PROJECT REPORT

On

"RECYCLING WASTE PAPER FOR THE ISOLATION, CHARACTERIZATION OF CELLULOSE NANOCRYSTALS AND SYNTHESIS OF CHITOSAN CELLULOSE NANOCRYSTALS GREEN COMPOSITE FILM FOR ANTIBACTERIAL STUDIES"

Submitted by

LIMA SEBASTIAN

[AM21CHE010]

In partial fulfillment for the award of the Postgraduate Degree in Chemistry

DEPARTMENT OF CHEMISTRY
AND
CENTRE FOR RESEARCH

ST. TERESA'S COLLEGE (AUTONOMOUS)
ERNAKULAM

2022-2023

ST.TERESA'S COLLEGE (AUTONOMOUS) ERNAKULAM

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M.Sc. CHEMISTRY PROJECT REPORT

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Year of Work	**: 2022-2023**

This is to certify that the project **"RECYCLING WASTE PAPER FOR THE ISOLATION, CHARACTERIZATION OF CELLULOSE NANOCRYSTALS AND SYNTHESIS OF CHITOSAN CELLULOSE NANOCRYSTALS GREEN COMPOSITE FILM FOR ANTIBACTERIAL STUDIES"** is a work done by **LIMA SEBASTIAN.**

ST. TERESA'S COLLEGE (AUTONOMOUS) DEPARTMENT OF CHEMISTRY ERNAKULAM

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Date: 03/06/2023

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DEPARTMENT OF CHEMISTRY
AND
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ST. TERESA'S COLLEGE (AUTONOMOUS)

ERNAKULAM

CERTIFICATE

This is to certify that the project work entitled "**RECYCLING WASTE PAPER FOR THE ISOLATION, CHARACTERIZATION OF CELLULOSE NANOCRYSTALS AND SYNTHESIS OF CHITOSAN CELLULOSE NANOCRYSTALS GREEN COMPOSITE FILM FOR ANTIBACTERIAL STUDIES**" is the work done by LIMA SEBASTIAN under the guidance of**, DR. USHAMANI M., ASSOCIATE PROFESSOR** Department of Chemistry and Centre for Research, St. Teresa's College, Ernakulam in partial fulfillment of the award of the Degree of Master of Science in Chemistry at St. Teresa's College, Ernakulam affiliated to Mahatma Gandhi University, Kottayam.

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Dr. USHAMANI M.

Project Guide

DECLARATION

I hereby declare that the project work entitled "**RECYCLING WASTE PAPER FOR THE ISOLATION, CHARACTERIZATION OF CELLULOSE NANOCRYSTALS AND SYNTHESIS OF CHITOSAN CELLULOSE NANOCRYSTALS GREEN COMPOSITE FILM FOR ANTIBACTERIAL STUDIES**" submitted to the Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous) affiliated to Mahatma Gandhi University, Kottayam, Kerala is a record of an original work done by us under the guidance of **DR. USHAMANI M., ASSOCIATE PROFESSOR**, Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam (Internal Guide). This project work is submitted in partial fulfillment of the requirements for the award of the Degree of Master of Science in Chemistry.

LIMA SEBASTIAN

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Last, but not least, I am grateful to our loving families and friends for the care, support, and concern they provide to follow our passion.

Lima Sebastian

Contents

List of Figures

Abbreviations	
Sugarcane bagasse	SCB
Cellulose Nanocrystals	CNC
Cellulose nanofiber	CNF
Bacterial cellulose	BC
Electrospun cellulose nanofiber	ECNF
Transmission electron microscope	TEM
Fourier transform infrared spectroscopy	FTIR
X-Ray diffraction	XRD
Scanning Electron Microscope	SEM
Selected area electron diffraction	SAED/SAD
Escherichia coli	E.coli

Chapter 1

Introduction

1.1 NANOTECHNOLOGY

Nanotechnology refers to the branch of science and engineering devoted to designing, producing, and using structures, devices, and systems by manipulating atoms and molecules at the nanoscale. This is done by either scaling up from single groups of atoms or by refining or reducing bulk materials. The National Nanotechnology Initiative (NNI) in the United States defines Nanotechnology as "a Science, engineering, and technology conducted at the nanoscale (1 to 100 nm), where unique phenomena enable novel applications in a wide range of fields, from Chemistry, Physics, and Biology, to medicine, engineering and electronics".[1] It is one of the most promising technologies of the twenty-first century.

Nanoparticle (or nanopowder or nanocluster or nanocrystal) is a microscopic particle with at least one dimension less than 100 nm. Undetectable by the human eye, nanoparticles can exhibit significantly different physical and chemical properties to their larger material counterparts. [2] Examples of nanoparticles are quantum dots, fullerenes, metal nanoparticles, graphene oxide, carbon nanotubes, etc. Owing to their very small size, nanoparticles have a very large surface area to volume ratio when compared to bulk material, such as powders, plates, and sheets. This feature enables nanoparticles to possess unexpected optical, physical, and chemical properties, as they are small enough to confine their electrons and

produce quantum effects. Nanotechnology is now widely considered to have the potential to bring benefits in areas as diverse as drug development, water decontamination, information and communication technologies, and the production of stronger and lighter materials[2]. The development of nano-phase materials from natural sources is an important eco-friendly approach to meet commercial applications in industries like refractories, ceramics, textiles medical, paints, and pigments[3]. A rapidly growing topic of nanotechnology study is the extraction of cellulose from natural sources and its conversion to nanocellulose.

1.2 CELLULOSE

The most prevalent source of polymeric raw materials on earth is cellulose, an intriguing and sustainable feedstock. Carbon, oxygen, and hydrogen are the three elements that make up the versatile carbohydrate known as cellulose, which has the chemical formula $(C_6H_{10}O_5)_n$. Cellulose is the major component in lignocellulosic biomass which is mainly localized in the plant cell wall at around 35–50%. It is composed of the linear homopolysaccharide of β-1,4- linked anhydrous-D-glucose units with the repeating unit of cellobiose. The monomer of cellobiose, named anhydroglucose unit, consists of three hydroxyl groups that form a strong hydrogen bond with the adjacent glucose unit in the same chain and with different chains, called intramolecular and intermolecular hydrogen bonding networks, respectively.[4] Its annual production is roughly estimated to be 1012 tons, but only a very small portion is exploited by various industries like textiles, paper, chemical, and materials industries. French Chemist Ansel Payen discovered Cellulose in the year 1838. Cellulose is the main component of paper, cardboard, and textiles made up of cotton or other plant fibers. It's also used to produce films and cellulose derivatives. It is chiral, tasteless, biodegradable, and has no odor.

In general, cellulose is a hard, fibrous, and water-insoluble substance that plays an essential function in keeping the structure of cell walls in plants. [3] Cellulose can be found in its purest form in plants, however, in wood, leaves, and plant stalks, it is found mixed with other materials such as lignin and hemicelluloses. Cellulose nanofibers have the potential to be used in multiple ways, notably as a reinforcement material in the development of nanocomposites. [4]

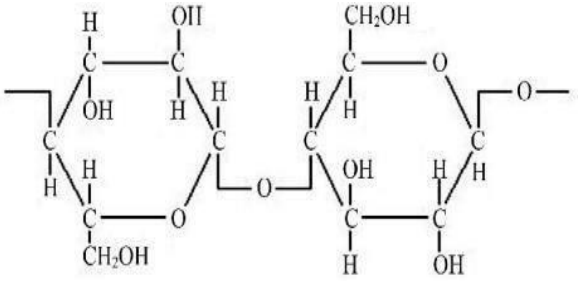

Fig 1: Structure of cellulose

Natural cellulose's shape, length, and diameter depend on where it came from and how it was extracted. Crystalline and amorphous domains both exist in natural cellulose. Depending on the extraction techniques used and the source, its crystallinity can change. In comparison to the crystalline region, the amorphous region is less dense and more likely to interact with other molecular groups. In contrast to the amorphous ones, the crystalline domains are more resilient to mechanical, enzymatic, and chemical processes. Different forms of cellulose, such as cellulose I, II, III, and IV, can be obtained depending on the molecule orientations, van der Waals, intra- and intermolecular contacts, isolation, and treatment method. These types of cellulose can also be changed to one another by heat or chemical processes.[3] Natural cellulose has a few restrictions that prevent it from finding broad use, including poor heat stability and non-compatibility.

Due to these restrictions, cellulose must be transformed into nanocellulose in order to create composites that can improve the necessary qualities. One of the most well-known green materials in recent years has been cellulose in the form of nanostructures. Nanocellulose possesses superior mechanical qualities, a high surface-to-volume ratio, a high aspect ratio, is renewable, and is biocompatible.[5] The many hydroxyl functional groups enable a variety of functionalization, resulting in the creation of distinctive materials with particular uses.[3]

1.3 NATURAL SOURCES OF CELLULOSE AND WASTE MATERIAL SOURCES

In general, natural sources of cellulose are obtained from biomass resources such as wood, herbaceous plants, grass, agricultural crops, animals, algae, bacterial sources, waste paper, cotton, etc. It is the major constituent of all plant materials including wood, cotton, flax, hemp, jute, ramie, cereal straws, rice straws, and sugarcane bagasse, forms about half to one-third of plant tissues, and is constantly replenished by photosynthesis. Thus it is the most abundant and renewable natural resource on earth [6]. Interestingly, apart from the plant kingdom, cellulose can also be derived from non-plant materials such as algae, sea creatures, bacteria, and fungi driven by the effort to obtain pure cellulose. Several studies have been conducted to produce cellulose from bacteria, in particular from Acetobacterxylinum. The sustainability, excellent mechanical properties, and interesting physical properties of cellulose nanomaterials have recently received a great deal of attention for modifying polymers and acting as both active and passive components in a wide range of potential products.[8]

Cellulose nanocrystals (CNCs) are one such cellulose nanomaterial and are considered as one of the most attractive renewable reinforcements isolated from natural biodegradable polysaccharides with promising properties and

a broad range of applications in several fields. In recent years many agricultural and industrial wastes have been brought to attention in the utilization and extraction of Cellulose Nanocrystals (CNCs). These wastes such as sugarcane bagasse, rice husk, and waste paper have been studied [6]. In published research, spherical CNC can be made from a variety of biomass sources, including cotton waste, eucalyptus pulp, empty fruit bunch fibers, and sago seed shells. [7,8] Most biomass waste contains low cellulose contents and requires pretreatment before CNC extraction.[7]

Out of various sources of cellulose available, we have chosen waste paper for the extraction of cellulose and its conversion to cellulose nanocrystals through the acid hydrolysis method. Waste paper is a competent raw material for the synthesis of CNC due to its abundant availability and high cellulosic content (60–70%) with comparatively fewer hemicelluloses (10–20%) and lignin (5–10%) without any harsh treatments. The production yields of nanocellulose were reported to vary from 1.5% to 64% depending upon the waste papers and treatments given.[9]

Fig 2: Different sources of cellulose

1.4 THE SOURCES-WASTE PAPER

The upward trend in literacy rate and increase in industrial development have led to an increase in demand for paper every year. Pulp and paper are the third largest industrial pollutant of air, water and soil. And it has been

estimated that the demand for paper in our country by 2025 would be close to 25 crore metric tons. The amount of waste paper generated by any academic institution is enormous which includes exam paper, record paper, tissue paper, and paper used for office work. Paper Waste is a severe problem in institutions and offices. Because of printing mistakes, junk mail, billings, and packaging, the paper may comprise up to 70% of the institution's and office's total waste. An average office employee would be using about 10,000 sheets of paper in a year. In addition to paper used for printing, institutions also consume other paper products, such as cardboard, envelopes, and wrappers, to name a few.

Chlorine-based bleaches are used during production, which releases toxic material into our environment. When paper rots, it emits methane gas which is twenty-five times more toxic than carbon dioxide. Through the production of paper, we are cutting down more trees which helps in absorbing toxic gases like carbon dioxide whose accumulation will adversely affect both environment and living beings. The waste generated from pulp and paper mills adversely affects the environment from different perspectives. The emissions from pulp and paper industries have a significant effect on the environment. The generated waste from the pulp and paper industry causes severe harm to aquatic life, disturbs the food chain, and also causes various health implications.[16]

As a cellulose biomass waste paper offers a possible source of raw material for the manufacture of CNCs. Unquestionably, the global production and consumption of several million tons of paper results in an enormous volume of wastepaper. Wastepaper continues to contribute significantly to municipal and industrial waste despite recycling efforts. Recycling waste paper produces lower grade paper because the fibres are cut shorter, and the quality of the paper is far worse than paper made from virgin pulps.

Since the maximum ratio of paper-to-paper recycling is reported to be 65%, this results in the production of large quantities of by-product which ultimately have to be disposed of. Finding alternate methods of recycling wastepaper is essential due to the increased expense of making paper from recycled paper and the disposal of waste fibres unfit for use. Wastepaper has the potential to be used as a raw material for the creation of cellulose nanocrystals (CNCs) because of its cellulosic composition. CNCs made from scrap paper would offer an option to recycle paper and perhaps solve the problem of byproducts produced during paper-to-paper recycling. About 70% of the cellulose in waste paper can be converted into CNCs through alkali pulping, bleaching, and acid hydrolysis. The created nano-cellulose crystals have potential uses in several industries. [6,8] The primary goal of our research is to separate cellulose nanocrystals, or CNCs, from waste paper. The trash record paper from our department was the primary component. About 70% of the cellulose in waste paper can be converted into CNCs through alkali pulping, bleaching, and acid hydrolysis. The created nano-cellulose crystals have potential uses in several industries [6,8] The primary goal of our research is to separate cellulose nanocrystals, or CNCs, from waste paper. The trash record paper from our department was the raw material used.

1.5. NANO CELLULOSE

Cellulosic materials with one dimension in the nanometer range (1 to 100 nm) are referred to generally as nanocellulose. It typically has a greater surface area than normal cellulose. Nanocellulose has unique properties such as low density, biodegradable, and good mechanical properties. It is also easily modifiable owing to its surface functionalities and typical morphology.[20] Depending on the production conditions, which influence the dimensions, composition, and properties, nanocellulose can be divided

into two main categories: (i) cellulose nanocrystals (CNC) and (ii) cellulose nanofibrils (CNF), also known as cellulose nanofibers. Bacterial cellulose (BC) and electrospun cellulose nanofibers (ECNF) are also considered as nanocellulose. However, CNC and CNF are much more common, since they are produced by the disintegration of cellulose fibers into nanoscale particles (top–down process), whereas BC and ECNF are generated by a build-up of nanofibers (bottom–up process) from low molecular weight sugars by bacteria or from dissolved cellulose using electrospinning, respectively.[23] Nanocellulose is an attractive material for various applications because it is based on abundant resources—economic, renewable, and commercially processable. Nanocellulose with small particle size and high crystallinity has better properties and is usually used as a nanofiller for some polymer materials. Nanocellulose is used in the various field such as a barrier in the separation process of hazardous waste, food wrappers that replace non- biodegradable plastics, and as nanocomposite to improve properties such as mechanical, thermal, and ionic conductivity properties of the polymer.[16]

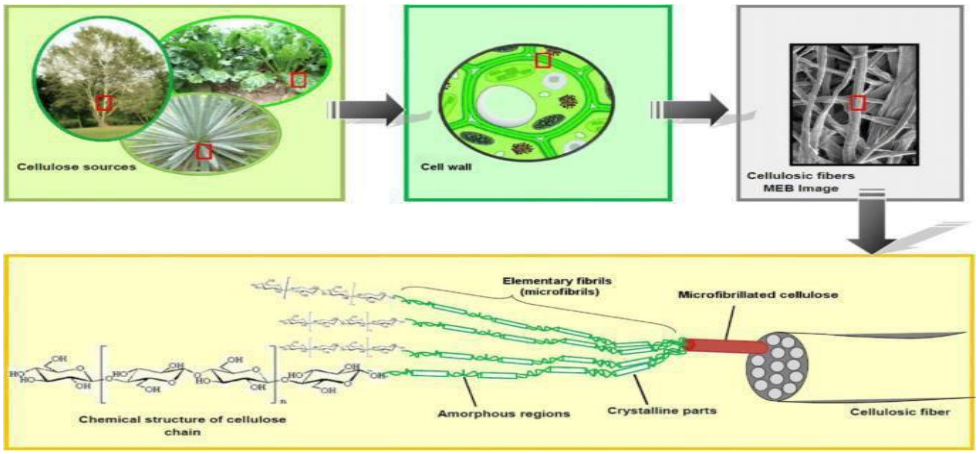

Fig 3: Microscopic view of Nano Cellulose

1.5.1 Cellulose Nanocrystal (CNC)

Cellulose nanocrystal has recently gained certain attention due to their characteristic features such as biodegradability, high aspect ratio, excellent mechanical properties, a large number of functional groups, and high thermal stability. They are rod-shaped structures with high crystallinity and hundreds of square meters per gram of the specific interface. This Nanocrystalline structure is long and regular rod-shaped as well as they are cost-efficient. They provide a suitable option for improving the mechanical properties of both synthetic and natural polymers. Availability and renewability are a few important characteristics of cellulose nanoparticles. The diameter of CNCs lies between 5 and 70 nm irrespective of their source of origin. Various extraction processes have been used for the production of CNCs but acid-hydrolysis is the most well-known, efficient, and widely used extraction method. The acid hydrolysis method used for obtaining the nanocrystals preferably dissolves the amorphous part, yielding cellulose nanocrystals with 5 nm diameter and 20–100 nm length. The length of Nano cellulose is in the range between 100 and 250 nm when they are derived from plant-based celluloses, and from 100 nm to several micrometers when they are obtained from celluloses of tunicates, algae, or bacteria.[8,17] It has high strength, modulus, large surface area, optical properties, etc. CNCs are hydrophilic in nature and can be functionalized with various materials to satisfy challenging requirements. Since they are defect free, the modulus of CNC is close to the theoretical value of cellulose. It is potentially stronger than steel. CNCs were first extracted by Alkali pulping and Bleaching treatment. It was isolated by acid hydrolysis under controlled conditions. [6]

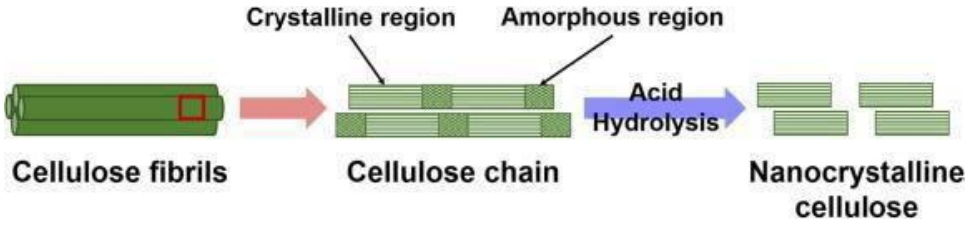

Fig 4: Schematic representation of CNC which can be extracted from cellulose chains.[39]

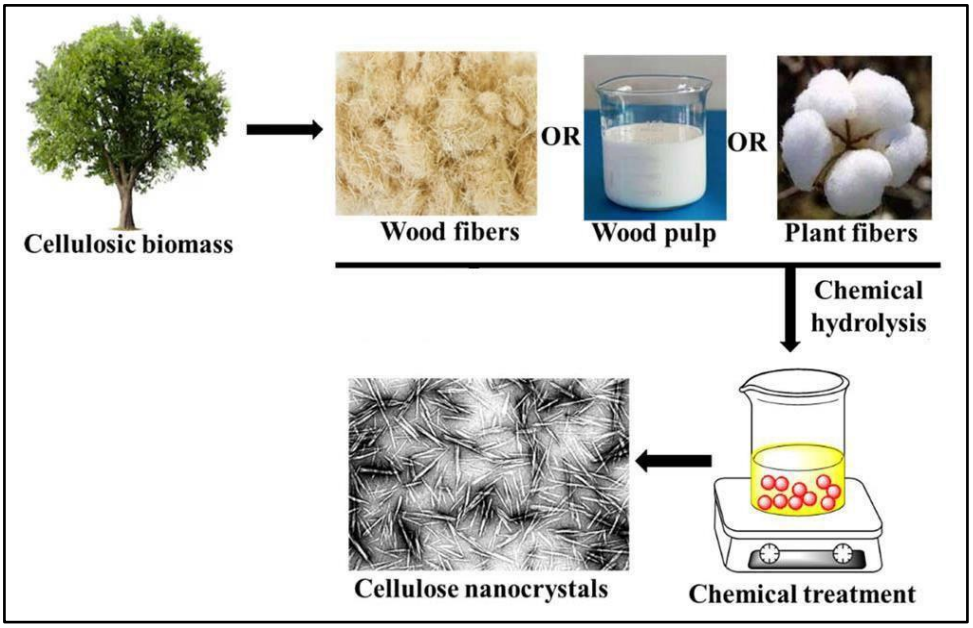

Fig 5: Synthesis of Cellulose Nano Crystals

1.5.2 Cellulose Nanofiber (CNF)

Cellulose Nanofibers are nanoscale cellulose with a diameter of about 5–60 nm and a length of many microns. The fibrils consist of both crystalline and amorphous sections. On the other hand, cellulose nanocrystals are needle-shaped and highly crystalline with some residual amorphous regions. CNF can be produced through several mechanical processes, such as high-pressure homogenization, Micro fluidization, refining, or grinding and the other less used mechanical treatments are electro-spinning, ultra-sonication, cry crushing, or Steam explosion. The main drawback of mechanical

processes is the high energy demand. Therefore, different chemical or enzymatic pre-treatments of pulp such as cationization, hydrolysis, TEMPO-mediated oxidation and Acetylation have been used to ease the mechanical treatment, reducing the energy consumption and obtaining the desired surface chemistry of the product. Cellulose Nanofibers are cellulose molecules that have a diameter of 5 to 60 nm and a length of many microns. Both crystalline and amorphous parts make up the fibrils. On the other hand, cellulose nanocrystals have some remaining amorphous regions and are needle-shaped in addition to being extremely crystalline. The important characteristics of CNFs include Light and strong, Ultra-fine fibers, Large specific area, Low thermal expansion, High gas barrier properties, environmentally friendly biomass material, Viscous in the presence of water etc.

Due to its morphology and characteristic physical properties, cellulose nanofiber is seen as a promising material for use in a wealth of fields, including filter material, high gas barrier packaging material, electronic devices, foods, medicine, cosmetics, and health care. [3,19]

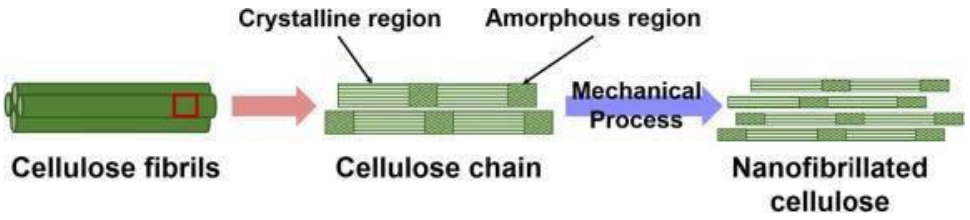

Fig 6: Schematic representation of nanofibrillated cellulose which can be extracted from cellulose chains using the mechanical process to cleavage the fiber into nanometer size in diameter [39].

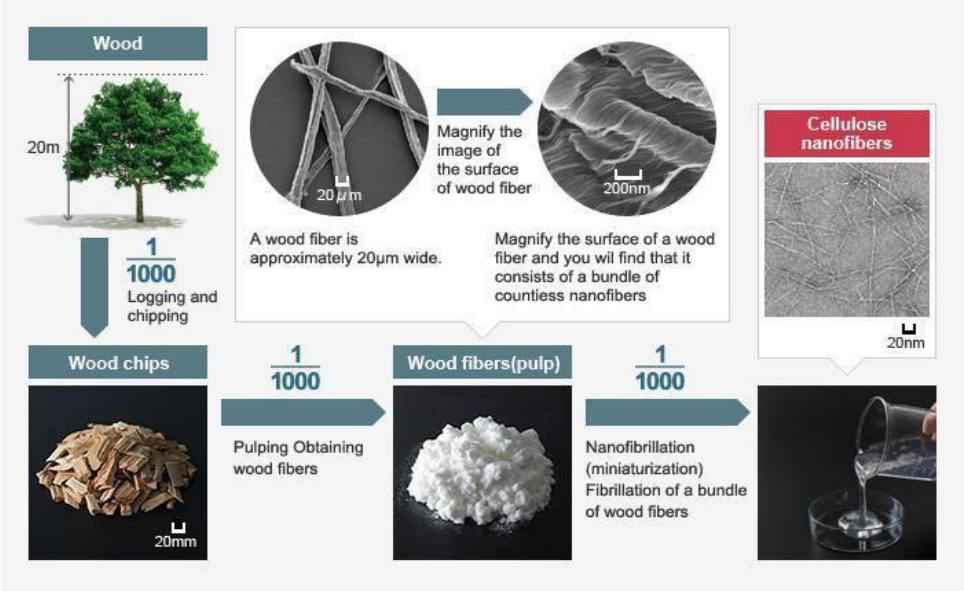

Fig 7: Synthesis of cellulose nanofiber

1.5.3 Bacterial Cellulose Nanocrystal (BNC)

BNC is a Nano-fibrillated biomaterial made by different species of acetic acid bacteria that exhibits a high degree of polymerization and crystallinity, excellent water-holding capacity, high purity, high transparency, excellent mechanical properties, and good biocompatibility, among other distinguishing characteristics. Due to these characteristics, BNC has a wide range of possible uses, including biomedical materials, functional paper, healthy foods, innovative materials, and textiles [20]. It incorporates meaningful structural elements and properties of the well-known plant cellulose with the special features of nanoscale materials. Several gram-negative, non-pathogenic bacterial species have been documented to manufacture Nano cellulose extracellularly, including Rhizobium, Xanthococcus, Pseudomonas, Azotobacter, Aerobacter, and Alcaligenes, however, the most prevalent BNC-producing strains are found in the genus Komagataeibacter. Bacteria produce the BNC through a process of dual coupled steps: polymerization and crystallization. In the bacterial

cytoplasm, glucose residues polymerize to β-1,4 glucan linear chains where they are extracellularly secreted. The developed chains are crystallized into` microfibrils; then certain numbers of microfibrils consolidate to materialize a highly pure 3D porous network of entangled nanoribbons of 20–60nm in width. Due to its extraordinary physical and chemical properties, including as eco-friendly manufacturing, low production costs, high mechanical capabilities, hydrophilicity, exceptional biocompatibility, and biodegradability, BNC has attracted increasing amounts of interest in recent years.[21,22] Bacterial cellulose is characterized by its high purity (with no accompanying substances like hemicelluloses, lignin, or pectin) and by an extremely high water content of 90% or greater.[24]

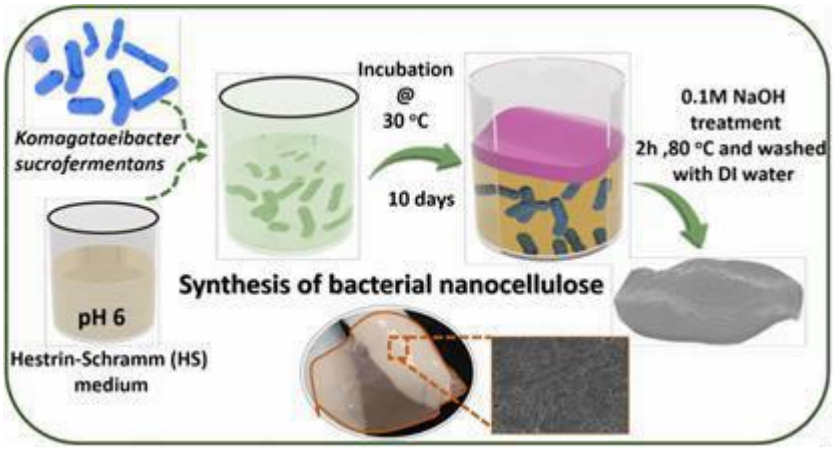

Fig 8: Synthesis of Bacterial Nano cellulose

1.5.4. Electro spun Cellulose Nanofiber (ECN)

The other possibility to produce cellulose with nanoscale/submicron lateral dimensions is electrospinning. Recently, the electrospun fibers from cellulose and its derivatives have gained evident interest. To produce Electrospun cellulose nanofibers (ECNF), cellulose is first dissolved in an appropriate solvent. Then, a high voltage is applied to a droplet of cellulose solution to overcome the surface tension and to form a jet of the solution. While it is passed through air, the solvent evaporates, forming a filament,

which is collected on an electrically grounded target. This method can be used to prepare fibers with a diameter of several tens of nanometers. However, ECNF starting from a few hundred nanometer to a few microns in diameter are commonly produced. Even so, they are often referred to as nanofibers. Recently, a lot of studies in this field have been devoted to the investigation of appropriate solvents for cellulose. Electrospinning can be also used to produce polymer composite fibers containing nanocellulose.[23] However, electrospun cellulose has some limitations, including low thermal stability and low solubility that greatly hinder its practical applications. Some efforts have been carried out to enhance the thermal stability, the mechanical property of cellulose, by blending polymer in recent years.[22]

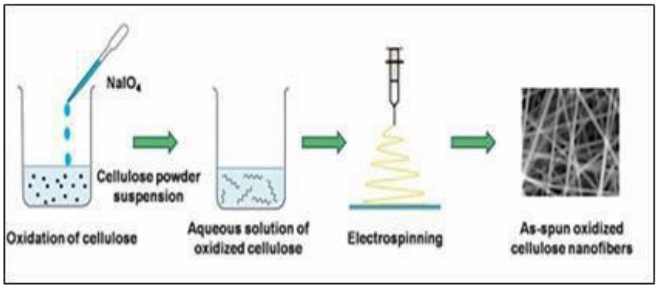

Fig 9: Synthesis of Electro spun cellulose Nano Fibers

1.6 CELLULOSE ISOLATION METHODS

The production of Cellulose Nano Crystals (CNCs) is one of the best methods to recycle waste paper. The characteristics of CNC, including crystallinity, yield, morphology, dimensions, surface chemistry, and chemical, physical and thermal properties, can be adjusted or a wide range of innovative applications, depending on the natural source, isolation techniques, and pre-and post-treatments.[3]

There are different routes for the isolation of nano cellulose which involve chemical routes, mechanical routes, physicochemical routes, and enzymatic methods. By using acid hydrolysis, nanocellulose can be isolated. However, before acid hydrolysis, a number of pre-treatment processes must be taken

to eliminate non-cellulosic components. An extensively researched chemical pre-treatment technique, alkali pre-treatment is based on the dissolution of lignin in the alkali solution. The hydroxides of sodium, potassium, calcium, and ammonium are among the different alkaline reagents that are frequently employed for alkali pre-treatment.

Among these sodium hydroxide was found to be the most effective. During the alkali pre-treatment procedure, a saponification reaction occurs that results in the breaking of the intermolecular ester bonds between hemicelluloses and lignin. As a result, lignin and hemicellulose fragments become solubilized in the alkali solution, bringing cellulose into contact with the enzymes. Additionally, the lignocellulosic structure is altered by alkali pre-treatment due to cellulose swelling, which lowers crystallinity and increases the degree of polymerization while increasing internal surface area. Additionally, during the alkali pre-treatment, the removal of acetyl groups and uronic acid replacements in hemicelluloses increases the accessibility of the carbohydrates to enzymatic hydrolysis.

The most popular inorganic acids used to make CNCs for acid hydrolysis are hydrochloric acid and sulfuric acid. However, investigations have shown that hydrolysis using hydrochloric acid has a poor dispersion ability, low thermal degradation, and weak oxidizing capacity. Contrarily, sulfuric acid hydrolysis produces the most stable cellulose solution due to the presence of the sulphate group on the crystallites, and as a result, the isolation of CNCs using sulphuric acid obtained better mechanical and thermal stability. Here, the hydrolysis procedure makes use of sulfuric acid. Cellulose was initially removed from the waste paper by alkali pulping and bleaching. After that, acid hydrolysis transformed it into nanocellulose crystals. Lignin, cellulose, and hemicellulose make up the majority of waste paper's three constituents. Among these, the cellulose component is extremely crystalline whereas the lignin and hemicellulose components are

comparatively amorphous. As a result, the cellulose crystals have two structures, crystalline area and amorphous region. The hemicellulose, lignin, wax, and other polymeric components found in the waste cotton cloths were removed using alkali treatment and bleaching. [6,17] The type and concentration of acid, temperature, duration, and acid-to-pulp ratio all have an impact on the features of CNCs that are produced.

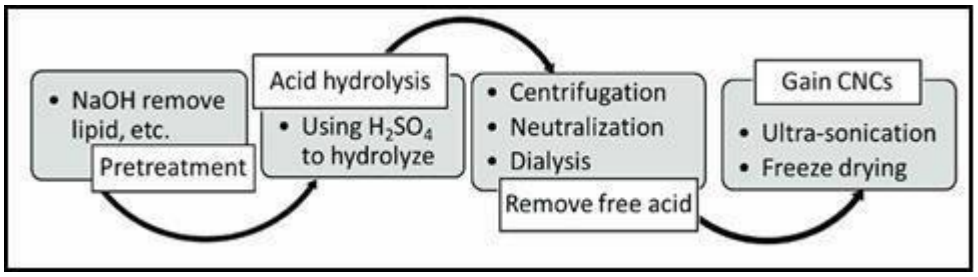

Fig 10: Isolation method

1.7 APPLICATION OF NANOCELLULOSE

There are a great number of potential applications of nanocellulose within different industries, viz. high-quality paper products; in cosmetics as a thickener and in the food industry as a stabilizer, fat replacer, and texturing agent; moldable lightweight, high-strength materials; composites for construction, vehicles, customer products, furniture; new materials for electronics and pharmaceutical applications. Industrial grades microcrystalline cellulose has a high proportion of sub-micron size colloidal microcrystals which are excellent stabilizing agents for water-based latex paints as well as for industrial coating and suspensions[6] . The emerging applications of CNCs in papermaking, polymer, food, and pharmaceutical industries, as well as in catalysis are discussed.

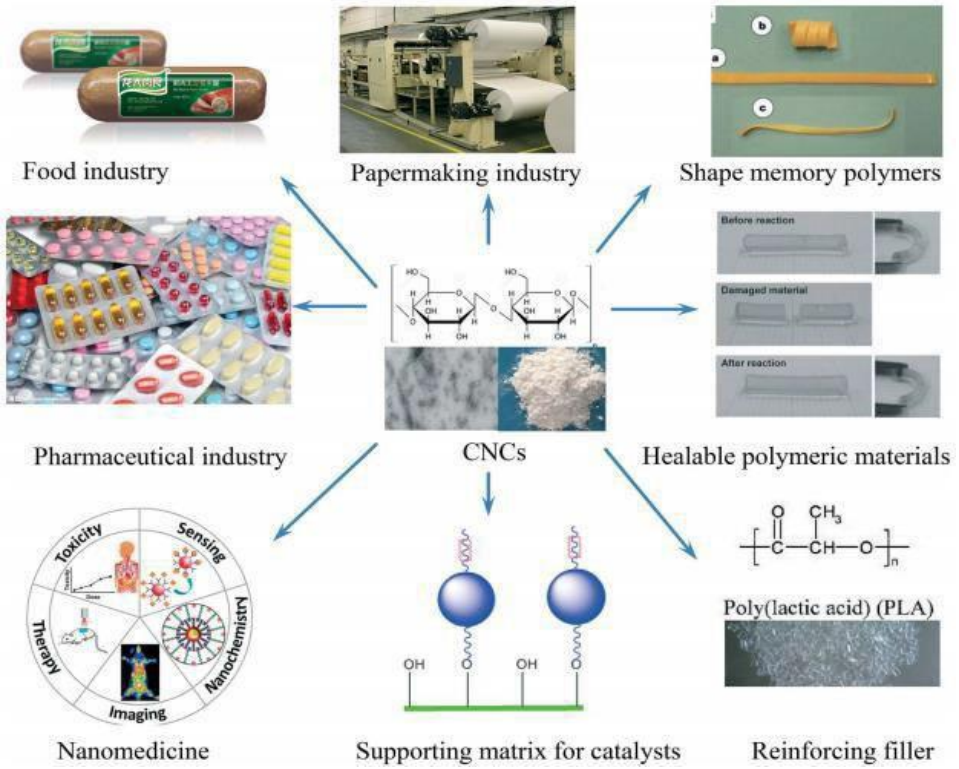

Fig11: Application of cellulose

1.7.1 Food Industry

Nano cellulose is of immense significance in the ongoing commercialization of nanotechnology. One such commercial industry is food packaging. This packaging technology is called Active Packaging which incorporates the use of nano-particles (Nano Cellulose) into polymer films to control microbial surface contamination of foods. From a packaging perspective, the motivations to apply Nano-scale particles are due to their Durability, Flexibility, Biodegradability, Transparency, and mechanical barrier property. Industries are very keen to develop Biodegradable, lightweight food-packaging materials, and nanoparticles serve their place. This kind of material can preserve the quality of food in terms of freshness and taste. Moreover, the shelf life of food will also increase which is quite important for both consumers and industries.[24]

As a food thickener, nanocellulose can be used as a low calorie replacement for carbohydrate additives, as a flavour carrier and suspension stabilizers. It can also be used to produce fillings, crushes, chips, wafers, soups, gravies, puddings etc. The food applications of CNCs were one of the earliest applications of nanocellulose due to the rheological behaviour of the nanocellulose gel.

1.7.2 Bio-medical Applications

The advancement in the area of nanomaterials, with outstanding features and various structures, has attracted more interest in their use in biomedical applications. Nano celluloses in particular show great promise as a cost-effective advanced material for biomedical applications because of their biocompatibility, biodegradability, and low cytotoxicity. Moreover, with their chemical functionality, they can be easily modified to yield useful products. Among the many types of nanocellulose materials, bacterial Nanocellulose has been a widely used cellulose in recent years for a wide range of biomedical applications, and it is successfully used in wound healing, tissue engineering applications, drug delivery, cartilage replacements, medical implants, etc. The coupling of Multidisciplinary fields such as life science, biology, physics, Chemistry, and engineering has long assisted the evolution of Nano biomaterials, which can be rationally designed from Biological or synthetic materials, for biomedical utilizations.[25]

1.7.3 Pharmaceutical

Cellulose has excellent properties of compaction when it is mixed with other pharmaceutical excipients, forming dense matrices that make the administration of therapeutic drugs easy. Nanocellulose offers potential advantages as an excipient in drug release. Its large surface area and negative charge suggest that higher quantities of therapeutical drugs can be

added to the surface of this material, showing the potential for a large quantity of charge and the optimal control of detoxification. The proven bio-compatibility of cellulose supports the use of nanocellulose for similar purposes. The hydroxide groups on the surface offer a site for surface modification to a broad range of chemical groups, using different methods. The surface modification can be used to tune the charge and drug release that are not normally linked with nano cellulose such as hydrophobic and non-ionized drugs

1.7.4 Paper-making industry

CNC has potential applications in the paper and paperboard industry where they can increase the fiber-fiber bond strength and thereby increase the strength of the paper. CNCs can also be used as a barrier in greaseproof types of papers and as a wet-end additive to enhance retention and dry and wet strength in commodity types of paper and board products. [24]

1.7.5 Catalysis

Immobilization of homogeneous catalysts on solid supports combines the advantages of homogeneous catalysis and heterogeneous catalysis. CNCs are ideal support matrix for catalysts because they display a well-defined size and high specific surface area, the high specific surface area of CNCs can: (1) support nano-catalysts directly; (2) be functionalized with other moieties to support the catalysts;9 (3) be grafted with the catalysts for selective catalysis. Table 4 shows the catalytic reactions in the presence of unsupported catalysts and CNC-supported catalysts. It is demonstrated that CNCs are excellent support matrices for the metal nanoparticles or the heterogenization of homogeneous catalysts. The CNC-supported nanocatalysts exhibited advantages over the supported nanocatalysts such as mild reaction conditions, excellent yields, short reaction time, easy work-

up procedure, product purity, and effortless separation and reusability of nanocatalysts.[26]

1.7.6 Reinforcing Filler for Polymers

The introduction of CNCs has a very significant impact on the mechanical properties of polymer nanocomposites over the corresponding matrix materials, such as the remarkable improvements in stiffness and strength. CNCs are attractive as reinforcing fillers due to the inexhaustible supply and high specific modulus (modulus/density).[27]

1.7.7 Healable polymeric materials

Self-healing materials with enhanced reliability, functionality, lifetime, and remarkable mechanical performance gained increasing interest from researchers in both academia and industry. Various stimuli-responsive attributes, such as the crack, heat, light, etc. can recombine the mechanically damaged network efficiently.

1.7.8 Sensors

Cellulose nanomaterials have also been exploited as sensors and stimuli-sensitive functional materials, including for use in biomedical applications. For example, modified CNCs with dual fluorescent labeling are used to create ratiometric pH-sensing nanoparticles. The emission spectra of the CNC suspension varied with pH, and the ratio of intensity at multiple wavelengths showed clear transitions as a function of pH. Peptide-modified CNCs in tandem with a cellulose membrane could be used as a biosensor wound dressing to detect a destructive protease

1.7.9 Nanocellulose in water treatment

Currently, researchers are exploring cost-effective and environment-friendly solutions for treating wastewater, which contains dyes, organic pollutants, and other toxic contaminants. In line with the solution for wastewater treatment, cellulose-based nano-composite material can serve

as a potential agent. It can act as a valuable resource to meet the industrial scalability and low carbon footprint profile because of its bio-renewable, environment-friendly, and inexpensive solutions.[8]

1.7.10 Composites

High mechanical performance composites can be produced by the incorporation of nanofillers into the polymers. Nanocellulose is the most appropriate material for composite preparation when compared to non-biodegradable nanofillers like carbon nanotubes, nano clays, etc. The introduction of CNCs into the polymeric matrix increases the tensile strength and decreases the elasticity. But its production is very difficult because of poor dispersion of CNC upon drying and low compatibility with hydrophobic groups. This can be overcome by the reinforcement of hydrophobic groups through surface modification and grafting. Nanocomposites can be used in several scientific areas and industries such as packaging, aerospace, adhesives, hydrogels, nano barriers, inks and painting, fire retardants, etc. [24,3]

In our work, we are making a composite thin film of CNC and Chitosan and assessing its potential application as an antibacterial agent.

1.8 CHITIN AND CHITOSAN

Chitin a naturally abundant mucopolysaccharide is a white, hard, inelastic, and nitrogenous compound that is a by-product of the fishery industry and is considered a regenerating raw material that is only second to cellulose in terms of abundance. Its natural abundance amounts to more than 1000 tons per year and about 70 % of which comes from marine species. Chitins are the main component in the shells of crustaceans such as shrimp, crab, and lobster, and is also found in the exoskeletons of mollusks and insects as well as in the cell walls of some fungi. Chitosan is mainly produced

commercially by deacetylation or removing acetyl groups from the chitin polymer by treatment with alkali.

Chitin is the second most ubiquitous natural polysaccharide after cellulose on earth and is composed of β(1→4)-linked2-acetamido-2-deoxy-βD-glucose1(N-acetylglucosamine). It is often considered a cellulose derivative, even though it does not occur in organisms producing cellulose. It is structurally identical to cellulose, but it has acetamide groups (−NHCOCH3) at the C-2 positions. Similarly the principle derivative of chitin, chitosan is a linear polymer of α (1→4)-linked2-amino-2-deoxyβ-D-glucopyranose and is easily derived by N-deacetylation, to a varying extent that is characterized by the degree of deacetylation and is consequently a copolymer of N-acetylglucosamine and glucosamine.

Chitin and chitosan are considerably versatile and promising biomaterials. The de-acetylated chitin derivative, chitosan is a more useful and interesting bioactive polymer. Despite its biodegradability, it has many reactive amino side groups, which offer possibilities for chemical modifications, the formation of a large variety of useful derivatives that are commercially available or can be made available via graft reactions and ionic interactions. Chitin is a white, hard, inelastic, nitrogenous polysaccharide found in the exoskeleton as well as in the internal structure of invertebrates. The waste of these natural polymers is a major source of surface pollution in coastal areas. The production of chitosan from crustacean shells obtained as food industry waste is economically feasible, especially if it includes the recovery of carotenoids. The shells contain considerable quantities of astaxanthin, a carotenoid that has so far not been synthesized and which is marketed as a fish food additive in aquaculture, especially for salmon. The chitinous solid waste fraction of the average Indian landing of shellfish ranged from 60,000 to 80,000 t. The three parts of our motherland, India,

are surrounded by ocean and its inner land is also very much rich with ponds, lakes, and lagoons. The proper utilization of those water resources (aquaculture) in terms of research in chitin and chitosan can bring economic and academic prosperity of the nation. Chitin and chitosan are now produced commercially in India, Poland, Japan, the US, Norway, and Australia. A considerable amount of research is in progress on chitin/chitosan the world over, including India, to tailor impart the required functionalities to maximize its utility.

Chitin and chitosan are naturally abundant and renewable polymers that have excellent properties such as biodegradability, bio-compatibility, non-toxicity, and adsorption. Various efforts have been made to prepare functional derivatives of chitosan by chemical modifications, graft reactions, and ionic interactions, and only a few of them are found to dissolve in conventional organic solvents. Chitosan is only soluble in aqueous solutions of some acids, and some selective N-alkylidinations and N acylation have also been attempted. Although several water-soluble or highly swelling derivatives are obtained, it is difficult to develop solubility in common organic solvents by these methods.

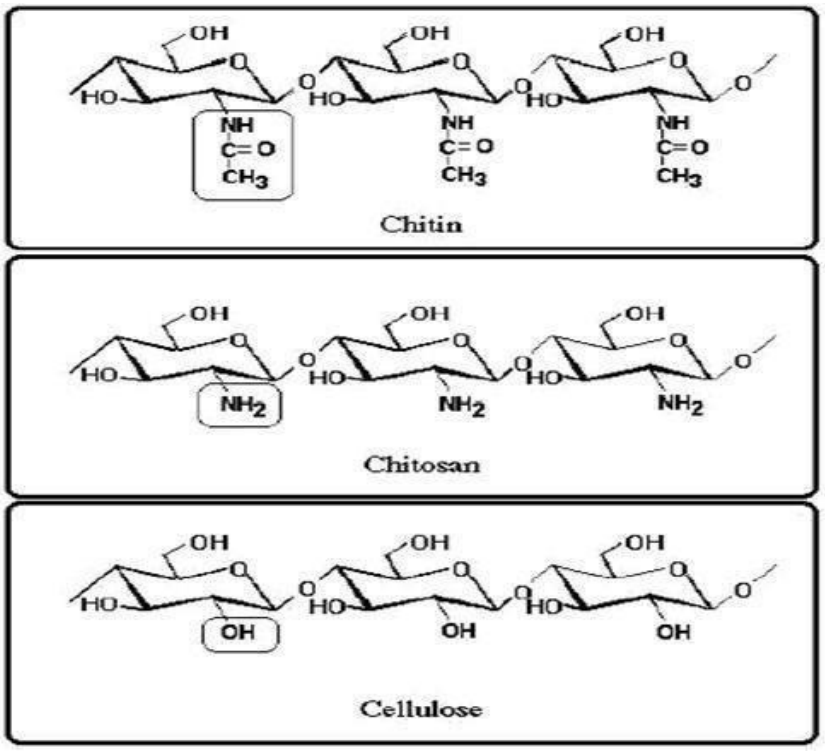

Fig 12: The structures of chitin, chitosan, and cellulose

Due to its key properties such as its biodegradability and biocompatibility, and its being mucoadhesive and non-toxic, chitosan is of great interest in many applications such as biomedicine, pharmacy, biotechnology, food industry, nanotechnology, etc. Chemical modification of chitosan is usually done in bulk, giving rise to randomly reacted units. When specific new functionalities are pursued, other approaches are preferred in which the reactions can be controlled stoichiometrically.

1.9 PROPERTIES OF CHITOSAN

The main characteristics of chitosan are its degree of acetylation and molecular weight. These have a determining effect on the chitosan's functional properties, from its solubility and materials-forming capacity to biodegradability and diverse bioactive attributes. Higher DDA chitosan films showed a greater crystallinity, a higher elastic modulus and tensile

strength and a lower swelling index than those with lower DDA. Deacetylation refers to the process of removal of acetyl groups from chitin and substitution of reactive amino groups (NH_2) and the degree of deacetylation (DDA) determines the content of free amino groups in the structure. So, it can be employed to differentiate between chitin and chitosan. DDA is considered an important property in chitosan as it affects the physicochemical and biological properties. Chitosan contains reactive amino and hydroxyl groups enabling it to chelate with metal ions and form complexes. Its biological properties include Biocompatible, Safe and non-toxic, binds to mammalian and microbial cells aggressively, Haemostatic, Fungi static, Spermicidal, Anti-tumor, Anti cholesteric, etc.

1.10 APPLICATIONS OF CHITOSAN

Due to its physical and chemical properties, Chitosan is being used in a vast array of widely different products and applications, ranging from pharmaceutical and cosmetic products to water treatment and plant protection. In different applications, different properties of Chitosan are required. These properties change with, e.g., degree of acetylation and molecular weight as well.

The use of chitosan has been postulated in numerous areas of biopharmaceutical research such as muco adhesion, permeation enhancement, vaccine technology, gene therapy, and wound healing. Recent applications of chitosan are in ophthalmic, nasal, sublingual, buccal, periodontal, gastrointestinal, colon-specific, vaginal, transdermal drug delivery and mucosal-vaccine and gene carrier. It can also be used in the pharmaceutical industry in direct tablet compression, as a tablet disintegrant, for the production of controlled release solid dosage form, or for the improvement of drug dissolution Chitosan derivatives were developed to improve not only biological activities but also water-soluble

properties, because the water-insoluble property was a major limiting factor for industrial application in spite of its unique biological aspects.

The improvement of structural properties of chitosan for a particular application can be easily brought about by chemical modification. Fortunately, chitosan is amenable to chemical modifications due to having of hydroxyl, acetamido and amine functional groups. For that reason, chemical modifications would not change the fundamental skeleton of chitosan and would keep the original physicochemical and biochemical properties while bringing new or improved properties. Chitosan and its derivatives find numerous applications in the field of tissue engineering, cancer diagnosis, ophthalmology,anti-thrombogenic and Haemostatic materials, antiageing properties, anti-tumor activity, water engineering, paper industry, textile industry, agriculture, photography, etc to name a few. Chitosan is reported to have antibacterial ability. The growth of *E. Coli* was inhibited in the presence of chitosan with a concentration greater than 0.025%. Chitosan is also able to inhibit the growth of other microbial species such as Fusarium, Alternaria, and Helminthosporium. The cationic amino groups of chitosan probably bind to anionic groups of microorganisms and prevent their growth. Low-molecular-weight chitosan after penetrating into bacterial cell walls, can bind with DNA and inhibit DNA transcription and mRNA synthesis. The natural antimicrobial characteristics of chitosan derivatives have resulted in their use in commercial disinfectants and topical antimicrobials.

Chitosan shows better film properties. Keeping in view of the ample potential of chitosan films, we have made an attempt to prepare green composite films of Chitosan-CNC. The physicochemical properties and antimicrobial properties of these green composite films were studied. A detailed comparative study of these green composite films with pure

chitosan films was also done. In our wor,k we are exploiting the antibacterial activity of Chitosan to synthesize a CNC-Chitosan thin film that could potentially be used in packaging.

1.11 CHARACTERISATION TECHNIQUES

1.11.1 XRD

One of the most popular methods for the characterization of nanoparticles is X-ray diffraction (XRD). The lattice parameters, phase, crystalline grain size, and crystalline structure are typically determined by XRD. When the conditions of Bragg's Law are satisfied, $n = 2d\sin\theta$, where n is an integer, the X-ray diffraction or constructive interference between elastically dispersed X-ray beams can be observed at particular angles 2θ when a crystal with an interplanar spacing of d (the crystal lattice constant) is exposed to an X-ray beam of a corresponding wavelength. The amount of crystallinity or amorphous content, phases present, phase concentrations, structure, and crystallite size and strain are all shown by the nanomaterial's powder diffraction pattern. The average crystallite size is determined by the peak widths in a given phase pattern; large crystallites produce sharp peaks, and an increase in peak width denotes a smaller crystallite size. The particle size of the crystal is estimated by using Scherrer's equation.[28]

1.11.2 FTIR

Fourier Transform infrared spectroscopy (FTIR) has been widely employed in research based on polysaccharides like cellulose because it accurately predicts the chemical changes that occur after the treatment using the chemical approach.[29]

The method is based on the measurement of electromagnetic radiation's absorption at wavelengths between 4000 and 400 cm-1 in the mid-infrared range. A recorded spectrum shows the location of bands associated with the strength and type of bonds, as well as certain functional groups, giving

information about the interactions and structures of molecules. In conjunction with conventional infrared spectroscopy, attenuated total reflection (ATR) sampling allows materials to be directly viewed in either a solid or liquid condition without the need for further preparation. An accessory for attenuated total reflection measures the changes that an internally reflected infrared beam experiences as it comes into touch with the selected sample. The chosen materials must make decent contact with the crystal's surface. The wave will change or weaken in the regions of the spectrum where the sample absorbs energy.[30]

1.11.3 TEM

The morphological and structural studies of cellulose microfibrils and nanocrystals incorporated information from spectroscopic and scattering analyses in addition to that obtained through TEM imaging and electron diffraction. The sample for TEM imaging has to be extremely thin in order to be transparent to electrons. Thus, preparing the sample is an essential step. The observed material's atomic number, density, and incident electron energy all affect the limiting thickness. For bulk materials, special sectioning techniques are needed to prepare such a thin specimen. However, because the thickness of each individual CNC is much lower than this 1 µm, it is much simpler to prepare TEM specimens, such as from diluted suspensions. [32] A transmission electron microscope can be divided into three major sections:

1. The electron gun is part of the microscope that fires electrons off toward the sample the user is magnifying.
2. Electromagnetic lenses that consist of coils of wires with electricity running through them.
3. Following, the electron beam enters the apparatus that creates images. The sample being scanned is at the system's beginning. To generate the

image, the electrons are focused onto a fluorescent or phosphorescent screen after passing through the sample and a new set of electromagnetic lenses.

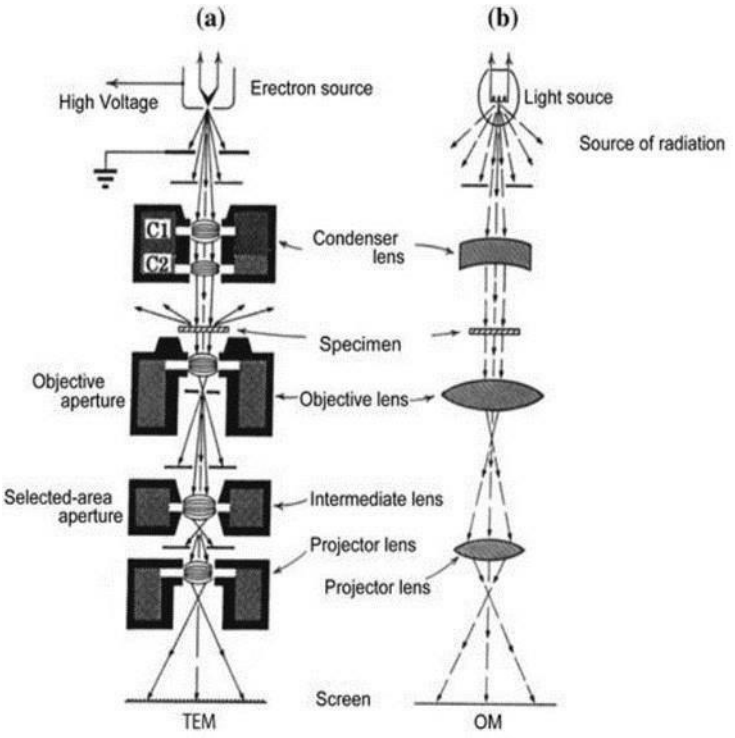

Fig 13: A schematic diagram of the Transmission Electron Microscope

1.11.4 SEM

Scanning electron microscope (SEM) is one of the most widely used techniques used in the characterization of nanomaterials and nanostructures. The signals that derive from electron-sample interactions reveal information about the sample including surface morphology (texture), and chemical composition of the sample.[34] Accelerated electrons in an SEM carry significant amounts of kinetic energy. This energy is dissipated as a variety of signals produced by electron-sample interaction when the incident electrons are decelerated in a solid sample. Due to very narrow electron beams, SEM micrographs have a large depth of field yielding a

characteristic three-dimensional appearance useful for understanding the surface structure of the sample. When an electron beam comes and hits the atoms of the specimen those atoms absorb their energy and give off their own electron- secondary electron. There is a detector to pick up the secondary electron which has a positive charge of it about 300 V. Secondary Electrons (SE) are emitted from very close to the specimen surface and can produce a very high-resolution image of the sample surface revealing details less than 1nm. The electron beam is generally scanned in a raster pattern and the beam's position is combined with the detected signal to produce an image of the surface. SEM requires more time-consuming sample preparation. The information obtained is visual and descriptive, it is usually not quantitative since only a few particles are seen in the viewing field at one time. However, when SEM is used with other techniques such as laser diffraction, it can provide valuable additional information on particle texture, which may help to explain agglomeration or flow problems. [35]

1.11.5 SAED

Selected area (electron) diffraction (abbreviated as SAD or SAED), is a crystallographic experimental technique typically performed using a transmission electron microscope (TEM). It is a specific case of electron diffraction used primarily in material science and solid-state as one of the most common experimental techniques. Especially with appropriate analytical software, SAD patterns (SADP) can be used to determine crystal orientation, measure lattice constants, or examine its defects. The SAD analysis is widely used in material research for its relative simplicity and high information value. Once the sample is prepared and examined in a modern transmission electron microscope, the device allows for a routine diffraction acquisition in a matter of seconds. If the images are interpreted

correctly, they can be used to identify crystal structures, determine their orientations, measure crystal characteristics, and examine crystal defects or material textures. The course of analysis depends on whether the diffractogram depicts a ring or spot diffraction pattern and on the quantity to be determined.

Software tools based on computer vision algorithms brought significant improvement to a routine quantitative analysis based on SAD in terms of accuracy, repeatability, and time efficiency. Compared to an expert evaluator, the computer is able to see features invisible to humans and process them in numbers and combinations never processable by humans. The analysis automation makes it available even for non-experts.[40]

1.11.6 STUDY OF WETTABILITY

(CONTACT ANGLE MEASUREMENTS)

The Contact angle is the angle, conventionally measured through the liquid, where a liquid-vapor interface meets a solid surface. It quantifies the wettability of a solid surface by a liquid via the Young's equation. A given system of solid, liquid, and vapor at a given temperature and pressure has a unique equilibrium contact angle. The equilibrium contact angle reflects the relative strength of liquid, solid, and vapor molecular interaction. Contact angles are extremely sensitive to contamination; values reproducible to better than a few degrees are generally only obtained under laboratory conditions with purified liquids and very clean solid surfaces. If the liquid molecules are strongly attracted to the solid molecules then the liquid drop will completely spread out on the solid surface, corresponding to the contact angle of 0° This is often the case for water on bear metallic or ceramic surfaces, although the presence of the oxide layer or contaminants on the solid surface can significantly increase the contact angle. Generally, if the the water contact angle is smaller than 90, the solid surface is considered

hydrophilic, and if the water contact angle is larger than 900 the solid surface is considered hydrophobic. Some materials with highly rough surfaces may have a water contact angle even greater than 150^0 due to the presence of air pockets under the liquid drop. These are called super-hydrophobic surfaces.

Fig 14: Contact angle Goniometer

The contact angle can be measured by different methods. They are the static sessile drop method, the pendant drop method, the dynamic sessile drop method. In the present study, we use the static sessile drop method, where the sessile drop contact angle is measured by contact angle Goniometer using an optical sub-system to capture the profile of a pure liquid on a solid substrate. The angle formed between the liquid-solid interface and the liquid-liquid interface is the contact angle. The current-generation system employs high-resolution cameras and software (Drop Image Advanced) to capture and analyze contact angles. Angles measured in such a way are often quite close to the advancing contact angle. [Z6] In this work, the water contact angles of the prepared films were measured before and after UV irradiation**.**

1.12 ANTIBACTERIAL ACTIVITY

Bacteria and fungi exist in every corner of the world, and their infection is one of the biggest global challenges to human health [41]. Notably, the overuse or abuse of antibiotics in the long-term lead to the emergence of multiple drug-resistant (MDR) bacteria, which increases the difficulty of infectious disease therapy. Also, fungi and bacteria can cause widespread plant diseases. Pesticides are used widely for the resistance of plant diseases, while their continuous over-use always leads to severe environmental problems. Non-functional cellulose showed a slightly better bactericidal effect on Gram-negative bacteria compared to Gram-positive ones. Biomaterials available for a wide range of applications are generally polysaccharides. They may have inherent antimicrobial activity in the case of chitosan. However, in order to have specific functionalities, bioactive compounds must be immobilized or incorporated into the polymer matrix, as in the case of cellulose.[42]

1.15 OBJECTIVES

- Recycling waste paper to produce a value-added product (CNC).
- Extraction of cellulose from the waste paper by alkali pulping, and bleaching.
- Preparation of CNC by acid hydrolysis.
- Characterization of raw material, extracted cellulose, and cellulose nanocrystals by XRD and FTIR.
- Morphology study of CNC by TEM.
- Determination of cellulose content using the Acid Detergent Method (ADF).
- Preparation of green composite thin film from CNC and chitosan.
- Characterization of thin film by SEM.
- Antibacterial study of chitosan-CNC composite.
- Study of wettability by contact angle measurement.

Chapter 2

Literature Review

Wan Hazman Danial and co-workers (2014), reported the preparation of cellulose nanocrystals (CNCs) from wastepaper, as an environment-friendly approach. Alkali and bleaching treatments were employed for the extraction of cellulose particles followed by controlled conditions of acid hydrolysis for the isolation of CNCs. (ATR FTIR) spectroscopy was used to analyze the cellulose particles extracted while Transmission electron microscopy images confirmed the presence of CNCs. The diameters of CNCs are in the range of 3 -10 nm with a length of 100 – 300 nm while a crystallinity index of 75.9% was determined from X-ray diffraction analysis. [44]

Pereira et al. 2011 from the Department of Materials and Technology, Brazil investigated sugarcane bagasse pulping and bleaching. A three- stage isolation process was utilized to obtain cellulose fibers. Crude, untreated SCB (sugarcane bagasse) was treated with a 10% (w/v) H_2SO_4 solution and then bleached in sodium chlorite to remove residual lignin. FTIR analysis revealed that bands at 1512 cm^{-1} and 1250 cm^{-1} were not present for bleached cellulose fibers which indicated a reduction in the lignin content of the cellulose fibers. The removal of lignin was confirmed by X-ray diffraction where a major diffraction peak for 2θ ranging between 22° and 23° was present, which corresponds to cellulose (002) crystallographic planes. The spectrum corresponding to the unmodified sugarcane bagasse showed diffraction peaks at 2θ angles 15.9º and 22.4º.

For crude cellulose fibers the same peaks could be observed at 15.9º and 23.2º but were of decreased intensity. Bleached cellulose fibers showed the same peaks observed at 16.2º and 22.9º but were of increased intensity. The TGA and DGT profiles of the fibers presented degradation peaks between 260 - 340°C from untreated to bleached cellulose fibers. The increase in degradation temperature for the treated fibers was attributed to the bleaching treatments. SEM micrographs showed that treatments were successful in the removal of wax, pectin, lignin, and hemicelluloses. Bleaching of the fibers reduced fiber length and fiber diameter. It was observed that the bleached cellulose fibers demonstrated higher thermal stability, crystallinity content increase, and flattened morphology when compared to crude cellulose fibers. [45]

Mukarram Zubair et al. (2021) studied cellulose, a biobased material that presents a massive opportunity for its application in the construction industry. Office paper waste (OPW) is cellulose-rich feedstock and a low- cost source to produce cellulose nanocrystals (CNCs). In this study, response surface methodology (RSM) was used for the production of CNCs from OPW using acid hydrolysis technique at different hydrolysis conditions; time (30–90min), temperature (40–50°C), The physicochemical properties of CNCs were analyzed using various techniques. The highest yield (> 83%) was achieved at the lowest process time, temperature, and acid ratio. The increase in process time and acid ratio from 30 to 90 min and 1:15 to 1:25 resulted in higher crystallinity of CNCs. However, higher process temperatures (50 °C) showed a reduction in crystallinity and length of CNCs. [46]

Meng Li et al. (2018) studied Starch nanocomposite films that were successfully prepared using the isolated cellulose nanofibers (CNFs) at loading from 5 to 20% by solution casting. The morphology, crystallinity, surface hydrophobicity, water vapor permeability, opacity, and mechanical test of nanocomposite films were studied. The CNFs were uniformly

distributed within these films up to 15% of CNFs. The incorporation of 20% CNFs in the composite film produced selective aggregation and, thus, inhomogeneity of the CNFs. The contact angle values increased from 49.46° (starch-only films) to 88.57° (CNFs loading at 20%). The incorporation of CNFs hinders the diffusion or permeability of water vapor through the composite. The addition of CNFs to starch films resulted in significant improvement in glass transition temperature (T_g), tensile strength and modulus of elasticity of nanocomposites films. [47]

Pratiksha Shrestha et.al (2021) studied on the Development of antibacterial bio composites reinforced with cellulose nanocrystals derived from banana pseudo stem. Crystalline nanocellulose (CNC) was derived from banana pseudo stem using acid hydrolysis method. CNC was characterized by scanning electron microscope, Fourier transformed infrared spectroscopy and X-ray diffraction. CNC was found in nanometric dimensions (18.79 ± 5.30 nm diameter and 202.12 ± 37.43 nm length) and exhibited high degree of crystallinity (81.67%). Chitosan-CNC based antimicrobial nanocomposites films were prepared by the incorporation of tetracycline and showed well-demarcated zone of inhibition against Staphylococcus aureus and Escherichia coli. Chitosan- CNC nanocomposite films showed significantly ($p < 0.05$) high tensile strength and good swelling properties in comparison to control CNC films. This study suggests that banana pseudostem can be used as a raw material for economic production of CNC and nanocomposites for biomedical applications.[48]

Cobb et al.'s 2018 contact angle measurement study It is a qualitative method of determining whether a surface is hydrophobic or hydrophilic. It is based on observations of the intermolecular interactions that occur when a drop of water comes into contact with a surface. It is mostly used to assess the wettability of a surface. Because fresh synthetic diamond surfaces have

a hydrophobic feature due to hydrogen termination, water contact angles are expected to be closer to 90°. However, if a diamond is oxygen-terminated, the surface slowly oxidises. Because water contact angles are frequently smaller than 65°, a hydrophilic characteristic can be observed. Contact angle measurements are said to be a quick and inexpensive procedure. [49]

Teixeira et al. 2011 from the National Nanotechnology Laboratory of Agriculture (LNNA), Embarq Agricultural Instrumentation, Brazil extracted and characterized nanocellulose whiskers from sugarcane bagasse. SCB fibers were extracted after alkaline peroxide pre-treatment followed by acid hydrolysis. The results showed that SCB could be used as a source to obtain cellulose whiskers and they had needle-like structures with an average length (L) of 255 ± 55nm and diameter (D) of 4 ± 2 nm, giving an aspect ratio (L/D) around 64. The samples were named SC and SCBW 75 due to the time used for acid hydrolysis. The produced whiskers were in the form of a stable suspension but the sample hydrolyzed for 75min had a brown discoloration due to a certain level of cellulose degradation. The whiskers obtained had a length (L) of around 255 ± 55 nm and diameters (D) of 4±2 and 8±3 nm for SCBW 30 and SCBW 75, respectively. The sample SCBW75 presented a decrease in crystallinity and a little change in the diffractograms profile, with the disappearance of the peak at $2\theta = 15.3°$.[50]

N.A. Sri Aprilia et al. 2021 from the Department of Chemical Engineering, Engineering Faculty, Universitas Syiah Kuala, Banda Aceh, Indonesia prepared nanocellulose from sugarcane bagasse by using homogeneous hydrolysis of hydrochloric acid (HCl) with and without ultrasonication. The homogeneous process by using ultrasonication for 30 minutes. Four nano cellulose samples were prepared with and without homogeneous with HCl concentrations of 10 and 20%. Nanocellulose characterization was analyzed by Fourier transform infrared spectroscopy

which indicated the presence of carboxyl groups from cellulose. The X- ray diffraction spectrum proved the presence of cellulose, with high crystallinity of 68 and 65% by ultrasonication and 53 and 48% without ultrasonication with increased HCl concentration. The sizes of crystalline nanocellulose were decreased with increased HCl concentration, even though with and without ultrasonication. Furthermore, the nanocrystalline size was reduced from 4.37, 4.15, and 3.94 nm. Finally, with scanning electron microscopy nanocrystalline cellulose analysis showed that the nano cellulose structure was like individual crystallites and was uniformly dispersed showing a needle-shaped structure.[51]

T.N. Mohammed Irfan and his co-workers in their current research showed that nanocellulose can be effectively separated from waste papers, a resource that is abundant, renewable, and inexpensive. Nanocellulose can be employed as an effective green filler in a PVA/starch matrix for packaging applications. Before the sulphuric acid treatment, the noncellulosic components were removed using sodium hydroxide and hydrogen peroxide, leaving behind fibers with a high cellulose content. Studies indicated that the recovered paper nanocellulose had a crystalline nature (74%) and strong thermal stability, as well as a nano fibrillar network-like structure. High aspect ratio materials can function well as reinforcement in polymers at extremely low filler loading levels to achieve percolation. EDX results confirmed that the cellulose was pure. The mechanical and water resistance characteristics of the films were improved when the PVA/starch matrix was crosslinked with isolated paper cellulose. The resulting composite film can thus serve as an alternative to packaging films made of synthetic polymers that are not biodegradable. [52]

Khan and his co-workers (2020) conducted a study on Chitosan-Nanocellulose Composites for Regenerative Medicine Applications and concluded that Cellulosic nanomaterials (CNs), owing to their exceptional

mechanical strength, ease of chemical modification, biocompatibility, and favorable interaction with chitosan, represent an attractive candidate for the fabrication of chitosan/ CNs scaffolds and hydrogels. The unique mechanical and biological properties of the chitosan/CNs bio-nanocomposite make them a material of choice for the development of next-generation bio-scaffolds and hydrogels for regenerative medicine applications. In this review, we have summarized the preparation method, mechanical properties, morphology, and cytotoxicity/ biocompatibility of chitosan/CNs nanocomposites for regenerative medicine applications, which comprises tissue engineering and wound dressing applications. This study aimed to characterize nanocellulose extracted from sugarcane bagasse (SCB) by acid hydrolysis 60% (w/w) H_2SO_4 at 45^0C. The effect of hydrolysis time (20, 30 and 40 min) on the structure and properties of the nanofibers was investigated. Fourier transform infrared spectroscopy (FT-IR), and X-ray diffraction (XRD) results indicated that the hemicellulose and lignin were removed extensively in the cellulose whiskers. The morphology and dimensions of the fibers and acid-released cellulose nanowhisker (CNW) were characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The results showed that SCB could be used as source to obtain cellulose whiskers and they had needle-like structures. Longer hydrolysis time produced a lower yield of nanofibers; whereas the degree of crystallinity increased from 38.22% to 65.37% with increasing hydrolysis time due to removal of amorphous cellulose.[53]

Chapter 3

MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter includes the characterization techniques, materials, and methods used for the synthesis and characterization of raw material, bleached samples, and Cellulose Nano Crystals (CNCs).

3.2 CHARACTERIZATION TECHNIQUES

3.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

This technique was used to manipulate structural changes on samples as a result of chemical modification by the identification of the functional groups. The changes in functional groups of the materials: Raw material, Bleached sample, and CNCs were investigated using FTIR spectroscopy using Nicolet. Is 50, FTIR (Thermo Nicolet, USA) spectrophotometer. The FTIR spectra of the samples were recorded in the transmittance mode in the range of 400 – 4000 cm^{-1}.

3.2.2 X-Ray Diffraction (XRD)

The crystallinity index of the material before and after chemical modification was analyzed using Shimadzu XRD-700 X-RAY Diffractometer. Raw material, bleached samples, and CNCs in the form of milled powder were placed on steel sample holders and leveled to obtain total and uniform X-ray exposure. The samples were analyzed at 25°C with

a monochromatic Cuk α radiation source -0.1539 nm with a 2Θ angle ranging from 10° to 60°.

3.2.3 Transmission Electron Microscopy (TEM)

Morphological properties and particle sizes of CNCs were determined using (Tecnai G2 20 S-twin) Transmission Electron Microscope. The samples were dispersed in a suitable medium and then placed on a copper grid coated with a carbon film.

3.2.4 Selected area electron diffraction (SAED)

selected area electron diffraction (SAED) is a crystallographic experimental technique that is performed by using a transmission electron microscope. In the SAED pattern, each obtained spot corresponds to a satisfied diffraction condition [36]. The crystalline nature of CNC was determined using (Tecnai G2 20 S-twin) Transmission Electron Microscope. The samples were dispersed in a suitable medium and then placed on a copper grid coated with a carbon film.

3.3 SYNTHESIS OF CELLULOSE NANOCRYSTALS USING WASTE PAPER

3.3.1 Chemicals Required

1. 10% Sodium hydroxide (NaOH)

2. 30% Hydrogen peroxide (H_2O_2)

3. 45% Conc. Sulphuric acid (H_2SO_4)

3.3.2 Materials Required

1. Waste paper

3.3.3 Apparatus Required

1. Magnetic stirrer

2. Hot Plate

3. Sonicator

3.3.4 PROCEDURE

ISOLATION OF CELLULOSE NANOCRYSTALS

This chapter co prehends the materials and methods used for the synthesis of CNC from waste paper. The method adopted is the acid hydrolysis method. The used waste paper was sourced from exam papers collected from our department. This was cut into small pieces for water treatment and boiled for 7 hours while distilled water was added periodically. It was then made to form a slurry, filtered, rinsed several times with distilled water, and dried at 60^0c. The dried sample was alkali pulped with 10% (w/v) NaOH at 80^0c for 5 hours. After neutralizing the sample by centrifuging and rinsing, with distilled water it was bleached with 5% NaOH (v/v) and 30% H_2O_2 (v/v) in a ratio of 1:1 at 50^0c for 3 hours. The slurry obtained is then filtered and washed with distilled water until neutral pH is achieved.

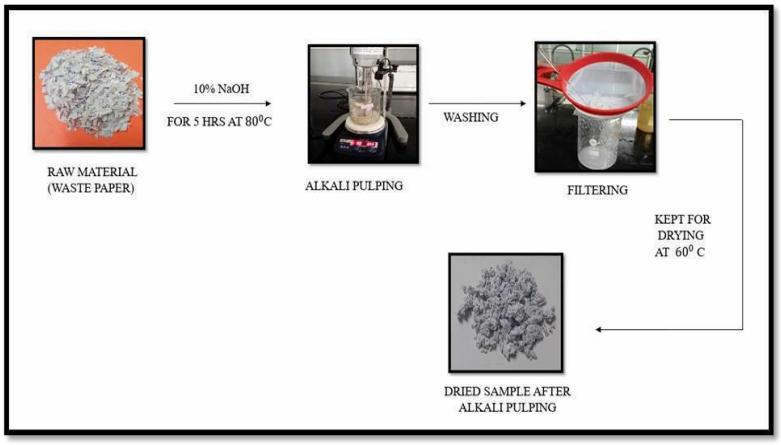

Fig 15: flow chart showing alkali pulping procedure

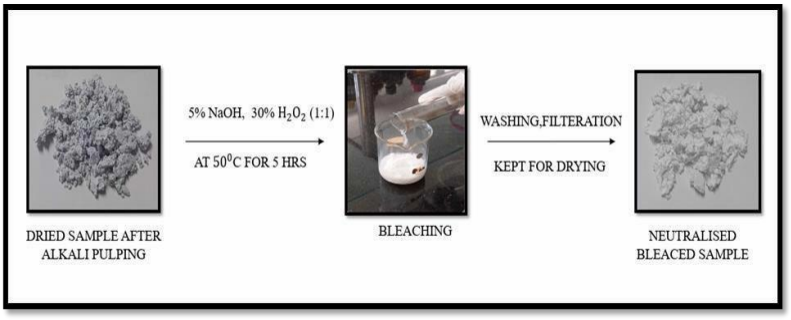

Fig 16: flow chart showing the bleaching procedure

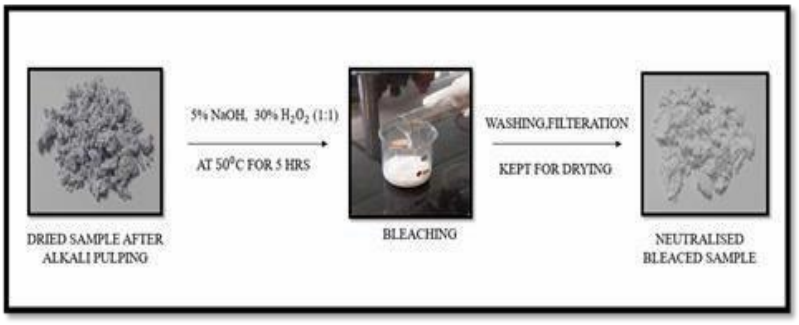

Fig 17: flow chart showing acid hydrolysis

PREPARATION OF CELLULOSE NANOCRYSTALS

The isolated cellulose was used to prepare cellulose nanocrystals by hydrolysis. The chemically purified cellulose from the waste paper was hydrolyzed with 45% (w/v) of H_2SO_4 at 50^0c with constant stirring for 3 hours. The mixture was diluted with distilled water followed by centrifugation to remove spent acid. The colloidal suspension was then sonicated in an ice bath for 30 min. This was then allowed to settle. The colloidal suspension formed is Cellulose nanocrystals (CNC).

3.4 DETERMINATION OF CELLULOSE (ADF- Acid Detergent Fibre method)

3.4.1 Reagents

1. Cetyl trimethyl ammonium bromide (C-TAB)
2. 0.5 M H_2SO_4
3. Octan-2-ol

3.4.2 Apparatus

1. Iodine flask
2. Sintered crucible
3. Suction filtration

3.4.3 Procedure

0.5 g (W_1) of the sample is weighed into a 250 ml iodine flask to this 100 ml C-TAB solution (dissolve 5 g of C-TAB in 500 ml of 0.5 M H_2SO_4) and a few drops of Octan-2-ol as an antiform agent. It is kept on a hot plate at 60^0C for 1 hour. Then it is filtered using a previously weighed sintered crucible (W_2) while it is hot using gentle suction. After this procedure, it is washed with boiled distilled water and acetone respectively until all the colors are removed. The sintered crucible is dried for two hours at 105^0C, cooled in a desiccator, and weighed(W_3). Add 72% H_2SO_4 (w/v) (cooled to 15^0C) till half of the sinter and make a smooth paste using the glass rod and

let the acid drain from the sinter crucible. Again refill with 72% H_2SO_4 and stir and repeat the procedure and let the acid drain. After 3 hours, filter the acid under a vacuum and wash the contents with hot water until free of acid. Rinse the stirring rod and wash the product with acetone. Dry the sinter at 105^0C for 2 hours, cool in a desiccator, and weighed (W_4).[37]

3.5 SYNTHESIS OF GREEN COMPOSITE THIN FILM FROM CNC AND CHITOSAN

1g Chitosan was dissolved in 100 ml 1% acetic acid and filtered. 100 ml of this 1% Chitosan solution was mixed with 10 ml of prepared CNC solution. It was thoroughly mixed for 2 hours. The uniform mixture obtained was solvent cast to obtain a thin film of Chitosan-CNC.

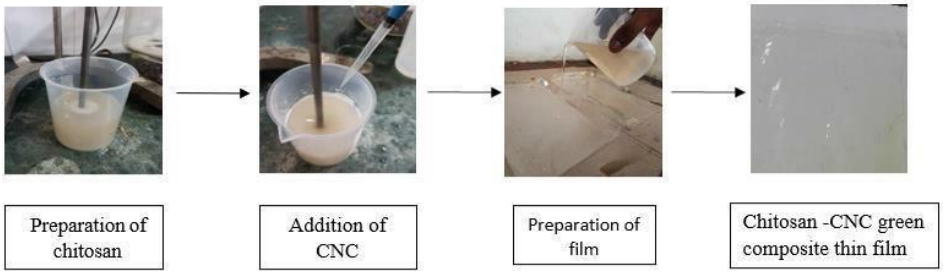

Fig 18: flow chart for preparation of green composite of chitosan and CNC

3.5 ANTIBACTERIAL STUDIES OF THIN FILM: DISC WELL DIFFUSION METHOD

The antibacterial activities of the Chitosan, chitosan-CNC composite, and CNC were assessed by agar well diffusion method. One ml of the fresh culture of *E.Coli* was inoculated in the sterile Petri dishes distinctly. Wells were made using a sterile cork borer into agar plates containing inoculums. Then, 100 µl of each test solution was added to respective wells. The test solutions were Chitosan(A), chitosan -CNC composite (B), CNC(C). Then, the plates were incubated at 37^0C for 24 hours. Antimicrobial activity was

detected by measuring the zone of inhibition (including the diameter of the wells) that appeared after the incubation period. Acetic acid was employed as a negative control. Tetracycline, an antibiotic was used as the standard.[33]

Chapter 4

RESULTS AND DISCUSSION

4.1 CHARACTERIZATION OF CELLULOSE NANOCRYSTALS

4.1.1 Physical Appearance

The physical appearance of the raw material which is obtained from waste paper, chemically purified cellulose, and nanocrystals prepared by hydrolysis methods are shown below.

RAW MATERIAL

CHEMICALLY PURIFIED CELLULOSE

CELLULOSE NANOCRYSTAL

The treatment of the raw material with NaOH and H_2O_2 changed the color and texture of the material. The white color indicates the removal of impurities and the presence of maximum cellulose content. Hydrolysis of the extracted cellulose with H_2SO_4 produced a stable colloidal suspension of Cellulose Nanocrystal.

4.1.2 XRD

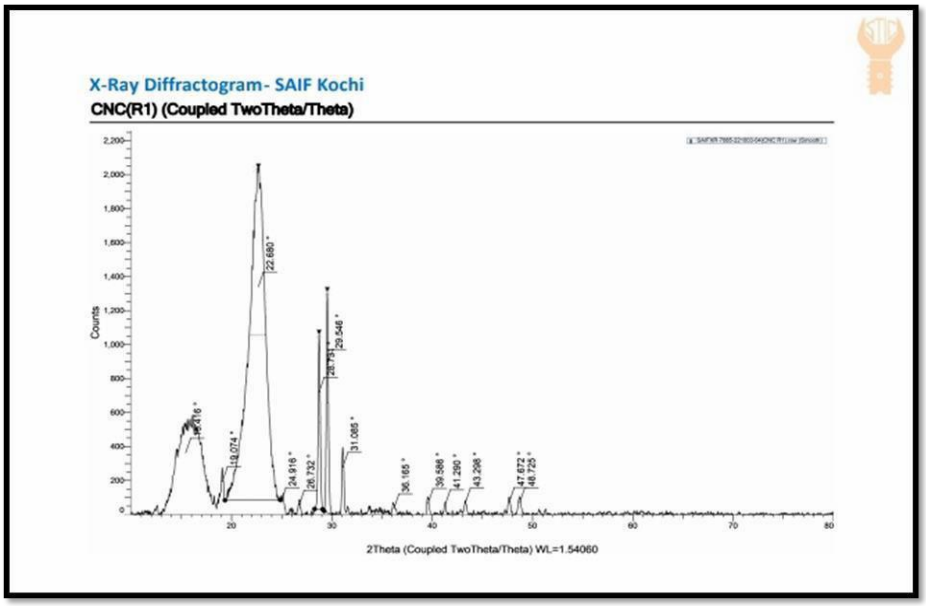

Fig 19: XRD pattern of the raw material.

It is 2θ angle ranging from 10° to 50°. These XRD spectra give peaks at 18.074° and 22.690°

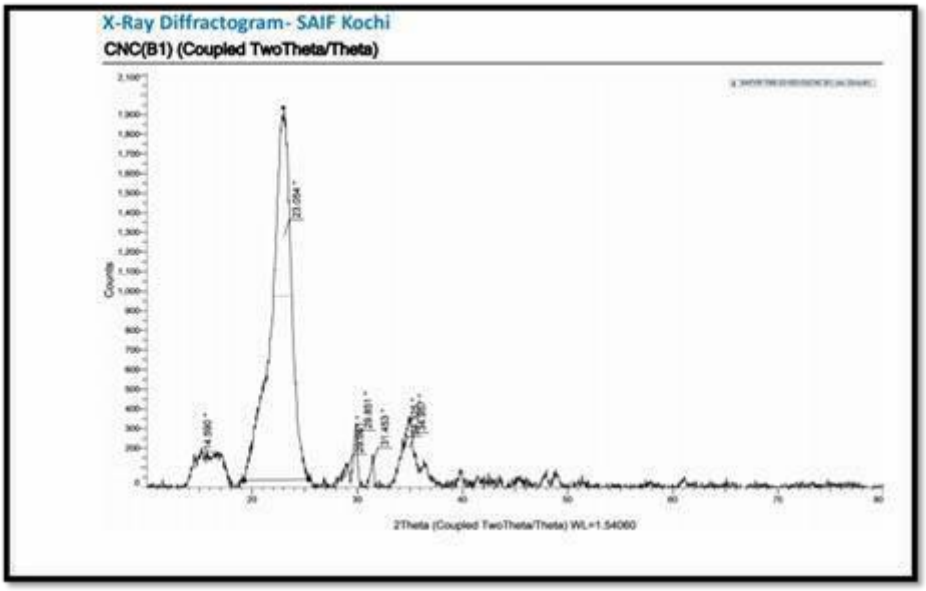

Fig 20: XRD patterns of the bleached sample

This sample shows two characteristic peaks at 14.590° and 23.054°

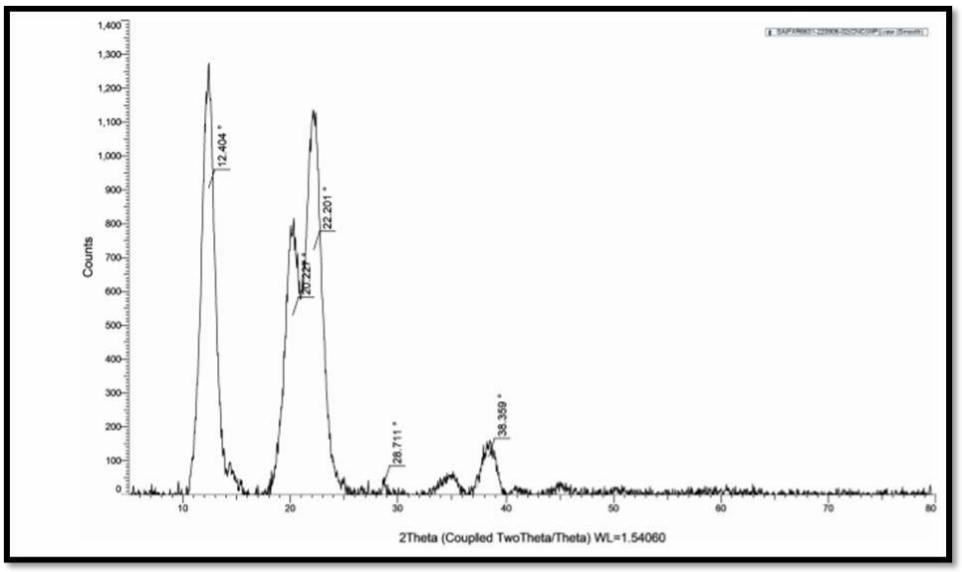

Fig 21: XRD pattern of CNC.

X-ray diffraction (XRD) is a useful tool for the determination of the cellulose crystalline structure. Fig. 21 shows the XRD spectra of the waste paper (raw material, bleached, and isolated CNCs). These results give similar diffraction peaks at 15.46^0, 22.680^0, and 29.54^0 corresponding to the (110), (200), and (004) crystallographic planes of the cellulose I lattice respectively. The samples presented high peak intensity around 2θ values of 23.054^0 which is correlated to the crystalline structure of cellulose. Also, from the XRD patterns, the presence of a board peak around 14^0 is characteristic of the amorphous arrangement. It was found that the crystal structure of cellulose remained unchanged during the process of alkali pulping and bleaching. XRD pattern of CNC shows highest diffraction peakat $2\theta=12.404^0$ and a doublet at 20.227^0. This shows a partial presence of cellulose type II.

4.1.3 FTIR

Infrared spectroscopy works based on the atomic vibrations in a molecule to be tested. The sample's FTIR spectra helped to determine their structural change before and after chemical treatment.

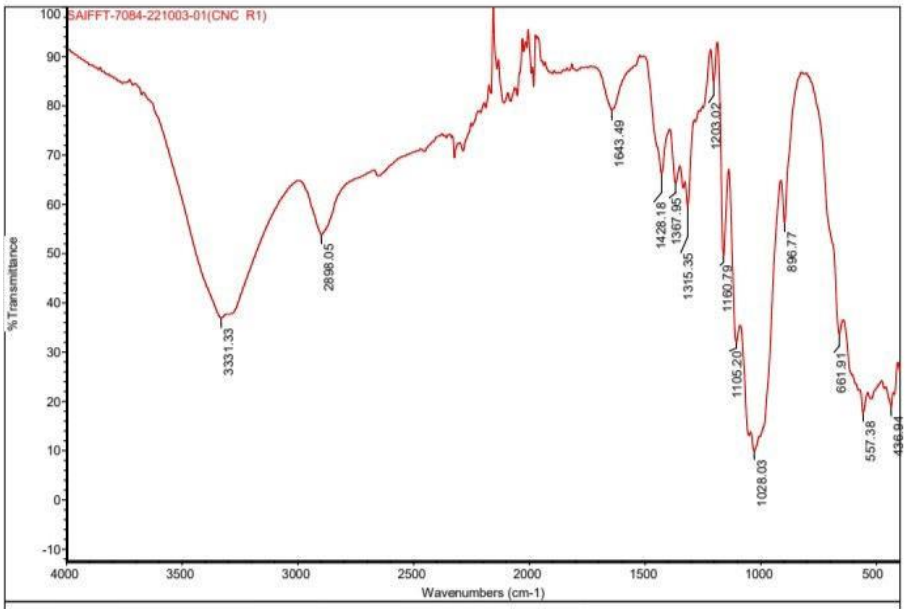

Fig 22: FTIR spectra of raw material

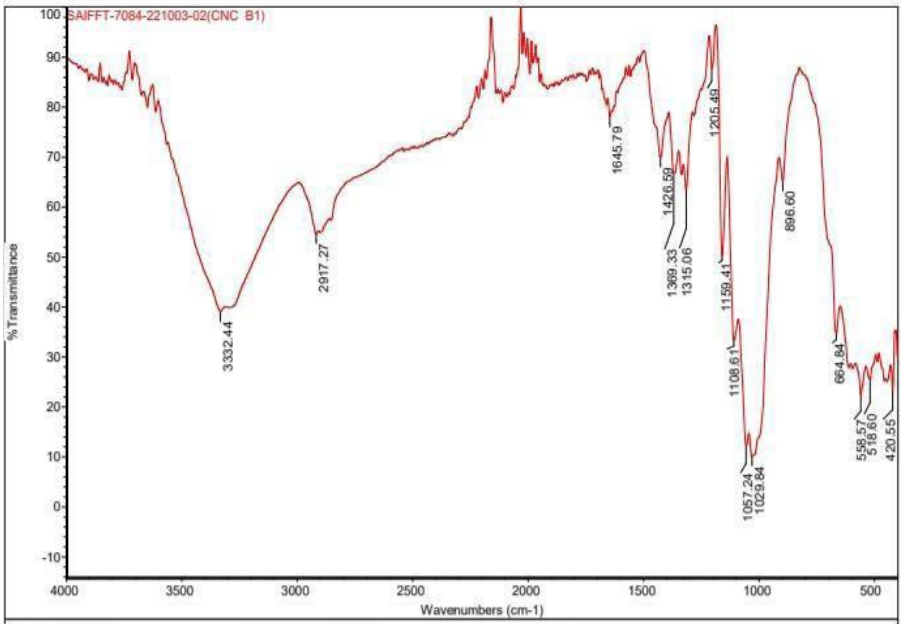

Fig 23: FTIR spectra of the bleached sample (cellulose)

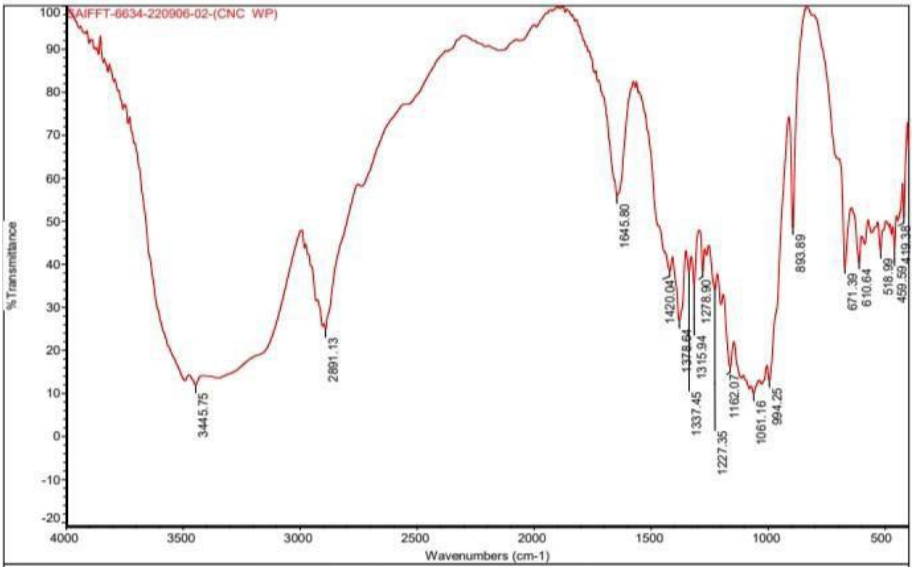

Fig 24: FTIR spectra of Cellulose nanocrystals

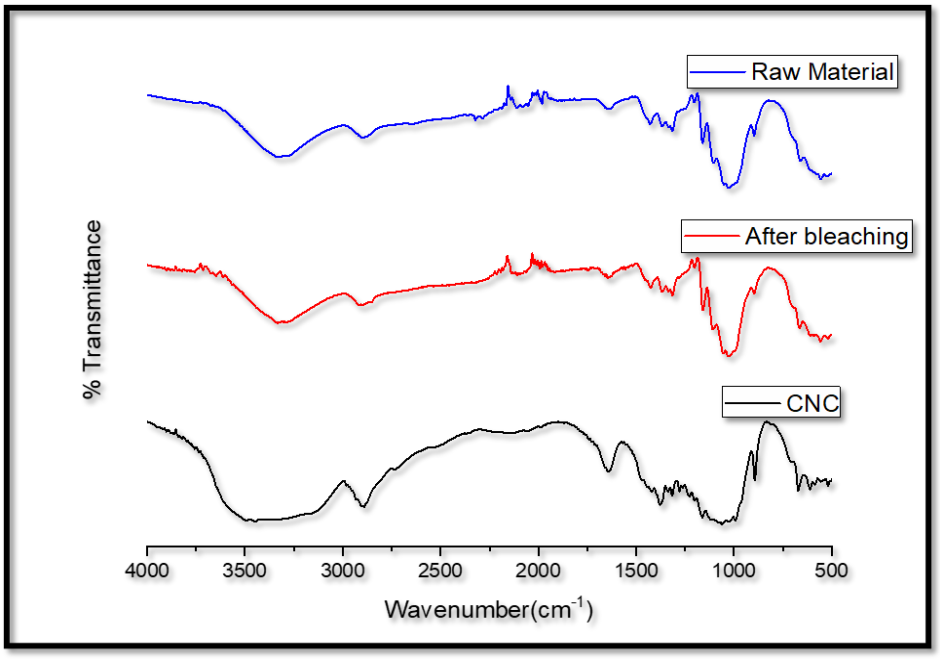

Fig 25: comparison graph of FTIR analyses of raw material, after bleaching sample and CNC

To compare the three different FTIR analysis graphs obtained for raw material, after the bleached sample (cellulose) and CNC (cellulose nanocrystals), we can see a peak at the range of 3500-3200cm^{-1} which represents the free OH stretching vibration. The peak at 2800-2900 cm^{-1} showing CH stretching vibration. In the 1650-1640cm^{-1} range, there is a peak that shows the OH bonding of absorbed water. All these peaks show an increase in intensity when the raw material is bleached to form cellulose nanocrystals. There is a peak that represents the glycosidic linkage of glucose units in cellulose at 890-900cm^{-1}. It is the most important peak in our sample which shows an increase in intensity from raw material to CNC. This indicates that our raw material is purely converting to cellulose. Near 1735cm^{-1} a peak that represents hemicellulose presence in both raw materials as well as in the bleaching sample. This peak is absent in the FTIR of CNC. This shows the elimination of hemicellulose. At 1263cm^{-1} shows a peak representing the ether linkage of lignin. And there is a diminishing effect when it reaches CNC confirming the removal of lignin. To conclude the analysis we can say that there is an overall increase in the peak representing cellulose and a decrease in the peak of hemicellulose, lignin, and other cellulosic components.

4.1.4 TEM

TEM was used to get information about the inner structure and hence determine the particle size of cellulose Nanocrystals. The chemical treatment of waste paper material with an alkali solution and further acid hydrolysis was expected to remove the hemicelluloses and lignin which form part of the amorphous region of the cellulosic region. The process aims to reduce the size of cellulose to the nanometer range while keeping the crystalline regions intact. The TEM imaging showed that the CNCs have a spherical shape with average 6-7 nm diameters.

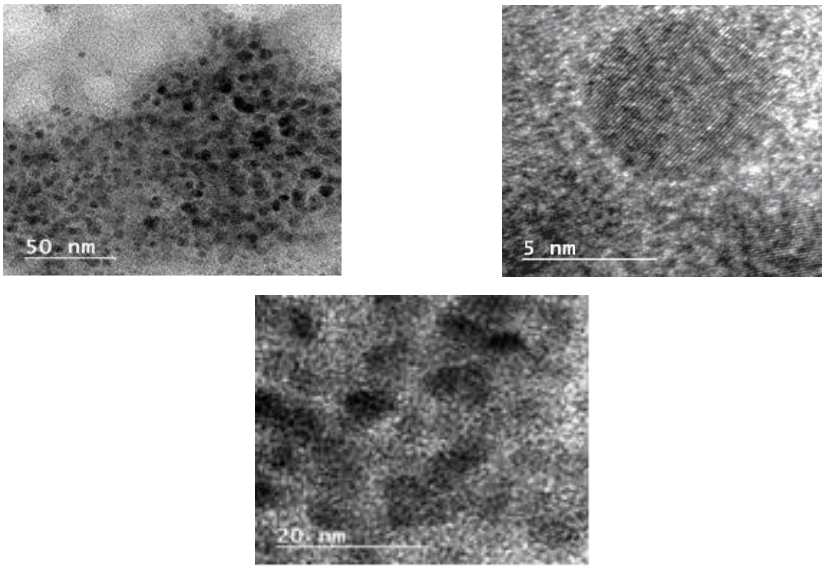

Fig 26: shows the TEM patterns of Cellulose Nanocrystals

4.1.5 SAED

Selected area electron diffraction (SAED) is a crystallographic experimental technique that is performed by using a transmission electron microscope. In the SAED pattern, each obtained spot corresponds to a satisfied diffraction condition. [36]

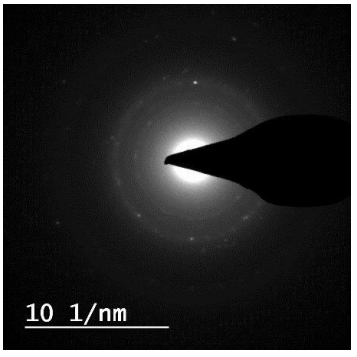

Fig 27: SAED pattern of CNC

The corresponding SAED pattern illustrates a series of bright spots which is due to the diffraction of crystallites of nanocrystals. This indicates the crystalline nature of nanocrystals.

4.2 DETERMINATION OF CELLULOSE

OBSERVATIONS AND CALCULATIONS

Table 1: Calculations for determining the percentage of cellulose in samples

WEIGHT	**RAW MATERIAL**	**AFTER ALKALI PULPING**	**AFTER BLEACHING**
WEIGHT OF THE SAMPLE (W_1)	0.5016	0.5046	0.5048
WEIGHT OF CRUCIBLE (W_3) (after 2 hrs of drying)	26.0313	22.9995	23.9066
WEIGHT OF CRUCIBLE (W_4)	25.6287	22.5285	23.4191
% OF CELLULOSE $\frac{W_3-W_4}{W_1} X100$	**80.26**	**93.34**	**96.57**

The percentage of cellulose contained was determined for three samples: raw material, an alkali pulped sample as well as a bleached sample by using the ADF method (Acid Detergent Fibre method). It was observed that there is an increase in cellulose content for synthesized CNC from raw material (80.26%) to extracted cellulose (96.57%). The bleached sample(cellulose)was found to have the most cellulosic content ie, about 96.57. Hence by conducting this procedure, we can conclude that CNC synthesized after the bleaching process became more cellulosic due to the diminishing amount of lignin, hemicellulose, and other non-cellulosic components.

4.2 ANTIBACTERIAL ACTIVITY ASSAY

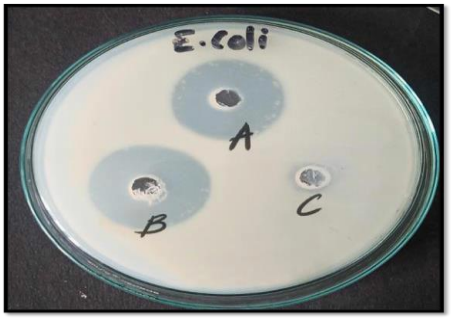

Fig 28: Antibacterial activity of synthesized cellulose nanocrystals, Chitosan, and Chitosan-CNC composite against *Escherichia coli.*

After incubation for 24 hours, it has been found that all three samples were bacteriocidal against *E.Coli* gram-negative bacterial stain. Chitosan showing the diameter of a zone of inhibition of 16 mm against *E.Coli* bacterial strain. For CNC the diameter of the zone of inhibition for *E.coli* shown is 6 mm. Chitosan-CNC composite showed an increased antibacterial activity than both Chitosan and CNC about 18mm in diameter in the zone of inhibition. Therefore it can be concluded that the antibacterial activity of CNC can be enhanced by the preparation of their composites with chitosan.[38]

Table 2: The study of antibacterial activity

SAMPLE	ZONE OF INHIBITION
A- CHITOSAN	16 mm
C- CNC	6 mm
B-COMPOSITE	18 mm

4.5 SEM

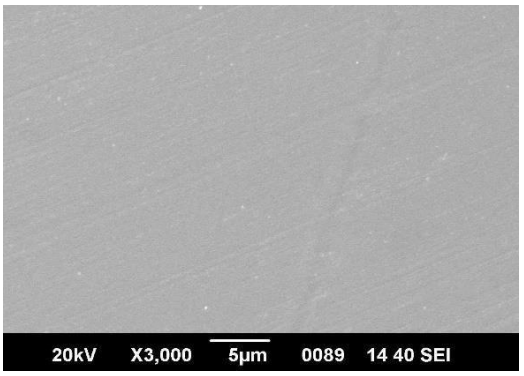

Fig 29: Surface view of chitosan film

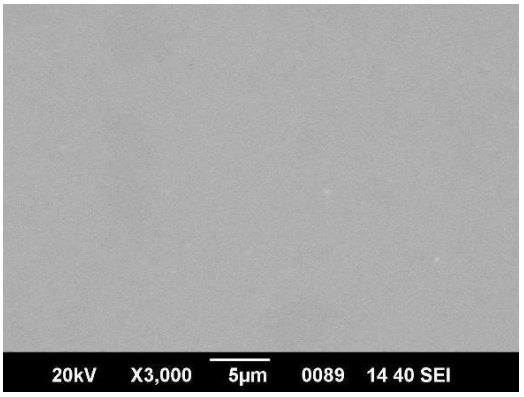

Fig 30: Surface view of chitosan-CNC composite thin film

The chitosan and chitosan-CNC films were analyzed by SEM to investigate their surface morphology. The SEM images were collected from a surface view. The chitosan film has a homogeneous surface and displayed a smooth morphology. This confirms that the CNC is uniformly embedded and forms a composite with chitosan and a homogeneous thin film is formed.

4.5 STUDY OF WETTABILITY (Contact Angle Measurement)

The water contact angle was measured on films prepared by hydrothermal method (0.1:1). The contact angle was about 80.4 which indicates the hydrophilicity of the films. This hydrophilic nature of the film is attributed to the self-cleaning property so that they can be used in several commercial sectors like self-cleaning windows, papers, fabrics, etc and it is a promising field for exciting research works.

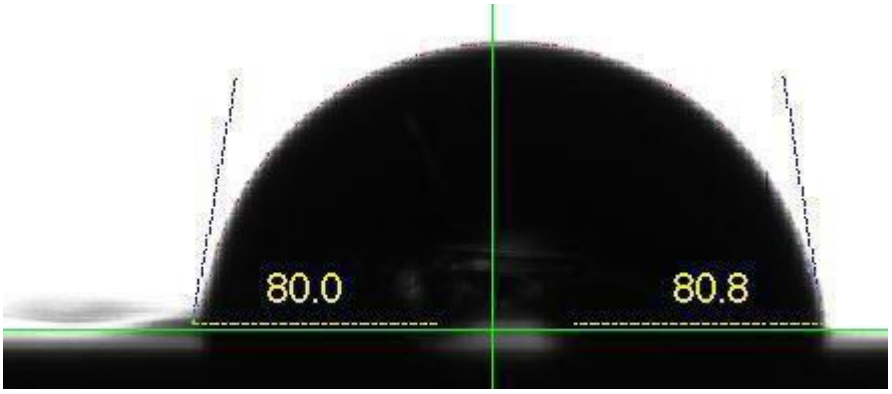

Fig 32: The water contact on CNC-Chitosan film

Chapter 5

Conclusions

The reuse, recycling, and recovery of various biomass waste for high-value-added products have received great attention in reducing the environmental impact and pollution. It is hoped that this study will drive further interest in the preparation of CNCs from renewable biomass sources and agro-waste products. In this project work, Cellulose nanocrystals were isolated from waste paper through acid hydrolysis method. CNCs have been employed in high-performance material due to their attractive properties such as light weight, excellent mechanical strength, biocompatibility, biodegradability, high surface area and tunable surface chemistry. The CNC solution was a milky white stable suspension of spherical-shaped nanocrystalline cellulose. The stability of the suspension was a result of the surface anionic repulsive charges of the crystals. The XRD, FTIR & TEM studies gave supporting evidence for the formation of nanocellulose. It is recommended that prior to acid hydrolysis bleaching of biomass is performed with the aim of removing as much of the non- crystalline and non-cellulosic components of biomass. This aids in reducing hydrolysis times and producing CNCs of uniform dimensions. The removal of non-cellulosic compounds was enhanced by the use Sulphuric acid during chemical treatment. Thorough rinses after each pre-treatment are strongly advised as traces of alkaline and bleaches interfere with and neutralize the acid used during the hydrolysis procedure. An increase in crystallinity was observed for the nano crystals in XRD which indicated the exposure of the crystalline phase after the successful elimination of the lignin and hemicelluloses. The FTIR analysis indicates the presence of different functional groups in the prepared

CNCs and also helped to determine the structural change after the chemical treatments. Determination of cellulose was done by Acid Detergent Fiber (ADF) method and an enhancement in the percentage of cellulose from 80.26 to 96.57 was observed from raw material to the bleached sample. This increase in the amount of cellulose further compliments the result obtained from FTIR and XRD characterization. Transmission Electron Microscopy (TEM) was used to study the morphology of synthesized Cellulose nanocrystals. The morphology of CNC was observed to be spherical in structure with an average diameter of 6-7 nm. The SAED pattern obtained confirms the crystalline nature of CNC.

A CNC-Chitosan thin film was fabricated. SEM characterization was taken to determine the surface morphology for both chitosan and chitosan CNC thin film, and was found to be uniform, homogenous, and smooth. A study of wettability was conducted for the thin film made from CNC- Chitosan composite for the determination of contact angle measurements and found that it is hydrophilic in nature. Antibacterial studies against gram-negative strain *E.Coli* for 3 samples of pure chitosan film, chitosan -CNC composite thin film as well as for CNC were conducted and it was found that Chitosan shows a zone of inhibition of 16mm against *E.Coli* bacterial strain. For CNC the diameter of the zone of inhibition was 6mm. But when CNC was composited with chitosan showed an increase in antibacterial activity than both Chitosan and CNC ie, about 18mm of diameter in the zone of inhibition. Therefore it can be concluded that the antibacterial activity of CNC can be enhanced by the preparation of their composites with chitosan owing to the synergestic effect of antibacterial activities of the individual components.

The findings of our study revealed the utilization of waste paper as the raw

material for the production of CNCs and its utilization for synthesis of an acterial thin film with chitosan.This innovative utilization of waste paper reduces the municipal waste load disposed of in a landfill, minimizes environmental pollution, and enhances the sustainable utilization of waste materials for producing value-added products.

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