GREEN SYNTHESIS OF IRON OXIDE NANOPARTICLES USING OCIMUM SANCTUM LINN EXTRACT

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Submitted by

SIVAPRASAD P (23520101014)

A P MEGHANA (23520101017)

ANJALI NAIR (23520101018)

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BISHOP MOORE COLLEGE , MAVELIKARA

CERTIFICATE

Certified that the dissertation entitled that **"Green Synthesis Of Iron Oxide Nanoparticles Using Ocimum Sanctum Linn Extract"** is a bonafide record of the project work accomplished by A P Meghana ,in partial fulfillment of the requirement for the Bachelor Degree in Chemistry of Kerala University is an authentic work carried out under my supervision and guidance during the period of 2022-2023

Dr. Priya Mary Abraham

Assistant Professor

Department of Chemistry

Bishop Moore College

Counter signed by ;

Dr. Siji K Mary

Head of the Department of Chemistry

Bishop Moore College

DECLARATION

I hereby declare that this project entitled **"Green Synthesis Of Iron Oxide Nanoparticles Using Ocimum Sanctum Linn Extract"** submitted to Kerala University in partial fulfillment of Bachelor's Degree in Chemistry is a bonafide record of the work carried out under the guidance of Dr.Priya Mary Abraham , Assistant Professor , Department of Chemistry , Bishop Moore College during the year 2022-2023 and no part of it has been submitted for any other degree or diploma.

SIVAPRASAD P (23520101014)

A P MEGHANA (23520101017)

ANJALI NAIR (23520101018)

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ABSTRACT

Recently, iron oxide nanoparticles have attracted much consideration due to their unique properties, such as superparamagnetism, surface-to-volume ratio, greater surface area, and easy separation methodology. Various physical, chemical, and biological methods have been adopted to synthesize magnetic nanoparticles with suitable surface chemistry. Iron oxide (Fe_2O_3) Nanoparticles were synthesized from Ferric Chloride ($FeCl_3$) and Sodium hydroxide (NaOH). In this study we synthesized with Ocimum sanctum Linn (Thulsi leaves) extract. Its properties were studied using characterization techniques like X-ray Diffraction analysis(XRD) and Scanning electron microscopy (SEM).

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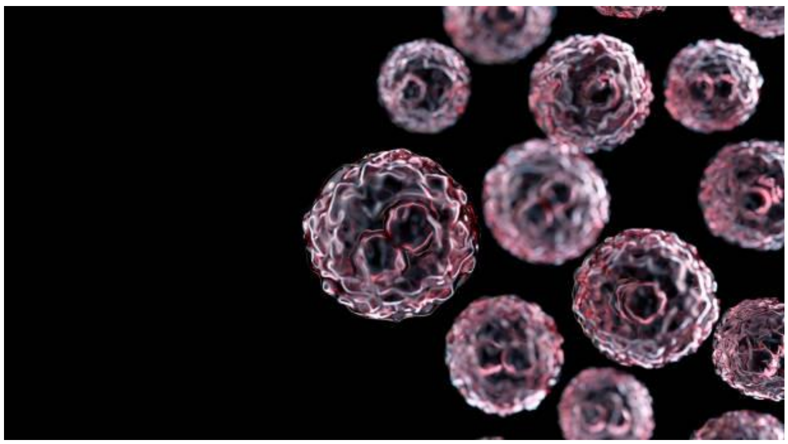
CHAPTER 1
INTRODUCTION

A nanoparticle is a small particle that ranges between 1 to 100 nanometers in size. Undetectable by the human eye, nanoparticles can exhibit significantly different physical and chemical properties to their larger material counterparts.



1.1NANOTECHNOLOGY

Nanotechnology refers to the branch of science and engineering devoted to designing, producing, and using structures, devices, and systems by manipulating atoms and molecules at nanoscale. Having one or more dimensions of the order of 100 nanometers (100 millionth of a millimeter) or less. In the natural world, there are many examples of structures with one or more nanometer dimensions, and many technologies have incidentally involved such nanostructures for many years, but only recently has it been possible to do it intentionally. Many of the applications of nanotechnology involve new materials that have very different properties and new effects compared to the same materials made at larger sizes. This is due to the very high surface to volume ratio of nanoparticles compared to larger particles, and to effects that appear at that small scale but are not observed at larger scales. The applications of nanotechnology can be very beneficial and have the potential to make a significant impact on society. Nanotechnology has already been embraced by industrial sectors, such as the information and communications sectors, but is also used in food technology, energy technology, as well as in some medical products and medicines. Nanomaterials may also offer new opportunities for the reduction of environmental pollution. But these new materials may also present new health risks

1.2 CLASSIFICATION OF NANOPARTICLES

1.2.1 ORGANIC NANOPARTICLES

Organic Nanoparticles are materials of two or more dimensions, with a size in the range of 1–100 nm. Nanoparticles show unique size dependent physical and chemical properties, for example, optical, magnetic, catalytic, thermodynamic and electrochemical. The chemical composition and the shape of a nanoparticle also influence its specific properties. The main groups of organic Nano carriers are liposomes, micelles, protein/peptide based and dendrimers. Dendrimers are highly branched synthetic polymers (<15 nm) with layered architectures constituted of a central core, an internal region and numerous terminal groups that determine dendrimer characteristics.

1.2.2 METAL BASED NANOPARTICLES

The metal-based nanoparticles such as silver, gold, copper, iron, zinc, platinum, and so on, received much attention in medicine. Faraday (1857) showed the metal nanoparticles can exist in solution. Much later, Kumar et al. (2018) studied the color and morphology of metallic nanoparticles. Currently, nanoparticles can be synthesized and improved by modifying the chemical groups, which help to bind the antibodies. Noble metal nanoparticles (Ag, Au, Pt) have been used for several biomedical applications. The noble metal nanoparticles have some unique properties that make it more valuable.

1.2.3 CARBON BASED NANOPARTICLES

Carbon nanomaterials are an extensive family of carbon allotropes, consisting of 0-dimensional fullerenes and quantum dots, 1-dimensional carbon nanotubes (CNTs), 2-dimensional graphene, and 3- dimensional nanodiamonds and nanohorns.

1.2.4 CERAMIC NANOPARTICLES

Ceramic nanoparticles are primarily made up of oxides, carbides, phosphates and carbonates of metals and metalloids such as calcium, titanium, silicon, etc. They have a wide range of applications due to a number of favorable properties, such as high heat resistance and chemical inertness.

1.2.5 SEMICONDUCTOR NANOPARTICLES

Semiconductor nanoparticles are known to possess a wealth of quantum phenomena and show unique size-dependent material properties. These materials possess properties between metals and nonmetals and therefore they have found various applications in the literature. With a change in the particle size, dramatic modifications to their electronic and optical properties take place due to the three-dimensional (3D) quantum confinement of electrons and holes when the size of the particle approaches the Bohr radius of an exciton.

1.2.7 POLYMERIC NANOPARTICLES

Polymeric nanoparticles are particles within the size range from 1 to 1000 nm and can be loaded with active compounds entrapped within or surface-adsorbed onto the polymeric core. Polymeric nanoparticles have shown great potential for targeted delivery of drugs for the treatment of several diseases.

1.3 DIFFERENT METHODS FOR THE SYNTHESIS OF NANOPARTICLES

The nanomaterials can be synthesized using two prominent approaches. They are top-down and bottom-up approaches. In top- down approaches, the bulk materials are mechanically machined and converted into fine particles in nano dimensions. In bottom-up approaches, the fine particles are assembled to build the nanomaterials through self-assembly or co-precipitation methods.

1.3.1 BALL MILLING THROUGH THE MECHANICAL METHOD

Ball milling is a mechanical method to fabricate nanomaterials. In this process, the materials are ground in a closed container. Small pebbles made of glass, ceramics, and stainless steel creates shear force during grinding. Bulk materials are subjected to place in the closed container. By grinding process, the bulk materials are converted to fine-tuned nanomaterials. Using this method, we can fabricate metallic hydrides and nitrides. These nitrides possess essential properties that provides a variety of applications.

1.3.2 PHYSICAL VAPOUR DEPOSITION (PVD) METHOD

Physical vapor deposition (PVD) is a process applied to the synthesis of ultra-thin films and surface coatings. It is used to produce metal vapor that can be deposited on the conductive layer as ultra-thin films and alloy coatings. The whole process is carried in a vacuum held in a vacuum chamber about 10−6 torr from a cathodic-arc source. In a clean atmosphere, vacuum deposition is held in the chamber and the metals are deposited as wider or sputtered in the localized area.. Using this method, we can synthesis nano-particles and allow to fabricate nano composites. The PVD includes evaporation and a sputtering process to fabricate thin films.

1.3.3 GREEN SYNTHESIS OF NANOPARTICLES

The three foremost conditions for the synthesis of nanoparticles are the selection of green or environment-friendly solvent, a good reducing agent, and a harmless material for stabilization. For the synthesis of nanoparticles, extensive synthetic routes have been applied in which physical, chemical, and biosynthetic routes are very common. Generally, the chemical methods used are too expensive and incorporate the uses of hazardous and toxic chemicals answerable for various risks to the environment. The biosynthetic route is a safe, biocompatible, environment-friendly green approach to synthesize nanoparticles using plants and microorganisms for biomedical applications.This synthesis can be carried out with fungi, algae, bacteria, and plants, etc. Some parts of plants such as leaves, fruits, roots, stem, seeds have been used for the synthesis of various nanoparticles due to the presence of phytochemicals in its extract which acts like stabilization and reducing agent.

In the biosynthesis of nanoparticles environmentally accepted "green chemistry" concept has been applied for the development of clean and environment-friendly nanoparticles which involves bacteria, fungi, plants, actinomycetes, etc., which is said to be "green synthesis". Biosynthesis of nanoparticles by using the above organisms epitomizes a green substitute for the invention of nanoparticles with innovative properties. In these syntheses, unicellular and multicellular organisms are allowed to react.Plants are known as chemical factories of nature which are cost-efficient and need little maintenance.Plants have revealed outstanding potential in heavy metal detoxifcation as well as accumulation by which environmental pollutants problem can be overcome because very small traces of these heavy metals are also toxic even at very low concentrations (Shahid et al. 2017). There are advantages for nanoparticle synthesis with plant extract as compared to some other biological synthesis such as by microorganisms as they can be done by complex actions of preserving microbial cultures. One advantage of plant-assisted nanoparticle synthesis is the kinetics for this route is ample higher than in other biosynthetic approaches equivalent to chemical nanoparticle preparation. Various parts of plants such as fruit, leaf, stem, root have been widely used for green synthesis of nanoparticles due to the excellent phytochemicals they produce. For nanoparticle synthesis, the part of the plant which has to be used in synthesis can be washed and boiled with distilled water. After squeezing, fltering, and adding respective solutions which nanoparticles we want to synthesize, solution color starts changing unveiled the formation of nanoparticles and we can separate these. Synthesis via natural plant extract is an environment-friendly and cheap process by which we can avoid any utilization of intermediate base groups.Literature suggested accumulation, detoxifcation, and phytoremediation of toxic metals by some plants, such as Thlaspi caerulescens, Maytenus founieri, Arabidopsis helleri, Sesbania drummondii, Acanthopanax sciadophylloides, Clethra barbinervis, and Brassica juncea. The use of these plants in heavy metal elimination from aqueous solutions has gained considerable attention due to its great potential for the removal of pollutants and toxicity from wastes in an eco-friendly method.Many nanoparticles such as gold, silver, zinc oxide, iron have been synthesized very easily by adopting a green approach . The phytocompounds present in the plant extract such as polyols, terpenoids, polyphenols are responsible for metallic ions bioreduction.

1.3.4. SOL-GEL METHOD

Sol-gel is a widely used method to prepare nanoparticles. The condensation and hydrolysis reactions are involved in the preparation of nanoparticles. Heat treatment is applied in the intermediate synthesis and is required to ensure the crystallinity of the nanoparticles. The alkoxides serve as a precursor to prepare oxide nanoparticles that interact through molecular forces (e.g., van der Waals forces or H-bonding) and are dispersed in a sol through evaporation or condensation. The precursor of alkoxide is hydrolyzed in the presence of a base or acid, resulting in a polymeric gel. The final product is determined by the rate of condensation and hydrolysis. For example, the smaller size nanoparticles made, the lower the hydrolysis rate. It is an apt process to synthesize composites, oxides, and ceramic nanoparticles with high purity homogeneous distribution. It has an advantage over the conventional oxide fusion method. For example, the xerogel nanocomposites made of iron or silica are produced by the direct mixture of iron III nitrate, using TEOS in a sol-gel formulation. The ferric iron is reduced to metallic iron in the presence of hydrogen gas under a temperature around 400–700 °C. The application of xerogel involves pressing a nanocomposite into a pellet on glass slides for electrical or magnetic conductivity. The advantage of the sol-gel method has high purity and achieves a uniform nanostructure at low temperature in the presence of ligand as a capping agent. The key downside of this method has the high level of impurities from reaction by-products and necessitating post treatment.

1.3.5. HYDROTHERMAL METHOD

Hydrothermal synthesis is one of the most commonly used methods for preparation of nanomaterials. It is basically a solution reaction-based approach. In hydrothermal synthesis, the formation of nanomaterials can happen in a wide temperature range from room temperature to very high temperatures. To control the morphology of the materials to be prepared, either low-pressure or high-pressure conditions can be used depending on the vapor pressure of the main composition in the reaction.

1.3.6. CHEMICAL CO-PRECIPITATION METHOD

The chemical co-precipitation method involves mixing of two different salts that end up with precipitation in an aqueous solvent, especially base. The parameters such as morphology, size, and composition are altered by changing the pH, temperature, ligands, precursor salt, and chemical ratios. The nanoparticles are stabilized by adding appropriate surfactant (e.g., oleic acid), ligand (e.g.,aptamer), polymer (e.g., poly(ethylene glycol acrylate)), and inorganic molecules (e.g., NaOH). Complex metals can synthesize by using this feasible method. For example, the synthesis of iron-chromite obtains by the mixture of iron (III) salt and chromate. In aqueous, the salts from metal ions are formed and then precipitated using ammonium base. The precipitate is decomposed at a high temperature to yield iron (III) chromite.

1.4 APPLICATIONS OF NANOTECHNOLOGY

Nanotechnology and nanomaterials can be applied in all kinds of industrial sectors. They are usually found in these areas:

- Electronics

Carbon nanotubes are close to replacing silicon as a material for making smaller, faster and more efficient microchips and devices, as well as lighter, more conductive and stronger quantum nanowires. Graphene's properties make it an ideal candidate for the development of flexible touchscreens.

- Energy

Nanotechnology lowers costs, produces stronger and lighter wind turbines, improves fuel efficiency and, thanks to the thermal insulation of some nanocomponents, can save energy.

- Biomedicine

The properties of some nanomaterials make them ideal for improving early diagnosis and treatment of neurodegenerative diseases or cancer. Some nanoparticles have also been used to enhance pharmaceutical products such as sunscreen.

- Environment

Air purification with ions, wastewater purification with nanobubbles or nanofiltration systems for heavy metals are some of its environmentally-friendly applications. Nanocatalysts are also available to make chemical reactions more efficient and less pollution.

- Food

Nanobiosensors could be used to detect the presence of pathogens in food. Nanocomposites to improve food production by increasing mechanical and thermal resistance and decreasing oxygen transfer in packaged products.

- Textile

Nanotechnology makes it possible to develop smart fabrics that are stain free and wrinkle free. Also it is possible for the production of stronger, lighter and more durable materials to make motorcycle helmets or sports equipment.

1.5 PHYSICAL AND CHEMICAL PROPERTIES OF NANOPARTICLES

Nanoparticles often have unique physical and chemical properties, For example, the electronic, optical, and chemical properties of nanoparticles may be very different from those of each component in the bulk. At the nanoscale, materials behave very differently compared to larger scales and it is still very difficult to predict the physical and chemical properties of particles of such a very small size. The principal parameters of nanoparticles are their shape, size, surface characteristics and inner structure.

Nanoparticles have different ways of interacting with each other. They can remain free or group together depending on the attractive or repulsive interaction forces between them. These interactions remain difficult to characterize. Nanoparticles suspended in gas tend to stick to each other more readily than in liquid.

1.6 NANOTECHNOLGY IN THE FUTURE

There are bright and dark spots in the future of nanotechnology. On the one hand, the sector is expected to grow globally, driven by technological advances, increased government support, increased private investment and growing demand for smaller devices, to name a few. However, the environmental, health and safety risks of nanotechnology and concerns related to its commercialization could hamper market expansion.

The United States, Brazil and Germany will lead the nanotechnology industry in 2024, with an important presence in the Top 15 Asian countries such as Japan, China, South Korea, India , Taiwan and Malaysia. The cosmetics sector will climb positions stealing third place from the biomedical sector in a ranking that will be led by electronics and energy, as it is now.

1.7 IRONOXIDE

Iron oxides are common natural compounds and can also easily be synthesized in the laboratory. There are 16 iron oxides, including oxides, hydroxides, and oxide-hydroxides. These minerals are a result of aqueous reactions under various redox and pH conditions. They have the basic composition of Fe, O, and/or OH, but differ in the valency of iron and overall crystal structure. Some of the important iron oxides are goethite, akaganeite, lepidocrocite, magnetite, and hematite.

1.8 IRON OXIDE NANOPARTICLES

Iron oxide (IO) nanoparticles consist of maghemite (γ-Fe_2O_3) and/or magnetite (Fe_3O_4) particles with diameters ranging from 1 and 100 nanometer and find applications in magnetic data storage, biosensing, drug-delivery etc. In nanoparticles, the surface area to volume ratio increases significantly. This allows a considerably higher binding capacity and excellent dispersibility of nanoparticles in solutions. Magnetic nanoparticles, with sizes between 2 and 20 nm display superparamagnetism, i.e their magnetization is zero, in the absence of an external magnetic field and they can be magnetized by an external magnetic source. This property provides additional stability for magnetic nanoparticles in solutions. Ironoxide nanoparticles have attracted considerable interest due to their superparamagnetic properties and their potential biomedical applications arising from its biocompatibility and non-toxicity. Recent developments in the preparation of ironoxide nanoparticles by thermal decomposition of iron carboxylate salts have significantly improved the quality of traditional ironoxide nanoparticles in terms of size tunability, monodispersity and crystalline structure. Using the proprietary monolayer polymer coating strategy, hydrophobic, organic ligand- coated ironoxide nanoparticles have successfully been converted into water soluble, bio-accessible ironoxide nanoparticles. The high stability of these water soluble ironoxide nanoparticles in harsh conditions of high pH and elevated temperature allow conjugation of these nanoparticles with other biomolecules. Additional biocompatible coatings for in vivo studies including polysaccharides (such as dextran) and lipid molecules have also been developed, resulting in nanoparticles consisting entirely of materials that have been approved by the United States Food and Drug Administration. Enhancement in the quality of both organic and water soluble ironoxide nanoparticles opens avenues of opportunities for development of ironoxide nanoparticles based applications, for example:

- As contrast agents for Magnetic Resonance Imaging (MRI)
- As drug carriers for target specific drug delivery
- As gene carriers for gene therapy
- As therapeutic agents for hyperthermiabased cancer treatment
- As magnetic sensing probes for in-vitro diagnostics (IVD)
- As Nanoadjuvant for vaccine and antibody production

1.9 APPLICATIONS OF IRONOXIDE NANOPARTICLES

Taking the advantage of their magnetic properties, iron oxide nanoparticles/nanopowder are widely used in biology and medicine mostly for separating biological products magnetically and delivering the particles to specific sites in drug delivery applications. Clinical applications of iron oxide nanoparticles as magnetic carries play an important role in diagnostics. In labeling materials in life science iron oxide nanoparticles appear to be so versatile and effective. Moreover, there are numerous reports on the use of iron oxide nanoparticles in fields like environmental remediation, food and agriculture, health care, energy, defense and aerospace technology, construction, textiles, automotive and electronics. There have been continuous progress and evolution in the quality, sensitivity, selectivity and versatility of iron oxides nanoparticles/nanopowder since their introduction to science and industry. The bare iron oxide nanoparticles/nanopowder might not be specific for sensitive applications. As a result, modification and coating strategies are so useful to improve the quality, sensitivity and, etc. There are ongoing and facile efforts to prepare monodispersed spherical iron oxides nanoparticles/nanopowder with particular shape, size and magnetic properties.

CHAPTER 2
AIMS AND OBJECTIVES

2.1 AIM

- Preparation of ironoxide nanoparticle with magnetic property from Ocimum Sanctum extract.
- Preparation of ocimum sanctum extract.
- Preparation of iron oxide nanoaprticle from the extract.

2.2 OBJECTIVES

The main objective of the study is the bio synthesis of fe_20_3 nanoparticles from tulsi leaves. The main scope of the study is the magnetic properties of these nanoparticles. Plant extracts are generally much more of interest in biosynthesis because their throughput in nanoparticle production is much higher than the use of microbes and plant parts are also easily available. Tulsi leaves are often used for their various medicinal properties. Nanomedicine has shown a promising potential in clinical usage against a number of human diseases (cancer, diabetes, hypotension, etc.), with the development of nanoscale materials for use in biological applications, notably cancer therapeutics. Among the numerous nanoscale materials for cancer diagnostics and treatment applications, magnetic nanoparticles have emerged the most potent, and their magnetic properties have been exploited in magnetic resonance imaging (MRI), biosensors applications. These magnetic nanoparticles in cancer treatment can be controlled to specific cancer sites for drugs to be delivered using external magnetics. Also, in the detection of cancer growth, MRI can be used to scan body parts or the materials can be designed by embedding them with receptors that are attracted to cancer cells.

Characterisation of the synthesized particles are also important to confirm the composition and size of the particles.

CHAPTER 3
LITERATURE REVIEW

Karin Andrade-Zavaleta et al., synthesized ironoxide nanoparticles by using Eucalyptus Globulus. The green synthesis of metal oxide nanoparticles is presented as an excellent sustainable alternative for achieving nanostructures, with potential applications. This research provides important information regarding the influence of the type of solvent used in extracting organic reducing agents from E. globulus on the ironoxide nanoparticles green synthesis protocol. A broad approach to characterization is presented, where UV-vis spectrophotometry suggests the presence of this type of nanoparticulate material. Likewise, the reduction mechanism was evaluated by FT-IR and the magnetic properties were evaluated by PPSM. In addition, characterizations were linked via elemental analysis (EDX), crystallographic characterization (XRD), electron microscopy (SEM/STEM), and Z potential to evaluate colloidal stability. The results show the influence of the type of solvent used for the extraction of organic reducing agents from E. globulus, and the effect on the synthesis of ironoxide nanoparticles. In addition, the nanostructure material obtained showed excellent efficiency in the remediation of agricultural soil, eliminating metals such as Cr-VI, Cd, and, to a lesser extent, Pb.

S. Kanagasubbulakshmi and K. Kadirvelu synthesized ironoxide nanoparticles by using Lagenaria Siceraria and evaluate its antimicrobial activity. Magnetic iron oxide nanoparticles with appropriate surface chemistry exhibit many interesting properties that can be exploited in a variety of biomedical applications such as magnetic resonance imaging contrast enhancement, tissue repair, hyperthermia, drug delivery and in cell separation. In this study unexplored Lagenaria siceraria leaves extract was found to be capable in green synthesis of Iron oxide nanoparticles (Fe_3O_4-nanoparticles) and their characteristics were studied by using UV-visible spectrophotometer, SEM, EDX, XRD, Zeta sizer, and FT-IR. Thus synthesised Fe_3O_4 nanoparticles were naturally stabilised, cubic shaped and in the size range of 30 mm - 100 nm. The phytochemicals present in the leaf has a main role as reducing agent that assists to the eco friendly synthesis of Fe3O4 nanoparticles with enhanced antioxidant property. Functional groups present on the nanoparticles are mainly –OH and –COOH (FT-IR) makes it hydrophilic hence nanoparticles does not need any further functional modification for applications. The antimicrobial property of synthesised Fe_3O_4-nanoparticles was evaluated against Gram negative - Escherchia coli, Gram positive- Staphylococcus aureus, The Zone of inhibition was found to be 10 mm for Escherchia coli, and 8mm for Staphylococcus aureus. Thus naturally stabilised Fe_3O_4 nanoparticles with herbal property can be used in various biological applications.

Ali Talha Khalil et al. synthesized ironoxide nanoparticles via aqueous extracts of Sageretia thea (Osbeck.). This was used as an effective chelating agent for the biosynthesis of iron oxide nanoparticles (IONP's) and extensively characterized through XRD, FTIR, Raman spectroscopy, Energy Dispersive Spectroscopy, HR-SEM/TEM and SAED. Antibacterial assays against five human pathogenic bacterial strains were carried out and minimum inhibitory concentrations were calculated. Pseudomonas aeruginosa (MIC: 7.4 μg/mL) was the most susceptible strain to biosynthesized ironoxide nanoparticles. All of the fungal strains showed susceptibility to the ironoxide nanoparticles. MTT cytotoxic assay was carried out against the promastigote and amastigote cultures of Leishmania tropica and their IC50 values were calculated as 17.2 and 16.75 μg/mL. The cytotoxic potential was further assessed using brine shrimps, and the IC50 was calculated as 16.46 μg/mL. Moderate antioxidant activities were reported. Human RBCs and macrophages were found to be biocompatible with biogenic ironoxidenanoparticles (IC50 > 200 μg/mL).

P. Karpagavinayagam , C. Vedhi synthesized ironoxide nanoparticles using avicennia mariana flower extract.There method describes a non-hazardous method for synthesizing iron oxide using a flower extract of Avicennia marina. UV–Vis absorption spectrum of iron oxide nanoparticles (FeO-NPs) display a peak in the region of 295– 301 nm. The FTIR spectrum of FeO-NPs shows bands at 3354 cm^{-1},1630 cm^{-1}, 1380 cm^{-1} and 610 cm^{-1}. The size and morphology of FeO-nanoparticles were explored using scanning electron microscopy (SEM) and atomic force microscopy (AFM), respectively. From the SEM results, the average size of FeO-nanoparticles is seen to be in the range of 30–100 nm. The grain size of FeO-nanoparticles was studied by XRD. Electrochemical studies were carried out to assess the redox behavior of FeO-nanoparticles. The prepared nanoparticles are used in industrial, dye degradation and to control the environment pollution.

Seerangaraj Vasantharaj, Selvam Sathiyavima, Palanisamy Senthilkumar,Felix LewisOscar ,Arivalagan Pugazhendhi synthesized ironoxide nanoparticles using extract of Ruellia tuberose. Utilizing plant sources as reducing agents will replace the use of toxic chemicals for nanoparticle synthesis. In the present study FeO nanoparticles were synthesized using Ruellia tuberosa (RT) leaf aqueous extract, further characterization of FeO nanoparticles was performed using UV–vis spectroscopy analysis showing visible peak at 405 nm. The Fourier transform infrared spectroscopy (FTIR) proved the presence of Fe metallic ions. The structural characteristic using Field emission scanning electron microscopy with energy dispersive x-ray spectroscopy (FESEM-EDX) and Transmission electron microscopy (TEM) analysis revealed hexagonal nanorods with agglomeration. Dynamic light scattering (DLS) calculated the average size of FeO nanopartiocles around 52.78 nm and differential scanning colorimetry (DSC) proved the stability of FeO nanoparticles till higher temperature of 165.52 °C. As an application part, the synthesized FeO nanoparticles showed potential antibacterial activity as individual and incorporating material over cotton fabrics against Gram negative and Gram positive pathogens. FeO nanoparticles showed higher antibacterial activity against Escherichia coli, Klebsiella pneumoniae and lesser antibacterial activity against Staphylococcus aureus. The photocatalytic ability of the synthesized FeO nanoparticles was demonstrated by the degrading crystal violet dye under solar irradiation upto 80%. Thus, FeO nanoparticles synthesized using Ruellia tuberosa could play a vital role in killing the bacterial pathogens and degrading dye for the bioremediation of wastewater from industrial and domestic sources.

CHAPTER 4

SYNTHESIS AND CHARACTERISATION TECHNIQUES

4.1 MATERIALS REQUIRED

- Ferric chloride ($FeCl_3$)

Here the ferric chloride pellets is purchased from Nice Chemicals. Tulsi extract is added drop wise to100 ml of aqueous solution ferric chloride, then stirred to mix well. Ferric chloride is an inorganiccompound with formula $FeCl_3$ which has numerous industrial, pharmaceutical and laboratorial uses. It is a orange to brown-black solid. It is slightly soluble in water. It is noncombustible.

- Sodium Hydroxide (NaOH)

The Sodium hydroxide pellet used is purchased from Nice Chemicals. Here the Sodium hydroxide is added drop by drop to the mixture of extract and Ferric chloride solution to maintain to pH. It also act as precipitating agent in the mixture. Caustic soda is the common name for sodium hydroxide. It is one of the strongest known alkali (water-soluble base). Caustic soda is commonly used for cleaning purposes, Detergents, paint removers, pH controller of the solution etc.

- Distilled water

Distilled water is water that has been boiled into vapor and condensed back into liquid in a separate container. Impurities in the original water that do not boil below or near the boiling point of water remain in the original container. Thus, distilled water is a type of purified water. Distilled water is a type of purified water that has gone through the process of distillation to remove impurities such as calcium, sodium, and magnesium. Here we used distilled water to wash the tulsi leaves and in centrifugation, to remove loosely bounded impurities.

- Ethanol

Ethanol is used in the centrifugation part that, added to get rid of the impurities in the colloidal solution before goes into centrifugation. Ethanol, also called ethyl alcohol, grain alcohol, or alcohol, a member of a class of organic compounds that are given the general name alcohols; its molecular formula is C_2H_5OH. Ethanol is an important industrial chemical; it is used as a solvent, in the synthesis of other organic chemicals.

- Whatman Filterpaper (1)

Whatman Grade 1 Qualitative Filter Papers are the most widely used filter paper for routine laboratory applications, qualitative analytical separations, and clarifying liquids. They are the standard grade cellulose filter papers for medium flow rates. These have applications with medium retention and flow rate. Extended range of sizes includes 10 to 500 mm diameter circles and 460 x 570 mm sheets.11 µm .The most widely used filter paper for routine applications with medium retention and flow rate. This grade covers a wide range of laboratory applications and is frequently used for clarifying liquids. Traditionally the grade is used in qualitative analytical separations for precipitates such as lead sulfate, calcium oxalate (hot), and calcium carbonate.In agriculture, it is used for soil analysis and seed testing procedures. In the food industry, Grade 1 is used for numerous routine techniques to separate solid foodstuffs from associated liquid or extracting liquid and is widely used in education for teaching simple qualitative analytical separations.

EQUIPMENTS

- Magnetic stirrer

A magnetic stirrer is a device widely used in laboratories and consists of a rotating magnet or a stationary electromagnet that creates a rotating magnetic field. This device is used to make a stir bar, immerse in a liquid, quickly spin, or stirring or mixing a solution. Here we used the stirrer for mix the extract with $FeCl_3$ and NaOH For 4 hours to get the precipitate.

- Ultracentrifugation Machine

An ultracentrifuge is a centrifuge optimized for spinning a rotor at very high speeds, capable of generating acceleration as high as 1 000 000 g (approx. 9 800 km/s2). The machine here used to centrifuge the precipitate mixed with ethanol and distilled water for 15 minutes at 4000 rpm.

- Muffle Furnace

A muffle furnace or muffle oven (sometimes retort furnace in historical usage) is a furnace in which the subject material is isolated from the fuel and all of the products of combustion, including gases and flying ash. Here used to dry the tulsi leaves at a temperature between 50-60 °C. Also used to dry the centrifuged product.

- Heating Mantle

Heating mantles are used for heating or tempering organic liquids placed in reaction kettles, round-bottomed flasks, or relevant reaction vessels required for the boiling, evaporation, distillation, or extraction process. Here we heated the dried tulsi with distilled water at a extent to 70°C for 40 minutes. After that the tulsi extract is collected.

- Thermometer

A thermometer is a device that measures temperature or a temperature gradient. A thermometer has two important elements: a temperature sensor in which some change occurs with a change in temperature; and some means of converting this change into a numerical value.

GLASS WARES

Beaker, measuring jars, round bottom flask, watch glass, glass rod, and crucibles were used for the synthesis.

4.2 SYNTHESIS OF IRON OXIDE NANOPARTICLES

4.2.1 Material Collection

Krishna Tulsi leaves were collected from kerala ,india .Tulsi leaves were Fresh collected from homes, and analytical grade chemicals were used without further purification. Of all the herbs used within Ayurveda, tulsi (Ocimum sanctum Linn) is preeminent, and scientific research is now confirming its beneficial effects. There is mounting evidence that tulsi can address physical, chemical, metabolic and psychological stress through a unique combination of pharmacological actions. Tulsi has been found to protect organs and tissues against chemical stress from industrial pollutants and heavy metals, and physical stress from prolonged physical exertion, ischemia, physical restraint and exposure to cold and excessive noise. Tulsi has also been shown to counter metabolic stress through normalization of blood glucose, blood pressure and lipid levels, and psychological stress through positive effects on memory and cognitive function and through its anxiolytic and anti-depressant properties. Tulsi's broad-spectrum antimicrobial activity, which includes activity against a range of human and animal pathogens, suggests it can be used as a hand sanitizer, mouthwash and water purifier as well as in animal rearing, wound healing, the preservation of food stuffs and herbal raw materials and traveler's health. Cultivation of tulsi plants has both spiritual and practical significance that connects the grower to the creative powers of nature, and organic cultivation offers solutions for food security, rural poverty, hunger, environmental degradation and climate change. The use of tulsi in daily rituals is a testament to Ayurvedic wisdom and provides an example of ancient knowledge offering solutions to modern problems. $FeCl_3$ and NaOH pellets were purchased from Nice Chemicals (p)Ltd. Distilled water used was distilled in Laboratory itself.

4.2.2 Aqueous Krishna Tulsi Leaf Extract Preparation

Freshly collected Krishna Tulsi leaves were washed thoroughly with distilled water to remove impurities weighed and transferred into the round bottom flask and it was mixed with water and refluxed for 40 minutes at 70 degree Celsius in a heating mantle. Then the extract was filtered using Whattman filter paper (No 1) several times and kept in sterile bottles under refrigerated conditions for further use .

4.2.3 Preparation Of Leaf Extract-Assisted Fe_2O_3 Nanoparticles

Freshly prepared Krishna Tulsi Leaf extract was added drop by drop to 350ml of an aqueous solution of $FeCl_3$ and stirred vigourously at room temperature . Then ,a few drops of NaOH were added into the homogeneous solution (Fig 1) under stirring to maintain an appropriate pH .The pale red colour of the solution changed to dark brown color after 4 hours of stirring ; then the precipitate settle down at the bottom of the beaker as colloidal particles. The colloidal solution is then centrifuged several times at a speed of 4000rpm for 15 minutes in the centrifuge machine and then washed several times with distilled water and ethanol to remove the loosely bounded impurities After the washing process is over the supernatant was collected and dried overnight in a hot air oven at 70 degree celsius .The dried dark brown samples were collected ,crushed and ground by mortar and pestle. The ground samples were calcinated in a muffle furnace at 700 degree Celsius for 3 hours .This process removes the organic and other impurities , finally a deep red colored iron oxide nanopowder is obtained .The dried samples were submitted to different characterization techniques to study physical and chemical properties.

<u>4.3 CHARACTERISATION TECHNIQUES</u>

<u>4.3.1 X-ray Diffraction</u>

X-ray diffraction analysis (XRD) is a technique used in materials science to determine the crystallographic structure of a material. XRD works by irradiating a material with incident X-rays and then measuring the intensities and scattering angles of the X-rays that leave the material .A primary use of XRD analysis is the identification of materials based on their diffraction pattern. As well as phase identification, XRD also yields information on how the actual structure deviates from the ideal one, owing to internal stresses and defects.

<u>Principle</u>

XRD finds the geometry or shape of a molecule using X-rays. XRD techniques are based on the elastic scattering of X-rays from structures that have long range order. The X-rays get diffracted by a crystal because the wavelength of X-rays is similar to the inter-atomic spacing in the crystals.

Bragg's law

XRD is based on Bragg's law. This Law was introduced by Sir W.H. Bragg and his son Sir W.L. Bragg. Bragg pointed out that X ray can takes place only at certain angle which are determined by the wave length of X-ray (λ), and the distance between the planes in the crystal (d). The equation which gives the relationship between interplannar distance in the crystal, angle of reflection, and wavelength of incident radiation is known as Bragg's law.

The components of the XRD are in general common and comprise of:

- Source of x-rays
- Sample stage
- Detector

(a) Source of x-ray ; X-ray tube is the common source of x-rays. It consists of an evacuated tube which contains a copper block anode. The cathode is a tungsten filament .When electric current is passed through the filament, electrons are generated which are move towards the anode under the highly voltage (30 – 150 kV). The accelerating electrons on striking the metal surface will knock out the electrons from the inner shells and the vacancies are created. These vacancies are filled by electrons from outer shells. In this process metal atoms emit x-rays.

(b) Sample stage ; Sample stage is also known as sample holder or a goniometer. The sample stage can be a simple needle that holds the crystal in place or fiber on which the crystal is mounted on an epoxy resin. The fibre is mounted on a brass mounting pin and then it is inserted into the goniometer head. The sample is centred in an optical arrangement such as microscope or video camera and making the adjustments along X, Y and Z directions in order to achieve the optimum view for the observer.

(c) Detector ; In earlier days photographic films were used for the recording of absorption pattern of diffracted beams. With the advances in detection technology more sensitive detectors are used in advanced instruments. Such detectors are gas filled transducers, scintillation counters and semiconductor transducers.

Instrumentation And Working Of XRD

X-ray diffractometers consist of three basic elements: an X-ray tube, a sample holder, and an X-ray detector.

X-Rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons toward a target by applying a voltage, and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X ray spectra are produced. These spectra consist of several components, the most common being $K\alpha$ and $K\beta$. $K\alpha$ consists, in part, of $K\alpha1$ and $K\alpha2$. $K\alpha1$ has a slightly shorter wavelength and twice the intensity as $K\alpha2$. The specific wavelengths are characteristic of the target material (Cu, Fe, Mo, Cr). Filtering, by foils or crystal monochrometers, is required to produce monochromatic X-rays needed for diffraction. $K\alpha1$ and $K\alpha2$ are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single-crystal diffraction, with $CuK\alpha$ radiation = 1.5418Å. These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X-rays impinging the sample satisfies the Bragg Equation, constructive interference occurs and a peak in intensity occurs. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor.

The geometry of an X-ray diffractometer is such that the sample rotates in the path of the collimated X ray beam at an angle θ while the X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of 2θ. The instrument used to maintain the angle and rotate the sample is termed a goniometer. For typical powder patterns, data is collected at 2θ from ~5° to 70°, angles that are present in X-Ray Scan.

Application

The most important application of X-ray diffraction is in the field of determination of crystal structure X-ray diffraction technique has enabled scientists to obtain deep insight into the structures of alloys and inter metallic compounds. Many metallurgical operations, which were until recently a matter of art, can now be studied by means of X-rays.

The X-ray pattern of an unknown substance may be used to identify it. Quantitative determination of impurities in crystals has also been made possible.

X-ray diffraction studies of such substances as rubber, fibres, silk, hair etc. have revealed the existence of repent, of ordered engagements as in a crystal. At present the technique of X-ray attraction studies of single crystals have developed to a stage where the structures of complex organic molecules have been established from such measurements.

The analytical applications of X-ray diffraction are numerous. The method is nondestructive and gives information on the molecular structure of the sample. Perhaps its most important use has been to measure the size of crystal planes. The patterns obtained are characteristic of the particulars compounds from which the crystal was formed. X-ray powder diffraction is most widely used for the identification of unknown crystalline materials. Determination of unknown solids is critical to studies in geology, environmental science, material science, engineering and biology.

Other applications include:

- Thin-film analysis

- Lattice parameter determination
- Purity/quality control of materials
- Determination of crystallinity of polycrystalline materials.

4.3.2 Scanning Electron Microscopy

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. In most applications, data are collected over a selected area of the surface of the sample, and a 2-dimensional image is generated that displays spatial variations in these properties. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20X to approximately 30,000X, spatial resolution of 50 to 100 nm). The SEM is also capable of performing analyses of selected point locations on the sample; this approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions (using EDS) crystalline structure, and crystal orientations (using EBSD). The design and function of the SEM is very similar to the EPMA and considerable overlap in capabilities exists between the two instruments.

Principle

Accelerated electrons in an SEM carry significant amounts of kinetic energy, and this energy is dissipated as a variety of signals produced by electron sample interactions when the incident electrons are decelerated in the solid sample. These signals include secondary electrons (that produce SEM images), backscattered electrons (BSE), diffracted backscattered electrons (EBSD that are used to determine crystal structures and orientations of minerals), photons (characteristic X-Rays) that are used for elemental analysis and continuum X-rays), visible light, and heat. Secondary electrons and backscattered electrons are commonly used for imaging samples:

secondary electrons are most valuable for showing morphology and topography on samples and backscattered electrons are most valuable for illustrating contrasts in composition in multiphase samples (i.e. for rapid phase discrimination). X-Ray generation is produced by inelastic collisions of the incident electrons with electrons in discrete ortitals (shells) of atoms in the sample. As the excited electrons return to lower energy states, they yield X-rays that are of a fixed wavelength (that is related to the difference in energy levels of electrons in different shells for a given element). Thus, characteristic X-rays are produced for each element in a mineral that is "excited" by the electron beam. SEM analysis is considered to be "non-destructive"; that is, x-rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to analyze the same materials repeatedly.

Components of SEM

The major components of the Scanning Electron Microscope include;

- Electron Source – This is where electrons are produced under thermal heat at a voltage of 1-40kV. the electrons condense into a beam that is used for the creation of an image and analysis. There are three types of electron sources that can be used i. e Tungsten filament, Lanthanum hexaboride, and Field emission gun (FEG)

- Lenses – it has several condenser lenses that focus the beam of electrons from the source through the column forming a narrow beam of electrons that form a spot called a spot size.

- Scanning Coil – they are used to deflect the beam over the specimen surface.

- Detector – It’s made up of several detectors that are able to differentiate the secondary electrons, backscattered electrons, and diffracted backscattered electrons. The functioning of the detectors highly depends on the voltage speed, the density of the specimen.
- The display device (data output devices)
- Power supply
- Vaccum system

Working of SEM

The source of the electrons and the electromagnetic lenses are from tungsten filament lamps that are placed at the top of the column and it is similar to those of the transmission electron Microscope.The electrons are emitted after thermal energy is applied to the electron source and allowed to move in a fast motion to the anode, which has a positive charge.The beam of electrons activates the emission of primary scattered (Primary) electrons at high energy levels and secondary electrons at low-energy levels from the specimen surface. The beam of electrons interacts with the specimen to produce signals that give information about the surface topography and composition of the specimen.The specimen does not need special treatment for visualization under the SEM, even air-dried samples can be examined directly. However, microbial specimens need fixation, dehydration, and drying in order to maintain the structural features of the cells and to prevent collapsing of the cells when exposed to the high vacuum of the microscope.The samples are mounted and coated with thin layer of heavy metal elements to allow spatial scattering of electric charges on the surface of the specimen allowing better image production, with high clarity.Scanning by this microscope is attained by tapering a beam of electrons back and forth over a thin section of the microscope. When the electrons reach the specimen, the surface releases a tiny staw of electrons known as secondary electrons which are then trapped by a special detector apparatus.When the secondary electrons reach and enter the detector, they strike a scintillator (a luminescence material that fluoresces when struck by a charged particle or high-energy photon). This emits flashes of light which get converted into an electric current by a photomultiplier, sending a signal to the cathode ray tube. This produces an image that looks like a television picture that can be viewed and photographed.The quantity of secondary electrons that enter the detector is highly defined by the nature of the specimen i.e raised surfaces to receive high quantities of electrons, entering the detector while depressed surfaces have fewer electrons reaching the surface and hence fewer electrons enter the detector. Therefore raised surfaces will appear brighter on the screen while depressed surfaces appear darker

Applications of SEM

- It is used in a variety of fields including Industrial uses, nanoscience studies, Biomedical studies, Microbiology
- Used for spot chemical analysis in energy-Dispersive X-ray Spectroscopy.
- Used in the analysis of cosmetic components which are very tiny in size.
- Used to study the filament structures of microorganisms.
- Used to study the topography of elements used in industries.

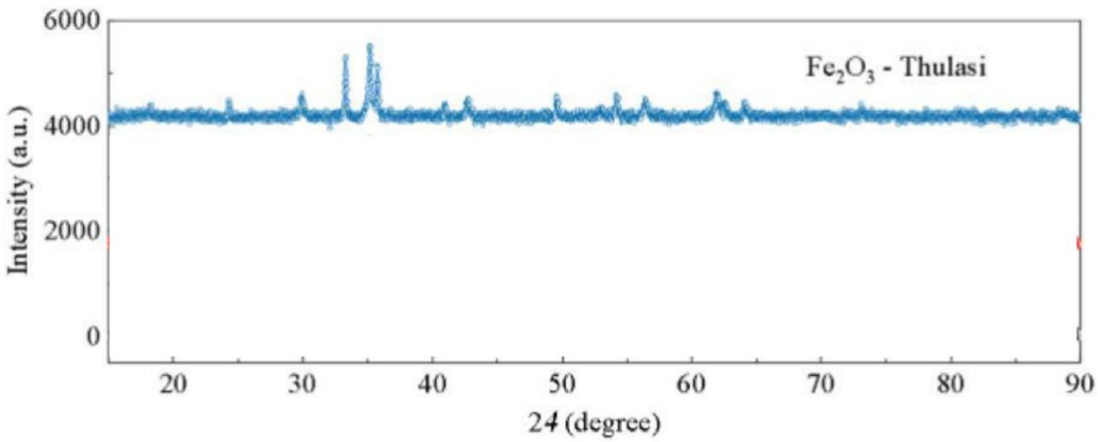
Advantages Of SEM

- They are easy to operate and have user-friendly interfaces.
- They are used in a variety of industrial applications to analyze surfaces of solid objects.
- Some modern SEMs are able to generate digital data that can be portable.
- It is easy to acquire data from the SEM, within a short period of time of about 5 minutes.

CHAPTER 5
RESULT AND DISCUSSION

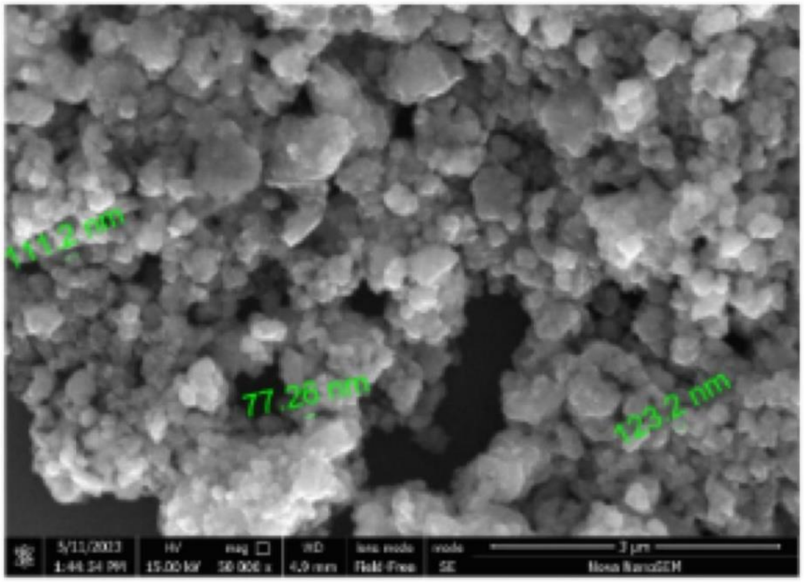
We synthesized magnetic iron oxide nanoparticles from Ocimum sanctum Linn (Thulsi) extract. Its properties were studied with different characterization methods. X-ray Diffraction (XRD) and Scanning electron microscopy (SEM) were the characterization methods used here.

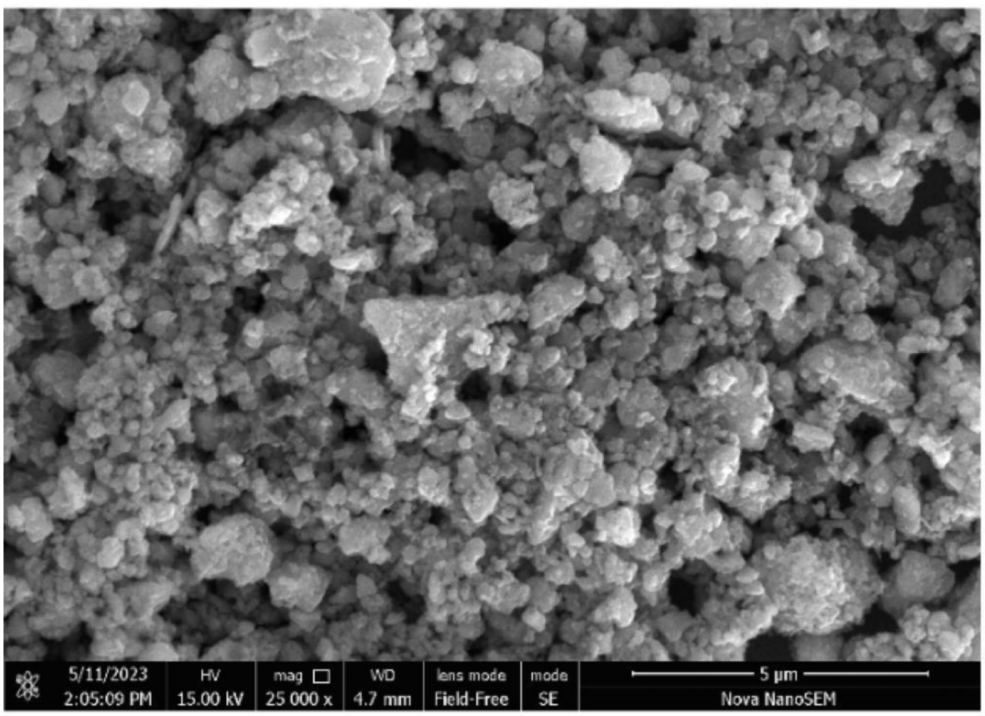
1.X-ray Diffraction (XRD)



X-ray Diffraction analysis were done to confirm the phase of fe_2O_3 nanoparticles. By refering to XRD pattern studied by `Mochamad Zainuri´ (May 2017) the peaks obtained at (113),(024),(012),(104),(110),(214),(018),(030),(116) belong to Hematite (α-Fe2O3) nanoparticles. By comparing to XRD peaks the synthesized particles show peaks at same position. This confirms that the iron oxide particle we synthesized is Hematite (α-Fe2O3).

2. Scanning Electron Microscopy (SEM)





The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens revealing information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. Here we used this characterization to identity the average particle size to confirm that we synthesized desired nanoparticles. As per surface images (fig 2.1) and (fig 2.2) the particles show a size range of size of 77.28 – 123.2 nm. The average particle is around 77 nm. This confirms that the synthesized Fe_2O_3 particles belong to nanosize reigme,hereby we can confirm that synthesized iron oxide is nanometer in size. Thus α-Fe_2O_3 particles were synthesized using a green synthesis method from thulsi leaves extract.

CHAPTER 6
CONCLUSION

Magnetic Iron oxide nanoparticles were biosynthesized by a chemical free method (from thulsi leaves extract). Thulsi leaves has a therapeutical application and is an excellent candidate in nanoparticle synthesis. The synthesized fe_2O_3 nanoparticles were magnetic in nature as we confirmed with a magnet. The particles were characterized using X-ray diffraction analysis (XRD) and scanning electron microscopy.

From X-ray diffraction analysis we confirmed the particle's phase is hematite (α-Fe2O3) From scanning electron microscopy studies we confirm the synthesized particle is nanometer in size as we got an average size of 77 nm. We can use these iron oxide nanoparticles for various relevant application such as, contrast agents for Magnetic Resonance Imaging (MRI), drug carriers for target specific drug delivery, gene carriers for gene therapy, therapeutic agents for hyperthermia based cancer treatments, magnetic sensing probes for in-vitro diagnostics (IVD)