalytic reduced in its reduction efficiency. The advantage of higher concentration is not limited, the disadvantage of using higher amount of catalyst was observed in 7mg at time taken for reduction increased past that of 5mg that is, 7mg took longer time of 13.9minutes compared to 5mg that took 6minutes for reduction of MB. Therefore, the catalyst amount of 5mg was taken for investigating the other parameters of the reaction.

This is consistent with other reports of catalytic reduction of dyes. The Au/CeO_2-TiO_2 catalyst, 423mg amount is taken for MB catalytic degradation studies. The catalytic $Au@polypyrorol/Fe_3O_4$ hollow capsule, 2mg was used in reduction MB (1).
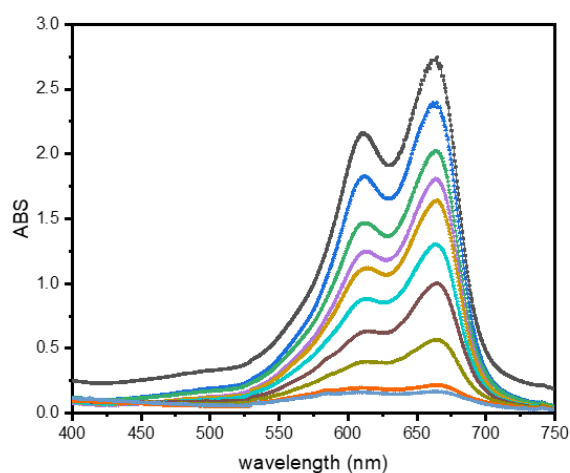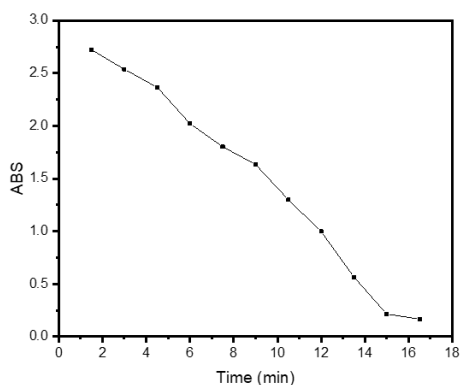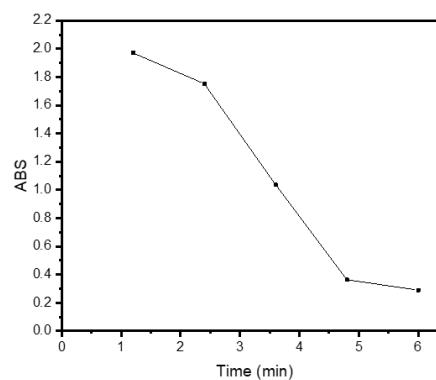

(a)
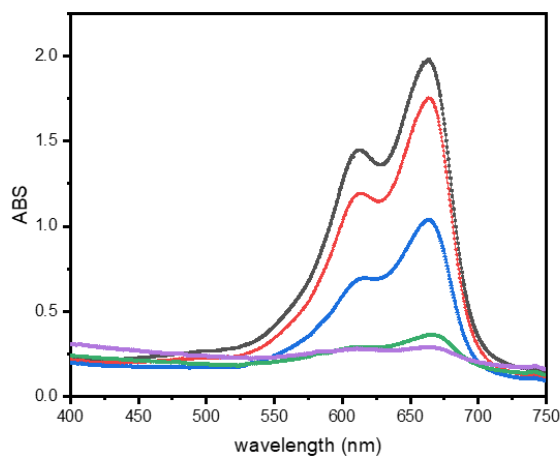
(b)
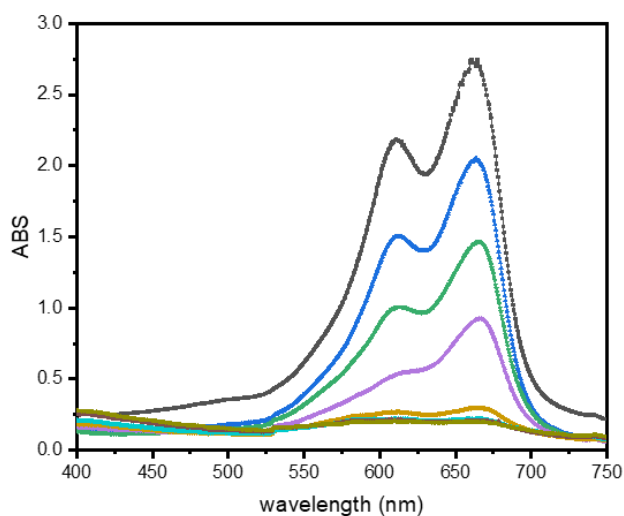
(c)

Figure-III; 10; plots showing variation of absorbance with wavelength, indicating reduction of methylene blue in presence of different masses of Ag-nanocomposites; 3mg fig(a), 5mg fig(b) and 7mg fig (c)
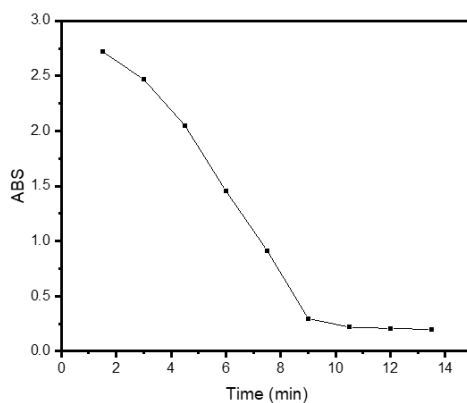
The plot relative absorption intensity with wavelength in a regular time intervals (1.5, 1.2, and 1.5) minutes reveal that complete reduction of methylene blue to leucomethylene blue was accomplished in times of (16.5, 6, 13.9) with respect to different masses (3, 5, 7) mg respectively**.**

(a)

(b)

(c)

Figure-III; 11**;** shows variation of maximum absorbance (663nm) with time thus indicating reduction of methylene blue in presence of NaBH$_4$ and with different masses of Ag-nanoparticles; that is 3mg fig(a), 5mg fig(b) and7mg fig(c)

On addition of different masses of Ag-nanocomposite in presence of NaBH$_4$ as a reducing agent followed by a time-dependent readings monitored using uv visible spectrometer, the intensity of absorbance at 663nm by methylene blue decreases with time, thus indicating reduction of methylene blue(blue) to leucomethylene blue(colourless).
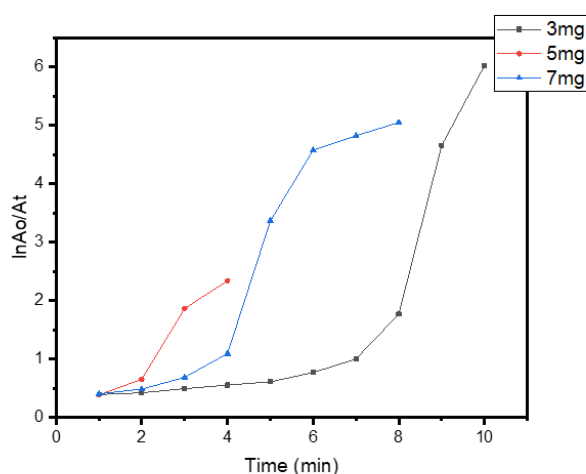
; plot of ln Ao/At versus reduction time in minutes.

Figure 9 indicates that reduction follows a pseudo-first order reaction kinetics with respect to MB as different masses of the Ag-nanocomposite were used; that is 3mg, 5mg and 7mg.

From fig 9, the results showed that the degradation of MB satisfactory followed a pseudo-first order kinetics. The plot of ln Ao/At versus was fairly linear fig 9, supporting the first-order nature of the kinetics (9).

Therefore, for degradation of MB, the Ag nanocomposite act as effective catalyst with regard of different masses of the catalyst.

III-6.2. Effect of concentration of $NaBH_4$.
The effect of change in concentration of $NaBH_4$(0.1, 0.15, 0.2) M on the reduction of MB was examined by using Ag nanocomposite catalyst, keeping the rest of the conditions identical (10^{-4}M MB and 5mg of Ag-nanocomposite). For reduction of MB under different concentrations of $NaBH_4$ that is; for 0.1M took only 6minutes for reduction; 0.15M took 7minutes; 0.2M took only 5minutes.

Table-III; 3; showing experiments carried out on reduction of methylene blue using different concentrations of $NaBH_4$

| Experiments	1	2	3

Concentration of methylene blue (M)	10^{-4}	10^{-4}	10^{-4}
Concentration of NaBH$_4$ (M)	0.1	0.15	0.2
Mass of the silver nanocomposite (g)	0.005	0.005	0.005
Time (min)	6	7.9	5.2

These experiments evidently reveal that NaBH$_4$ concentration variation (0.1, 0.15, 0.2) M, virtually changes the absorbance decrease rate. In other words, NaBH$_4$ 0.1M concentration is the optimum for reduction reaction.

When the concentration of NaBH$_4$ was more than 0.15M, the catalytic efficiency kept unchanged. The advantage of higher concentration is limited and there are significant disadvantages of using higher concentration of NaBH$_4$ beyond its optimal level would not actually promote the efficiency of the process.
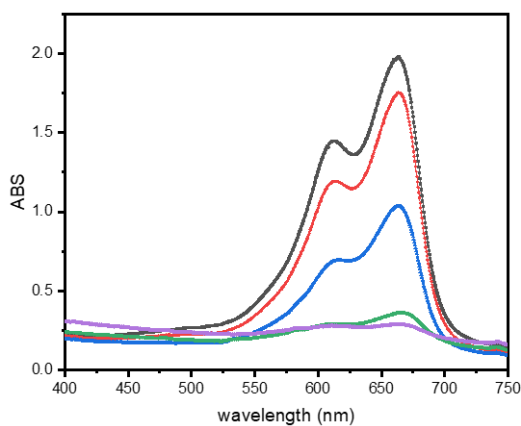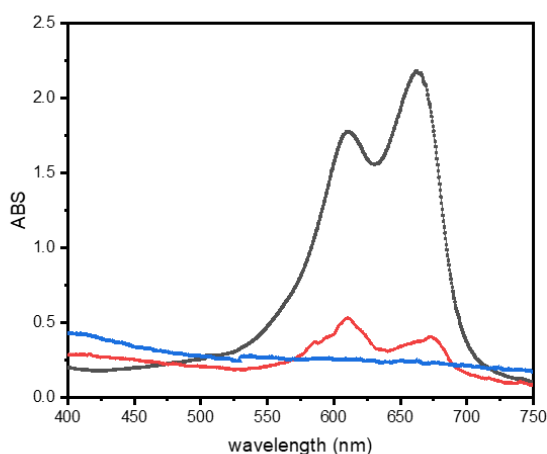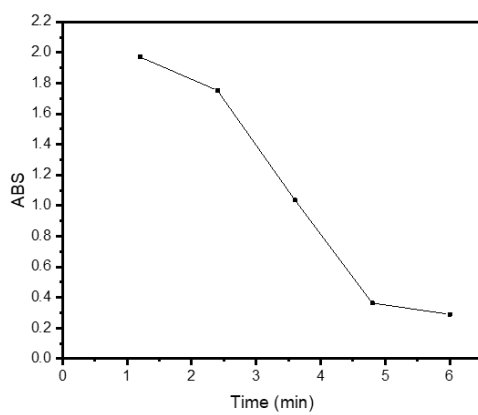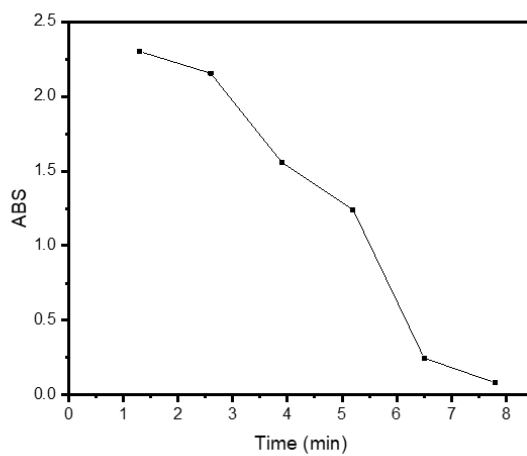

(a)
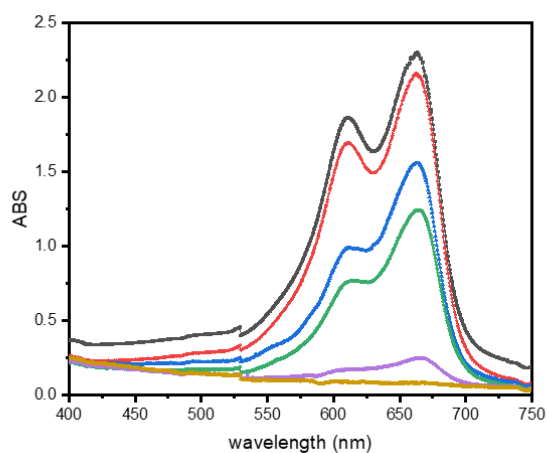
(b)

Figure-III; 13; plots showing variation of absorbance with wavelength, indicating reduction of methylene blue in presence of different concentrations of NaBH$_4$; 0.1M fig(a), 0.15M fig(b) and 0.2M fig (c)
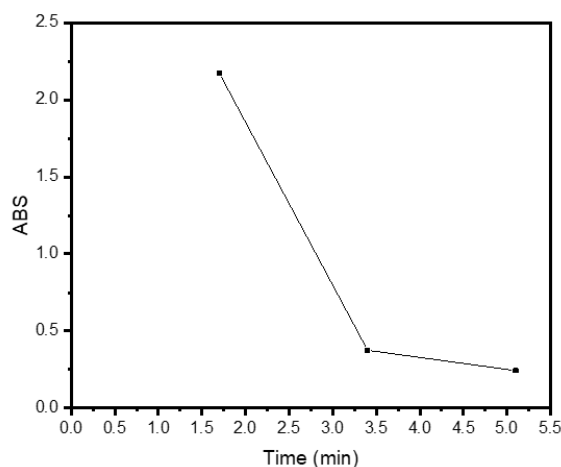
The plot relative absorption intensity with wavelength in a regular time intervals (1.2, 1.3 And 1.7) minutes reveal that complete reduction of methylene blue to leucomethylene blue was accomplished in times of (6, 7.9, 5.2) with respect to different concentrations of NaBH$_4$ (0.1, 0.15, 0.2) M respectively

(a)

(b)

(c)

Figure-III; 14;shows variation of maximum absorbance (663nm) with time thus indicating reduction of methylene blue in presence of Ag-nanocomposite with different concentrations of NaBH$_4$; that is 0.1M fig(a), 0.15M fig(b) and 0.2M fig(c)

On addition of different concentrations of NaBH$_4$ in presence of Ag-nanocomposite as a catalyst followed by a time-dependent readings monitored using uv visible spectrometer, the intensity of absorbance at 663nm by methylene blue decreases with time, thus indicating reduction of methylene blue(blue) to leucomethylene blue(colourless).

Therefore; when working with high concentration of NaBH4; it is mean that it is possible to make pollution because it is toxical in certain concentration, that's why we work with 0.1M and not 0.2M.

III-6.3. Effect of the concentration of MB
The effect of concentration of MB is investigated by varying the dye concentration and keeping other parameters constant. By increasing the concentration of MB, the time for the maximum absorbance value of MB to decrease, increases as shown in the table below. In other words, the higher concentration of MB somehow deactivates the catalytic sites, and catalytic efficiency kept constant. Here, the advantage of high concentration is limited and there are significant disadvantages of using higher concentration of dye.

Table-III; 4; showing experiments carried out on reduction of methylene blue using different concentrations of methylene blue

71

| Experiments	1	2	3
Concentration of methylene blue (M)	$2*10^{-5}$	$5*10^{-5}$	$8*10^{-5}$
Concentration of $NaBH_4$ (M)	0.1	0.1	0.1
Mass of the silver nanocomposite (g)	0.005	0.005	0.005
Time (min)	6.9	16.5	21.5

The reduction of MB suffered a setback as the concentration of MB was increased to ($5*10^{-5}$, $8*10^{-5}$) M, reduction was achieved after 21.5minutes, this shows that surface of the Ag-nanocomposite has been saturated with the dye molecules and the reducing agent may not have good contact with the surface.
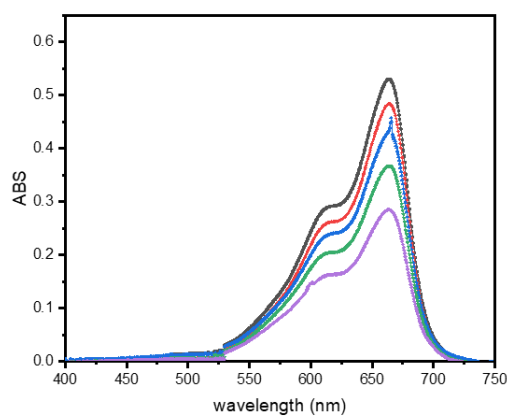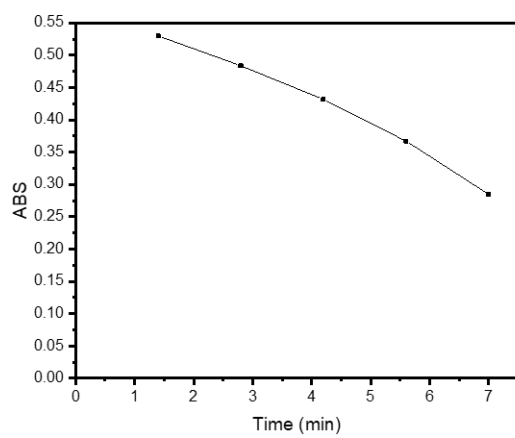

(a)
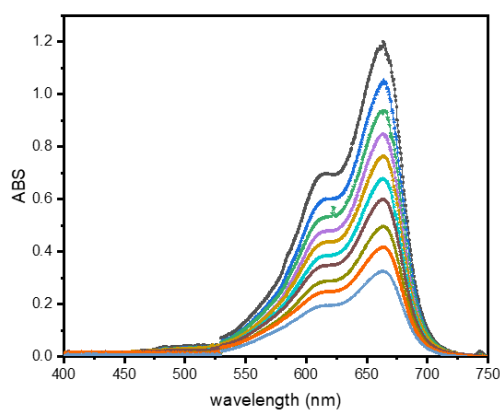
(b)
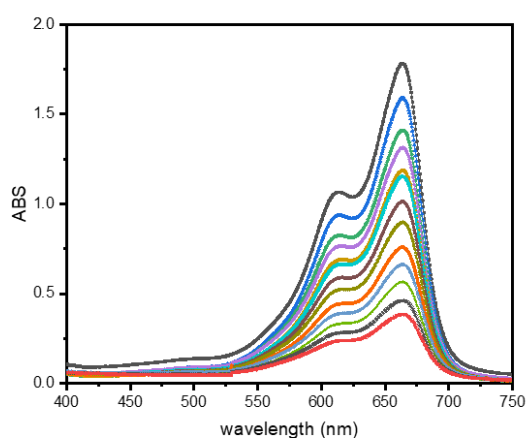
(c)

Figure-III; 15; plots showing variation of absorbance with wavelength, indicating reduction of methylene blue in presence of different concentrations of methylene blue ;$2*10^{-5}$ M fig(a), $5*10^{-5}$ M fig(b) and $8*10^{-5}$ M fig (c)

The plot relative absorption intensity with wavelength in a regular time intervals (1.4, 1.5 And 1.5) minutes reveal that complete reduction of methylene blue to leucomethylene blue was accomplished in times of (6.9, 16.5, 21.5) with respect to different concentrations of methylene blue (2, 5, 8) $*10^{-5}$ M respectively
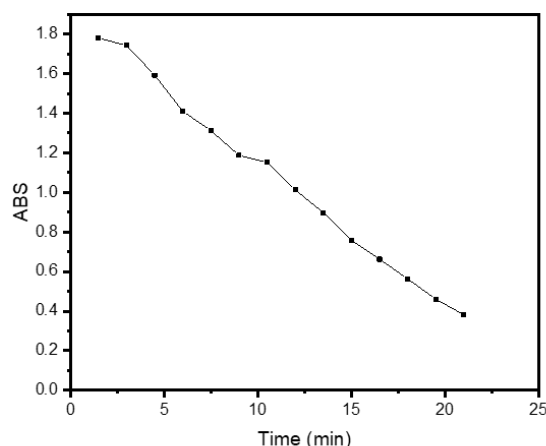
(a)
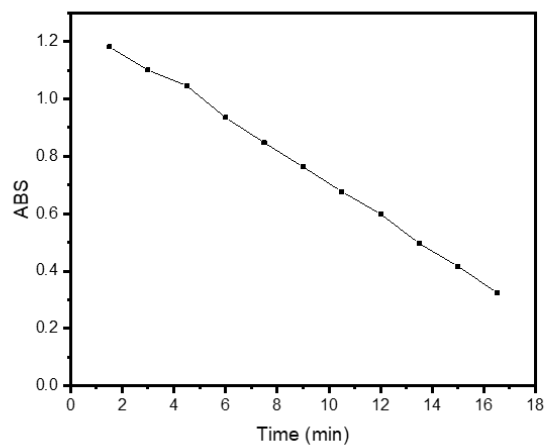
(b)

(c)

Figure-III; 16; shows variation of maximum absorbance (663nm) with time thus indicating reduction of methylene blue in presence of Ag-nanocomposite and NaBH$_4$ with different concentrations of methylene blue; that is $2*10^{-5}$ fig(a), $5*10^{-5}$ M fig(b) and $8*10^{-5}$ M fig(c)

On using different concentrations of methylene blue in presence of Ag-nanocomposite as a catalyst and NaBH$_4$ as a reducing agent followed by a time-dependent readings monitored using uv visible spectrophotometer, the intensity of absorbance at 663nm by methylene blue decreases with time, thus indicating reduction of methylene blue(blue) to leucomethylene blue(colourless).

Therefore; increase in concentration of methylene blue lead to increase in degradation time because at high concentration of methylene blue lead to increase in the number of molecules of methylene blue and that's why the degradation decreases.

III-7. Conclusion

The method I used for biosynthesis of AgNPs and its composite is inexpensive, eco-friendly and non-toxic. The support used the synthesis of the nanocomposite is believed to have enhanced properties of AgNPs and it also prevented agglomeration of the nanoparticles. The Ag-nanocomposite was able to show great catalytic activity of reduction(degradation) of methylene blue(blue) to leucomethylene blue. The parameters; mass of the Ag-nanocomposite, concentration of sodium borohydride and concentration of methylene blue, showed that's important to put them into consideration during the reduction of methylene

blue(blue) to leucomethylene blue(colourless) as they varied in the time dependent uv visible spectroscopy analysis of reduction of MB.

III-8. REFERENCES

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General Conclusion

In conclusion, the synthesis nanocomposite utilized as a degradation agent for methylene blue, with NaBH4 as the reduction agent, has demonstrated remarkable effectiveness in degrading methylene blue. The study involved testing the nanocomposite with varying masses, concentrations of methylene blue, and concentrations of NaBH4, yielding consistently impressive results.

The findings of this research provide substantial evidence that the nanocomposite exhibits powerful degradation capabilities for methylene blue. By utilizing NaBH4 as the reduction agent, the nanocomposite successfully initiated the degradation process, leading to the efficient breakdown of methylene blue molecules.

The experimentation involved systematic variations in mass, concentration, and reactant concentration, allowing for a comprehensive evaluation of the nanocomposite's performance. Throughout the tests, the nanocomposite consistently demonstrated excellent degradation efficiency, further establishing its effectiveness as a degradation agent.

These results have significant implications for potential applications in wastewater treatment, environmental remediation, and various other areas where the removal of methylene blue is crucial. The nanocomposite's remarkable degradation power can potentially lead to more efficient and sustainable solutions for treating methylene blue-contaminated water sources.

However, further research and experimentation are necessary to optimize the synthesis process, fine-tune the nanocomposite's composition, and investigate its long-term stability and recyclability. Additionally, exploring the nanocomposite's performance against other dye molecules and evaluating its potential toxicity or side effects are important areas for future investigation.

Overall, the synthesis nanocomposite demonstrated outstanding potential as a degradation agent for methylene blue, highlighting its capacity to contribute to the development of effective and environmentally friendly solutions for dye removal in various applications.