Fabrication of Pectin and Chitosan based Bioplastic Films Incorporated with ZnO Nanoparticles for Food Packaging Application

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Dedicated to my Beloved Parents,

for their Endless Efforts, Support,

Love, and Encouragement

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List of Abbreviations

NPs	Nanoparticles
FTIR	Fourier Transform Infrared Spectroscopy
UV	Ultra Violet
VIS	Visible
XRD	X-Ray Diffraction
SEM	Scanning Electron Microscopy
mL	Milliliter
µL	Microliter
µm	Micrometer

Abstract

The demand for biodegradable and environmentally friendly food packaging stems from growing environmental concerns about traditional plastics, which contribute to pollution and take centuries to decompose. Bioplastics provide a sustainable alternative that reduces reliance on fossil fuels while minimising environmental effect. In this study, pectin was utilised as a matrix, chitosan as a co-biopolymer, and glutamic acid and glycerol as natural plasticisers to create bioplastics by hydrogen bonding and electrostatic interactions, which were accompanied by weak vander Waal's forces. The FTIR study showed the presence of hydrogen bonding and electrostatic interactions, whilst the XRD pattern revealed the bioplastics' semi-crystalline structure. Their swelling ratio was between (30-335 ± 20%) and mass loss (53-75 ± 5%), making them sensitive to hydrolysis after a 24-hour incubation in water. The reported tensile modulus ranged from 0.1 to 3.30 N/mm^2, with A3+3% NPs having the highest (3.30 N/mm^2), which can be attributed to the greater plasticization effect in the A1+3% NPs. After 7 days of dipping in soil, the samples containing ZnO nanoparticles showed a degradation of up to 9.92% and were eco-friendly according to eco-toxicity assays, making them suitable for use in food packaging applications. As a result, these pure biobased polymers could serve as beneficial materials for future food packaging application.

Chapter 1 INTRODUCTION

1.1 Polymers:

Polymers composed of lengthy chains of repeating units called monomers, which bind together to form complex structures with a wide range of properties, polymers are useful and necessary materials. These molecules are present in both natural and artificial forms, and they are essential to many industrial, biological, and daily processes. Proteins, DNA, and cellulose are examples of natural polymers that are essential to life because they serve as the building blocks of living things and help maintain the structure and functionality of tissues and cells. Engineered for specific purposes, synthetic polymers such as plastics, nylon, and polyester have transformed numerous industries by offering lightweight, flexible, and long-lasting materials that can be moulded into an infinite variety of shapes and forms. Polymers have special qualities that make them useful in a variety of industries, from electronics and medical to building and packaging. These qualities include strength, flexibility, and resistance to chemicals and wear. Due to their flexibility to be customised to fit specific requirements, polymers are now an essential part of modern industry and technology, providing answers to problems with material performance, energy efficiency, and sustainability.[1] [2] [3] [4]

1.1.1 Types of polymers:

Both natural and synthetic polymers, each with their own special qualities and uses, can be broadly divided into two categories.

1.1.1.1 Natural polymers:

Natural polymers are substances that occur naturally and are essential components of biological systems. For instance, proteins polymers composed of monomers of amino acids perform a variety of functions that are necessary for the structure and operation of living things, including catalysing biochemical events and supporting cellular structure. DNA, or deoxyribonucleic acid, is another important naturally occurring polymer. Made up of nucleotide monomers, DNA is the genetic material that all living things contain. Furthermore, the carbohydrate polymer cellulose, which is present in plant cell walls, is essential for preserving the structural integrity of plants and is also utilised to make paper and textiles.[5] [6] [7]

1.1.1.2 Synthetic polymers:

Synthetic polymers are man-made materials that have been specifically designed to fulfil demands across a range of industries. Probably the most well-known synthetic polymer, plastics are created from petrochemical monomers and found in an incredible range of products, from household goods and packaging to car components and medical equipment. Synthetic polymers including polyethylene, polystyrene, and polyvinyl chloride (PVC) are frequently found in popular products. Another synthetic polymer that is frequently utilised in the textile industry to create sturdy garments and fabrics is nylon. Polyester is prized for its strength, resistance to wrinkles, and ability to dry quickly; it is also used in textiles. Furthermore, synthetic polymers are essential in high-performance applications where particular properties such high strength, flexibility, or biocompatibility are needed, like in electronics, aircraft, and medical equipment.

In addition, polymers can be categorised according to their structure, place of origin, and reaction to heat.[8] [9]

Thermoplastic polymers are perfect for recycling and reshaping because they can be melted and remoulded several times. Examples are the polymers polyethylene and polypropylene, which are frequently used in automobile parts and packaging. Thermosetting polymers are materials that are utilised in adhesives, coatings, and composite materials where a strong, heat-resistant substance is required. Once cured or hardened, they cannot be remelted. Phenolic and epoxy resins are two examples.Elastomers are a type of polymers that have elastic characteristics, or the ability to stretch and revert to their original shape. They are another significant category. The most widely used elastomer is rubber, which is found in gaskets, tires, and seals, among other things.[10] [11]

These divisions show the variety and adaptability of polymers, which are designed for a broad range of functions, from commonplace objects to specialised industrial applications, highlighting their vital role in contemporary society.

1.2 Synthetic polymers and environmental concerns:

Despite being immensely helpful and adaptable, synthetic polymers are very harmful to the environment because of their extensive use, tenacity, and disposal difficulties. The main problem is that they don't biodegrade. Because most synthetic polymers, including plastics, are difficult for the environment to break down, garbage builds up in landfills, seas, and other ecosystems. The degradation of this persistent pollution can take hundreds or even thousands of years, during which time species may suffer significant harm. Plastic waste can be consumed by animals who mistake it for food, which can result in obstructions, starvation, or even death. Moreover, a particular source of concern is microplastics, which are minute pieces of broken down plastic that can be consumed by a range of creatures, making their way up the food chain and perhaps affecting human health.[12] [13] [14]

The release of harmful chemicals during the manufacture, application, and disposal of synthetic polymers is another significant environmental problem. Hazardous materials that pose threats to human health and the environment are frequently used in the production process. These materials can contaminate air and water. Certain plastics have the potential to release toxic compounds like phthalates and bisphenol A (BPA), which are endocrine disruptors and can have negative health impacts on both people and animals, when they are heated or exposed to sunlight.[15] [16]

Another issue with the prevalent way of disposing of synthetic polymers is incineration. Although it lessens the amount of waste produced, it may also emit dangerous pollutants, such as carbon dioxide and other greenhouse gases that contribute to climate change. Dioxins and furans, two extremely toxic byproducts of incineration that can have long-term effects on the environment and human health, are examples of toxic byproducts.[17] [18]

Recycling presents several difficulties even though it is a partial answer. Not all synthetic polymers can be recycled readily, and even those that can frequently see quality degradation with each recycling cycle, which results in downcycling the process of using the material to make goods of worse quality. Efficient management of polymer waste is made more difficult by the contamination of recyclable materials and the inadequate recycling infrastructure in numerous places.[19,20]

Additionally, the manufacture of synthetic polymers is mostly dependent on fossil fuels, which exacerbates environmental damage and depletes non-renewable resources. Plastics production involves the extraction, processing, and transportation of natural gas and oil, which has a substantial negative impact on the environment due to greenhouse gas emissions, habitat damage, and oil spills.[21] [22]

In general, the environmental issues surrounding synthetic polymers highlight the need for improved waste management techniques, more recycling, the creation of biodegradable substitutes, and a change to more environmentally friendly production techniques in order to lessen their influence on the environment.[23] [24] [25]

1.3 Why biopolymers:

The significance of biopolymers in mitigating environmental issues and promoting sustainable development is becoming more widely acknowledged. Biopolymers are a more sustainable alternative to synthetic polymers made from fossil fuels because they are made from renewable biological sources like plants, animals, and microbes. One of their biggest benefits is that biopolymers can naturally decompose in the environment, which lessens the amount of persistent plastic trash that has grown to be a global environmental catastrophe. Because of its propensity to decompose, it lessens the damage that plastic pollution causes to wildlife, ecosystems, and human health by avoiding long-term contamination and the production of microplastics.

The transition to a circular economy where materials are made to be securely recycled, reused, or returned to the environment is also supported by the usage of biopolymers. Since the resources used to produce biopolymers are renewable, their production can be incorporated into agricultural systems, using organic waste and crops to produce materials that are a part of a closed-loop system. This lessens the need for finite fossil fuels and the carbon footprint left by the manufacture and disposal of traditional plastics. Biopolymers have the potential to provide functional advantages in a range of applications, in addition to environmental benefits. For example, certain biopolymers, such polylactic acid (PLA) and polyhydroxyalkanoates (PHAs), have qualities that allow them to be used in packaging, agriculture, and medical devices. PLA is made from sugarcane or corn starch and is utilised in 3D printing, disposable dinnerware, and biodegradable food packaging. PHAs are made by microbes and are biocompatible and biodegradable in the human body, making them useful in drug delivery systems, medical implants, and biodegradable plastics. Moreover, the creation of biopolymers spurs material science innovation, resulting in new goods with specialised qualities to fulfil niche markets across a range of sectors. For instance, the food industry is using biopolymer based packaging more and more to extend product shelf life, cut waste, and preserve sustainability. By reducing their negative effects on the environment and improving soil health, biopolymer based products in agriculture, such as slow-release fertilizers and biodegradable mulches, help to promote more environmentally friendly farming methods.

The developing bioeconomy, which emphasizes the utilization of biological resources to produce goods and services in an environmentally friendly manner, also heavily relies on biopolymers. This change could lead to lower greenhouse gas emissions, more robust and sustainable global economies, and new business opportunities. Furthermore, the usage of biopolymers is in line with the rising demand from consumers for environmentally friendly goods, as more people look for substitutes for traditional plastics that worsen pollution and deplete resources.

In conclusion, the potential of biopolymers to provide a viable substitute for conventional synthetic polymers that has major positive effects on the environment, the economy, and society is what makes them so important. In order to address some of the most urgent environmental issues of our time, they are an essential part of the shift towards more sustainable production and consumption patterns because of their biodegradability, renewable origins, and capacity to support a circular economy.

1.4 Biopolymers - a step towards green chemistry:

Green chemistry, which emphasises the design of products and processes that minimize environmental effect and increase sustainability, has made tremendous progress with the development of biopolymers. Biopolymers provide a more sustainable option than conventional synthetic polymers since they are made from renewable biological sources like plants, algae, and microbes. Conventional synthetic polymers are usually sourced from non-renewable fossil fuels and provide serious environmental concerns. Green chemistry principles are in line with the manufacture and application of biopolymers. These concepts include the use of renewable feedstocks, the reduction of hazardous compounds, and the design for degradation.

The capacity of biopolymers to degrade naturally is one of their main contributions to green chemistry. Biopolymers are made to naturally decompose, as opposed to traditional plastics, which can linger in the environment for millennia. This helps to prevent the buildup of plastic waste and the environmental risks that come with it. When it comes to problems like microplastic contamination, ocean pollution, and landfill overflow, biodegradability is crucial. Biopolymers lessen the ecological footprint of materials and promote a cleaner, healthier world by breaking down into non-toxic byproducts.

Additionally, compared to the synthesis of conventional polymers, the manufacturing of biopolymers frequently uses less energy and harmful chemicals. As a result, manufacturing operations have a smaller total environmental impact and emit fewer greenhouse gases. Utilising waste and agricultural byproducts as raw materials to produce biopolymers promotes waste valorisation, which converts things that would otherwise be thrown away into useful resources. This contributes to a more circular economy by encouraging the efficient use of biomass and conserving resources.

Additionally, biopolymers foster innovation in material science, which results in the creation of new goods that uphold green chemistry principles while satisfying the needs of diverse industries. For example, biopolymer-based packaging materials provide a less carbon footprint and more environmentally friendly option to traditional plastic packaging by minimizing reliance on petroleum-based resources. Biopolymers are utilized in the medical profession to create biocompatible materials that improve patient care and lessen the environmental impact of medical waste. These products include biodegradable implants, medication delivery systems, and wound dressings.

In summary, biopolymers promote the use of renewable resources, lessen environmental harm, and provide sustainable substitutes for synthetic polymers, all of which are hallmarks of green chemistry. The use of biopolymers is a critical step towards a more sustainable future where materials are created with environmental stewardship in addition to utility, as industry and consumers prioritize sustainability more and more.

1.5 Pectin:

Pectin is a naturally occurring biopolymer that is mostly present in the cell walls of fruits and some vegetables. There, it serves as a structural element by giving the cells stiffness and assisting in their binding. From a chemical perspective, pectin is a polysaccharide, a complex carbohydrate primarily made up of galacturonic acid units. Citrus fruits, apples, and pears are particularly rich in it and are frequently utilized as sources for commercial pectin extraction. The food sector uses pectin extensively, particularly in the making of jams, jellies, and other fruit-based goods. It is an indispensable gelling agent in these applications because of its special capacity to create a gel in the presence of acid and sugar. This gelling quality stabilizes the product and keeps liquid and solid components from separating, which improves shelf life while also improving food texture and consistency.

Pectin is used extensively in the pharmaceutical and cosmetic industries in addition to its culinary purposes. Pectin is utilized in pharmaceuticals as a natural thickening agent, an emulsion stabilizer, and a dietary fiber source in a range of health supplements. It is a well-liked component of functional foods and nutraceuticals due to its possible health benefits, which include improving digestion, decreasing cholesterol, and stabilizing blood sugar. Furthermore, because pectin forms protective coatings over wounds and is biocompatible, it is used in wound healing solutions.

Because of its and stabilizing qualities, pectin is utilized in skin care formulations in the cosmetics sector. It assists in giving creams and lotions a silky, gel-like texture, and its natural source satisfies consumer need for more sustainable and environmentally friendly beauty products. Pectin is a desirable constituent in many different industries due to its natural, biodegradable, and non-toxic nature. This is especially true given the growing need for sustainable and natural materials. Its value as a functional element and a contribution to sustainability and health is highlighted by its positive characteristics and adaptability.

Figure 1.1: Sources of Pectin

1.6 Chitosan:

Chitin, which is found in the exoskeletons of crustaceans like prawns and crabs as well as the cell walls of fungi, is the natural biopolymer that is used to create chitosan. It is created by partially removing the acetyl groups from chitin by treating it with an alkaline substance. Chitosan gains its distinctive qualities from this process, including biocompatibility, biodegradability, and antibacterial activity, which make it extremely important for a range of uses. Because chitosan may form protective barriers, promote healing, and regulate medication release, it is employed in tissue engineering, drug delivery systems, and wound dressings in the biomedical industry. It is also used in water treatment, where its capacity to bond with contaminants and heavy metals makes it a more environmentally friendly method of water filtration. In the food business, chitosan is utilised to stop contamination in food packing and serves as a natural preservative that prolongs the shelf life of perishable items.

Because it is made from sustainable resources like fish waste, chitosan is more appealing to a wider range of industries. Because of its adaptability, it can be formulated for a variety of applications, from agriculture to cosmetics, where it offers antibacterial and moisturising properties to skincare and haircare products. However, the source and manufacturing method of chitosan can have an impact on its quality, which can alter how well it performs in various applications. Notwithstanding these difficulties, chitosan's contribution to sustainable technology, health, and environmental preservation is only growing, making it a crucial component of both present-day and future advancements.

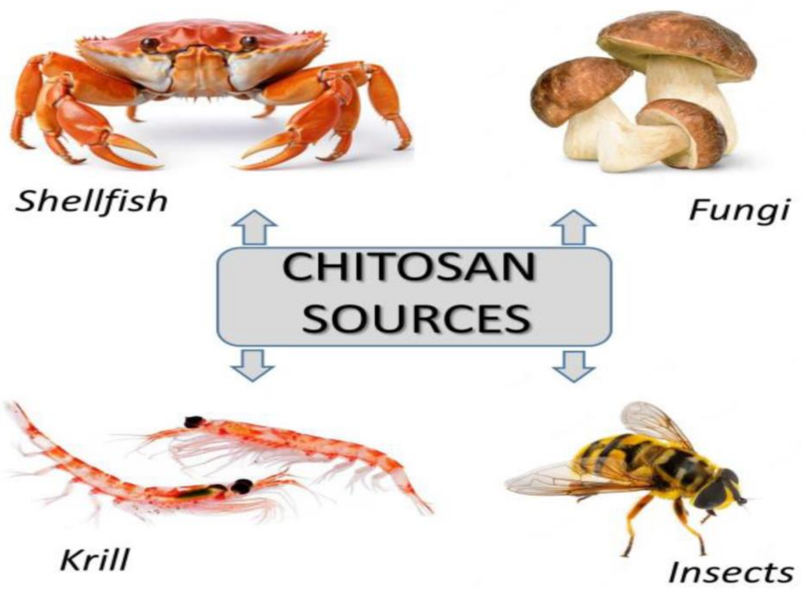


Figure 1.2: Sources of Chitosan

1.7 Biopolymer blended films:

In order to generate a film with improved or customised qualities, two or more distinct biopolymers can be combined to make composite materials known as biopolymer blended films. These films are generally utilised in applications that require biodegradable and environmentally friendly materials, such as packaging, agriculture, medicine, and other fields. Researchers and manufacturers can acquire a balance of qualities, such as mechanical strength, flexibility, barrier properties, and biodegradability, by blending multiple biopolymers, which would not be possible with a single biopolymer.

These films' biopolymers can originate from a variety of natural sources, such as:
-Polysaccharides: These include chitosan, cellulose, and starch.
-Proteins: Such as casein, gelatin, and soy protein.
-Lipids: Fatty acids and waxes, for example.
-Polyesters: Such as polyhydroxyalkanoates (PHA) and polylactic acid (PLA).

1.7.1 Uses of biopolymer blended films:

Biopolymer blended films are used in many different industries because of their eco-friendliness and adaptability. They provide biodegradable substitutes for conventional plastics in the food packaging sector, acting as efficient oxygen and moisture barriers to increase shelf life. These films are used in agriculture as seed coats that protect and promote germination, as well as mulch to keep soil moisture in the soil and keep weeds out. Wound dressings and medication delivery systems are two medical applications that benefit from their biocompatibility and controlled release characteristics. Their use in skincare and hair care products improves texture and hydration, which benefits the cosmetics sector. Biopolymer blended films are also used in consumer products like reusable bags, building for protective barriers and insulation, and environmental applications including water filtering and pollution control. This adaptability emphasises their contribution to the advancement of sustainability while fulfilling certain functional needs in a range of industries.

1.8 Nanoparticles:

Nanoparticles are defined as materials with two or more dimensions and a size range of one to one hundred nm. Due of their extensive surface area and electrical characteristics, NPs have Biochemistry, photochemistry, and electrochemistry all greatly benefit from them . Currently, there is a lot of study being done in the technology, research, and nanocrystal fields. They already exist on an industrial and financial level. The use of nanomaterials in industry has the potential to reduce eco-toxicity as well as lab toxicity, allowing for the measurement and management of variables that impact the environment and human health. Nanoparticles are helping a wide range of industries, including transportation, energy, agriculture, medicines, cosmetics, and consumer products, to fulfil the demands of a wide range of new uses in the industry. It's exciting to think of using "smart delivery systems" to deliver nanoparticles in biomedicine. P. Ehrlich, the 1906 Chemistry Nobel laureate, referred to nanoparticles as "magic bullets." The scientific community is very interested in metal nanoparticles because they provide a link between atomic and bulk structures. These materials' physicochemical properties include high surface area, high reactivity, and controllable pore size and shape. Metallic nanocrystals can now be incorporated into a wide range of commercial, medical, and home goods in light of current developments in nanotechnology.

1.8.1 Importance of being nano:

Because of their intriguing characteristics and countless possible applications in contemporary technology, nanoparticles are highly sought after by scientists. This is a question regarding the fundamental property changes at the macro/micro and nanoscales. The material's physical and chemical properties change when nanoparticle size is decreased, increasing surface area per unit mass. The materials' thermal stability and catalytic performance might be impacted by the high surface atom and energy concentrations. Their lower melting point is a result of their two bigger area to volume ratios. These materials have a higher mechanical strength than their bulk counterparts because to crystal imperfections.

1.8.2 Iron nanoparticles:

Iron is abundant in the Earth's crust, accounting for 4 percent of all metals. It originates from mineral deposits such as magnetite and haematite. It's a good-ductile, malleable, and robust metal. Nano-iron particles are iron metal particles that have a diameter of less than 100 nm. Manufacturing can be carried out in a number of ways, including by using dry progressions like vapour deposition techniques or wet chemical processes like colloid chemistry or sol-gel methods. Compared to bulk iron, nanostructured iron is even more reactive to oxygen and water. This makes them an excellent source of antioxidants. Furthermore, they are widely used in the sciences in a variety of contexts, such as anti-oxidants, larvicidal and bactericidal therapies, and much more.

1.8.3 Silver nanoparticles:

One well-known antibacterial substance is silver. It has a lengthy and fascinating history in the treatment of human illness as an antibiotic. It can be applied to bone prosthesis, orthopaedic reconstruction, wound healing, and water purification, among other things. Moreover, surgical instruments and cardiac gadgets can be made with it. Ionisable silver can now be added to textiles for clinical usage to lower the risk of nosocomial infections and enhance personal hygiene thanks to advancements in biotechnology. Infectious disorders linked to healthcare and antibiotic resistance have been major concerns in recent years; these nanoparticles have been suggested as a viable remedy for these problems.

1.8.4 Zinc nanoparticles:

Zinc oxide (ZnO), one of the most significant metal oxide nanoparticles (NPs), has attracted attention from scientists recently. The catalytic, photochemical, antibacterial, and UV filtering characteristics of ZnO NPs are thought to combine to make them a perfect option for wastewater treatment. ZnO NPs are especially helpful in wastewater treatment because of their many beneficial qualities, which include catalysis, photochemistry, therapeutic benefits, fungicidal activity, antibacterial activity, and UV filtration.

1.9 Classification of nanoparticles:

Depending on their morphology and spherical shape, nanoparticles can be categorised and classified in a variety of ways. Moreover, they could be put into groups according on how they aggregate. Based on their number of proportions, nanomaterials can be categorised as one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) structures.

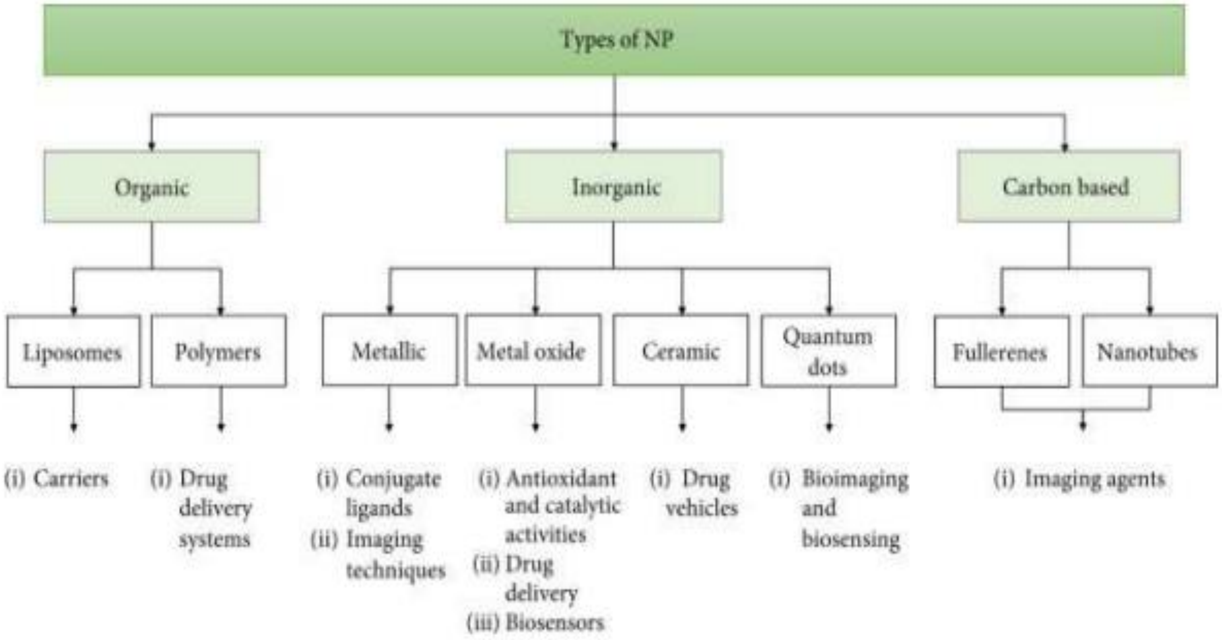


Figure 1.3: Classification of Nanomaterials

1.9.1 Composition:

Materials can be categorized based on their composition, whether they are mixed or single. They exist in nature as a blend of many substances. Today, there are numerous approaches to the production of pure single-composition materials.

1.9.2 Morphology:

The following categories of nanoparticles have been established based on their morphological features: sphericity, flatness, and the differentiation of particles with high and low aspect ratios. High aspect ratio nanoparticles include, among others, diameter-dependent, helical, zigzag, and belt-shaped nanotubes and nanowires. On the other hand, spherical, oval, cubic, prism, helical, or pillar-like groupings of many particles that exist as powders, suspensions, or colloids are examples of small-aspect ratio morphologies.

1.9.3 Dimensionality:

The classification of structures at the nanometre scale was developed as follows: 0D (nanopores and nanoparticles), 1D (layered or laminate structures), 2D (filamentary structures longer than the cross-sectional dimensions), and 3D (nanostructured materials) dimensional structures.

1.9.4 Nanoparticle Uniformity and Agglomeration:

Because magnetic nanoparticles have a propensity to cluster, aerosols, suspensions, and colloids of nanoparticles fall within this category. The size of the agglomeration that forms determines how nanoparticles behave as larger particles, unless their surfaces are covered with non-magnetic materials.

1.9.5 Source:

Nanoparticles can originate from a wide range of natural and artificial causes, such as intentional or unintentional particles. Based on where they came from and how they were made, they are divided into two categories: (a) naturally occurring nanoparticles and (b) artificially created nanoparticles. Previous studies have demonstrated that NPs can occur naturally and can be created by processes including biomineralisation and biodegradation.

1.10 Synthetic material:

NPs are categorised as nanoparticles based on carbon, lipid, semiconductor, ceramic, polymeric, and metal, and they can be driven from a wide range of materials.

1.11 Fabrication of nanoparticles:

To create NPs, two approaches can be utilised: top-down (physical method) and bottom-up (chemical and green ways). NPs can be produced using a variety of techniques, including biological, physical, chemical, photochemical, electrochemical, radiolytic, and sonolytic ones.

1.12 Approaches for synthesis:

There are essentially two methods for creating nanoparticles: the top-down and bottom-up approaches.

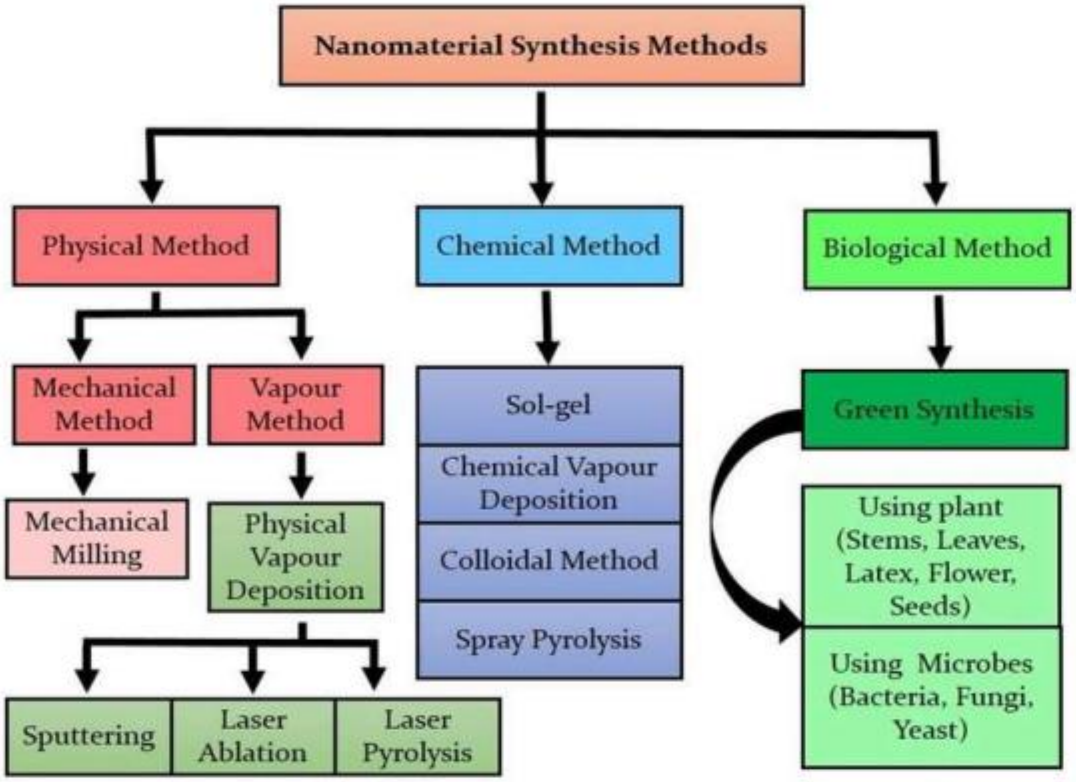


Figure 1.4: Methods of Nanomaterial Synthesis

1.12.1 Bottom-up approach:

This method starts with atoms and molecules and works its way up to nanoparticles. This procedure comprises disassembling a material into its component parts, including the assembly that yields nanoparticles. Nanoscale material forces can be used to connect smaller units into larger stable molecules during the assembly process. The creation of quantum dots through epitaxial growth and the synthesis of nanoparticles through colloidal dispersion are two instances of bottom-up methods. As examples of bottom-up approaches to nanoparticle synthesis, consider sediments, reduction processes, sol gels, green synthesis, and biosynthetic procedures.

1.12.2 Top-down approach:

This method utilises bigger structures that are modifiable externally during the nanoparticle synthesis. The nanoscale version is constructed upon a larger-scale structure as a foundation. This method cannot produce nanoparticles on a large scale. The etching by the mask and ball millings 6 are two general examples of the top down method. The most widely used top-down approach in recent years is photolithography. It produced structures smaller than 100 nanometres and was widely used in the manufacturing of computer chips.

1.13 Factors affecting formation of NPs:

As will be covered below, a number of variables affect the size, shape, and rate at which nanoparticles form. Using walnut green husk extract as a chelating agent to verify the influence of concentration, the shape, crystal formation, particle size, and magnetic properties of green generated nanocomposites NiFe2O4/Fe2O3/CeO2 were investigated and evaluated using analytical techniques. The particles were between 60 and 140 nm in size. The intrinsic characteristics of a nanoparticle are greatly influenced by its size, shape, content, and structure. The morphology of nanoparticles (NPs), including their size and form, can be influenced by several reaction conditions, including pH, reagent concentration, incubation temperature, duration, and others. Accelerating the synthesis and final conversion into nanoparticles was achieved by increasing the concentration of metal ions and the quantity of plant extract. The firmness of the NPs rises with the concentration of the reducing agent, and these two variables combine to effectively regulate firmness. Temperature has a significant impact on how NPs develop. The likelihood of smaller NPs forming increases with temperature. The improved productivity of NPs was amply displayed at higher temperatures.The pH is another crucial factor to take into account because it may be changed to create a variety of NPs. Raising the pH of the reaction media may have prevented further nucleation and produced smaller NPs . The influence of pH on the generation of nanoparticles was investigated by varying the pH of the reaction mixture .An alkaline pH promoted the production of NPs while an acidic pH hindered it. Decahedral and spherical NPs were produced when the medium's pH was raised. As the experiment goes on longer, the stability of NPs gets better. NPs were produced fast using the tulasi extract.Temperature and reaction duration affect particle size.Physical parameters are also very important; these include salt and reducing agent concentrations as well as the sites where NPs originated.

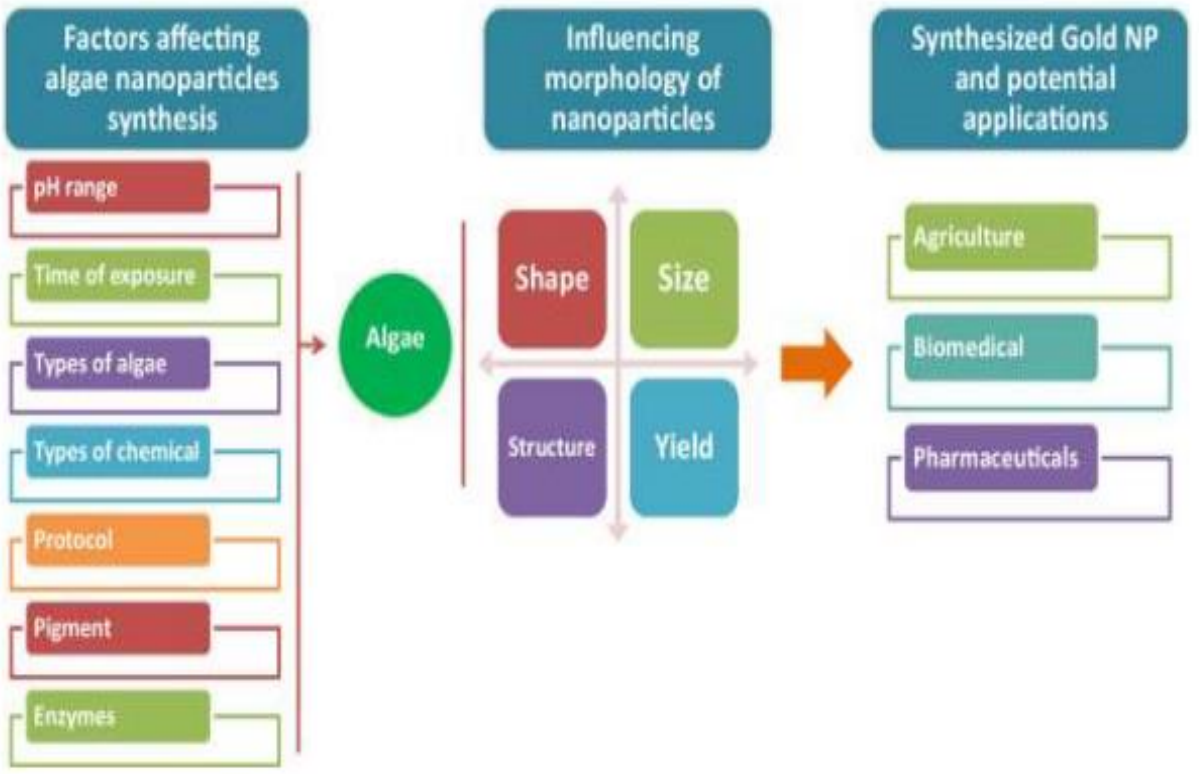


Figure 1.5: Factors Affecting Formation of NPs

1.14 Green Synthesis of nanoparticles:

Using a bottom-up method, the process of creating nanoparticles from plants is known as "green synthesis." Plant extracts are used by scientists to create stable nanoparticles. Plant extracts function as a metal-reducing agent. This approach to producing nanoparticles from plants is both economical and effective. It is the most effective and fastest method for creating nanoparticles. The "caging agent" that lowers metal ions is comprised of amino acids, alkaloids, terpenoids, flavonoids, and other phenolic compounds that are present in plants. There are numerous uses for these plant-derived nanoparticles in the biological sciences.For the manufacturing of nanoparticles, plants are regarded as "green nano factories". Scientists from several fields, including biology, chemistry, and engineering, are keen to create nanoparticles (NPs) from plants and explore their potential applications. Because they are readily available, safe, and nontoxic, contain a large number of metabolites that can aid in the reduction of iron ions, and can produce nanoparticles more quickly than microorganisms, plant extracts are excellent for this kind of application. A photobiological method of producing nanoparticles was introduced as a result of the increased interest in cutting prices, time, waste, and other factors. This resulted in the creation of easy and environmentally favourable ways to produce nanoparticles. Green nanoparticles manufactured from plants and their parts have been produced before. Different kinds of nanoparticles have already been produced using plants and their parts. Originally, metal nanoparticles were made from the entire plant. To create NPs, one can utilise extracts, whole plants, plant dry mass, or pure phyto-compounds like cellulose, glucose, and starch.

1.15 Role of plant biomolecules in green synthesis of nanoparticles:

Plant-derived primary metabolites such as reducing sugars, protein peptides, and amino acids are crucial for the stabilization and reduction of metallic nanoparticles. Polysaccharides also play a role in nanoparticle synthesis. In the production of nanoparticles, beta D-glucose acts as a reducing agent, while starch serves as a stabilizer. Aloe vera contains natural phytochemicals that function as both capping and reducing agents, making it ideal for nanoparticle formation. Rosmarinic acid, the predominant polyphenol in C. aromaticus, is likely involved in the reduction of metal ions (M^+) to nanoparticles (M^0), contributing to its antioxidant properties. Dioscorea bulbifera tuber, rich in flavonoids and phenolic acid derivatives, is another excellent source for nanoparticle production. Flavonoids play a vital role in the reduction process, facilitating the conversion of M^+ to M^0. Pulicaria glutinosa extract contains phenolic compounds that are key to reducing iron ions. Additionally, the high concentration of carbazoles in curry leaf has demonstrated significant antioxidant activity. In Astragalus gummifer aqueous extract, hydroxyl and carbonyl groups contribute to the reduction of metal ions, with proteins in the plant extract capping the monovalent metal ions to prevent further aggregation. Various organic compounds in crude plant extracts, including pigments, alkaloids, polyphenols, and proteins, are believed to aid in nanoparticle formation. The natural abundance of metabolites with negatively charged functional groups in plant extracts may account for the reduction of metal ions and the stability of the produced nanoparticles. Green chemistry and engineering offer significant social and economic benefits, which have led to increased support from governments and scientific communities for sustainable solutions.

1.16 Possible mechanism involved in the green synthesis of NPs:

Nanoparticles can be produced both in living plants and in sundried biomass, and various researchers have suggested different mechanisms to understand this process. Initially, nanoparticles accumulate when metal ions are reduced by reducing agents or enzymes attached to the cell wall, which mediate this reduction process. The creation of spherical nanoparticles from the fungus *P. glomerata* involves activation, nucleation, and reduction mechanisms.

During the green synthesis of nanoparticles, when leaf extract is added to aqueous $MgCl_2/FeCl_2$, ionization occurs as follows:

- $FeCl_2\ (aq) \leftrightarrow Fe^{2+}\ (aq) + 2Cl^-\ (aq)$
- $MgCl_2\ (aq) \leftrightarrow Mg^{2+}\ (aq) + 2Cl^-\ (aq)$

Biomolecules in plants act as electron donors, while metal ions serve as electron acceptors. Chlorophyll pigments function as stabilizing agents between donor and acceptor molecules. These biomolecules enable the reduction of iron ions to metal by serving as the main reducing agent in the reaction. Various oxidizing and reducing agents are employed for both oxidation and reduction processes. The $-CH_3$ group in plant extract interacts with metal ions, contributing to nanoparticle formation and stability.

According to this study, reduced cofactors or biosynthetic products play a crucial role in the reduction of salts to nanoparticles. The reduction of M^+ to M^0 can be explained by the glycolysis mechanism:

- $Glucose + 2ADP + 2Pi + 2NAD^+ = 2\ Pyruvate + 2ATP + 2NADH + 2H^+$

Nicotinamide adenine dinucleotide, or NAD, is an eco-enzyme that is produced during glycolysis and is found in all living cells. By absorbing electrons from other molecules, NAD functions as an oxidising agent and can reduce metal ions to nanoparticles. Antioxidant metabolites and enzymes in plants work together to protect cellular components from oxidative damage. The green synthesis of metal nanoparticles involves reduction, capping, and stabilization, thanks to the presence of enzymes, proteins, sugars, and other biomolecules in plant extracts.

While different phytoconstituents may follow different mechanisms, ion reduction remains the primary process. The exact method by which plants produce nanoparticles is still not fully understood. Biomolecules are believed to play a direct role in the reduction of ions and nanoparticle formation. Although biochemical mechanisms emphasize the importance of phytoconstituents in green synthesis, the molecular mechanisms behind nanoparticle green synthesis remain largely unknown.

1.17 Use of *Camellia sinensis* leaf extract for the synthesis of ZnO nanoparticles:

Camellia sinensis, better known as the tea plant, is highly valued for its leaves, which are packed with bioactive compounds like polyphenols, flavonoids, catechins, and tannins. These compounds have potent antioxidant, antimicrobial, and anti-inflammatory properties, making the leaves useful not only for traditional tea production but also for various biomedical and industrial applications. The rich content of these phytochemicals in *Camellia sinensis* leaves has sparked interest in green chemistry, particularly for the eco-friendly synthesis of metal nanoparticles, such as zinc oxide (ZnO) nanoparticles.

The synthesis of ZnO nanoparticles using *Camellia sinensis* leaves involves an environmentally friendly approach that takes advantage of the natural reducing and stabilizing agents found in the leaf extract. In this process, the bioactive compounds in the tea extract reduce zinc ions (Zn^{2+}) to form ZnO nanoparticles, while also acting as capping agents to prevent clumping, thus controlling the size and shape of the nanoparticles. This method is more beneficial than traditional chemical synthesis methods because it avoids the use of toxic chemicals, minimizes environmental impact, and often results in nanoparticles with improved biocompatibility.

ZnO nanoparticles produced using *Camellia sinensis* leaf extract offer a variety of beneficial properties, including high surface area, photocatalytic activity, and strong antimicrobial effects. These qualities make them suitable for a wide range of applications, including UV protection in cosmetics, antibacterial coatings and wound healing in biomedical fields, pollutant degradation in environmental applications, and the development of sensors and other devices in electronics. This green synthesis approach not only aligns with the principles of sustainable chemistry but also opens up new possibilities for the use of natural resources in advanced material science.

1.18 Applications:

Biopolymer films infused with nanoparticles offer the benefits of both eco-friendly, biodegradable biopolymers and the enhanced functionalities of nanoparticles, resulting in a wide array of applications across multiple industries. In food packaging, these films provide superior antimicrobial and barrier properties, helping to extend shelf life and ensure food safety. In the medical field, they are utilized in wound dressings, drug delivery systems, and antimicrobial coatings, offering protection and controlled release of therapeutic agents. For environmental applications, they are effective in water treatment and air purification, as nanoparticles aid in breaking down pollutants. In agriculture, these films safeguard crops from pathogens and improve soil quality. Additionally, they are used in cosmetics for better ingredient delivery and in electronics for flexible, sustainable devices. This innovative approach results in multifunctional materials that meet the demands of modern technology and sustainability.

1.19 Antibacterial activity:

Nanoparticles have the potential to serve as alternatives to conventional antibiotics. An aqueous solution of iron nanoparticles demonstrated antibacterial activity against ***E. coli***. The green synthesis of nanoparticles enhances their suitability for antimicrobial research.

1.19.1 Mechanism:

Nanoparticles rupture the bacterial cell membrane and enter the cell, where they interact with proteins, leading to their denaturation, and cause damage to DNA and RNA, ultimately resulting in cell death.

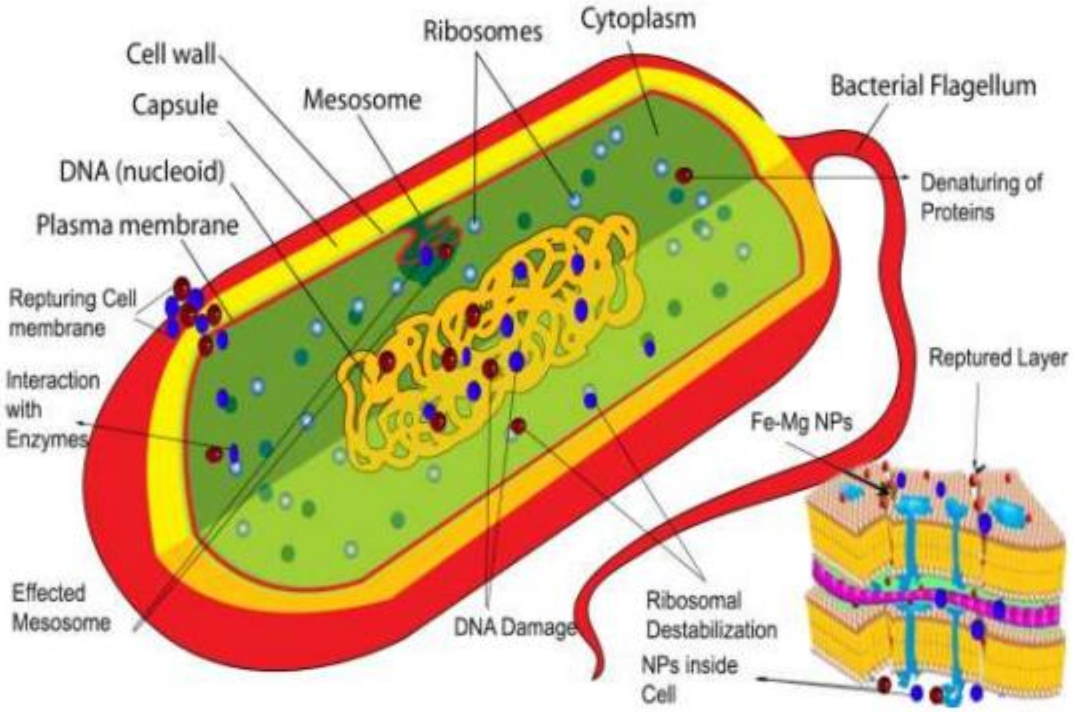


Figure 1.6: Action of Nanoparticles on Bacteria

1.20 Food packaging:

Food packaging must prioritize safety protocols since human health is the top priority. Traditional food packaging materials are vulnerable to oxidation and microorganisms, so it's essential to develop packaging materials that are resistant to both.

Chapter 2 LITERATURE REVIEW

2.1 Biopolymers

Aqsa Arooj et al. stated that by strengthening their characteristics through natural plasticisers, heat crosslinking, and hydrogen bonding interactions, bioplastic films derived from starch, pectin, and chitosan provide environmentally beneficial options for food packaging. These films are completely biodegradable and environmentally sustainable due to their amorphous qualities. Mechanical tests that demonstrate their appropriateness for packing applications include toughness, elongation at break, and tensile modulus. Notably, the synthesis of these bioplastics is sustained by the use of pectin derived from banana peels, which adds to their environmental friendliness.[26]

S. Chodijah et al. studied that the properties of biodegradable plastic films are improved by pectin derived from banana peels, which also helps to increase the films' tensile and water resistance. Citric acid is used to aid lessen browning, which enhances the films' quality even further. These bioplastics can be used to package food, and edible films made of pectin provide a useful and environmentally favourable substitute for more sustainable packaging options.[27]

Raffaele Porta et al. investigated the application of transglutaminase (TGase) in the synthesis of biodegradable films using natural resources, with a focus on its function in protein crosslinking in edible films. Because of their improved functional qualities, it emphasises the potential of TGase-crosslinked films for a variety of applications, especially in food packaging and coatings.[28]

Danila Merino et al. stated that the interaction between chitosan and pectin molecules improves overall performance, and the produced biocomposites for food packaging show better characteristics and biodegradability. The films maintained their transparency and antioxidant capacity even after losing their antibacterial activity, which makes them appropriate for food packaging applications where these qualities are crucial.[29]

Teresa I.A. Gouveia et al. stated that for food packaging, pectin films with plasticisers like ChCl or NADES are being developed. These films exhibit better water resistance and mechanical qualities, particularly when glycerol is employed as the plasticiser. These films' quality is further improved by the application of compression moulding, underscoring its potential for usage in environmentally friendly packaging.[30]

Gabriela Abdalla et al. concluded that lemon oil-infused chitosan films for fruit preservation showed antibacterial activities; the films' thermal characteristics and crystallinity were influenced by the release of essential oils. The antibacterial activity of the lemon oil was increased by the nanoemulsified form's improved interactions with the polymer matrix. The inclusion of lemon oil decreased crystallinity, which affected the films' thermal stability even though the films' mass loss after storage showed no discernible variations.[31]

2.2 Nano Technology

Ananda et al. investigated that the word "nanotechnology" is used today to refer to a broad spectrum of global research projects. It is the investigation of materials on a nanoscale. Clusters of atoms make up nanoparticles, which typically have a diameter of less than 100 nm. Nanotechnology is swiftly becoming the most revolutionary scientific discipline and will have a huge impact on many other fields in the future. It is focused on creating nanomaterials with different sizes, shapes, and chemical compositions as well as exploring how they could be used in human applications.[32]

Husen and siddiqi believe that since nanotechnology has so many novel and profitable uses, it is currently one of the most engaging and intriguing areas of research. Numerous industries, including food, agriculture, electronics, medicine, cars, information and communication technology, energy, textiles, and construction, have been prompted to invest and restructure their futures as a result of nanotechnological advancements.[33]

Manivasagan et al. investigated that in the current century, nanotechnology is predicted to have a significant positive impact on a number of critical scientific fields, including energy, paints, industries, medicine, and electronics. Therefore, one key component of green chemistry that integrates biotechnology and nanotechnology is the production of nanoparticles. Microbes and plants can also facilitate the production of nanoparticles with desired size and surface characteristics. In actuality, a wide range of biological characteristics, including antioxidant, antibacterial, anticancer, anti-biofueling, antimalarial, and antiparasitic, can be found in nanostructures produced by microorganisms and plants.[34]

Liz-Marza and Kamat stated that nanotechnology can be created by starting with nanoparticles. Nanomaterials, nanoscale particles, nanoscale materials, nanosized particles, nanosized materials, nanoobjects, and nanostructured materials are only a handful of the terminology used to characterise these microscopic objects. Extensive study has been conducted recently on noble metal nanoparticles (NPs).[35]

2.3 Green Synthesis

Kavitha et al. proposed that utilising extract from fifty distinct plants with a wide range of biomolecules, nanoparticle biosynthesis is able to stabilise nanocrystals and speed up the reduction process. The expanded nature of plants during the nano revolution has made bio and green production of nanomaterials very interesting in the search to create stable nanoparticles. They came to the conclusion that using green-synthesised nanoparticles can replace traditional techniques, which have a number of disadvantages.[36]

Kaushik et al. provided a view on environmentally friendly synthesis of nanoparticles that is less toxic, more economical, and uses less energy than other standard processes that are rife with issues such the use of dangerous solvents for health, toxic byproducts, and energy waste. To create nanomaterials in this sense, they employed a variety of plant extracts, bacteria, yeast, fungi, and algae.[37]

Vithiya and Sen prepared a review on the biosynthesis of nanoparticles. With an emphasis on green synthesis, they provide a comparison of several synthetic techniques. Additionally, their research highlighted the shortcomings of current techniques and looked into the potential for biological synthesis of nanoparticles in the future, briefly describing their uses.[38]

Shah et al. stated that the fields of biotechnology and nanobiotechnology have recently come together to form a new discipline called nanobiotechnology, that involves the use of diverse plants, algae, yeast, fungi, viruses, and bacteria to generate nanoparticles with distinct advantages over previously used approaches. They showed how the characteristics of nanoparticles are very different from those of the larger-scale counterpart of the same material.

Their review aimed to provide an overview of current developments in the biosynthetic approach of nanoparticle production.[39]

Mohanpuria et al. investigated that reduction/oxidation processes are most frequently employed in Ag nanoparticle production. Because the reaction happens in a single step, molecules having both capping and reducing characteristics are chosen.[40]

2.4 Green Synthesis of ZnO Nanoparticles

Shabnam Fakhari et al. discovered that using zinc acetate/nitrate and *Laurus nobilis* leaf extract as precursors, zinc oxide nanoparticles (ZNPs) were created. The ZNPs were identified by unique absorption peaks in UV-Vis spectroscopy at 350 nm. The high purity, structure, and average particle sizes of 21.49 nm and 25.26 nm were validated by XRD examination. The ZNPs' spherical forms, which varied depending on the precursors employed, were shown by SEM. ZnO production and the presence of phytochemicals were confirmed by FT-IR measurement, and the excellent purity of the synthesised ZNPs was further confirmed by EDX analysis.[41]

Happy Agarwal, S. et al. stated that ZnO nanoparticles (ZnO NPs) can be synthesised environmentally friendly by using a range of biological sources, such as algae, plants, fungus, and bacteria. Particularly, plant extracts function as stabilising and reducing agents to create very stable and pure nanoparticles. For the production of ZnO NP, algae species such as *Sargassum muticum* are also utilised. We examine the effects of conventional heating and microwave irradiation on the characteristics of synthesised nanoparticles. The structure, stability, and purity of these biosynthesised nanoparticles are examined using a variety of characterisation techniques.[42]

Marina Bandeira et al. investigated that using a variety of biological substrates, green synthesis techniques for ZnO nanoparticles (ZnONPs) are environmentally benign and encourage the creation of stable nanoparticles with improved characteristics. Green chemistry principles are applied in these procedures, and the biological extracts that are utilised determine the mechanism of ZnONP production. Neutral pH conditions are ideal for synthesising high-quality ZnONPs, while pH fluctuations play a major influence. The review also highlights the sustainable and environmentally friendly character of these approaches by summarising several synthesis methodologies and their mechanisms.[43]

Gunalan Sangeetha et al. stated that ZnO nanoparticles with tunable diameters ranging from 25 to 45 nm and unique polydispersity have been created using aloe vera leaf extract. These nanoparticles' UV-Vis spectra revealed absorption peaks between 358 and 375 nm, and surface plasmon resonance (SPR) had an impact on their optical characteristics. The size and shape of the nanoparticles were mostly regulated by the leaf broth, which had an excitation wavelength of 325 nm. These ZnO nanoparticles, which were created with the aid of aloe vera, have special qualities and might be used in cosmetics and biomedicine.[44]

R. Sharmila Devi et al. stated that ZnO nanoparticles with diameters between 30 and 35 nm are produced quickly, steadily, and environmentally friendly through the green synthesis of ZnO nanoparticles utilising *Hibiscus rosa-sinensis* leaf extract. The size and form of the synthesised nanoparticles were ascertained by SEM analysis, while XRD analysis verified the presence of ZnO by distinctive peaks. An effective way to produce ZnO nanoparticles with regulated structure and characteristics is provided by this plant-based technology.[45]

Sunday Adewale Akintelu et al. investigated that plant extracts are used in the biological synthesis of zinc oxide nanoparticles, or ZnO NPs, which is becoming more popular due to its environmentally friendly method and wide range of uses in textile, medical, and agricultural fields. Plant extracts are essential for lowering and stabilising ZnO NPs, which are identified by methods such as XRD, TEM, and SEM to ascertain their characteristics. Apart from their antibacterial properties resulting from the induction of reactive oxygen species (ROS), ZnO nanoparticles hold considerable promise in a number of industries. Although physical and chemical synthesis techniques are explored, a sustainable substitute for creating ZnO NPs with a variety of uses is plant-extract-based synthesis.[46]

Mahesh Midatharahalli Chikkanna et al. discovered that the utilisation of agricultural waste materials as reducing agents in the green synthesis of zinc oxide nanoparticles (ZnO NPs) presents an economical and sustainable approach. Strong antibacterial action against *Salmonella* and *Bacillus* species is demonstrated by the synthesised ZnO NPs, which are distinguished by tiny, spongy, flower-like structures. This environmentally friendly method emphasises ZnO NPs' potential for use in antibacterial therapies.[47]

2.5 Polymer Composites

Basheer Aaliya et al. stated that the potential of biopolymer composites to improve plastic products while lowering carbon emissions and promoting environmental sustainability is drawing attention. Biopolymer-reinforced natural fibres have better mechanical qualities, which makes them appropriate for applications requiring load carrying. The goal of research is to find novel natural fibres and enhance fibre processing for usage in composites that can decompose naturally. These materials exhibit considerable promise in global markets, with growing uses in the automotive and medical sectors.[48]

Kishor Kumar Sadasivuni et al. discovered that they are biodegradable and renewable, biopolymers provide excellent replacements for synthetic polymers. These materials' mechanical qualities and commercial worth are greatly improved by reinforcement, particularly when fillers such nanometals are included. Biopolymer composites are becoming more and more significant in a variety of industries due to their versatility and uses in the fields of building, electronics, and medicine.[49]

Sung-Bin Park et al. find out that biopolymer composites are improving applications such as bone and cartilage regeneration, and are becoming increasingly important in tissue engineering and regenerative medicine. Nanomaterials have been added to hydrogels to enhance activities including cardiac cell coupling, while gelatin-based nanoparticles have been shown to exhibit pH-responsive behaviour in drug administration. The value of raw biopolymers is increased by these functional composites, which makes them suitable for barrier membranes in tissue regeneration and other medicinal applications.[50]

I.T. Norton et al. stated that weak interfaces in biopolymer composites can lead to void development prior to failure, whereas strong interfaces improve reinforcing but lessen the strain associated with failure. Shear effects, kinetics, and phase behaviour all affect the droplet deformation and phase inversion that mixed biopolymer systems display. These characteristics are important for the production of food, which emphasises the necessity for more study to maximise material performance.[51]

Yabin Guo et al. investigated that composites based on chitosan have a variety of functional characteristics that make them excellent choices for biomedical applications. The combination of chitosan and other biopolymers has produced some noteworthy innovations, such as injectable, self-healing, and antibacterial properties. Reactive radical species are also employed in these composites to generate covalent bonds, which further improves their biofunctional qualities and makes them an important tool in the medical domain.[52]

Elke Scholten et al. stated that emulsifiers may become less stable due to environmental stressors, which will weaken emulsions. Fibrillar assemblies, on the other hand, improve stability and regulate the release rates of encapsulated components by reinforcing emulsion surfaces and forming gel-like structures. Food architecture can be changed by modifying the ways in which ingredients interact with one another. Emulsion stability is further improved by methods such as layer-by-layer assembly, which regulate interfacial strength and permeability.[53]

2.6 Antibacterial Application

Lemire et al. explored that composites made of metal can be employed as antibacterial agents in place of antibiotics. Recent research has shown that metal nanoparticles can harm microorganisms through oxidative stress, protein malfunction, and membrane damage, among other effects. Toxicological principles control chemical antibacterial action, and the range of metals was covered in this context.[54]

Lee et al. stated that iron nanoparticles in an aqueous solution against E. coli that shown antibacterial action. It was discovered that activity increased under de-aerated circumstances and that a high concentration of nanoparticles was needed in air saturation conditions. The cell membrane was damaged by nanocrystals, and oxidative stress was brought on.[55]

Naseem and Farrukh et al. examined the antibacterial capabilities of iron nanoparticles, which were created by dissolving sieved powder of Lawsonia inermis and Gardenia jasminoides plant leaves in deionised water, employing a well-diffusion approach to combat Salmonella enterica, Escherichia coli, Proteus mirabilis, and Staphylococcus aureus.[56]

Awwad et al. identified nanoparticles as a possible substitute for traditional antibiotics. It was discovered that the reaction rate was influenced by the content of the extract from Olea europaea leaves, the concentration of metal salt, temperature, and time. The produced nanomaterials were examined using SEM, XRD, AAS, FT-IR, and UV-Vis spectroscopy, and their antibacterial efficacy was tested against waterborne pathogens such as Listeria monocytogenes, Shigella, and Staphylococcus aureus.[57]

Vergheese and Vishal found that the nanoparticles' environmentally friendly manufacturing made them appropriate for antibacterial research. The size, shape, functional group, crystal structure, and composition of the nanoparticles were assessed using FT-IR, XRD, SEM-EDX, UV-Vis, and GC-MS methods. Nanoparticles were examined for antibacterial action against both gram-positive and gram-negative bacteria, and the results showed that they were effective.[58]

Abdel-Aziz et al. claimed that silver and its antibacterial qualities are well-known and are becoming more significant in this field. They attempted to synthesise nanoparticles and evaluate their antibacterial and antioxidant capabilities using green extract from Chenopodium murale leaves. The size of the nanoparticles was found to vary between 30 and 50 nanometres by a variety of analytical methods. Nanomaterials exhibited more activity in comparison to plant extracts.[59]

2.7 Antioxidant Activity

Valgimigli et al. aimed to classify nanomaterials according to the mechanism of action of their antioxidants and examined the techniques for estimating antioxidant potential by outlining the chemistry of these mechanisms. When searching for higher-quality antioxidants, one of the most promising frontiers is represented by nanoparticles.[60]

Divya and Anand sought to determine whether the ethanolic extract of Terminalia catappa Linn possessed any antioxidant qualities. According to their research, T. catappa leaf extract exhibits antioxidant characteristics, which may account for the plant's therapeutic benefits. The plant material was extracted using Soxhlet extraction, using ethanol solvent. Using the DPPH, nitric oxide, and H2O2 tests, the reducing power was determined. In all investigations, the ethanol-based T. catappa extract was revealed to have concentration-dependent antioxidant power.[61]

Patra et al. stated that the antibacterial and antioxidant capabilities of nanoparticles made from maize ear leaf extract were examined. Their antibacterial and antioxidant qualities make them formidable candidates for usage in pharmaceutical and medical applications.[62]

Mitiku and Yilma focused on silver nanoparticles made from an aqueous extract of Moringa stenopetala leaves and investigated their potential as antioxidants and antimicrobials. FT-IR, XRD, and UV spectroscopy were employed to analyse the generated nanomaterials. Nanocrystals' antimicrobial and antioxidant qualities were proven.[63]

Mahdi-pour et al. found that different portions of the L. Cammara extract showed good antioxidant activity and might be used medicinally. To test antioxidant activity, they employed the Griess-Ilosvay method, xanthine oxidase antioxidant activity, and DPPH essay.[64]

Thilagavathi et al. stated that the antioxidant activity of Ag nanoparticles made by bioreducing AgNO3 and different amounts of plant extract from Limonia acidissima was investigated. The creation of nanoparticles was verified by UV visible analysis, and SEM was used to characterise the particles. According to the phosphomolybdenum technique, the nanomaterial exhibited strong antioxidant properties.[65]

Proestos et al. investigated that plant oils and extracts from across Greece that have been reflux-prepared with methanol for their antioxidant capabilities. DPPH and the ABTS essay were used to confirm antioxidant activity. The Rancimat test demonstrated a clear defence against lipid oxidation, while Folin-Ciocalteu demonstrated the presence of antioxidants in the extract.[66]

2.8 Water uptake and Mass loss

Iryna Yakimets et al. the elastic characteristics of biopolymer films are strongly influenced by their water content, and this effect varies based on the particular substance. The elasticity of hydroxypropyl cellulose (HPC) sheets decreases exponentially with an increase in water content, resulting in the lowest elasticity modulus and maximum plastic deformation. Gelatin films, on the other hand, are more hydrophilic than HPC and cassava films. Variations in the impact of water content on the elasticity of different biopolymer films are highlighted by the fact that water-biopolymer interactions are impacted by structural differences and can be reliably predicted by water sorption models.[67]

Jong-Whan Rhim & Perry K.W. Ng stated that clay addition particularly enhanced the mechanical and barrier qualities of chitosan-based nanocomposite films. After different treatments, soy protein-based films showed comparable improvements. The performance of nanocomposite materials was further improved by the organophilicity of montmorillonite

(MMT), which made it more compatible with organic polymers. Tensile strength and water vapour barrier qualities were also significantly improved in thermoplastic starch (TPS)-MMT hybrids.[68]

Farhad Garavand et al. discovered that biopolymer films' mechanical, thermal, and physicochemical characteristics are greatly enhanced by crosslinking, which also raises the films' melting and thermal stability temperatures. When compared to nanomaterial reinforcement, this technology is more economical, and natural crosslinking agents provide a healthier and more environmentally responsible option. Thus, crosslinking offers a useful and effective way to improve the performance of biopolymer films.[69]

E. Kristo et al. investigated that higher tensile strength and Young's modulus were observed in pullulan films, whereas sodium caseinate films showed greater elongation at break. Beeswax lamination led to higher elongation at break and decreased tensile strength, but it also dramatically decreased water vapour permeance. Pullulan and sodium caseinate proportions had a detrimental impact on film characteristics, and their ratios had an impact on tensile strength. Water vapour permeability was also significantly influenced by film thickness, particularly in pullulan-sodium caseinate films. In general, the performance of these composite films is greatly influenced by the selection of components, their ratios, and the thickness of the film.[70]

Ho Jin Kang et al. stated that the moisture sensitivity of films was affected by both high-pressure homogenisation and glycerol content, with the latter having a greater impact than the former. Higher glycerol content films showed increased moisture sensitivity and water vapour permeability, especially those with 50% glycerol. The film viscosity and storage modulus (G') of potato peel-based films were influenced by homogenisation pressure and the number of passes, but the main factor affecting the water uptake and overall moisture sensitivity was the content of glycerol.[71]

2.9 Food Packaging

Llorens, A et al. stated that food contact polymers are enhanced with metallic-based micro- and nano-structured components to prevent plastic photodegradation and to increase mechanical and barrier properties. Colloidal silver zeolites (Silver Zeolites) and heavy metals in elemental nanoparticle form have additional antibacterial qualities. They are used to preserve food and clean work surfaces in industrial environments. Using additional crucial components in active food packaging, like oxygen scavenging or ethylene oxidation, prolongs the shelf life of food.[72]

Ibrahim, S. et al. created a composite of ZnO and TiO2 nanoparticles with polystyrene for use in food packaging using the sol gel technique. Antimicrobial effectiveness against yeast, fungi, Pseudomonas aeruginosa, and Staphylococcus aureus has been reported. This implies that the existing nanocomposite belongs in the category of active packaging materials and that it ought to be suggested for usage in these kinds of applications.[73]

Dey, A. et al. stated that when silver nanoparticles were synthesised utilising bacterial isolate and their mechanical and food packaging qualities were assessed, the PS/Ag nanocomposites showed antibacterial efficacy against test pathogens that were gram-positive, gram-negative, yeast, and fungal. These nanocomposites films can be used to make food packaging materials.

Chapter 3 MATERIALS & METHODOLOGY

The present study entitled as "Fabrication of pectin and chitosan based bioplastic films incorporated with ZnO nanoparticles for food packaging application" was performed at National University of Sciences and Technology, Islamabad. The materials, methodologies and experiments have been elaborated and given as under.

3.1 Materials

3.1.1 Equipment used

Water distillation assembly, Analytical Balance, Vacuum filtration, Electromagnetic stirrer, Sonicator bath, Rotary evaporator, Electric furnace, Oven, Incubator, UV-Vis spectrometer, Autoclave, FTIR, Scanning Electron Microscope, XRD, EDX analysis.

3.1.2 Apparatus/Glassware used

Beaker, Test tube, Conical flask, Vial, Volumetric flask, Petri dish, Measuring flask, Measuring cylinder, Stirrer, Funnel, Petri dish.

3.1.3 Materials/Chemicals used

Distilled water, *Camellia sinensis* leaves extract, Ethanol, Zinc acetate dihydrate, Pectin, Chitosan, L-glutamic acid, Glycerol, Whattman no.1 filter paper.

3.2 Preparations

3.2.1 Preparations of *Camellia sinensis* leaf extract

Took leaves of *Camellia sinensis*, washed them thoroughly and shade dried them for 5 to 7 days. Ground the leaves to fine powder after they got dried. 100 mL of distilled water and 5 g of dry green tea leaf powder were combined, and the mixture was magnetically swirled for two hours at 80 °C. Place it in the refrigerator once it has cooled to room temperature and been filtered with Whattman No. 1 filter paper.

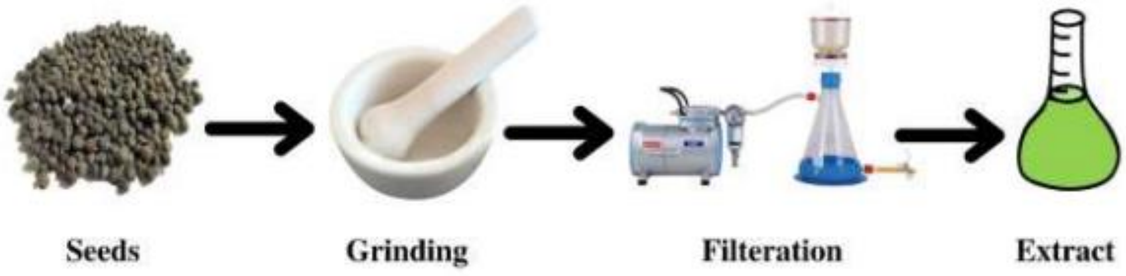


Figure 3.1: Preparation of *Camellia sinensis* Leaf Extract

3.2.2 Synthesis of zinc oxide (ZnO) NPs

Throughout the trials, distilled water was utilised and 90% pure zinc acetate dihydrate was procured from Himedia. After dissolving 0.2 M of zinc acetate dihydrate in 70 mL of distilled water, the mixture was agitated for a short while. 100 mL of distilled water and 5 g of dry green tea leaf powder were combined, and the mixture was magnetically swirled for two hours at 80 °C. This green tea extract was cooled to room temperature and filtered using Whatman No. 1 filter paper. Thirty millilitres of the resulting homogenous mixture was then added to the previously made zinc acetate solution. Pale white ZnO nanoparticles were produced by drying the reaction solution at 60 0C for an entire night. These nanoparticles were then calcined at 100 0C for a single hour and stored in airtight vials for subsequent processing.

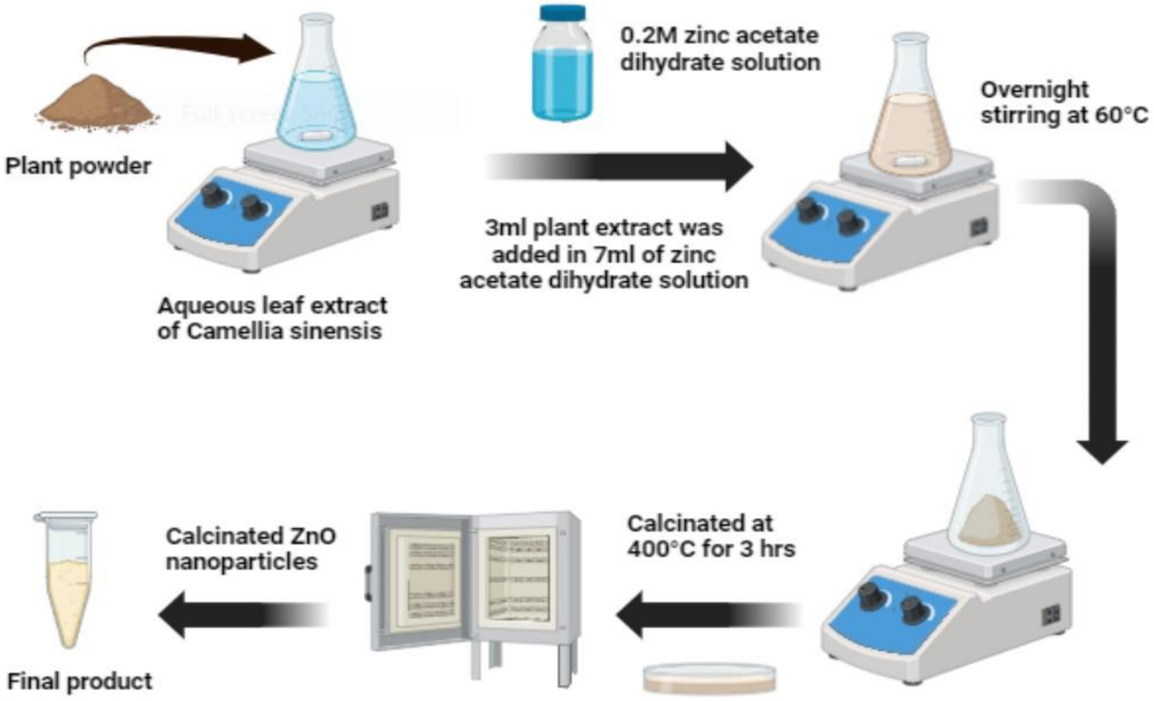


Figure 3.2: Synthesis of ZnO Nanoparticles

3.2.3 Fabrication of Bioplastic Films

In a typical experiment, 40 mg of chitosan was dispersed in 5 mL of distilled water in a 10 mL glass vial, then 20 mg of glutamic acid, 40 mg of pectin, 10 µL of glycerol and 1 mg of ZnO nanoparticles was added to it under constant stirring. The mixture is heated to a clear, homogenous, viscous solution on a hotplate at a temperature between 40 and 45 ^{0}C. For twelve hours, the solution was heated to around 40 ^{0}C on a hotplate that was set over a glass petri dish. Using a spatula or tweezer, the produced film was taken out of the glass petri dish. The following section discusses the varying mechanical and thermal activities that were displayed by the various ratios of pectin to chitosan and the various percentages of nanoparticles.

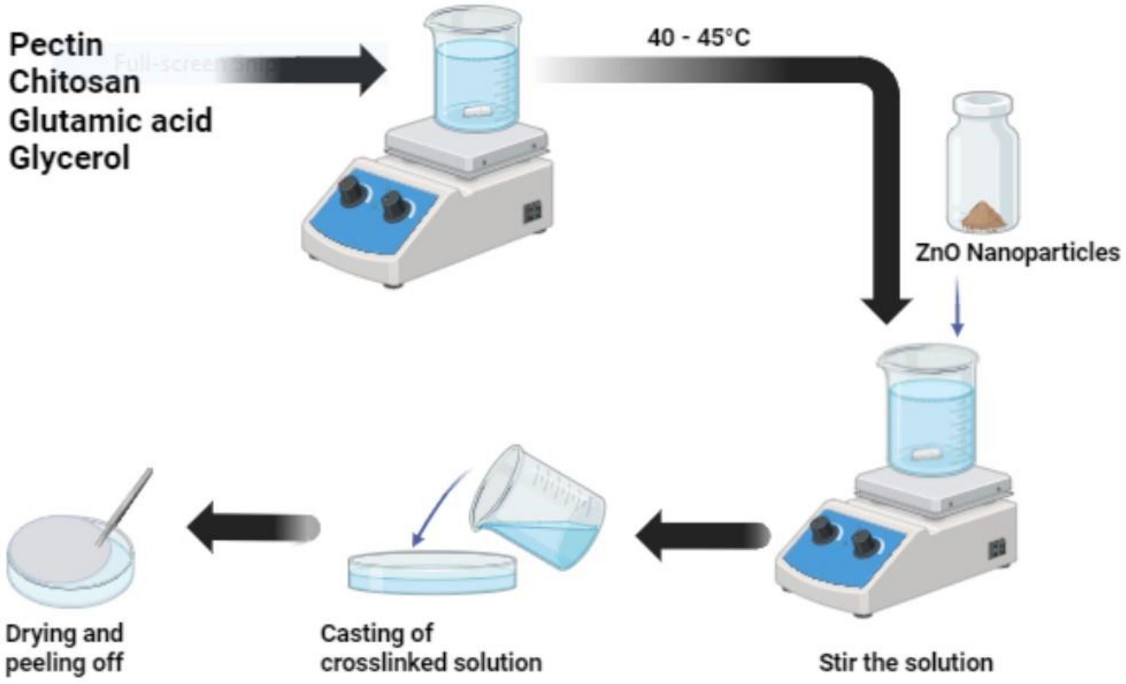


Figure 3.3: Fabrication of Bioplastic Film

3.3 Applications

The synthesized bioplastic films were screened for following activities.

3.3.1 Antibacterial Activity

Antibacterial activity of synthesized bioplastic films was determined by agar disk diffusion method as described by Ruparelia et al. [74]. The antibacterial activity was checked for five different bacteria i.e., *Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Methicillin-resistant Staphylococcus aureus* and *Staphylococcus aureus*.

Composite discs were made by dissolving a solution of all the nanoparticle samples and a plant extract at a concentration of 50 µg/ml in dimethyl sulfoxide. Nutrient agar medium was employed for the antibacterial investigation. After heating, 14 g of nutritional agar was dissolved in 100 ml of double-distilled water. After heating the combination still further to produce a clear solution, it was autoclaved for 20 minutes at 121°C to sterilise it. Nutrient agar was added to petri plates to create the culture medium, which was then allowed to solidify for a full day. After the culture had grown, the inhibitory zones were measured and the bacteria were injected and maintained in an incubator at 37 °C for 24 hours.

3.3.2 Antioxidant Activity

3.3.2.1 DPPH free radical scavenging assay

Antioxidant activity was measured by modified spectrophotometric method of Brand-Williams et al., (1995) [75]. Dispersing nanoparticles and films in ethanol yielded solutions with concentrations of 5, 10, 15, and 20 µg/ml. Similarly, green tea leaf extract was produced in ethanol at the same concentration. The preparation of DPPH (2,2-dipheny1-1-picrylhydrazyl) solution involved dissolving 0.06 g of DPPH in 150 ml of ethanol and letting it sit for 30 minutes. Four sets, with four test tubes each, were labeled as A, B, C and D. To each test tube, 3 ml of the DPPH solution was added. Test tubes A, B, C, and D were filled, respectively, with 0.1 ml of the produced nanoparticle samples A, B, and C at concentrations of 5, 10, 15, and 20 µg/ml and the same concentration of green tea leaf extract solution. The change in color was detected by using UV-Visible technique at 517 nm.

By applying following formula, percentage scavenging was calculated. The positive reference standard was ascorbic acid.

$$\text{\%age Scavenging} = \frac{\text{Absorbance of Control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

3.3.2.2 Total Antioxidant Assay

Phosphatemolybdenum assays were used to measure total antioxidant activity [76]. After mixing together 20 ml of distilled water with 1 ml of 0.6 M of sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate and adding water to make volume 50 ml, the phosphomolybdate reagent solution was prepared. Test tubes containing distilled water and molybdate reagent solution were individually loaded with the hydroalcoholic extract of all samples in concentrations of 5 mg/ml, 10 mg/ml, 15 mg/ml and 20 mg/ml. Incubation time was 90 minutes at 95 ^{0}C.

3.3.3 Biodegradation Assay

The biodegradation assay involved immersing the bioplastic samples in soil for varying durations, such as 24 hours, 72 hours, 120 hours, and 168 hours, and quantifying the deterioration in terms of mass loss (%).Degradation in the soil environment is driven by microorganisms that secrete catalytic agents, such as hydrolase enzymes, which cleave hydrolytic bonds in biopolymers to form new biomass, metabolites, and gas release [77]. It's crucial to remember that while calculating biodegradation rates, weight loss (%) is only taken into consideration as a secondary variable; CO2 evolution is favoured. But unlike in the case of perfect/controlled laboratory testing circumstances, the field experiments in this instance are more difficult since multiple bacteria are involved and the tested bioplastic is not the only source of carbon.[78] [79]

3.4 Food Packaging

Apple slices and lemon were packed in prepared sheets and were kept for 24 hours and 3 days respectively and results were noted.

Chapter 4 RESULTS & DISCUSSION

For analysis of all the samples various characterizations i.e., XRD, SEM, EDS, FTIR and Raman were implemented. Nanoparticles of ZnO and bioplastic films were prepared in the ratios of 1:1, 1:2 and 1:3 with 1%, 2% and 3% of ZnO nanoparticles incorporated in them. Among all, the one with 1:1 and 3% of nanoparticles in them exhibited promising results, so results of 3% are mostly discussed here in detail.

4.1 X-Ray Analysis

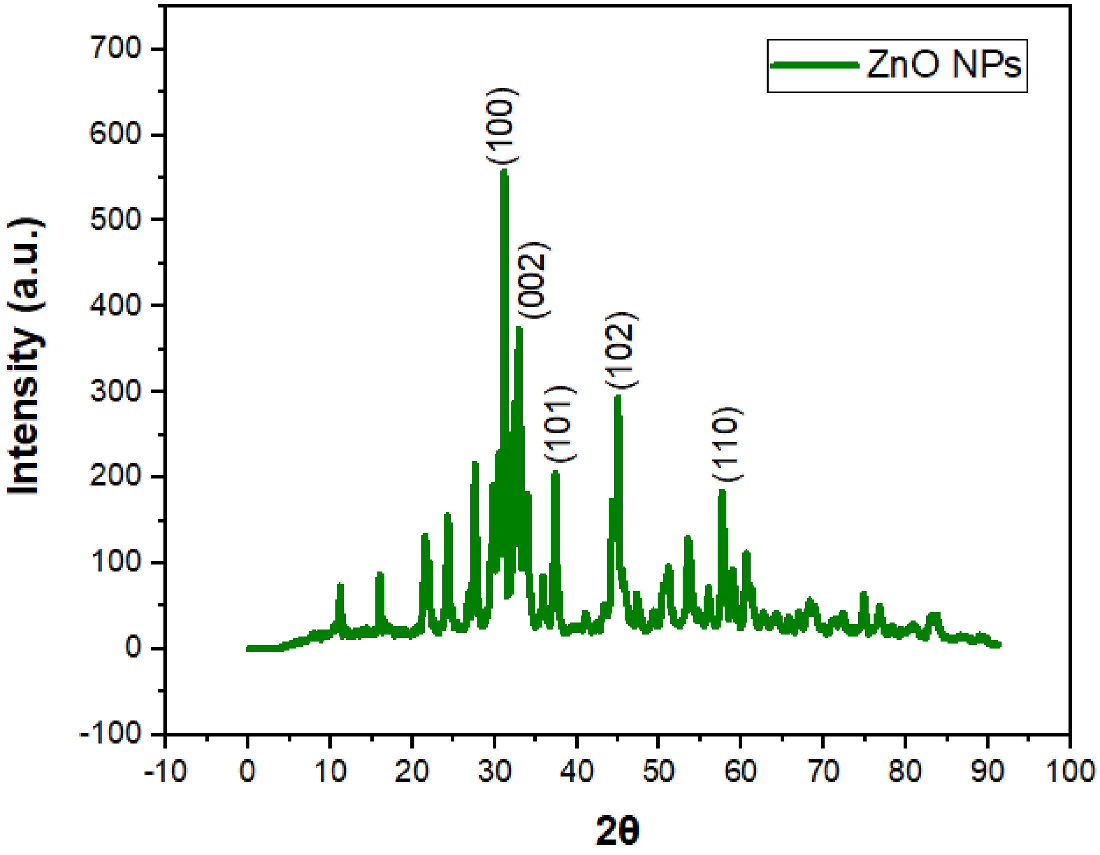
The structural analysis of nanoparticles and fabricated films was done by X-Ray Diffraction using CuKα source and wavelength 1.54 Å.

4.1.1 Zinc oxide (ZnO) Nanoparticles

An X-ray diffraction (XRD) pattern of zinc oxide (ZnO) nanoparticles usually reveals a hexagonal wurtzite crystal structure. ZnO's distinctive diffraction peaks are clearly defined and match up with particular crystalline planes.
The most noticeable peaks in the ZnO XRD pattern are often seen at 2θ values of about 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, 62.9°, 66.4°, 68.0°, and 69.2° of elevation. The diffraction from the wurtzite structure's lattice planes is represented by these peaks.
Based on the conventional ZnO hexagonal phase (JCPDS card no. 36-1451), the lattice planes associated with these peaks are as follows:

- The peak at 31.7° corresponds to the (100) plane.
- The peak at 34.4° corresponds to the (002) plane.
- The peak at 36.2° corresponds to the (101) plane.
- The peak at 47.5° corresponds to the (102) plane.
- The peak at 56.6° corresponds to the (110) plane.
- The peak at 62.9° corresponds to the (103) plane.
- The peak at 66.4° corresponds to the (200) plane.
- The peak at 68.0° corresponds to the (112) plane.
- The peak at 69.2° corresponds to the (201) plane.

The great crystallinity of ZnO nanoparticles is confirmed by these distinct, sharp peaks. The positions and intensities of the peaks reveal information about the size and strain of the particles, which can be further examined using the Debye-Scherrer equation to determine the crystallite size. The diffraction peaks may broaden in addition to these main peaks, which usually happens because of the particles' nanoscale size. The ZnO nanoparticles' lattice strain or smaller crystallite sizes may be indicated by the widening. As a result, the XRD pattern is essential for verifying ZnO's hexagonal wurtzite structure and evaluating the size and calibre of the nanoparticles.[80]



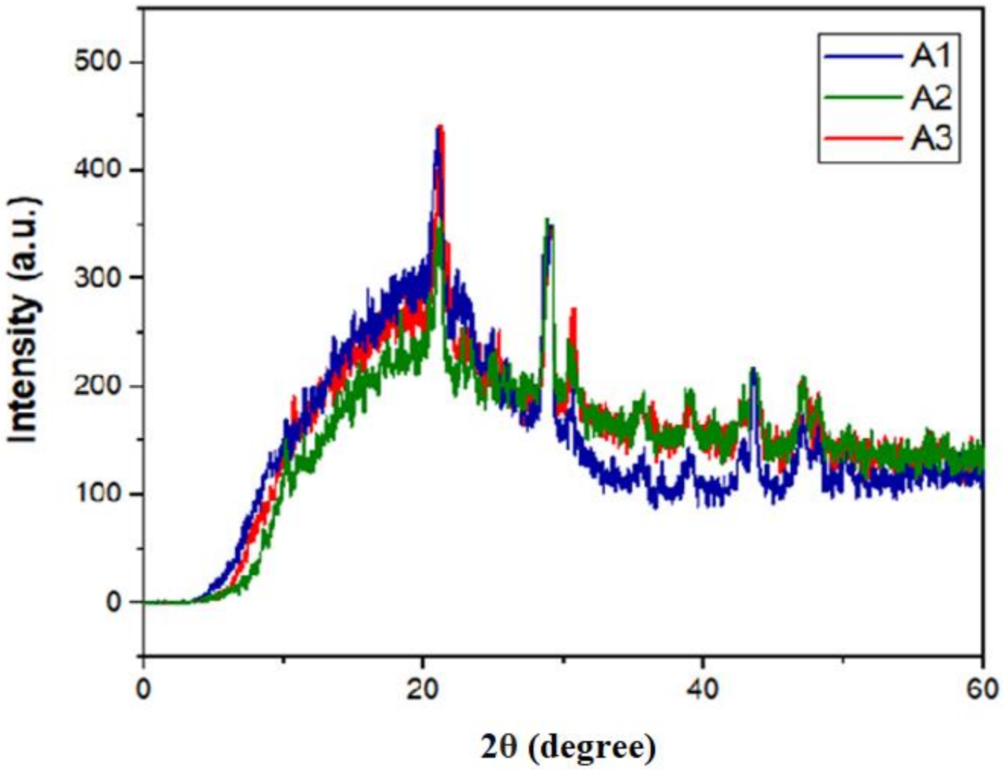
Graph 4.1: XRD Pattern of ZnO Nanoparticles

4.1.2 XRD Pattern of Bioplastic Films (Without Nanoparticles)

For the 1:1 chitosan to pectin ratio, chitosan's inherent semi-crystalline nature dominated. Chitosan is known to form crystalline regions even without covalent bonding, mainly through hydrogen bonding between its chains. As a result, somewhat sharper peaks are observed at specific 2θ values in the XRD pattern, corresponding to the crystalline domains of chitosan. The peaks are found around $2\theta=20^o$, representing the (100) and (101) planes of chitosan. Since pectin is largely amorphous, the overall pattern showed broad features, with the sharp peaks emerging from chitosan's ordered packing.

For the 1:2 chitosan to pectin ratio, the increased presence of pectin, which is highly amorphous, lead to a more dominant amorphous pattern. However, some sharp peaks still persist due to chitosan's residual crystallinity. The sharpness and intensity of the peaks is less pronounced compared to the 1:1 ratio, as the higher amount of pectin disrupts chitosan's crystalline regions. The broadening of peaks indicates a reduction in crystallinity, with fewer and weaker sharp peaks.

In the 1:3 ratio, where pectin becomes more dominant, the XRD pattern shifted towards an even more amorphous structure. Pectin does not typically form crystalline regions, so the contribution from chitosan is significantly reduced. Any sharp peaks from chitosan's crystallinity fainted. The pattern mainly consist of a broad hump at $2\theta=20^o$, indicative of an amorphous material with minimal crystallinity.[81] [82]



Graph 4.2: XRD of Bioplastic Films (Without Nanoparticles)

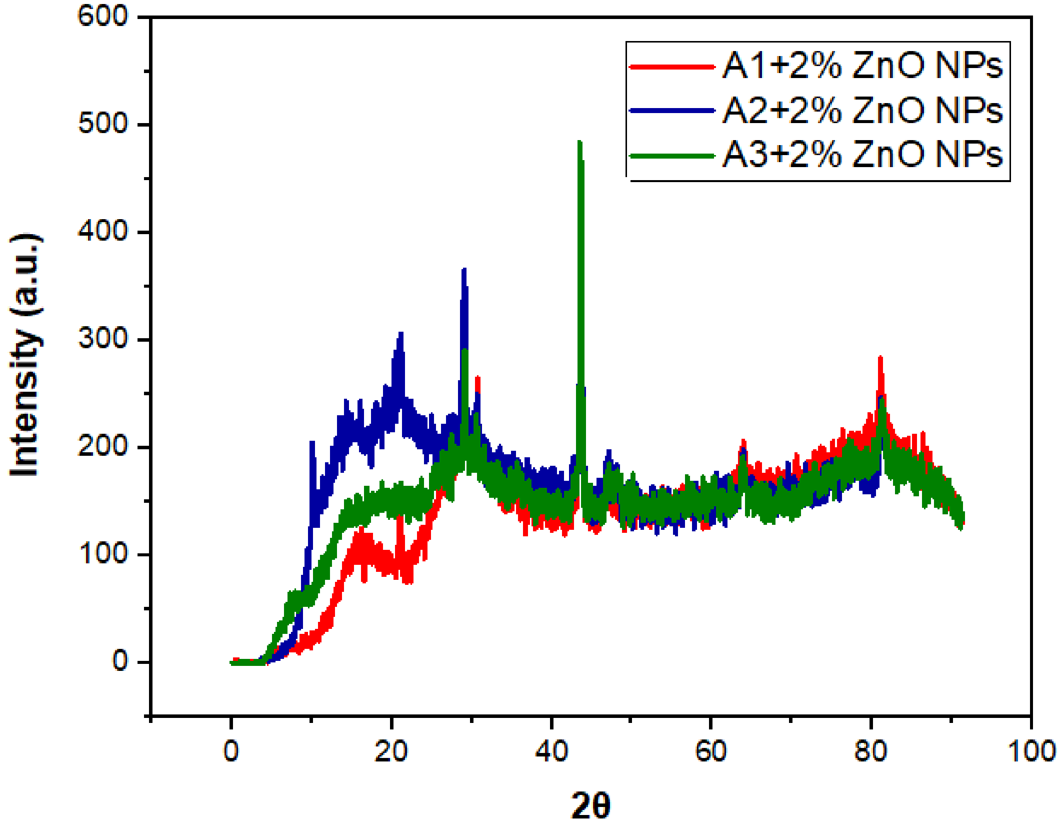
4.1.3 XRD Pattern of Bioplastic Films (With 2% Nanoparticles)

The XRD pattern of bioplastic films made from chitosan and pectin in ratios of 1:1, 1:2 and 1:3, with glutamic acid, glycerol and 2% zinc oxide nanoparticles, would reflect a combination of the structural contributions from each component.

For the 1:1 chitosan to pectin ratio, chitosan, being largely amorphous, would contribute to a broad hump around 2θ=20º, reflecting its disordered molecular structure. In contrast, pectin which tends to exhibit crystalline characteristics, showed some sharp peaks corresponding to its more ordered regions. The incorporation of ZnO nanoparticles introduced distinct sharp peaksat specific 2θ values, such as 31.7º, 34.4º and 36.2º, corresponding to the (100), (002) and (101) planes of ZnO's wurtzite's structure. These peaks stand out against the broad background of chitosan and the somewhat sharper contributions from pectin.

In the 1:2 ratio, the XRD pattern shifted towards increased crystallinity due to the higher pectin content. The presence of more pectin resulted in more noticeable sharp peaks corresponding to its crystalline structure. The overall pattern leaned more toward a semi-crystalline structure with the crystalline peaks from pectin and ZnO dominating, though still blended with the amorphous hump from chitosan.

In the 1:3 ratio, with pectin now being the major component, the XRD pattern showed an even stronger crystalline contribution from pectin. With sharp peaks becoming more prominent. Chitosan’s broad, amorphous hump is still present but less significant compared to the previous ratios. The ZnO nanoparticles continued to display their characteristic sharp peaks, but the overall pattern appeared more crystalline due to the dominant pectin content. The presence of glutamic acid and glycerol have a plasticizing effect, potentially reducing the overall crystallinity, but the sharp ZnO peaks would remain prominent.[80] [81] [82]



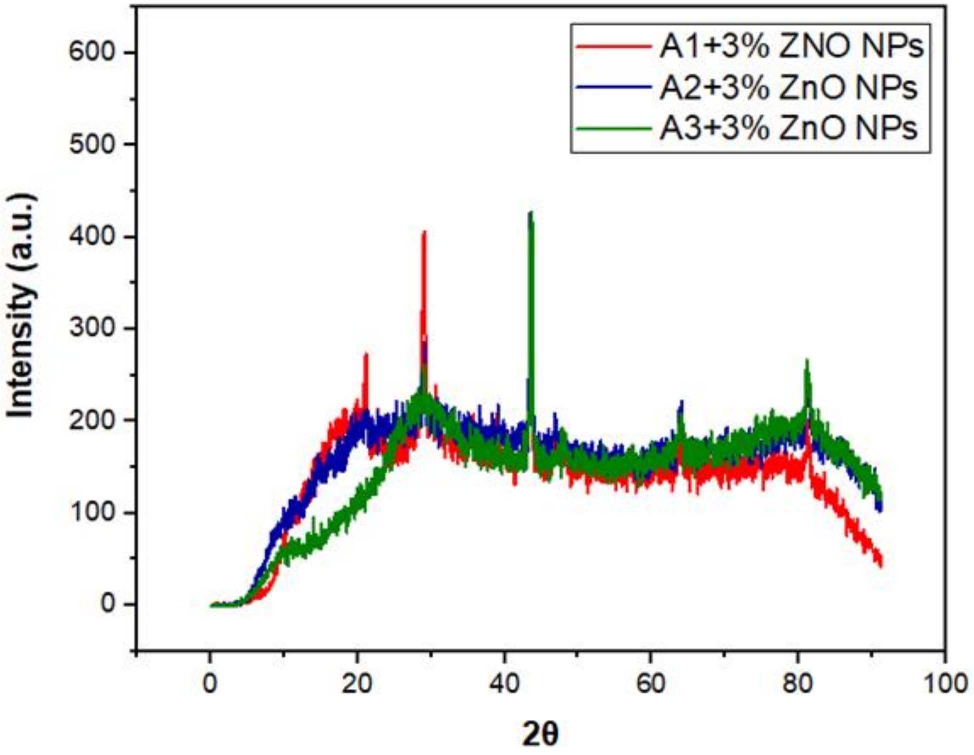
Graph 4.3: XRD of Bioplastic Films (With 2% Nanoparticles)

4.1.4 XRD Pattern of Bioplastic Films (With 3% Nanoparticles)

In the 1:1 ratio, chitosan's broad amorphous hump near 20º blended with pectin's sharper crystalline peaks. The ZnO peaks are distinct and well-defined, superimposed on this mix of amorphous and crystalline features.

In the 1:2 ratio, pectin's crystalline peaks became more pronounced, while chitosan's amorphous contribution is decreased. The ZnO peaks remained strong and sharp.

In the 1:3 ratio, pectin's crystalline peaksdominated the pattern, with chitosan's amorphous background becoming minimal. ZnO's sharp peaks are still prominent, further enhancing the crystalline appearance of film.[80] [81] [82]

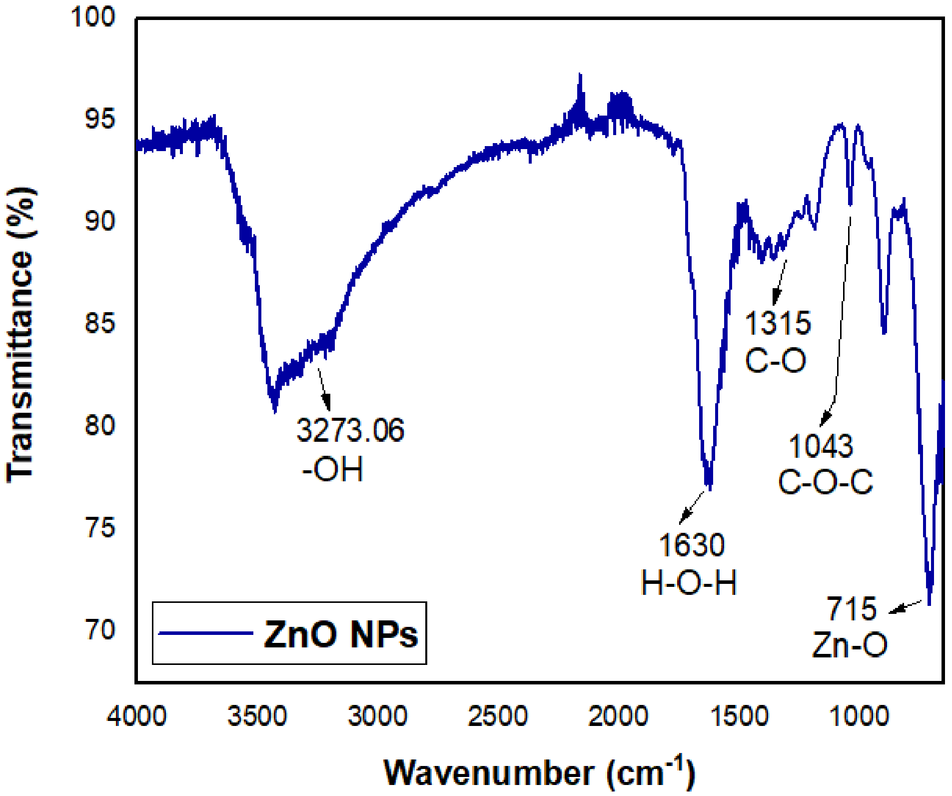


Graph 4.4: XRD Pattern of Bioplastic Films (With 3% Nanoparticles)

4.2 FTIR Analysis

4.2.1 FTIR of ZnO Nanoparticles

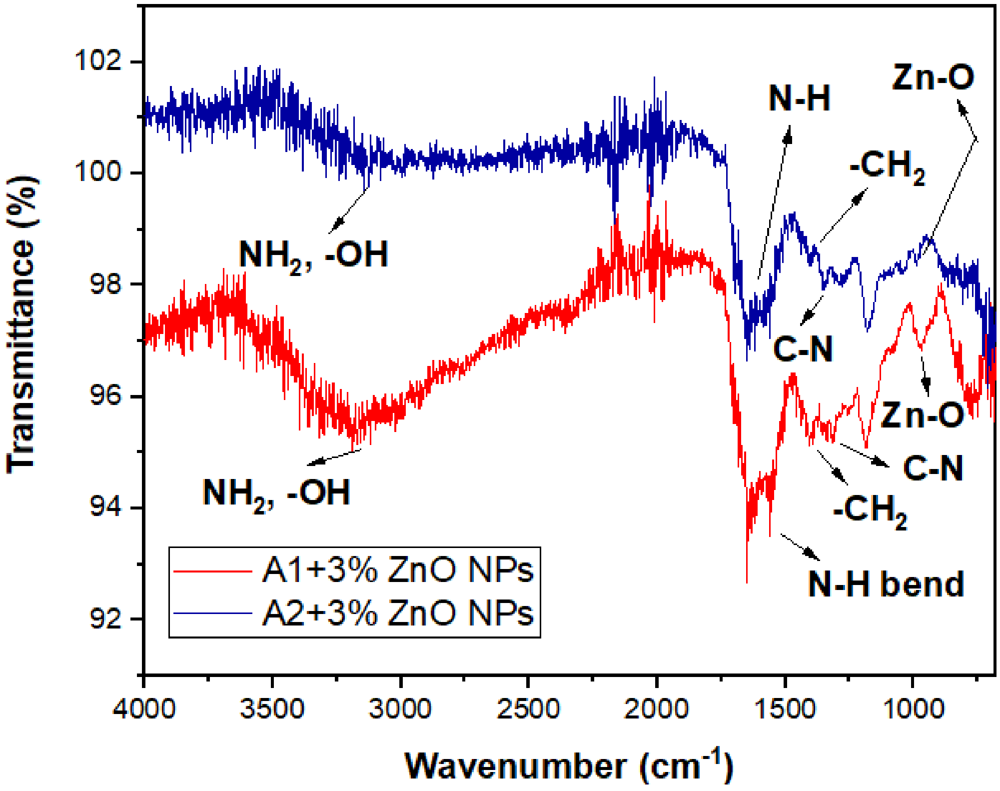
The FTIR spectra of ZnO nanoparticles shows prominent bands at 3273, 1630, 1315, 1043, and 715 cm^{-1}, which can be explained as follows. The band at 3273 cm^{-1} represents O-H stretching vibrations, indicating the presence of hydroxyl groups or adsorbed water molecules on the nanoparticle surface. The 1630 cm^{-1} band is due to H-O-H bending, confirming the existence of surface moisture. The peak at 1315 cm^{-1} is most likely caused by C-O stretching vibrations, possibly from surface-adsorbed carboxylate groups or organic pollutants. The band at 1043 cm^{-1} may represent C-O or C-O-C stretching, implying organic residues or adsorbed ambient CO_2. The band at 715 cm^{-1} represents Zn-O bond vibrations, slightly displaced due to nanoparticle size or surface contacts.[83]



Graph 4.5: FTIR Spectrum of ZnO Nanoparticles

4.2.2 FTIR Spectrum of Bioplastic Films

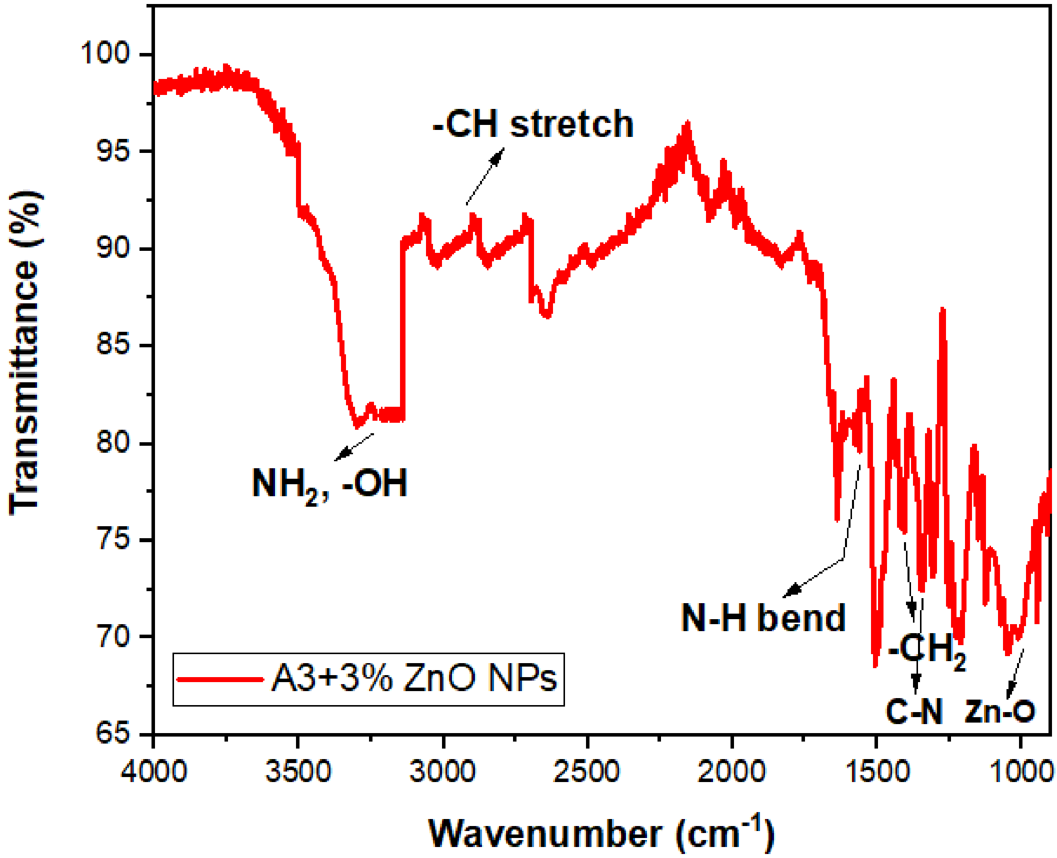
The FTIR spectrum of bioplastic films containing chitosan and pectin in 1:1, 1:2, and 1:3 ratios, integrating 3% zinc oxide nanoparticles, shows distinct interactions between film components and ZnO nanoparticles, with significant bands at 3300, 1562, 1402, 1335, and 972 cm^{-1}. The broad band at 3300 cm^{-1} corresponds to O-H and N-H stretching, indicating hydrogen bonding from chitosan, pectin, and maybe adsorbed water. The band at 1562 cm^{-1} corresponds to N-H bending vibrations, typical of the amine groups in chitosan. It may also reflect interactions between chitosan and ZnO. The 1402 cm^{-1} band is due to C-H bending or asymmetric stretching of carboxylate groups in pectin. The peak at 1335 cm^{-1} is likely due to C-N stretching vibrations, probably caused by amine groups reacting with ZnO. The band at 972 cm^{-1} could imply Zn-O stretching, indicating the existence of ZnO nanoparticles in the film structure.[84] [85]



Graph 4.6: FTIR Spectrum of Bioplastic Films

4.2.3 FTIR Spectrum of Bioplastic Film

The FTIR spectrum of the film with a 1:3 chitosan to pectin ratio and 3% zinc oxide nanoparticles exhibits strong bands at 3300, 2921, 2852, 1562, 1402, 1335, and 972 cm^{-1}, showing different chemical interactions. The broad band at 3300 cm^{-1} represents O-H and N-H stretching vibrations, showing hydrogen bonding from chitosan, pectin, and possibly adsorbed moisture. The bands at 2921 and 2852 cm^{-1} correspond to C-H stretching vibrations from aliphatic chains, maybe from pectin or leftover organic matter. The 1562 cm^{-1} peak is due to N-H bending caused by chitosan's amine groups, which may interact with ZnO nanoparticles. The band at 1402 cm^{-1} indicates C-H bending or asymmetric stretching of carboxylate groups, likely from pectin. The 1335 cm^{-1} signal indicates C-N stretching from chitosan's amine groups, which may interact with ZnO. The 972 cm^{-1} band shows Zn-O stretching, indicating the presence of ZnO nanoparticles in the film.[84] [85]

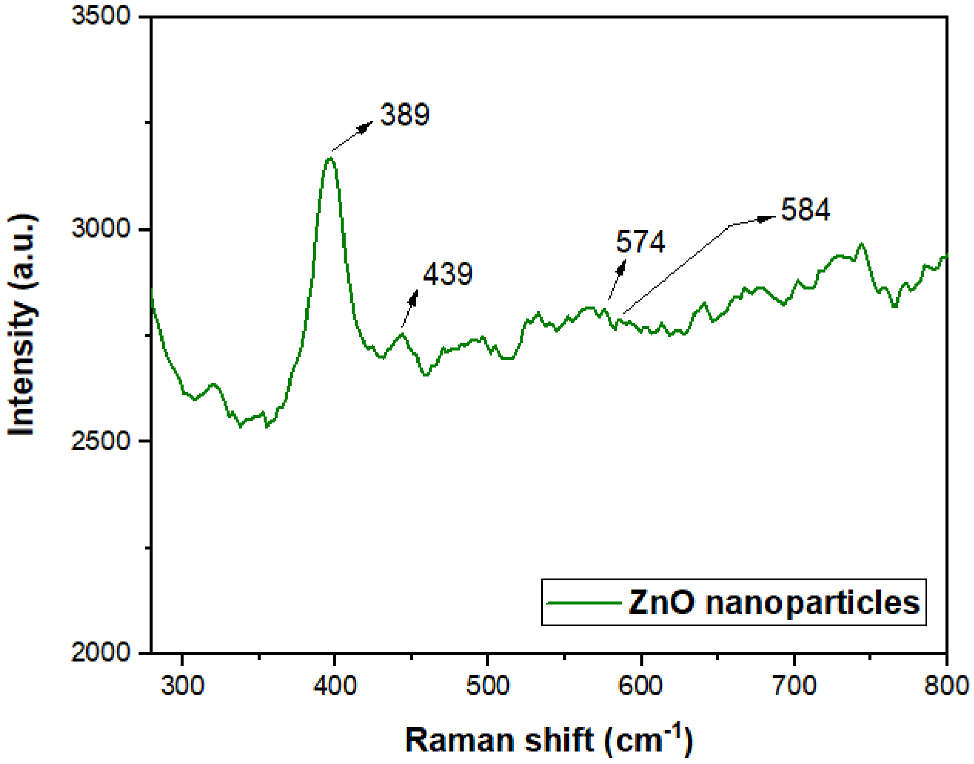


Graph 4.7: FTIR Spectrum of Bioplastic Film

4.3 Raman Analysis

4.3.1 Zinc oxide (ZnO) Nanoparticles

The Raman spectra of zinc oxide nanoparticles showed peaks at 389, 439, 574, and 584 cm⁻¹, which correspond to distinct vibrational modes of ZnO's wurtzite structure. The peak at 389 cm⁻¹ corresponded to the E_2 (low) mode, which is related with vibrations of heavier zinc atoms. The peak at 439 cm⁻¹ is indicative of the E_2 (high) mode, indicating the vibrations of oxygen atoms in the ZnO lattice. It is a hallmark of ZnO's wurtzite structure. The peaks at 574 and 584 cm⁻¹ are likely connected to A_1 (LO) or E_1 (LO) modes, which are longitudinal optical phonon modes. They may suggest defects, oxygen vacancies, or surface disorder in the ZnO nanoparticles.[86]



Graph 4.8: Raman Spectrum of ZnO Nanoparticles

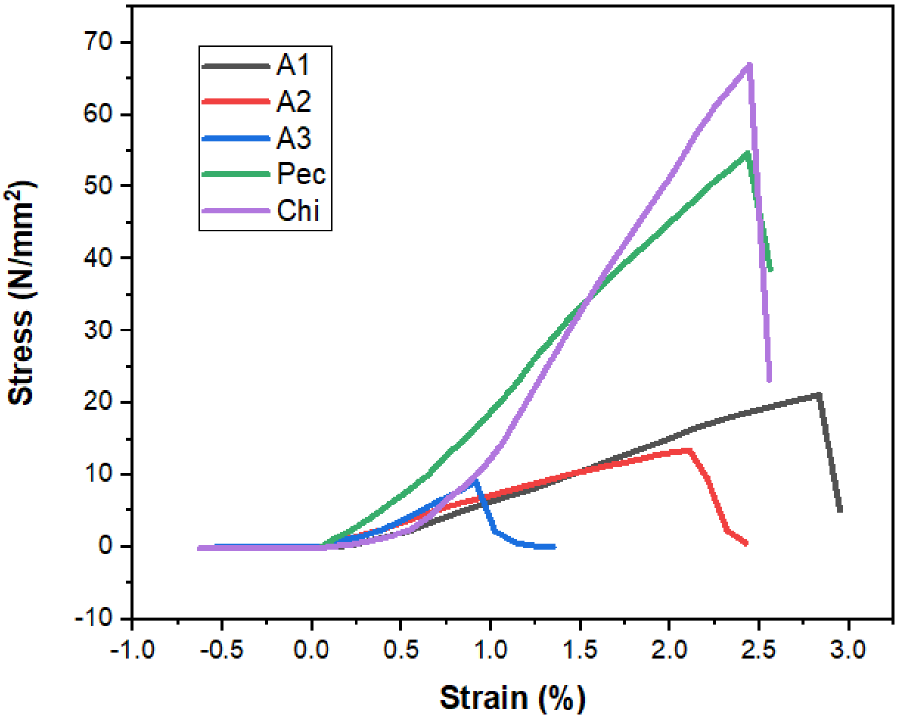
4.4 Mechanical Testing

4.4.1 Stress-Strain Curves of Bioplastic Films (Without NPs), Pectin and Chitosan

The mechanical properties of the films, as measured by their modulus of elasticity and ultimate tensile strength, exhibit unique trends. The modulus of elasticity values for films A1, A2, and A3 are 0.245, 0.243, and 1.05 N/mm², respectively. These films are moderately flexible compared to the blank pectin and blank chitosan films, which have substantially higher moduli of 18.20 and 21.66 N/mm². This shows that the blank films are much stiffer and less deformable than the A-series films.

A1 has the highest ultimate tensile strength among the A-series films at 21.14 N/mm², followed by A2 at 13.28 N/mm² and A3 at 8.76 N/mm². This suggests that A1 is more resistant to breakage during stress. Blank pectin and blank chitosan have significantly greater tensile strengths (53.56 N/mm² and 66.24 N/mm², respectively), indicating superior mechanical strength and stiffness compared to composite films.

The lower modulus and tensile strength in the A-series films show that the addition of additives or changes has boosted flexibility while decreasing mechanical strength. Blank pectin and chitosan, on the other hand, have a higher modulus and tensile strength, indicating that they keep structural integrity and strength better, making them more rigid but less flexible.[87]

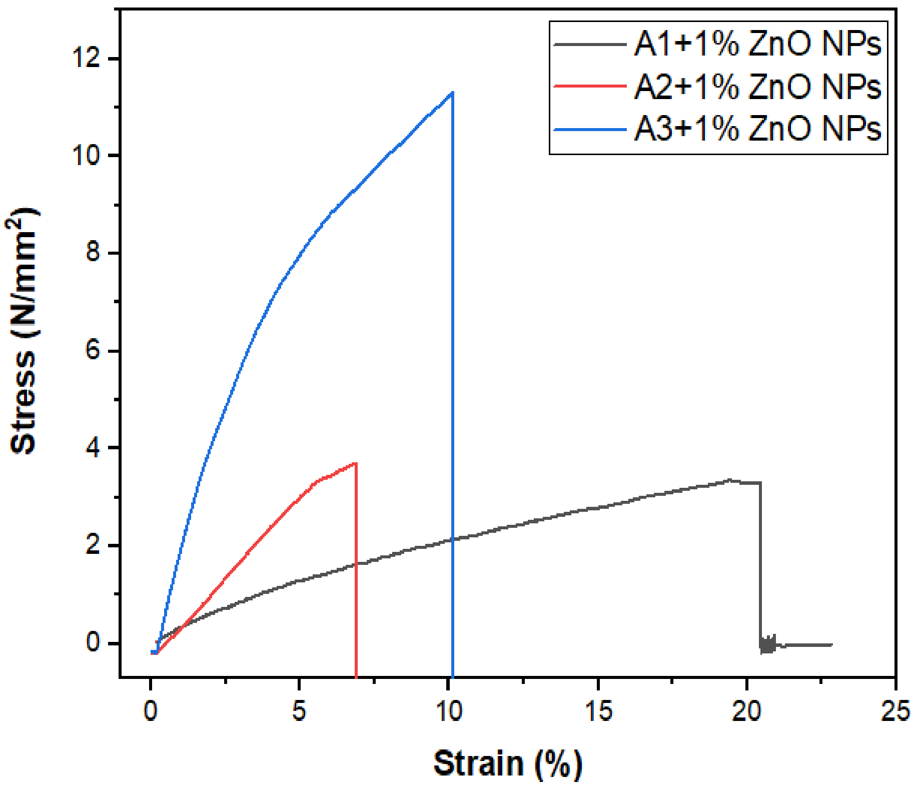


Graph 4.9: Stress-Strain Curve (A1, A2, A3, Pec, Chi)

4.4.2 Stress-Strain Curves of Bioplastic Films (With 1% Nanoparticles)

The mechanical properties of films A1, A2, and A3 with 1% zinc oxide nanoparticles differ significantly from their non-ZnO equivalents in terms of modulus of elasticity and ultimate tensile strength. A1, A2, and A3 had modulus of elasticity values of 0.22, 0.69, and 1.98 N/mm², suggesting increased stiffness due to nanoparticle interaction with the film matrix. A3, having the highest modulus, is the stiffest and least flexible, whereas A1 remains the most flexible with the lowest modulus.

A1 has the lowest ultimate tensile strength at 3.31 N/mm², followed by A2 at 3.57 N/mm² and A3 with the maximum strength at 11.1 N/mm². This pattern indicates that ZnO nanoparticles help to improve the mechanical strength of the films, particularly in A3, which has much higher tensile strength. A3's higher modulus and tensile strength indicate that it can tolerate more stress before breaking than A1 and A2. Overall, the addition of ZnO nanoparticles improves stiffness and strength, particularly in A3, while A1 and A2 remain more flexible with lower mechanical strength.[88]



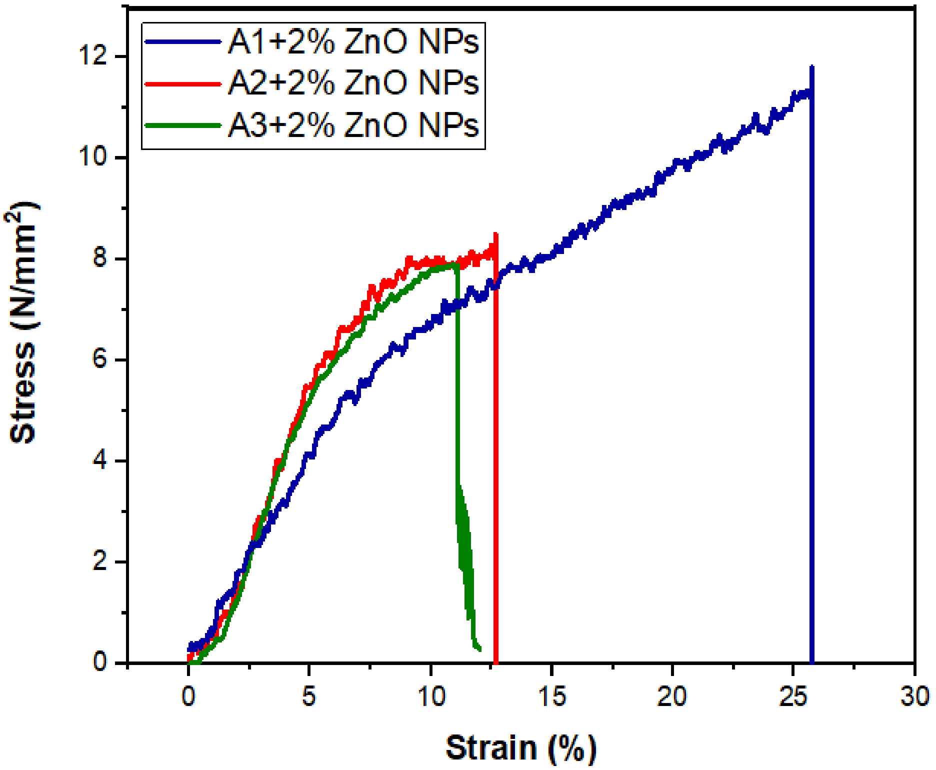
Graph 4.10: Stress-Strain Curve of Films (With 1% Nanoparticles)

4.4.3 Stress-Strain Curve of Bioplastic Films (With 2% Nanoparticles)

The mechanical characteristics of films A1, A2, and A3 with 2% zinc oxide nanoparticles indicate further improvement in modulus of elasticity and ultimate tensile strength. A1, A2, and A3 have modulus of elasticity values of 0.79, 1.19, and 1.32 N/mm², suggesting higher stiffness than their 1% ZnO equivalents. A3 remains the stiffest, while A1, while still more flexible, exhibits a considerable increase in stiffness over its 1% equivalent.

A1 has the highest ultimate tensile strength (11.28 N/mm²), followed by A2 (8.12) and A3 (7.84 N/mm²). The higher tensile strength of A1 indicates that it can absorb more stress before breaking, implying that the addition of 2% ZnO greatly reinforced this film. In contrast, A2 and A3 have significantly lower tensile strengths, but they are still enhanced over their earlier states.

Overall, adding 2% ZnO nanoparticles increases stiffness and strength in all films, with A1 having the best balance of flexibility and mechanical strength, while A2 and A3 are stiffer but significantly weaker in terms of tensile strength.[88]



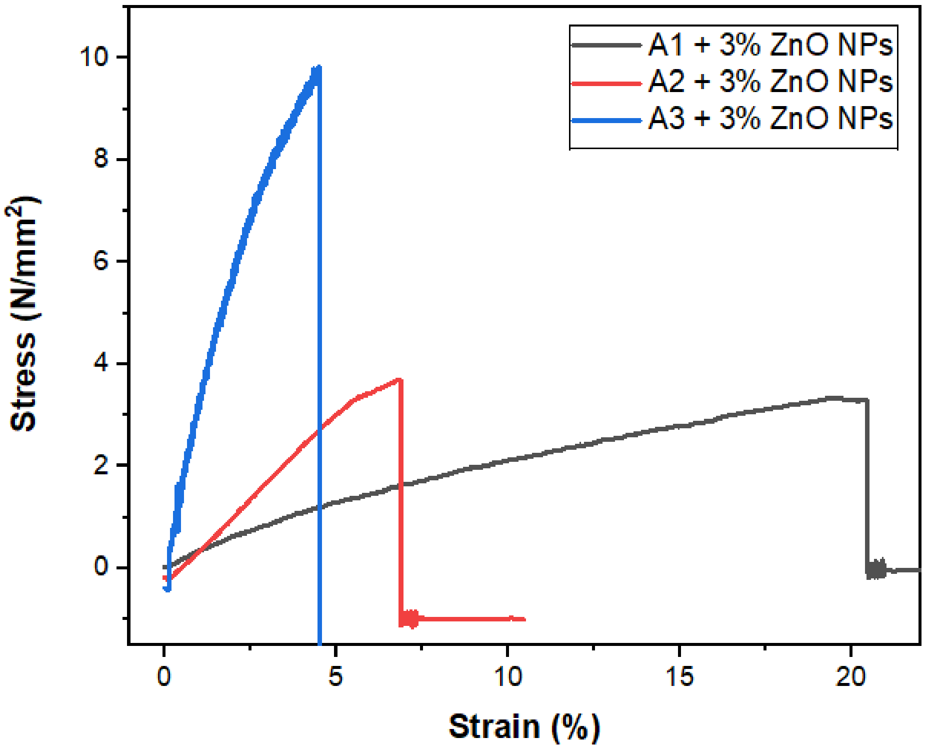
Graph 4.11: Stress-Strain Curve of Films (With 2% Nanoparticles)

4.4.4 Stress-Strain Curve of Bioplastic Films (With 3% Nanoparticles)

The mechanical characteristics of films A1, A2, and A3 containing 3% zinc oxide nanoparticles exhibit significant changes in modulus of elasticity and ultimate tensile strength. The modulus of elasticity for A1, A2, and A3 are 0.19, 0.70, and 3.30 N/mm², demonstrating a range of stiffness. A1 remains highly flexible, with the lowest stiffness, whereas A3 exhibits a considerable rise in rigidity, indicating a much stiffer structure than A1 and A2.

A3 has the highest ultimate tensile strength (9.56 N/mm²), indicating its ability to absorb higher stress before breaking. The 3% ZnO inclusion significantly improves mechanical strength. A1 and A2 exhibit lower tensile strengths (3.27 N/mm² and 3.36 N/mm², respectively), indicating that these films are more flexible but have a lower capacity to resist stress.

Overall, the addition of 3% ZnO nanoparticles improves the stiffness and strength of A3, making it the most rigid and strong of the three. A1 and A2 maintain greater flexibility at the expense of reduced mechanical strength, making A3 better suited to applications needing greater rigidity and tensile strength.[88]



Graph 4.12: Stress-Strain Curve of Films (With 3% Nanoparticles)

4.5 Water Uptake and Mass Loss Studies

4.5.1 Water Uptake

The water uptake percentages indicate that the films' ability to absorb water differs greatly depending on the formulation and the presence of zinc oxide nanoparticles (ZnO NPs). Here's one explanation of these values:

A1, A2, and A3 (without ZnO NPs) exhibit increased water uptake (30.61%, 52.94%, and 65.82%), implying that the composition or ratio of components in these films influences their water absorption. The higher the water uptake, the more hydrophilic the film becomes. A3 appears to have the strongest affinity for water, which could be owing to changes in pectin, chitosan, glutamic acid, or glycerol ratios.

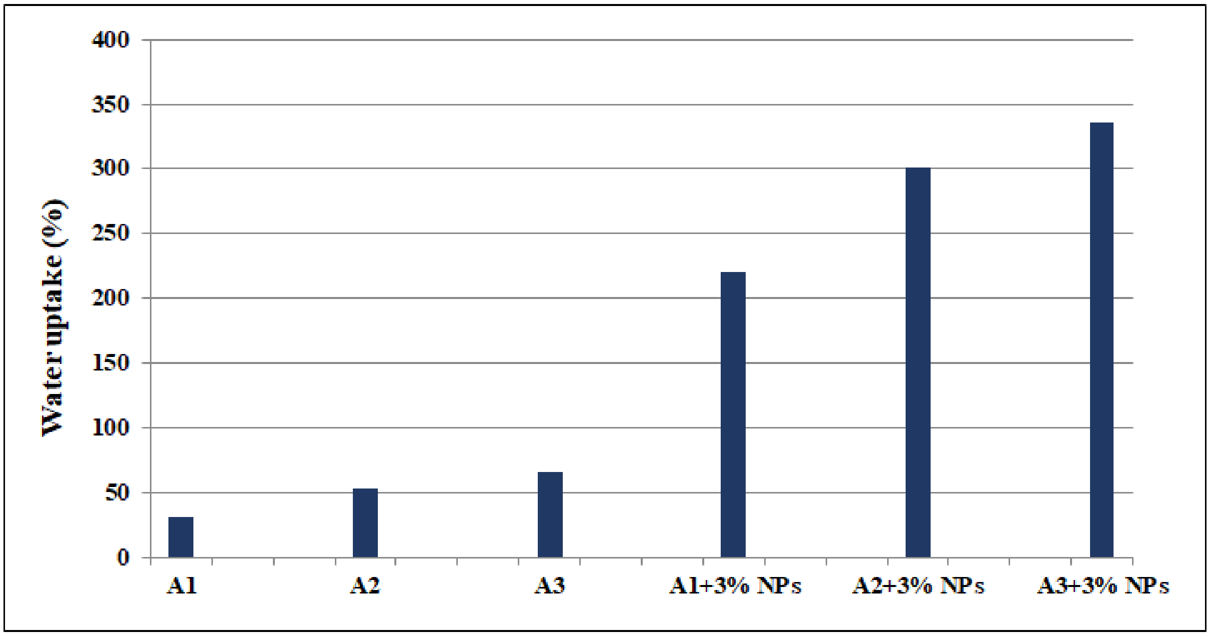
A1+3% ZnO NPs, A2+3% ZnO NPs, and A3+3% ZnO NPs exhibit considerably increased water absorption (220.28%, 301.56%, and 335.44%, respectively), showing that the inclusion of ZnO NPs improves the films' ability to absorb water. ZnO NPs could change the porosity or structure of the films, allowing more water to enter or improving interactions with the hydrophilic groups in the bioplastic matrix. This could potentially indicate a destabilising effect on the film network, leading it to expand more in water.The trend of increased water uptake in ZnO films shows that the nanoparticles influence the film's structural or surface properties, making it more hydrophilic or permeable.

4.5.2 Mass Loss

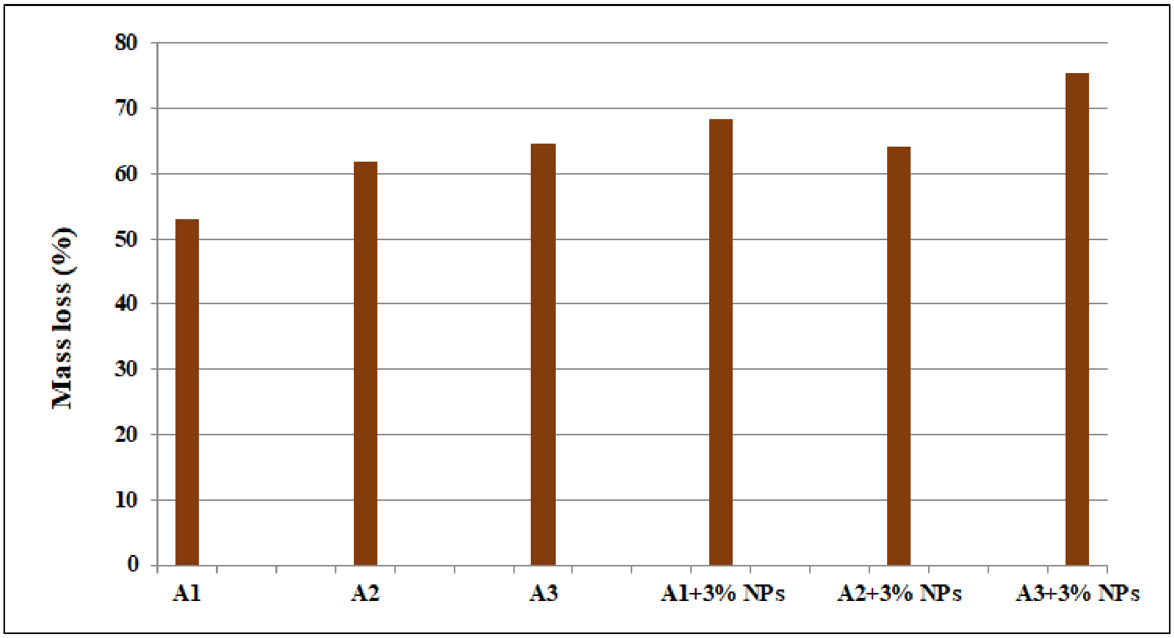
The mass loss numbers represent how much material was dissolved, degraded, or leached from the films during water immersion and subsequent drying. This is what the evidence suggests: A1, A2, and A3 (without ZnO NPs) exhibit increasing mass loss, ranging from 53.12% to 64.58%. This tendency most likely reflects changes in the films' composition or structure, with A3 being the most prone to mass loss, potentially due to its increased water intake (as previously seen). Higher water intake may cause more widespread swelling, resulting in more material loss during drying.

A1+3% ZnO NPs, A2+3% ZnO NPs, and A3+3% ZnO NPs show comparable but generally larger mass loss (62.29%, 64.10%, 75.55%) than their equivalents without ZnO NPs. The introduction of ZnO nanoparticles appeared to make the films more vulnerable, notably A3+3% ZnO NPs, which lost the most mass (75.55%). The nanoparticles may change the film's structure, making it more prone to disintegration or degradation after water immersion.

Overall, the inclusion of ZnO NPs appears to contribute to increased mass loss, which could be attributed to how the nanoparticles alter the stability or crosslinking of the film matrix.[89]



Graph 4.13: Water Uptake (%) Studies



Graph 4.14: Mass Loss (%) Studies

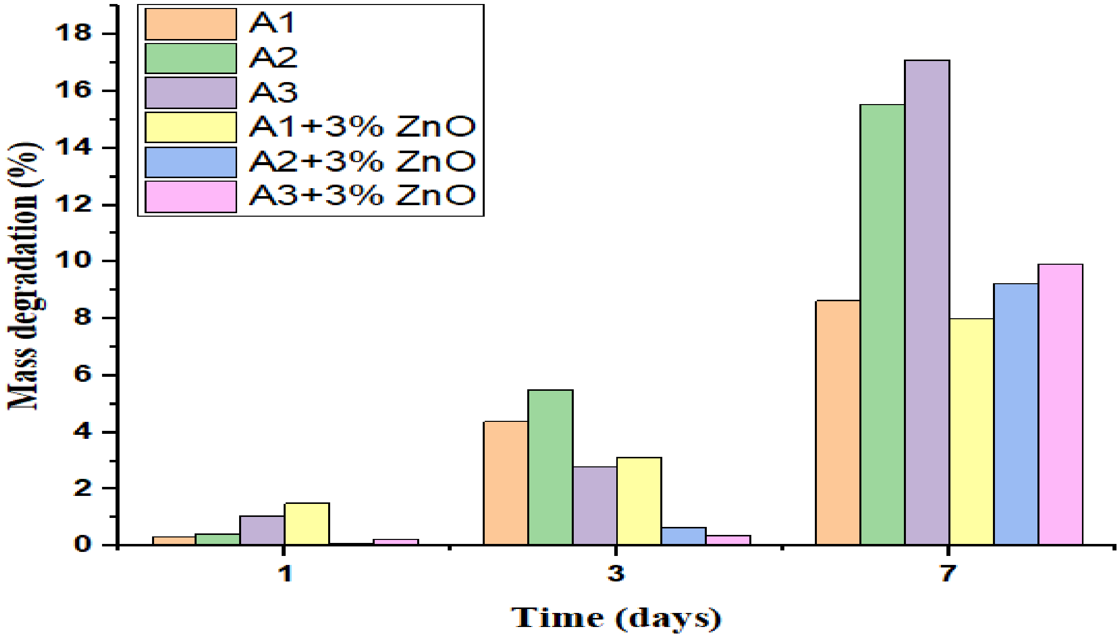
4.6 Biodegradability Test in Soil

The biodegradability test results show how different film compositions decompose in soil over time. Here is an interpretation of the findings:
After 1 day, the biodegradability values for all films are rather low, indicating that the initial breakdown process in soil is delayed. However, film A3 and A1+3% ZnO NPs degrade slightly faster (1.06% and 1.5%), indicating that these compositions are more vulnerable to microbiological or environmental conditions that cause deterioration. The ZnO nanoparticles in A1+3% NPs may interact with the film matrix to promote initial biodegradation.

After 3 days, all films' degradation percentages increase, with A2 having the highest biodegradability (5.5%) among non-ZnO films. This shows that A2's composition becomes more degradable in soil with time. The ZnO NP-incorporated films continue to degrade more slowly, with A2+3% ZnO NPs showing negligible breakdown (0.65%), which may indicate that ZnO NPs prevent or delay microbial activity or natural breakdown processes.**After 7 days**, the degradation rate increases dramatically, particularly in films A2 and A3 (15.55% and 17.08%). These two films have the maximum biodegradability, which could be attributed to their composition that promotes microbial decomposition. However, films containing ZnO NPs show substantial degradation at this point, while it is still less than that of non-ZnO films. The addition of ZnO NPs in films A1+3%, A2+3%, and A3+3% appears to have a twofold effect: initially delaying biodegradation, but after a week, the films continue to degrade at rates comparable to non-ZnO films (8.02%, 9.25%, 9.92%).

In conclusion, the inclusion of ZnO NPs appears to slow biodegradation initially but does not completely prevent it. Films lacking ZnO NPs decay faster, especially after a few days, with A3 demonstrating the fastest degradation after 7 days. This trend shows that ZnO NPs may provide some initial protection against environmental breakdown, perhaps extending the films' lifespan during short-term exposure. [89]

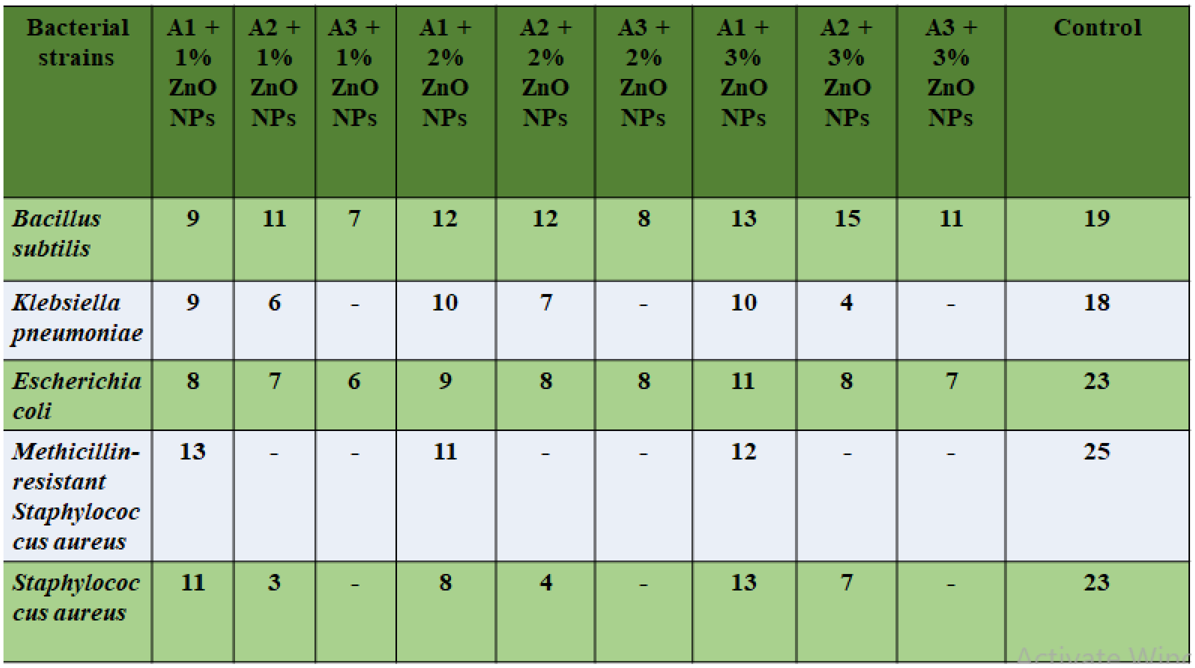
Figure 4.1: Biodegradability Test in Soil



Graph 4.15: Biodegradability Test in Soil for 1, 3 and 7 days

4.7 Antibacterial Activity

The synthesized bioplastic films were test against different gram positive and gram negative microorganisms such as *Bacillus subtilis, Klebsiella pneumoniae, Esherichia coli, Methicillin-resistant Staphylococcus aureus* and *Staphylococcus aureus.* Bioplastic films show maximum zone of inhibition of 15 mm against *Bacillus subtilis* by A2+3% NPs, 10 mm against *Klebsiella* *pneumonia* by A1+3% NPs, 11 mm against *Escherichia coli* by A1+3% NPs, 13 mm against *Methicillin-resistant Staphylococcus aureus* by A1+1% NPs, 13 mm against *Staphylococcus aureus* by A1+3% NPs. The details of activity are given in table below.[90]



Bacterial strains	A1 + 1% ZnO NPs	A2 + 1% ZnO NPs	A3 + 1% ZnO NPs	A1 + 2% ZnO NPs	A2 + 2% ZnO NPs	A3 + 2% ZnO NPs	A1 + 3% ZnO NPs	A2 + 3% ZnO NPs	A3 + 3% ZnO NPs	Control
Bacillus subtilis	9	11	7	12	12	8	13	15	11	19
Klebsiella pneumoniae	9	6	-	10	7	-	10	4	-	18
Escherichia coli	8	7	6	9	8	8	11	8	7	23
Methicillin-resistant Staphylococcus aureus	13	-	-	11	-	-	12	-	-	25
Staphylococcus aureus	11	3	-	8	4	-	13	7	-	23

Figure 4.2: Antibacterial assay of bioplastic films with zone of inhibition (in mm)

4.8 Food Packaging

Food packaging test was done in order to check the tendency of the prepared films to store the food. For this purpose, as in literature, a polystyrene packing was synthesized by dissolving small polystyrene balls in toluene and then casting this solution in a glass petri dish and letting it stick to the petri dish so that it can be used as a packing material. The same is done by preparing solutions of my films (with and without nanoparticles). Apple slices packed in bioplastic fims (with nanoparticles), bioplastic films (without nanoparticles) and polystyrene showed clear difference. Similarly, lemon packed in these three different films also showed difference. The study showed that after 2 days, the apple and lemon slices in the polystyrene packing started degrading gradually, as shown in the figure below.[91] [92]

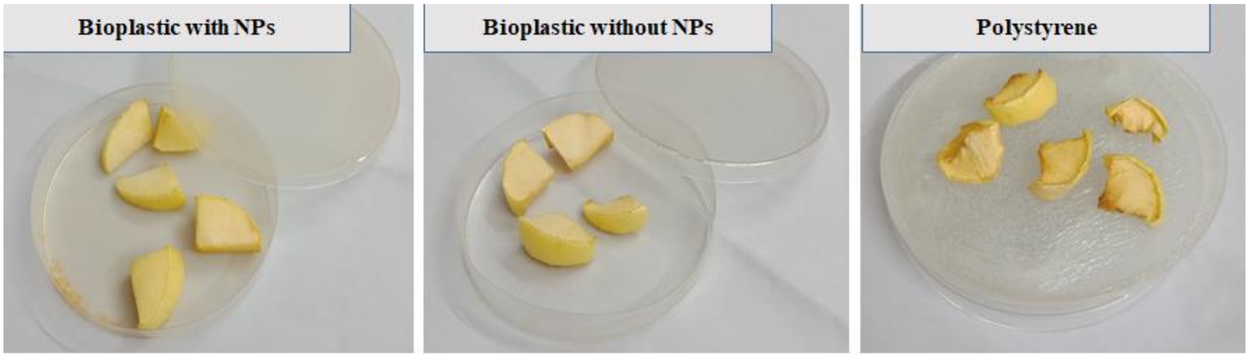


Figure 4.3: Packaging comparison with Apple slices

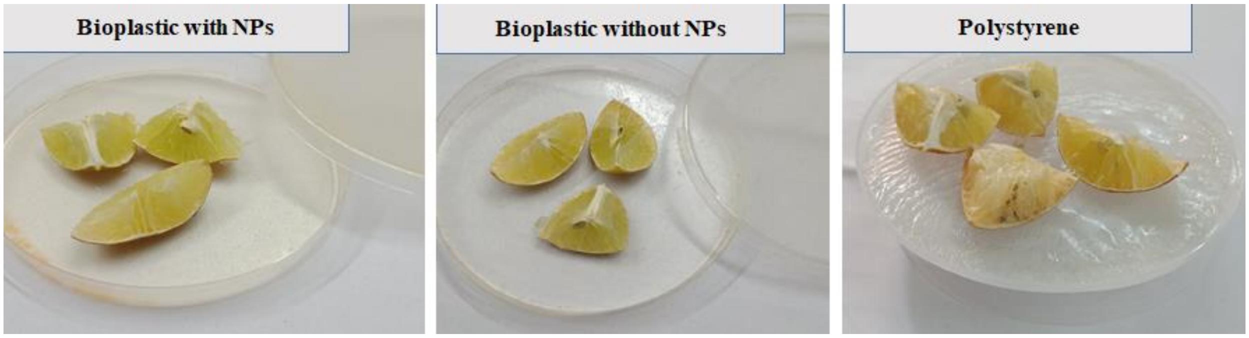


Figure 4.4: Packaging comparison with Lemon slices

Chapter 5 CONCLUSION

I've combined electrostatic interactions and non-covalent hydrogen bondings to generate bioplastics from pure biobased sustainable ingredients using a green synthesis approach. Chitosan was used as a co-biopolymer along with pectin biopolymer as the matrix material to create stable bioplastics. Two renewable plasticisers were used: glycerol and glutamic acid. Following a 24-hour water incubation period, the resultant films show water uptake (30–335 ± 20%) and mass loss (53–75 ± 5%). The semi-crystalline nature of all the films and the preservation of their connections via hydrogen bonds and electrostatic interactions were confirmed by both FTIR and XRD analysis. The bioplastics produced from waste inexpensive source materials have mechanical strengths ranging from 0.1 to 3.30 N/mm^2, with A1+3% NPs exhibiting the largest plasticization effect and A3+3% NPs having the highest mechanical strength. The film's properties were also susceptible to hydrolytic degradation after being immersed in water for 24 hours; it showed 30 to 335% water absorption. After seven days in the soil, they showed up to 9% degradation and passed an ecotoxicity test, indicating they were safe for the environment. As a result, these bioplastic films may prove to be a practical renewable resource for use in the future for food packaging purposes.

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