CHAPTER I

NECESSITY OF RESEARCH WORK

I.1. OBJECT, SCOPE, AND APPLICATION OF THE RESEARCH WORK

Research is necessary because it improves treatments and services not only for current generation, but also those for future generations. The primary goal of the research is to correct and improve hypotheses, leading to the acceptance of certain scientific truths. Scientific research is defined as research that uses systematic and scientific methods to collect, evaluate, and perceive data in order to provide us with the knowledge that we need to solve problems and make decisions. Life itself necessitates investigation. We now understand how things are, how they change, and what causes such occurrences. Today's students, thankfully, are becoming more interested in science and technology, and research is gaining traction in their minds.

Nanotechnology has recently attracted the attention of researchers because it allows for the modification of substances at the nanoscale level. Nanotechnology, as defined by the National Science Foundation, is the ability to comprehend, modify, and regulate matter at the atomic and molecular levels. Subfields of nanotechnology include wet nanotechnology, green nanotechnology, and nanoengineering. Improved transistors, water filtration, drug delivery, biological and chemical sensors, reducing the size and power drain of memory storage and computing elements in a computer, and light-emitting diodes are some of the more exciting applications of nanotechnology. Chemists recently integrated DNA-based rolling motors into computer functions, paving the way for molecular robots. These DNA-based motors combine computational power with the ability to burn fuel in a controlled manner.

Researchers have developed a scavenger nanoparticle that may provide a competitive advantage for fuel cell systems over battery systems. By supervising stem cells as they form bone tissue, nanotechnology has the potential to improve the body's regenerative capacity. Despite numerous research challenges, nanotechnology has already produced new materials and is advancing in a wide range of fields. Organic, inorganic, and carbon-based nanoparticles are the three types of nanoparticles. Metallic nanoparticles garner the most attention among the various types of nanoparticles. It has precious metals or magnetic metals in it, such as nickel oxide and iron oxide. For the synthesis of nanoparticles, there are two approaches: top-down and bottom-up. Metallic nanoparticles with unique physical and chemical properties, such as zinc oxide, are used in cosmetics and biomedical applications. [1]. Furthermore, ZnO nanoparticles have been reported to be non-toxic to the human body. As a result, ZnO nanoparticles with proper functionality may offer the possibility of treating cancer diseases. ZnO nanoparticles can also be used to deliver zinc for diabetic treatment and have the potential to increase crop yield. [2]. We created ZnO nanoparticles from *Azadirachta indica* leaves extract to investigate their antifungal and seed germination properties.

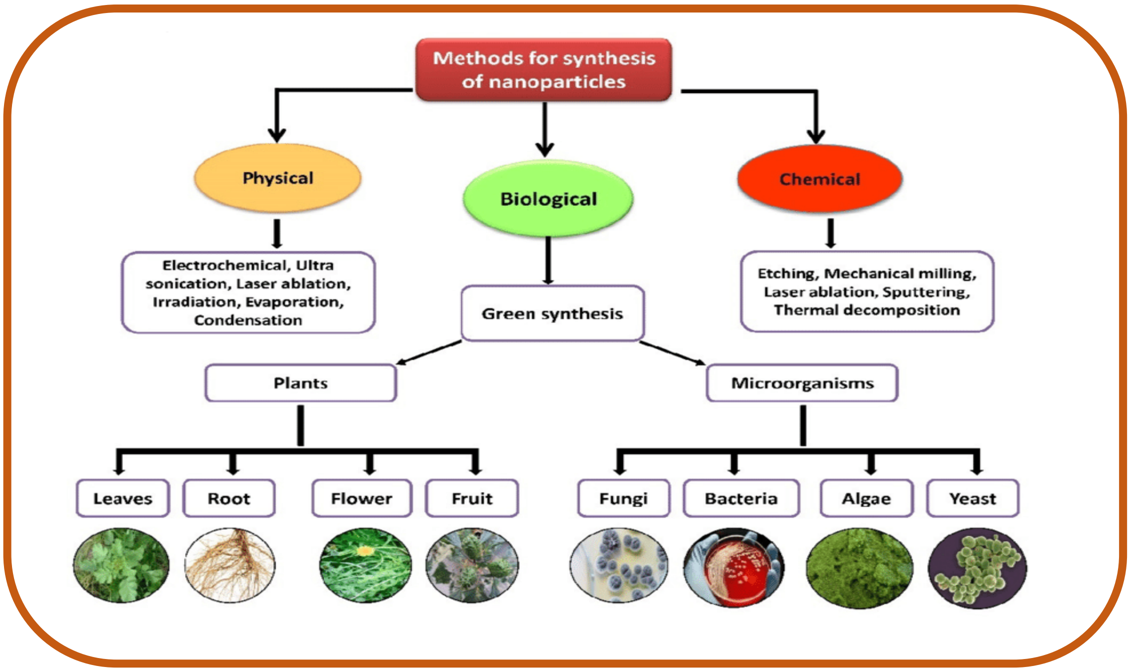


Fig. I.1. Methods of nanoparticle synthesis

Magnetic iron oxides, particularly α-Fe_2O_3, have a wide range of biomedical applications, including drug delivery, protein immobilisation, and thermal therapy[3]. They are highly catalytic, magnetic, antimicrobial, and antioxidant. They can also be used to detect pollutants, as explosives, MRI contrast agents, nano pesticides, composite fillers, and fuel additives. Iron oxide nanoparticle applications and synthesis have piqued the interest of nanotechnology researchers over the last decade. Much research has been conducted on the synthesis and applications of iron oxide nanoparticles. Different synthesis paths have been developed, but green synthesis remains a major challenge in the field of synthesis. The main difficulty in the synthesis of iron oxide nanoparticles is reproducibility. As a result, we attempted to make iron oxide nanoparticles using a green method of precipitation. The synthesized nanoparticles were also investigated for various biomedical applications and catalytic activities.

Nickel oxide nanoparticles, among other nanomaterials, have piqued the interest of scientists due to their intriguing properties. It's found in micro batteries, electrochromic batteries, microwave absorbers, solar cells, alkaline battery cathodes, inductors, gas or temperature sensor devices, transparent heat mirrors, and other applications. NiO is a p-type semiconductor with a limited visible absorption band. There have been few studies on the biosynthesis of nickel oxide nanoparticles in comparison to chemical synthesis. The traditional synthesis method has several drawbacks, including pollution, complexity, high costs, and hazardous materials. As a result, there is a strong desire to develop eco-friendly methods. We developed NiO nanoparticles from *citrus limon* and *citrus limetta* peel extract in response to the need for environmentally friendly nanoparticle synthesis. Green nanomaterials have a high dimension, polydispersity, and stability. As a result, nanomaterials have unique properties that allow for a diverse range of applications.

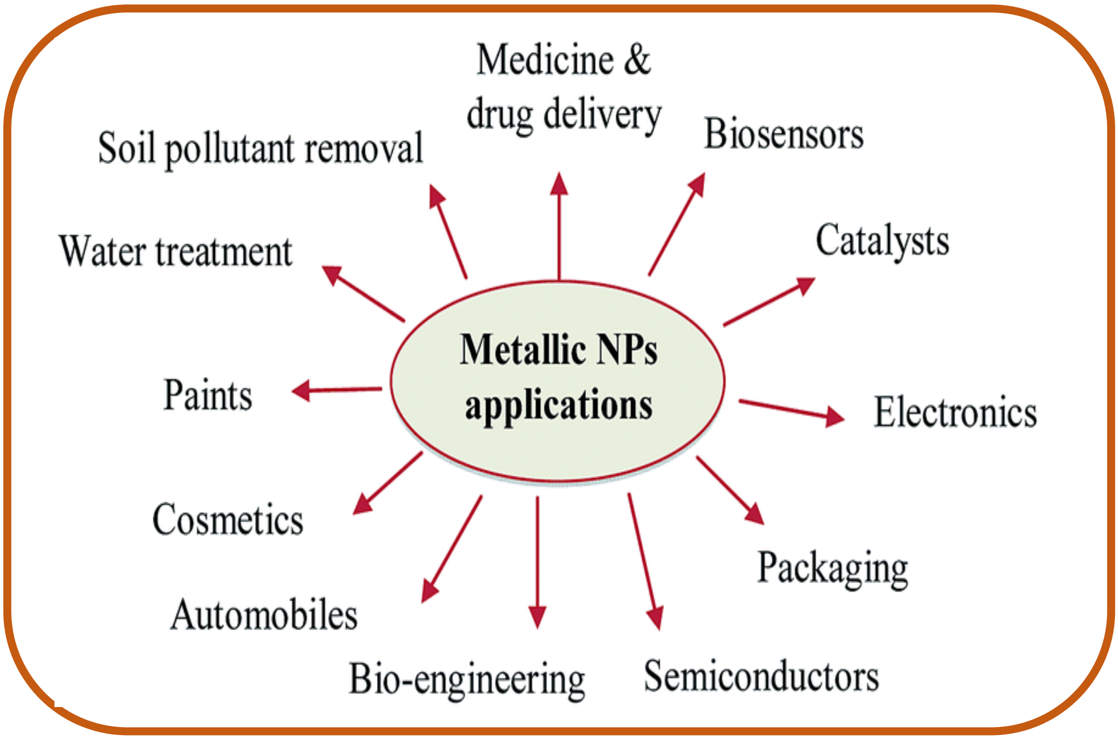


Fig. I.2. Applications of metal nanoparticles

Doping, a method of improving the properties of nanostructures by incorporating specific entities on nanomaterials, tunes the properties to perform their required function. Doping of appropriate entities is critical from the standpoint of specific applications, and in some cases, doping can overcome the limitation of nanomaterial use. [4]. During this journey, we created copper and nickel doped zinc oxide nanoparticles. Doping has been shown to improve optical, chemical, and physical properties, as well as bacterial activity. Doping results in a defect in the ZnO lattice due to differences in the ionic radius of the host and guest moieties, as well as charge compensation. [5]. Doped materials demonstrated improved antifungal and seed germination activities.

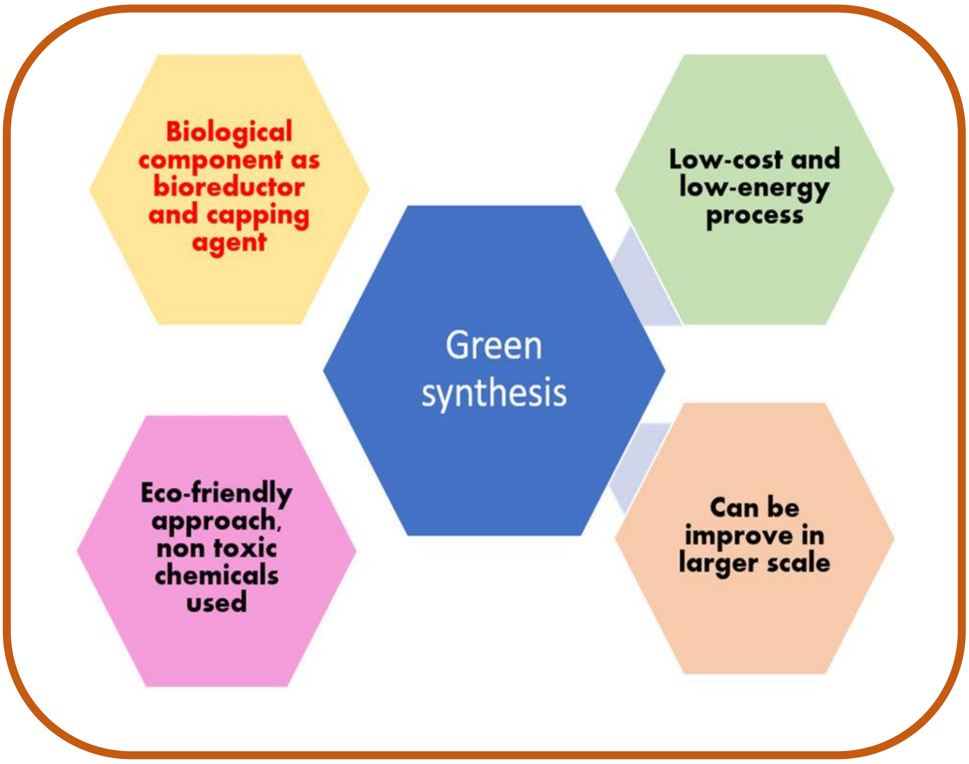
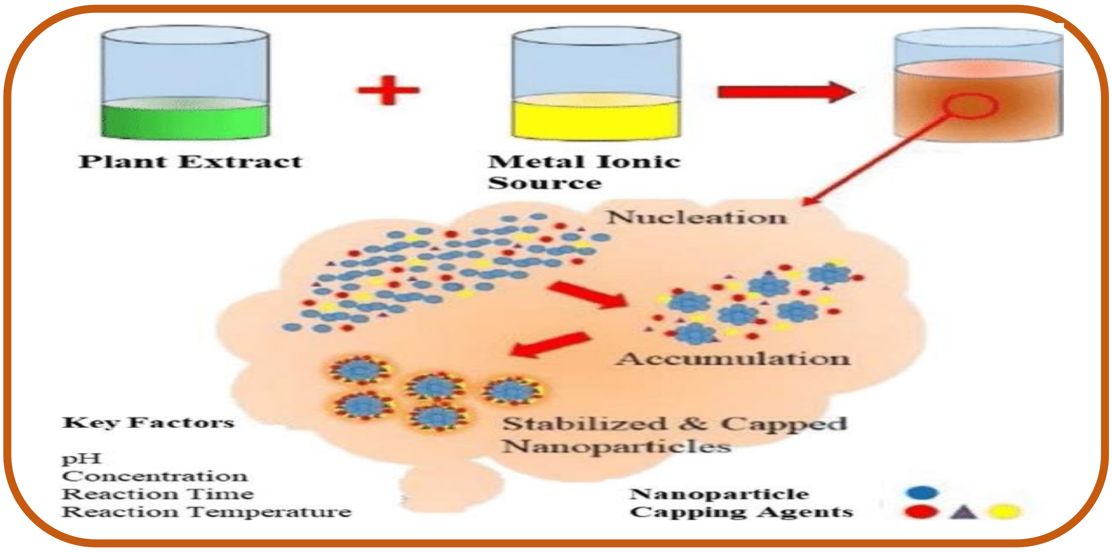


Fig. I.3. Key merits of Green Chemistry

"Green synthesis" has gotten a lot of attention for its ability to synthesise a wide range of nanomaterials such as metal/metal oxide and hybrid nanoparticles. It is thought to be a useful technique for reducing the harmful effects of traditional methods used in laboratories and industry by developing dependable, long-lasting, and environmentally friendly synthesis procedures.[6]. Safer solvent systems and natural resources must be used to achieve this goal. Because of the availability of phytochemicals such as aldehydes, flavones, amides, terpenoids, carboxylic acid, and ascorbic acids, green synthesis methodology has captivated the entire attention for the synthesis of metal or metal oxide nanoparticles. These phytochemicals have a reducing and stabilising effect. Various biological sources, such as algae, fungi, bacteria, yeast, and plant extracts, are now being used to synthesise nanoparticles. [7], [8]. As a result, we focused our research on the environmentally friendly synthesis of metal/metal oxide nanoparticles.



Scheme I.1. Schematic diagram of mechanism of nanoparticle synthesis

Deoxyribonucleic acid is essential for all living organisms and viruses to follow genetic instructions such as growth, reproduction, functioning, and development. Because the interaction of nanoparticles with DNA molecules can cause cell damage, research into the interaction of nanoparticles with DNA is critical. Nanoparticles can interact with DNA in both covalent and non-covalent ways. UV-Visible spectroscopy is the most effective spectroscopic technique for determining the type of interaction. If there is a hyperchromic shift, there will be noncovalent interaction; if there is a hypochromic shift, there will be covalent interaction. The similarity in size of nanoparticles and biomolecules facilitates this interaction. [9]. These DNA-nanoconjugates have the potential to be used as drug carriers and gene delivery vehicles in biomedical applications. The interaction of CT-DNA and nickel oxide nanoparticles was thoroughly investigated in the current study.

The growing demand for industries and factories increases the amount of waste produced and the amount of water consumed. Because most dyes are non-biodegradable and toxic to living organisms, dye degradation has become increasingly important in recent years. Many of the dyes that mix with drinking water can be teratogenic, carcinogenic, or mutagenic. Methylene Blue is a hetero polyaromatic dye that is widely used in the textile industry for dyeing cloth and leather. It also has clinical applications. Reverse osmosis, precipitation, ion exchange technology, sonolysis, biodegradation methods, radiolysis, and the Fenton process are the most commonly used methods for purifying drinking water.

The reaction system consists of hydrogen peroxide and an iron system that works in an acidic medium, whereas the Fenton-like reaction system contains Fe^{3+}. Fe^{2+}/Fe^{3+} and can function in a neutral medium. One disadvantage of the Fenton process is that it cannot be eliminated from the environment. As a result, environmentally sustainable water treatment methods are required, and Fenton-like catalysts may be a better substitute in this regard.

Pathogenic bacteria found in food included *E. coli*, *Salmonella sp*., *Klebsiella sp*., *Salmonella typhimurium*, *Bacillus megaterium*, *Staphylococcus aureus*, and *Bacillus subtilis* causes for a significant risk to human. Metal oxide nanoparticles are effective pathogen-removing antimicrobial agents. Metals may not have antibacterial properties in their salt or oxide forms, but they can be tuned in the nano form.[10]. Metal oxide nanoparticles have effective connectivity with microbial protein and DNA, preventing microbial replication and causing the electron transport chain to break down, resulting in cell death[11]. As a result, we use a precise preparation of metal oxide nanoparticles that can be used to inhibit bacterial growth in our research. Nanoparticles do have bacterial potential via two mechanisms: (a) Toxicity of free metal ions (b) Production of reactive oxygen species Gram-positive bacteria have a higher IC50 value than Gram-negative bacteria because their peptidoglycan layer is thicker. Because of increased antibiotic resistance in microorganisms, there is a high demand for new antimicrobials with the potential to reduce healthcare costs, and green synthesised nanomaterials meet this demand. The use of synthetic antimicrobials in various fields such as cosmetics, medicine, textiles, and the environment has resulted in a rapid increase in demand for these new antimicrobials.

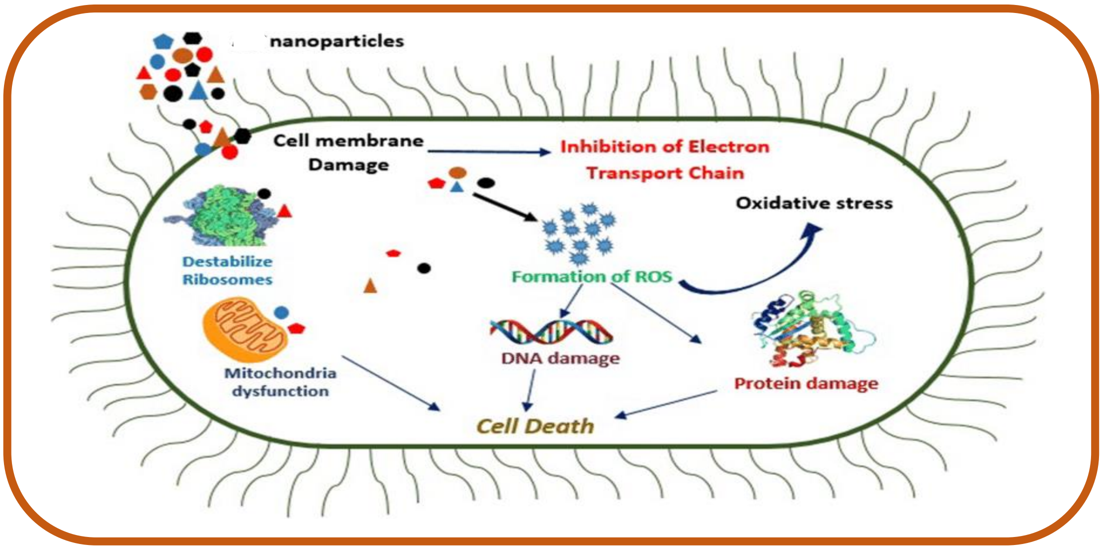


Fig. I.4 Various modes of action of nanoparticle on bacteria

Life on Earth is unimaginable without oxygen, and the action of oxygen on living organisms is obvious, so the search for antioxidants is difficult to put an end to. Different metabolic processes in living organisms produce free radicals. Aside from affecting cell function, it can also harm protein, fat, carbohydrates, and nucleic acid [12]. The cell develops an intercellular antioxidant system to prevent intercellular oxidation. Recently, butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) have been linked to adverse health effects. [13]. In the search for non-toxic, effective, and natural antioxidants, nanomaterials have emerged as a viable alternative to synthetic antioxidants. Nanoparticles of silver, gold, iron, and nickel oxide, for example, may have antioxidant properties. Antioxidants counteract the effects of oxidants by providing hydrogen ions to reactive species. The liophilic DPPH radical is used to determine antioxidant activity in vitro study, and this assay requires little time, chemicals, and samples. DPPH has a maximum absorbance at 517 nm and gradually decreases in absorbance as the concentration decreases. Antioxidant activity is produced by the surface reaction of nanoparticles and DPPH radicals.

It produces carcinogenic aflatoxin and can contaminate food sources when grown on them. Similarly, different *Rhizopus* sources cause agricultural losses, particularly in food crops. Rhizopus causes leak diseases in food crops as well as pole rot. Another morphological fungi, *Fusarium solani*, causes head blight, scab, and crown rot in food crops. Fungicides can be used to control fungi, but they can also be harmful. In this case, phytosynthesized nanomaterials may be a better option. We attempted to synthesise metal oxide nanoparticles with antifungal properties, and doping on nanomaterials improved their antifungal properties.

As a result, the goals, scope, and applications of this research journey are

- The purpose of environmental nanoparticle synthesis is to reduce waste and use environmentally friendly methods. Other advantages of green nanoparticle synthesis include low cost, reproducibility, ambient conditions, and non-hazardous materials.
- The precipitation method for synthesis of nanoparticles is used for complete precipitation of metal ions. Besides this, nanoparticles with higher surface area can be synthesized.
- Metal ions are completely precipitated using the precipitation method for nanoparticle synthesis. Furthermore, nanoparticles with a larger surface area can be created.
- This research work brings to light various types of interactions through the use of physiochemical methodologies.

- The use of biosynthesized nanoparticles as Fenton-like catalysts, antibacterial, and antifungal agents opens up new avenues for future research. They can also be used for biofertilizers and drug delivery systems.
- Theoretical DFT studies also confirm the reliability of our practical work.

I.2. CHOICE OF DYE MOLECULES, METAL SALTS, BIOACTIVE MOLECULES, CHEMICALS, AND SOLVENTS FOR RESEARCH

Dye molecule

Methylthioninium chloride salt ($C_{16}H_{18}ClN_3S$)

Metal salts

Zinc nitrate hexahydrate ($Zn(NO_3)_2 .6H_2O$)

Nickel nitrate hexahydrate ($Ni(NO_3)_2 . 6H_2O$)

Copper Sulphate ($CuSO_4$)

Ferrous Sulphate ($FeSO_4$)

Biological compounds

Calf thymus DNA

Lemmon

Mosambi

Chemicals

Ethylene glycol

Sodium hydroxide

Hydrochloric acid

Solvent

Water

Dimethyl sulfoxide

Ethanol

Acetone

I.3. METHODS OF INVESTIGATION

The following methods of investigation was used in our research work

UV-Vis Spectroscopy

Powder X-Ray Diffraction

FTIR spectroscopy

Scanning Electron Microscopy

Dynamic light scattering

Zeta Potential

Field emission scanning electromicroscopy

Energy dispersive X-ray analysis

Antibacterial study

Antifungal study

Seed germination study

Antioxidant activity