PROJECT REPORT

On

“ISOLATION, CHARACTERIZATION AND APPLICATION OF CHEMICALLY PURIFIED CELLULOSE AND ITS NANOCRYSTALS FROM SUGARCANE BAGASSE”

Submitted by
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AM20CHE009

In partial fulfillment for the award of the
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DEPARTMENT OF CHEMISTRY
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M.Sc. CHEMISTRY PROJECT REPORT

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This is to certify that the project "**ISOLATION, CHARACTERIZATION AND APPLICATION OF CHEMICALLY PURIFIED CELLULOSE AND ITS NANOCRYSTALS FROM SUGARCANE BAGASSE**" is the work done by**HELEN SANDRA T P.**

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Submitted to the Examination of Master's degree in Chemistry

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CERTIFICATE

This is to certify that the project work entitled "**ISOLATION, CHARACTERIZATION AND APPLICATION OF CHEMICALLY PURIFIED CELLULOSE AND ITS NANOCRYSTALS FROM SUGARCANE BAGASSE**" is the work done by**HELEN SANDRA T P** under the guidance of **Dr.USHAMANI M, ASSOCIATE PROFFESOR,** Department of Chemistry and Centre for Research, St. Teresa's College, Ernakulam in partial fulfilment of the award of the Degree of Master ofScience in Chemistry at St. Teresa's College, Ernakulam affiliated to Mahatma Gandhi University, Kottayam.

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DECLARATION

I hereby declare that the project work entitled "**ISOLATION, CHARACTERIZATION AND APPLICATION OF CHEMICALLY PURIFIED CELLULOSE AND ITS NANOCRYSTALS FROM SUGARCANE BAGASSE**"submitted to 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 me under the guidance of **Dr.USHAMANI M, ASSOCIATE PROFFESOR,** Department of Chemistry and Centre for Research, St. Teresa's College (Autonomous), Ernakulam This project work is submitted in the partial fulfillment of the requirements for the award of the Degree of Master of Science in Chemistry.

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Abbreviations	
Sugarcane bagasse	SCB
Cellulose Nanocrystals	CNC
Cellulose nanofiber	CNF
Bacterial cellulose	BC
Electrospun cellulose nanofiber	ECNF
Lignocellulosic biomass	LCB
Transmission electron microscope	TEM
Fourier transform infrared spectroscopy	FTIR
X-Ray diffraction	XRD
Energy dispersive X-ray analysis	EDAX
Scanning Electron Microscope	SEM

Chapter1

INTRODUCTION AND LITERATURE REVIEW

1.1 NANOTECHNOLOGY

Nanotechnology is concerned with development and utilization of structures and devices withorganizational features at the intermediate scale between individual molecules and about 100 nm where novel properties occur as compared to bulk materials.Nanotechnology involve the creation and manipulation of materials at the nanometre scale, either by scaling up from single groups of atoms or by refining or reducing bulk materials[1].It is recognized as an emerging technology of the 21st century, in addition to the already established areas of information technology and biotechnology. This is due to the scientific convergence of physics, chemistry, biology, materials and engineering at nanoscale, and importance of the control of matter at nanoscale on almost all technologies[2]. 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[1].

In nanotechnology, nanoparticles research is an important aspect due to its innumerable applications.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**.** The definition given by the European Commission states that the particlesize of at least half of the particles in the number size distribution must measure 100 nm or below. Most nanoparticles are made up of only a few hundred atoms. 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, plate and sheet. 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 [3].

Development of nano phase materials from the natural sources is an important eco-friendly approach to meet the commercial applications in industries like refractories, ceramics, textile, biomedical, paints and pigments[3].Extraction of cellulose from natural sources and its conversion to nanocellulose is a fast developing area of research in nanotechnology.

1.2CELLULOSE

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 homopolysaccharideof β-1,4- linked anhydro-D-glucose units with the repeating unit of cellobiose (Fig.1.1). The monomer of cellobiose, named anhydroglucose unit, consists of three hydroxyl groups which form strong hydrogen bond with the adjacent glucose unit in the same chain and with the different chains, called asintramolecular and intermolecular hydrogen bonding networks, respectively[4].It is tasteless, colorless polysaccharide and remain immiscible in aqueous solvent and other organic ones. The presence of multiple –OH functional groups on it result into interlinking of the chains by H-bonding due to which it is gaining fibrous properties and high tensile strength as well. The presence of many –OH functional groups make it more viable for the chemical modification by required functionalities[5] .

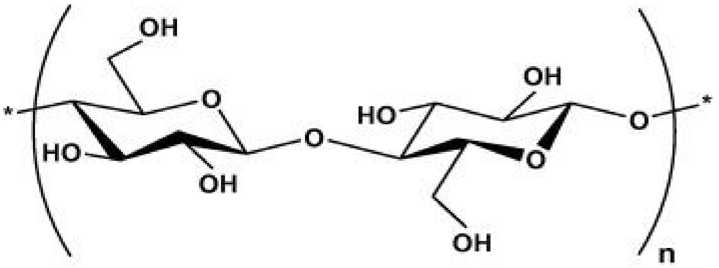

Fig.1.1. Schematic of cellobiose[4].

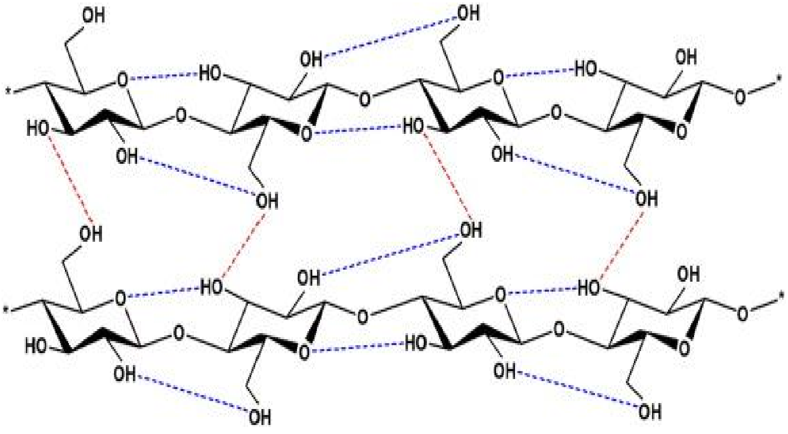

Fig.1.2. Intramolecular (---) and intermolecular (---) hydrogen bonding networks in cellulose structure[4].

Cellulose is crystalline in nature as compared to other polysaccharide like starch, dextrin which are amorphous[5]. 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.Cellulose has low density, biodegradability, and great mechanical properties according to the kind of cellulose available. Natural fiber is made up of cellulose that is arranged into fibrils parallel to each other. Each fiber is a composite where rigid cellulose microfibrils are embedded in a soft matrix of the cell wall, consisting mainly of hemicellulose and lignin[7]. 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]. Despite its appealing properties (higher purity and crystallinity than plant cellulose), the associated cost involved in the production needs to be taken into account if mass production is needed. The widespread availability and continuous supply of cellulose from plant fibres seems to offer the cheaper option[9].

1.3 NATURAL SOURCES OF CELLULOSE

Cellulose is the most abundant biopolymer on earth and is the chain of glucose residues that can be obtained easily from nature[10]. They are also referred to as cellulosic fibers, related to the main chemical component cellulose, or as lignocellulosic fibers, since the fibers usuallyalso contain a natural polyphenolic polymer, lignin, in their structure[11]. The source of the cellulose determines not only its size and properties, but also the energy consumption of the extraction process to produce nanocellulose[12].

Depending on preparation methods of nanocellulose, the hierarchical and multi-level organization of plants makes the extraction of different types of cellulosepossible. Cellulose is mainly produced in wood of forest resources, non-wood lignocellulose and agricultural residues or by-products which includes hemp, flax, jute, ramie, kenaf, cotton, leaves of sisal and abaca, corncob,risk husk, sugarcane bagasse, apple pomace, grape pomace, passion fruit peels, tea leaf, banana fiber, groundnut shell and plum seed shell.These cellulose plant sources are abundant in quantities around the world and are therefore readily available, nearly inexhaustible and cheaply sourced, resulting in the CNC production cost is comparatively low[13].The most common type of plant fibre used for cellulose fabrication is wood pulps due to its relatively high cellulose purity, durable and ductile networks, and good physical properties compared with other plant-based sources[14].

Cellulose can be extracted from animals such as tunicate, prawns and crabs. CNFs with cross-sectional dimensions of 8×20 nm measured by atomic force microscopy (AFM) was obtained from tunicate (Halocynthiapapillosa)[10].In recent decades bacterial cellulose(BC), is gaining more attention due to the unique self-assembling of secreted fibrils into nanostructured biomaterial possessing exceptional biophysical characteristics that are suitable for a variety of biomedical applications . Moreover, BC belongs to the category of materials that have been generally recognized as safe. BC is produced by the fermentation of certain bacterial species including Gram-negative bacteria species such as Acetobacter (reclassified as Komagataeibacter), *Rhizobium, Agrobacterium, Pseudomonas, Salmonella, Alcaligenes,* as well as Gram-positive species such as *Sarcinaventriculi*[29].Bacterial species, such as *Acetobacterxylinum* (presently *Gluconacetobacterxylinus* or *Komagataeibactermedellinensis*) and *Gluconacetobactermedellinensis*, were investigated for their potential as adsorbent, catalyst, and membrane. BNC-based adsorbent, catalyst, and aerogel membrane were applied for the removal of copper and lead, dye removal, and membrane distillation[12][15].

Out of various sources of cellulose available ,we are choosing sugarcane bagasse for the extraction of cellulose andits conversion to cellulose nanocrystals through acid hydrolysis method.

1.4 SUGARCANE BAGASSE

The sugarcane plant, *Saccharumofficinarum*, is used to produce sugar. The plant has 3 main sections namely the green tops, the millable stem and the roots. For sugar production the stem or stalk is milled and crushed at the front end of the mill where the sugarcane juice is extracted. The dry fibrous residue which is left over is called sugarcane bagasse. Sugar cane bagasse is the by-product obtained after sucrose extraction from the sugar cane plant.Bagasse is also a by-product of the Brazilian sugar cane where it is burned in cogeneration facilities .It contains 40-50% cellulose, much of which is in the crystalline structure. Another component in sugarcane bagasse is hemicellulose as much as 25-35% which is an amorphous polymer and mainly composed of xylose, arabinose, galactose, and mannose. The rest is mostly lignin with about 18-24%[16].Worldwide production of sugarcane bagasse is about 1.4 billion(10^9) tonnes per year .

Bagasse is composed primarily of bast which is the outer lining of the stems and pith and the internal soft component of the stem after the removal of the juices. Conventionally sugarcane bagasse is separated into pith and refined fibre. Around 6 to 7% of the sugar industry bagasse is used to produce animal feed, paper and furfural products; 2% as pith in the production of animal feed, 4 to 5% as refined fibre by two South African paper mills while the net use of bagasse for furfural production is negligible. Due to its highly heterogeneous nature, bagasse is first treated before it is used in the paper making industry. The pith is believed to have a detrimental effect on paper making as it clogs the mat and retards draining of water from paper during paper production. A process called "depithing" is used to remove pith from the bagasse. Depithing is believed to remove 30% of the shortest fibre material from the bagasse. Currently bagasse is effectively used in the production process of paper although the mill receives no direct income from the bagasse; the savings in electricity costs are an indirect benefit to them[17].

Bagasse has been reported to cause tremendous environmental pollution in the areas located with sugar industries since they are scattered all over making the scenery of these areas to look ugly. The bagasse wastes produce bad odors when it ferments making the environment not conducive to reside on. Furthermore, these wastes cost the industries on storage space and disposal costs. The sugar industries generate huge quantity of bagasse during the manufacture of sugar from cane. This phenomenon results to environmental distress, also triggering numerous environmental extortions instigating damage to the land and its surroundings[18].

Sugarcane bagasse has high proportion of cellulose, which can be readily isolated from the other components namely lignin and hemicellulose, by pulping. Bagasse provides an ideal opportunity for producing value-added products from such an inexpensive source of biomass[19]. The extraction of nanocellulose from sugarcane bagasse is easier than that of wood because cellulose microfibrils are organized in the less tightly bound manner in the primary cell of agriculture fibers compared to the secondary wall of the wood.Thus SCB can be used as a source for extraction of cellulose from it[7].

1.5 NANOCELLULOSE

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].

Cellulose nanomaterials, especially from plant sources, can be categorized as either cellulose nanocrystals (CNCs) or cellulose nanofibrils (CNFs). Bacterial cellulose (BC) and electrospun cellulose nanofibers (ECNF) are also considered as nanocellulose[21]. CNCs are discrete, rodshaped cellulose particles typically with high crystallinity and are primarily produced through acid hydrolysis of native cellulose. It is considered to be an easy and fast process to produce CNC. A strong acid such as H_2SO_4 and HCl is practically used to break the glycoside bond in cellulose.Typically, CNCs have nanometer-scaled diameters with lengths of hundreds to thousands of nanometers, and they often contain surface charge groups and can thus be colloidally stable. Conversely, CNFs are typically network structured nanoscaled fibers that can be produced from countless methods usually involving some kind of chemical treatment followed by mechanical refining. Mechanical methods could produce cellulose nanofibrils (CNF) which have both crystalline and amorphous phase. Different techniques have been employed to produce CNF, including high pressure homogenization, grinding and ultrasonic techniques [22].The morphology of CNFs widely varies from process to process, and they can often have a wide distribution of diameters and lengths[8].

Nanocellulose is an attractive material for various applications because it is based on abundant resources—economic, renewable, and commercially processable. Nanocellulose with a small particle size and high crystallinity has a betterproperties and usually used as 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 which replace the non-biodegradable plastics, and as nanocomposite to improve properties such as mechanical, thermal, ionic conductivity properties of the polymer[16].

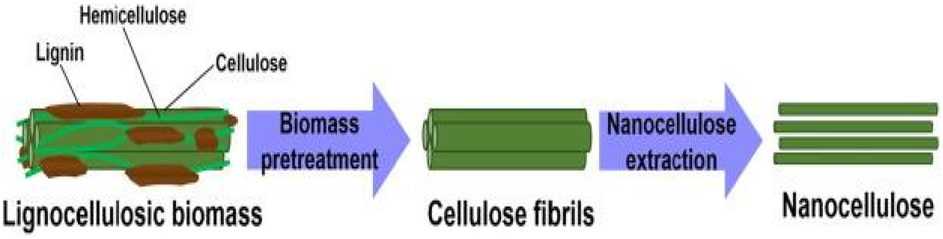

Fig.1.3.Schematicof nanocellulose extractionfrom lignocellulosicbiomass[4].

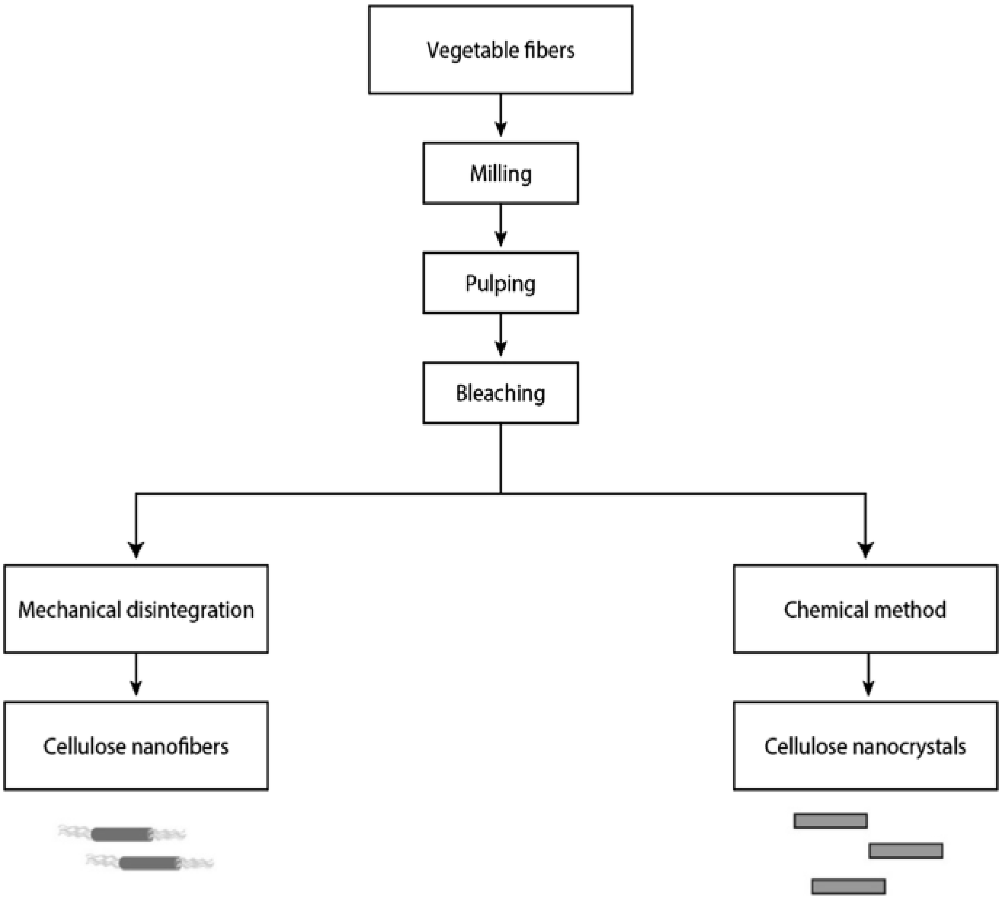

Fig.1.4.Classification of Nanocellulose

1.6 CLASSIFICATION OF NANOCELLULOSE

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 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].

1.6.1 CELLULOSE NANOFIBERS

Cellulose nanofibers/Microfibrillatedcellulose(CNF) is known as cellulose microfibrils, are found embedded in a matrix of hemicelluloses and lignin in the cell wall. In the crystalline domains, the cellulose chains are arranged in a way such that each longmolecule isconnected by hydrogen bonds to the neighboring chains forming a highly ordered crystalline form. Every molecule of these chains is made of glucose rings joined together without foldings. Even the density and the modulus of the two materials are very similar.

The isolation of CNFs can beperformed by a wide variety of mechanical techniques such as refining, grinding, high pressure homogenization, cryocrushing, etc. Production of CNFs from wood pulp using homogenization was first reported in 1983. Thereafter, numerous investigations in this area were done. In addition, different pretreatments can be utilized before mechanical processes in order to reduce energy consumption as well as to make the surface hydrophobic.The raw materials play a considerable role in energyconsumption during the production process, they showless impact on the characteristics of CNFs. In combination with a suitable matrix polymer, CNFnetworks show considerable potential as an effective reinforcement for high-quality specialty applications ofbio-based composites. Recently, because of its eminent properties such as the high strength, flexibility and aspect ratio, several groups of researchers have focused on the preparation of CNFs[11].

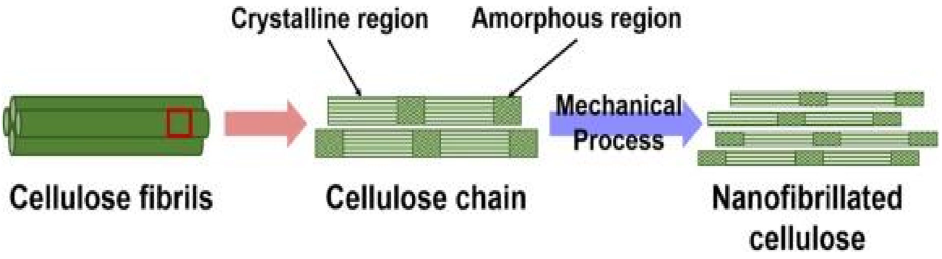

Fig. 1.5. Schematic of nanofibrillated cellulose which can be extracted from cellulose chains using mechanical process to cleavage the fiber into nanometer size in diameter[4].

1.6.2 CELLULOSE NANOCRYSTALS

Cellulose nanocrystals(CNCs) are needle-like cellulose particles having at least one dimension equal or lessthan 100 nm with highly crystalline nature. 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. It has been observed that the performance of CNCs as a nano-reinforcing agent influenced with the variation of morphology and properties of these nanoparticles which depend mainly on original source of cellulose and extraction methods[11].

The degree of crystallinity and aspect ratio, i.e. length to diameter (L/d) ratio, are very important parameters which determine the CNC properties. Aqueous suspensions of CNCs produced by sulfuric acid hydrolysis are more stable than hydrochloric or phosphoric acid hydrolysis. This is due to the introduction of negatively charged sulfate groups on their surface that prevent their agglomeration by electrostatic repulsion. Also, TEMPO (2,2,6,6- tetramethyl-1- piperidinyloxy)-mediated oxidization of CNC enhances the negative charges on the surface. In the last decades, CNC gained more attention due to its desirable properties such as mechanical strength, high specific surface area, biodegradability and broad chemicalmodifying capacity[21].

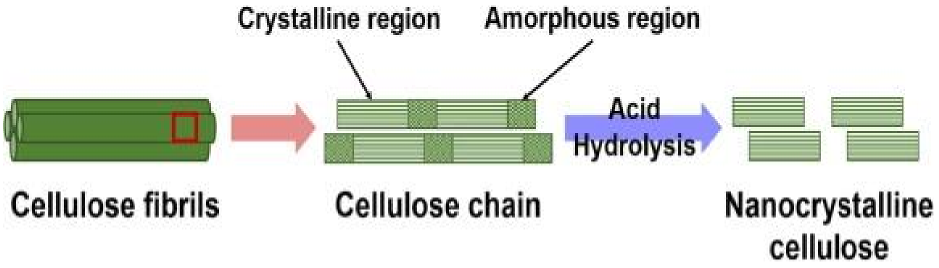

Fig.1.6. Schematic of CNC which can be extracted from cellulose chains[4].

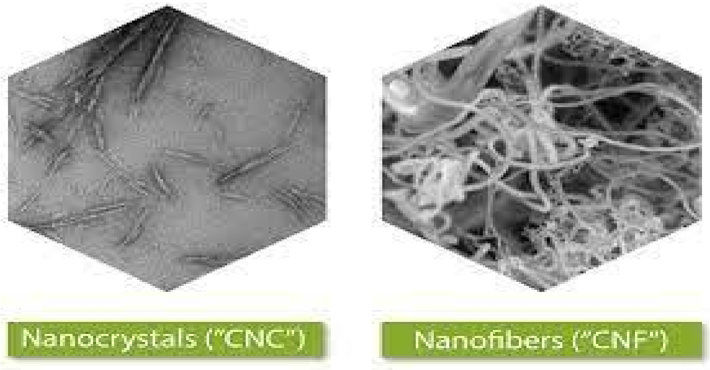

Fig.1.7.Cellulose nanocrystals and Cellulose nanofibers.

1.6.3BACTERIAL NANOCELLULOSE

Cellulose biosynthesis takes place not only in plants, but also in bacteria such as Acetobacter, Acanthamoeba, and Achromobacter spp., algae such as Valonia, Chaetamorpha spp., and fungi. By selecting the substrates, cultivation conditions, various additives, and finally the bacterial strain, it is possible to control the molar mass, the molar mass distribution, and the supramolecular structure. Thus, it is possible to control important cellulose properties, and also the course of biosynthesis (e.g., kinetics, yield, and other metabolic products). Materials also known as bacterial cellulose, microbial cellulose, or bio-cellulose are formed by aerobic bacteria, such as acetic acid bacteria of the genus Gluconacetobacter, as a pure component of their biofilms. These bacteria are widespread in nature where the fermentation of sugars and plant carbohydrates takes place. In contrast to CNF and CNC materials isolated from cellulose sources, Bacterial cellulose(BC) is formed as a polymer and nanomaterial by biotechnological assembly processes from low-molecular-weight carbon sources, such as D-glucose. Although identical to cellulose of plant origin in terms of molecular formula, bacterial cellulose is quite different. The degree of polymerization (DP) is very high, with DP values of 2000–8000. Crystallinity is also high, with values of 60%–90%. 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].

1.6.4 ELECTROSPUN CELLULOSE NANOFIBERS

The other possibility to produce cellulose with nanoscale/submicron lateral dimensions is electrospinning. Recently, the electrospun fibers from cellulose and its derivativeshave 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 diameter of several tens of nanometers. However, ECNF starting from few hundreds of nanometer to 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 investigation of appropriate solvents for cellulose. Electrospinning can be also used to produce polymer composite fibers containing nanocellulose[23].

1.7 PRETREATMENT TECHNIQUES FOR EXTRACTION OF CELLULOSE

1.7.1MILLING

Milling is employed to reduce the crystallinity and particle size LCB. Milling can reduce the particle size upto 0.2 mm. However, biomass particle of size < 0.4 mm has no remarkable effect on the rate and yield of hydrolysis. Depending upon the type of motorized equipment operated the different milling methods are two-roll milling, ball milling, rod milling, hammer milling, vibratory milling, colloid milling, and wet disk milling. The reduction in particle size and crystallinity is determined by the type of milling method adopted, processing time and also the type of biomass used.

1.7.2 EXTRUSION

Extrusion is one of the most commonly used physical pretreatment method applied to LCBs. The action of one or two screws that spin into a tight barrel, which is furnished with temperature control forms the basis of this method. The raw materials are passed through the barrel under high temperature (>300°C) where the recalcitrant structure of the lignocellulose disrupts due to the combined effects of high temperature and the shear forces caused by the rotating screw blades in the barrel. Extrusion machines are mainly classified into single-screw extruders (made of one single solid piece) and twin-screw extruders (made of small pieces called screw elements arranged cylindrically). The screw configuration is an important factor affecting the decomposition of LCB.

1.7.3 ULTRASONICATION

Ultrasonication pretreatment is based on the principle of cavitation through the employment of ultrasonic radiation. The cavitation generates shear forces that cleaves the complex network structure of LCB and promotes the extraction of desired compounds such as, cellulose, hemicellulose, and/or lignin. Several factors influencing the sonication treatment includes ultrasound frequency, sonication duration, sonication power and temperature. It has been seen that the choice of solvents (dilute aqueous solutions of inorganic acids or alkalis, organic solvents or ionic liquids) is critical in determining the optimum conditions for ultrasonication pre-treatments.

1.7.4 ALKALI PRETREATMENT

Alkali pre-treatment is a widely studied chemical pre-treatment method which is based on the solubilization of lignin in the alkali solution. The various alkaline reagents used commonly for alkali pre-treatment are the hydroxides of sodium, potassium, calcium and ammonium. Among these sodium hydroxide was found to be the most effective. A saponification reaction takes place throughout the alkali pre-treatment process which causes cleavage of the intermolecular ester linkages between hemicelluloses and lignin. This results in solubilisation of lignin and hemicellulose fragments in the alkali solution and brings the cellulose in the interaction of enzymes. Also, alkali pre-treatment changes the lignocellulosic structure via cellulose swelling that leads to reduction in crystallinity and degree of polymerization thereby increasing internal surface area. In addition, removal of acetyl groups and uronic acid substitutions in hemicelluloses during alkali pre-treatment also increases the accessibility of the carbohydrates to enzymatic hydrolysis.

1.7.5 ACID PRETREATMENT

Acid pre-treatment of LCBs is based on the susceptibility of the glucosidic bonds between hemicellulose and cellulose to acid. Hydronium ions which originates from the acid catalyst cause breakdown of the long cellulose and hemicellulose chains into sugar monomers. Both, inorganic acids such as sulfuric acid ,phosphoric acid, nitric acid and hydrochloric acid and organic acids such as formic acid, maleic acid, and oxalic acid are used. Acid pre-treatment can be used either as concentrated acids (30–70%) at low temperature (< 100°C) or as dilute acids (0.1–10%) at high temperatures (100–250°C). Studies reveal that among the acids, dilute sulphuric acid (H_2SO_4) is the most extensively used to pretreat LCBs.

1.7.6 STEAM EXPLOSION

Steam explosion is the most commonly employed and effective pre-treatment method, which is typically a combination of both mechanical forces and chemical effects applied to LCBs. In this technique, the biomass is subjected to high-pressure saturated steam (0.69–4.83 MPa) at a temperature of 160–260°C to let water molecules penetrate the substrate structure. The pressure is then suddenly reduced to let the water molecules escape in an explosive way. This rapid release of pressure causes explosion of the bulk LCB into splitted fibers. Besides, the high temperature and pressure enhance the breakdown of the glycosidic bonds in cellulose and hemicellulose and cleavage of hemicellulose-lignin bonds. During this treatment, the hydrolysis of hemicellulose into glucose and xylose monomers liberate acetic acid which catalyze the further hydrolysis of hemicelluloses, and; hence the process is also termed as autohydrolysis[25].

1.8APPLICATIONS OF NANOCELLULOSE

There are a great number of potential applications of nanocellulose within different industries, viz. high quality paper products; in cosmetics as thickener and in food industry as stabilizer, fat replacer and texturing agent; moldable light weight, high strength materials; composites for construction, vehicles, customer products, furnitures; 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].

1.8.1 PAPER AND PAPERBOARD

CNC have potential application in the paper and paperboard industry where they can increase the fibre-fibre bond strength and thereby increasing the strength of the paper. CNCs can also be used as a barrier in greaseproof type of papers and as a wet-end additive to enhance retention, dry and wet strength in commodity type of paper and board products.

1.8.2FOOD

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.

1.8.3HYGIENE AND ABSORBENT PRODUCTS

Different applications in this field include but are not limited to:

- super water absorbent (e.g. material for incontinence pads material)
- nanocellulose used together with super absorbent polymers
- nanocellulose in tissue, non-woven products or absorbent structures
- antimicrobial films

1.8.4MEDICAL, COSMETIC AND PHARMACEUTICAL

The use of nanocellulose in cosmetics and pharmaceuticals was also early recognized. A wide range of high-end applications have been suggested:

- freeze-driednanocellulose aerogels used in sanitary napkins, tampons, diapers or as wound dressing.
- the use of nanocellulose as a composite coating agent in cosmetics e.g. for hair, eyelashes, eyebrows or nails.
- a dry solid nanocellulose composition in the form of tablets for treating intestinal disorders.
- nanocellulose films for screening of biological compounds and nucleic acids encoding a biological compound.
- filter medium partly based on nanocellulose for leukocyte free blood transfusion.

•powderednanocellulose has also been suggested as an excipient or bulkingagent in pharmaceutical compositions. An excipient is a natural or synthetic substance formulated alongside the active ingredient of a medication, included for the purpose of bulking-up formulations that contain potent active ingredients.

•nanocellulose in compositions of a photoreactive noxious substance purgingagent.

•elasticcryo-structured gels for potential biomedical and biotechnologicalapplication[17].

1.8.5 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 is 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.

1.8.6 OPTO-ELECTRONICS

Another exciting application for cellulose nanomaterials is liquid crystal displays (LCDs) and other electro-optical devices. Cellulose nanocrystal suspensions display liquid crystal behavior, and the CNCs can be aligned under an electric field, making them potentially suitable for optical devices or displays. By altering the electrical signal through the CNC suspensions, the orientation of the CNCs and thus transmittance of light can be controlled.

1.8.7NANOCELLULOSE IN WATER TREATMENT

Currently, researchers are exploring cost-effective and environmentfriendly solutions for treating wastewater, which contains dyes, organic pollutants, and other toxic contaminants. In line with the solution about 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].

1.9 ZINC BASED NANOMATERIALS

Zinc nanomaterials represent a versatile class of nanoproducts and nanoenabled devices as these nanomaterials can be synthesized in quantum amounts through economically affordable processes/approaches. Further, these nanomaterials exhibit potential targeted antimicrobial properties and low to negligible phytotoxicity activities that well-qualify them to be applied directly or in a deviant manner to accomplish significant antibacterial, antimycotic, antiviral, and antitoxigenic activities against diverse phytopathogens causing plant diseases. The photo-catalytic, fluorescent, and electron generating aspects associated with zinc nanomaterials have been utilized for the development of sensor systems (optical and electrochemical biosensors), enabling quick, early, sensitive, and on-field assessment or quantification of the test phytopathogen[26].

1.10 ZINC SULFIDE NANOPARTICLES

The stable cubic form of zinc sulphide (ZnS) is known also as zinc blende or sphalerite. The hexagonal form is known as the mineral wurtzite. The cubic form of ZnS has a band gap of about 3.54 electron volts but the hexagonal form has a band gap of about 3.91 electron volts.

ZnS nanoparticles possess anomalous physical and chemical properties such as enhanced surface to volume ratio, the quantum size effect, surface and volume effect and macroscopic quantum tunneling effect, more optical absorption, chemical activity and thermal resistance, catalysis, and the low melting point. ZnS nanoparticles can be exploited for versatile applications including field emitters, electroluminescence, electro catalyst, biosensors[27].

1.11CHARACTERIZATION TECHNIQUES

1.11.1 TRANSMISSION ELECTRON MICROSCOPY

A transmission electron microscope (TEM) is a special type of microscope that uses electrons for magnification. The magnification in a standard optical microscope is limited by the wavelength of visible light. Electrons have a much smaller wavelength, which allows electron microscopes to achieve extreme magnification. An average TEM can achieve a maximum magnification level of about one million times.

In order to understand how a TEM uses electrons to create magnified images, we need to understand how the instrument operates. A TEM can be broken down into three major sections shown in figure 1.8.

1. The electron gun is the part of the microscope that fires electrons off toward the sample the user is magnifying. There are multiple types of electron guns, but the most common uses a heated tungsten filament to create the electrons. The electrons are then drawn toward an anode at one end of the electron gun. A hole in the anode allows electrons to pass through it and be fired toward the sample.
2. Once the electrons leave the electron gun they enter the condensersystem, which consists of one or two lenses. These lenses are not the typical glass type you think about but are instead electromagnetic lenses that consist of coils of wires with electricity running through them. They create a magnetic field that focuses and constricts the electrons into a thin beam.
3. The electron beam then enters the image producing system. At the beginning of this system is the sample being imaged. The electrons are transmitted through the sample and into another series of electromagnetic lenses that focus the electrons onto a fluorescent or phosphorescent screen to create the image.

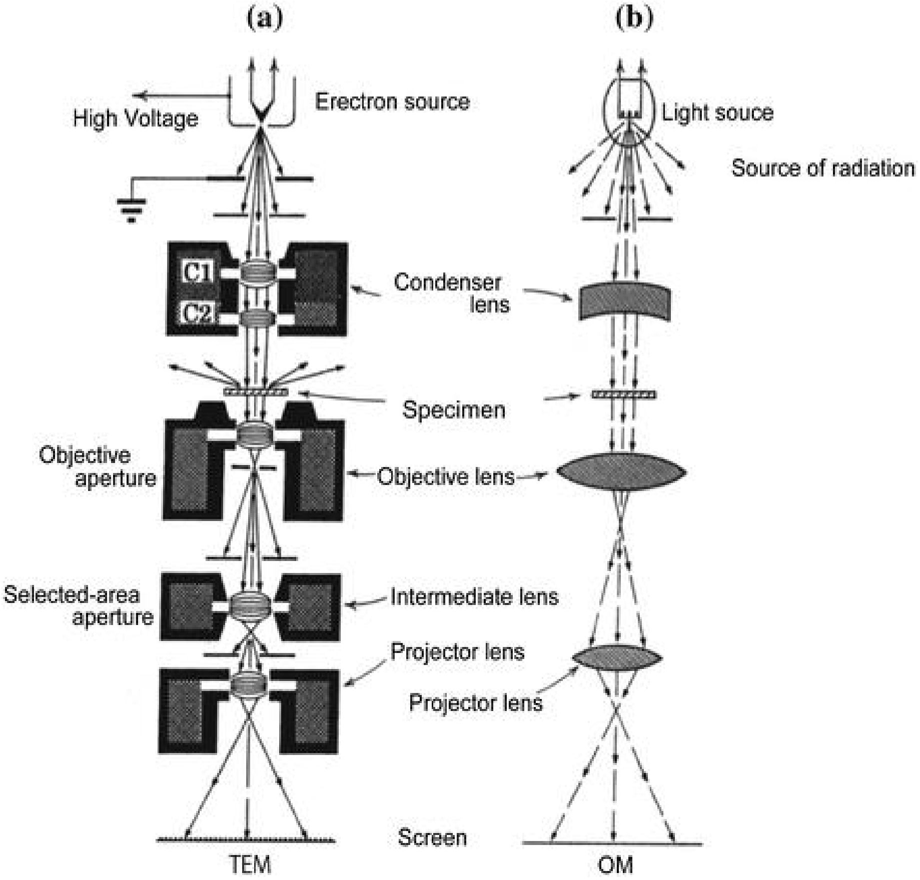

Fig. 1.8. A schematic diagram of transmission electron microscope

1.11.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY

Fourier-transformed infrared spectroscopy (FTIR) is a technique based on themeasurement of the absorption of electromagnetic radiation with wavelengths within the mid-infrared region (4000-400 cm^{-1}). If a molecule absorbs IR radiation, the dipole moment is somehow modified and the molecule becomes IR active. A recorded spectrum gives the position of bands related to the strength and nature of bonds, and specific functional groups, providing thus information concerning molecular structures and interactions.Attenuated total reflection (ATR) is a sampling technique used alongside traditional infrared spectroscopy, which ultimately qualifies samples to be observed directly in either solid or liquid state, without additional preparation.An attenuated total reflection accessory functions by quantifying the changes that happen to an internally-reflected infrared beam, once it comes into contact with the chosen sample. To do this, an infrared beam is focused onto a crystal with a high refractive index at a set angle.The resulting internal reflections create a transient wave that reaches beyond the outer surface of the optically dense crystal, and then into the sample which is held in contact with it.This initial wave only protrudes by a couple of microns (between 0.5 μ and 5 μ) beyond the surface of the crystal, and into the sample itself. Therefore, there must be decent contact made between the chosensample and the surface of the crystal. In the parts of the spectrum where the sampleabsorbs energy, the wave will be either altered or attenuated[28] .

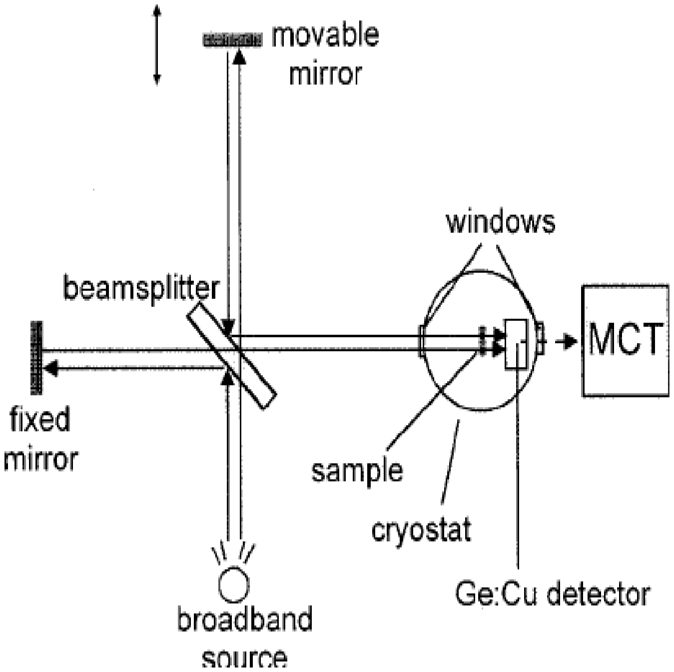

Figure 1.9. A schematic diagram of FTIR spectrometer.

1.11.3X-RAY DIFFRACTION

X-ray diffraction (XRD) is one of the most extensively used techniques for the characterization of nanoparticles. Typically, XRD provides information regarding the crystalline structure, nature of the phase, lattice parameters and crystalline grain size. When a crystal with an interplanar spacing d (crystal lattice constant) is irradiated by X-ray beam with a comparable wavelength λ, the X-ray diffraction or the constructive interference between elastically scattered X-ray beams can be observed at specific angles 2θ whenthe Bragg's Law is satisfied, $n\lambda = 2d\sin\theta$ where n is any integer. Samples are often prepared as smear or compact flat or in capillary and are exposed to a monochromatic beam of X-ray which is diffracted by the nanomaterial and data on the diffracted beam is collected at an angle (2θ) with respect to the incident beam. A powder diffraction pattern supplies information from the nanomaterial on phases present, phase concentrations, structure, as well as the degree of crystallinity or amorphous content followed by details on crystallite size/strain. The peak widths in given phase pattern provides information on the average crystallite size where large crystallites give rise to sharp peaks and any increase in the peak width indicates a reduced crystallite size. The particle size of the crystal is estimated by using Scherrer's equation. An advantage of the XRD techniques, commonly performed in samples of powder form. The broadening of XRD peaks was mainly caused by particle/crystallite size and lattice strains other than instrumental broadening. The XRD-derived size is usually bigger than the so-called magnetic size, due to the fact that smaller domains are present in a particle where all moments are aligned in the same direction, even if the particle is single domain [29] .

1.11.4 ENERGY DISPERSIVE X-RAY ANALYSIS

Energy Dispersive X-Ray Analysis (EDX), referred to as EDS or EDAX, is an X-ray technique used to identify the elemental composition of materials. EDX systems are attachments to Electron Microscopy instruments (Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM)) instruments where the imaging capability of the microscope identifies the specimen of interest. The basic principle of EDAX is a generation of X-rays from a specimen through the electron beam. To produce characteristic X-rays from an object, it is bombarded by either a highly energetic beam of charge carriers (electrons or protons) or X-rays. The atoms of the sample contain ground state (unexcited) electrons in discrete energy levels or electron shells bound to the nucleus. An electron from an inner shell may beexcited by an incident beam, thereby removing it from its shell and generating a hole where electron was present before excitation. This hole can be occupied by an electron of a higher-energy shell. The difference in energies of the higher- and lower- energy shells is emitted as an X-ray. Quantitative measurement of the energy and number of these X-rays can be done with energy-dispersive analysis of X-rays. Since the energy of these X-rays is characteristic feature of energy difference between two shells and atomic structure of discharging element, EDAX can be employed to identify the elemental composition of an object. The data generated by EDX analysis consist of spectra showing peaks corresponding to the elements making up the true composition of the sample being analysed. Elemental mapping of a sample and image analysis are also possible.Applications include materials and product research, troubleshooting, de-formulation, and more [30].

1.11.5 SCANNING ELECTRON MICROSCOPE

Scanning electron microscope (SEM) is one of the most widely used techniques used in characterization of nanomaterials and nanostructures. The signals that derive from electron-sample interactions reveal information about the sample including surface morphology (texture), chemical composition of the sample. [31] Accelerated electron in a 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 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 electron beam comes and hits the atoms of 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 on it about 300 V. Secondary Electrons(SE) are emitted from very close to specimen surface and can produce very high resolution image of 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 [30].

1.12 LITERATURE REVIEW

Sun et al. 2004 from the College of Forestry in the North-Western Science and Technology University of Agriculture and Forestry, Yangling, China isolated cellulose from sugarcane bagasse. Varied concentration of alkali, alkali peroxide, acidic sodium chlorite and an 80% acetic acid and 70 % nitric acid mixture were utilized to yield pure cellulose fractions. The resulting cellulose fraction was subjected to acid hydrolysis and characterized. FT-IR studies showed a decrease in lignin-associated absorbance at 1600 and 1510 cm^{-1}.Slight acetylation was achieved using the acetic-nitric acid mixture, which was evident by an appearance of a band in acetyl ester bands at 1745 (C=O ester), 1374 cm^{-1} (-C-CH_3), and –CO- stretching band at1261 cm^{-1}.^{13}C-NMR studies revealed a decrease in the crystallinity of the cellulose via the presence of peaks located up field at 62.4 and 64.8 ppm. These peaks were attributed to the C-6 in cellulose. The study revealed a decrease in the amorphous cellulose content. Though the amorphous content was removed, no notable increase in crystallinity was observed. The ^{13}C-NMR results revealed that the crystallinity of cellulose decreased with the treatment of SCB with acidified sodium chlorite followed by alkali extraction or with an acetic acid-nitric acid mixture extraction under the condition given. Thermal decomposition studies revealed that cellulose preparations with a higher purity were more thermally stable. The TGA curves showed that the decomposition of the cellulose ranged between 205 °C to 305 °C. The DSC thermograms showed exothermic peaks at 205 °C and 430 °C. These peaks were attributed to the thermal disintegration of the cellulose polymer. The study revealed that alkali and acetic-nitric acid mixture delignification of SCB was a viable method for the isolation of cellulose from SCB. Slight acetylation and degradation of the close fibers was also noted.

Troedec et al. 2008 from the GEMH-ENSCI Group d'Etude des MatériauxHérérogenés, EcoleNationaleSupérieure de CéramiqueIndustrielle, Limoges Cedex, France studied the influence 30 of various chemical treatments on the composition and structure of hemp fibers using sodium hydroxide (NaOH), ethylene diamine tetra acetic acid (EDTA), polyethylenimine (PEI), calcium hydroxide ($Ca(OH)_2$) and calcium chloride ($CaCl_2$) as chemicals of interest. Each considered treatment modifies either the chemical nature of the surface of natural fibers, or the surface state, like the charge or the conformation of polymers. NaOH treatments at pH 14 was found to successfully remove waxes and oils on the surface of the hemp fibers. Similar results were obtained with PEI. However, digestion of the fibers in $Ca(OH)_2$ solution resulted in the deposition of calcium containing nodules on the surface of the fibers. Immersion of the fibers in a neutral $CaCl_2$ solution did not have any notable effect on the surface of the hemp fibers. The use of EDTA a strong calcium chelating agent complexes with the calcium in pectin aggregates and promotes separation of the fibers. Thermal degradation studies of the treated fibers all showed that the treatments resulted in a change in the degradation temperature of the fibers after treatment. All treatments result in the removal of amorphous cellulose and leave a more ordered and temperature resistant molecule. $Ca(OH)_2$ and EDTA resulted in a decrease in degradation temperatures indicating that the cellulose was more easily degraded by these two treatments. NaOH treatments easily remove the amorphous cellulose fraction and hence increased the degradation temperature to 410 °C . XRD studies show that treatments with PEI, NaOH and EDTA all increase the crystallinity index. This is due to the fact that these treatments remove the amorphous cellulose from the polymer. PEI forms stable amine carbonate salts with the carbonyl groups from cellulose degrading the amorphous cellulose. NaOH treatments hydrolyses the amorphous cellulose fraction which results in an increased crystallinity index. $Ca(OH)_2$ and $CaCl_2$ treatments do not increase the crystallinity index of the hemp fibers. With FTIR analysis the band at 1732 cm^{-1} , characteristic for hemicelluloses, has a low absorbance value for PEI and $Ca(OH)_2$ and is absent for NaOH treatment. The absorption band located at 1505 cm^{-1} is present for all treatments. This peak is for lignin and it is evident that none of the treatments could successfully remove lignin.

Teixeira et al. 2011 from the National Nanotechnology Laboratory of Agriculture (LNNA), Embraqa Agricultural Instrumentation, Brazil extracted and characterized nanocellulose whiskers from sugarcane bagasse. SCB fibres 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 hydrolysed for 75min had a brown discolouration due to a certain level of cellulose degradation. The whiskers obtained had a length (L) of around 255 ± 55 nm and the 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 $2\theta = 15.3°$.

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 fibres. Crude, untreated SCB was treated with a 10% (w/v) H_2SO_4 solution 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 fibres which indicated a reduction in the lignin content of the cellulose fibres.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 fibres the same peaks could be observed at 15.9º and 23.2º but were of decreased intensity. Bleached cellulose fibres showed the same peaks observed at 16.2º and 22.9º but were of increased intensity. The TGA and DGT profiles of the fibres presented degradation peaks between 260 - 340°C from untreated to bleached cellulose fibres. The increase in degradation temperature for the treated fibres 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 fibres reduced fibrelength and fibre diameter. It was observed that the bleached cellulose fibres demonstrated higher thermal stability, crystallinity content increase, and flattened morphology when compared to crude cellulose fibres.

Fazli et al. 2012 attended the 2012 2nd International Conference on Environment Science and Biotechnology in Singapore where they presented their work titled Nano Crystalline Cellulose Production and

Its Application in Novel Food Packaging. Cotton linter was used as a substrate for the production of cellulose nanocrystals using chemical methods. Alkali treatment with subsequent acid hydrolysis using a solution of 65% w/w for 3 hours was used to isolate the nanocrystals. After ultrasonication for 15 minutes at the cellulose nanocrystals suspension was ready.

Maiti et al. 2013 from the Department of Chemistry, Nanjung University of Information Sciences and Technology, China prepared and characterized nanocellulose with a new shape from three different precursors. China cotton, South African cotton and waste tissue papers were used to produce nanocellulose by acid hydrolysis route. No chemical pre-treatment were done for the production of nanocellulose from these precursors. All were subjected to a 47% sulphuric acid solution which was vigorously stirred at 60°C for 2 hours. The resulted suspension was centrifuged and washed with deionized water several times to reduce acid concentration. The suspension was finally neutralized with 0.5 N NaOH solutions and again washed with distilled water. The prepared nanocellulose suspension was freeze-dried to get nanocellulose powder. The approximate ranges of diameter of CNC, cotton nanocellulose, and TNC, tissue nanocellulose were from 30 to 60 nm and 10 and 90 nm respectively. However, TEM image of SANC, South African cotton nanocellulose, showed smaller and finer particles of completely different shape from other samples. The diameter range of SANC, South African Cotton nanocellulose, aggregates was from 2 and 10 nm, was smaller than those from CNC and TNC, ranging from 30 to 60 nm and 10 to 90 46 nm, respectively.DLS measurements showed the particle size was larger for CNC and TNC in comparison to that observed in particle size analysis study due to the high agglomeration affinity of CNC and TNC. Two samples showed an increase in % crystallinity when converted to CNC. China Cotton showed a 10% increase from 82.4 -92.4 %, South African cotton 7.6 % from 90.2 – 97.8 % .Only Waste Tissue paper experienced a decrease in % crystallinity from 90.7 – 89.9%. Waste tissue paper is generally made from paper pulp. This paper pulp was used to undertake several chemical processes to remove the amorphous portion. Due to removal of this amorphous portion of that paper pulp by means of different chemical processes high crystallinity was observed in case of waste tissue paper. But when waste tissue paper was subjected to acid hydrolysis for the generation of TNC, the highly ordered crystalline structure was affected and that resulted in a little lower crystallinity. The nanocellulose (CNC, SANC, and TNC) exhibited distinct endothermic changes within the range of temperature when studied. TGA and DTG revealed that major degradation temperature shifted to higher range of temperature in case of nanocellulose than corresponding precursor. The higher thermal stability of the nanocellulose can be ascribed to their higher flexibility, hence higher possibility of entanglements of the nanofibrils. The major degradation peak temperatures were observed at 360 °C, 358 °C and 367 °C for CNC, SANC and TNC respectively which appeared as higher than the values of their respective raw materials i.e. 338 °C, 290 °C and 353 °C respectively .

Ponce-Reyes et al. 2014 from the Departmento de IngenieriaBioquimica, Prolongacion de Carpio y Plan de Ayala prepared cellulose nanoparticles from agave waste and studied their morphology and structural characterization. Dried agave fibres were milled to size less than 2.36 μm. These were subjected to a 3 hour 5 M NaOH treatment at 80 °C with constant agitation. After successive rinsing with deionized water, the resulting solids were treated with DMSO at 80 °C for 3 hours. An HCl: H_2SO_4: H_2O with ratio 1:3:6 was used to hydrolyse the cellulose at 80°C for 3 hours. The resulting milky white suspension was then neutralized with 2N NaOH solution and dialyzed. SEM images showed cellulose nanoparticles of 55 heterogeneous sizes. DLS showed the average particle size obtained to be 97 ± 30 nm within a range of 31-198 nm. XRD studies showed the crystallinity indices for MCC and CNP to be 77 % and 39.4% respectively (Ponce-Reyes et al. 2014).

Wulandari et al. 2016 from Inorganic and Physical Chemistry Research Division, Institute Teknologi Bandung, Indonesia prepared nanocellulose from sugarcane bagasse by acid hydrolysis method and studied their morphology and structural characterization.Sugarcane bagasse was bleached with 0.735% (w/v) sodium hypochlorite for 6 hours with constant stirring at 45 °C to remove the lignin. The residue was washed with distilled water until a neutral pH. The neutral residue was refluxed with 17.5% sodium hydroxide for 3 hours with constant stirring at 45 °C to remove hemicellulose. The residue of this process was also washed until reach a neutral pH, and it was dried at room temperature. Isolated cellulose from sugarcane bagasse was hydrolyzed with sulfuric acid with a ratio of cellulose to sulfuric acid 1:25. The hydrolysis process was quenched by adding 10-fold excess distilled water (250 ml) to the reaction mixture. A colloidal suspension which produced was centrifuged,dialyzed and sonicated to homogenize the generated nano-cellulose. There are two conditions used in this report to produce nano-cellulose, that are sulfuric acid concentration of 60% of at 40 °C for 5 minutes for nano-cellulose A and 50% at 40 °C for 10 minutes for nano-cellulose B. Nanocellulose B has better properties than nano-cellulose A. Nanocellulose B has the average diameter of 111 nm with the maximum distribution of 95.9 nm. The crystallinity index of nano-cellulose B is higher than the isolated cellulose in the amount of 76.01%. The result of TEM analysis shows that nano-cellulose B has a spherical morphology(Wulandari et al. 2016).

KittiyaPlermjai et al. 2018 from College of Nanotechnology ,Thailand extracted nanocellulose from sugarcane bagasseand studied their morphology and structural characterization.This study represents the cellulose extraction from sugarcane bagasse using ball-milling process together with acid hydrolysis. The production of ball-milling-assisted acid hydrolysis expected high surface area and high crystalline. The ball milling technique increased the surface of cellulose and acid hydrolysis increase the crystallinity of cellulose because hydrolysis removes the amorphous region of cellulose, giving fast formation of cellulose microfibrils. Morphology of bagasse, cellulose and nanocellulose were characterized by scanning electron microscopy (SEM).The chemical composition of extracted cellulose and nanocellulose was investigated by Fourier- transformed infrared spectroscopy that verifying the removal of lignin and hemicellulose during cellulose isolation process from sugarcane bagasse. The crystalline structure of cellulose and nanocellulose were characterized by X-ray diffraction (XRD), indicating that the nanocellulose prepared by ball milling with acid hydrolysis has higher crystallinity than nanocellulose prepared by ball milling.

N.A. Sri Aprilia et al. 2021 from Department of Chemical Engineering, Engineering Faculty, UniversitasSyiah 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 nanocellulose 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 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 nanocellulose structure was like individual crystallites and was uniformly dispersed showing a needle-shaped structure.

Chapter2

MATERIALS AND METHODS

2.1 INTRODUCTION

This chapter includes the characterization techniques, materials and methods used for the synthesis and characterisation of CNC and ZnS nanoparticles.

2.2 CHARACTERIZATION TECHNIQUES

2.2.1 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; SCB, CPC and CNCs were investigated using FTIR spectroscopy using Nicolet, iS50, FTIR (Thermo Nicolet, USA) spectrophotometer. The FTIR spectra of the samples were recorded in the transmittance mode in the range of 4000 cm^{-1} to 500 cm^{-1}.

2.2.2 X-RAY DIFFRACTION (XRD)

The crystallinity index of the SCB before and after chemical modification was analyzed using Shimadzu XRD-700 X-RAY Diffractometer. SCB, CPC and CNCs in 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 CuKα radiation source λ = 0.1539 nm with a 2 theta angle ranging from 10° to 60°.

2.2.3 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.

2.2.4 SEM

Scanning electron microscope (SEM) is one of the most widely used techniques used in characterization of nanomaterials and nanostructures. The signals that derive from electron-sample interactions reveal information about the sample including surface morphology (texture), chemical composition of the sample. [27] Accelerated electron in a 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 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 electron beam comes and hits the atoms of 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 on it about 300 V. Secondary Electrons(SE) are emitted from very close to specimen surface and can produce very high resolution image of 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 [29] .

2.2.5 EDAX

Energy Dispersive X-Ray Analysis (EDX), referred to as EDS or EDAX, is an X-ray technique used to identify the elemental composition of materials. EDX systems are attachments to Electron Microscopy instruments (Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM)) instruments where the imaging capability of the microscope identifies the specimen of interest. The basic principle of EDAX is a generation of X-rays from a specimen through the electron beam . To produce characteristic X-rays from an object, it is bombarded by either a highly energetic beam of charge carriers (electrons or protons) or X-rays. The atoms of the sample contain ground state (unexcited) electrons in discrete energy levels or electron shells bound to the nucleus. An electron from an inner shell may be excited by an incident beam, thereby removing it from its shell and generating a hole where electron was present before excitation. This hole can be occupied by an electron of a higher-energy shell. The difference in energies of the higher- and lower- energy shells is emitted as an X-ray. Quantitative measurement of the energy and number of these X-rays can be done with energy-dispersive analysis of X-rays. Since the energy of these X-rays is characteristic feature of energy difference between two shells and atomic structure of discharging element, EDAX can be employed to identify the elemental composition of an object. The data generated by EDAX analysis consist of spectra showing peaks corresponding to the elements making up the true composition of the sample being analysed. Elemental mapping of a sample and image analysis are also possible.Applications include materials and product research, troubleshooting, de-formulation, and more [29] .

2.3. SYNTHESIS OF CELLULOSE NANOCRYSTALS(CNCs)

MATERIALS

- ☐ Sugarcane bagasse
- ☐ Nitric acid (HNO_3)
- ☐ Sodium hypochlorite (NaClO)
- ☐ Sulfuric acid (H_2SO_4)
- ☐ Acetic acid(CH_3COOH)
- ☐ Sodium hydroxide(NaOH)

- ☐ Distilled water

METHODS

ISOLATION OF CHEMICALLY PURIFIED CELLULOSE(CPC)

Sugarcane baggase(SCB) was primarily washed with distilled water to remove any unwanted particles. It was then sun-dried before grinding into small pieces using a blender. The powdered bagasse was oven dried at 105° C for about 5 h and then stored at room temperature in air tight polythene bags. Briefly, 17.5g of the bagasse powder was mixed with 250 mL 6% (w/v) of HNO_3 for 2 h in a hot water bath placed at 80°C then washed with deionized water until neutral. The solution was then refluxed with 200 mL of 1% NaOH at constant stirring for 2 h at 80°C in a hot water bath. It was further washed with distilled water and bleached with 200mL of sodium hypochlorite 5% (w/v).It is then washed till neutral pH. Acetic acid was further added to the lignocellulosic extract and stirred for 2 h at 80°C. The residue was then washed with deionized water until a neutral pH and left to dry.

PREPARATION OF CELLULOSE NANOCRYSTRALS

The isolated cellulose was used to prepare cellulose nanocrystals by hydrolysis. The chemically purified cellulose from SCB was hydrolyzed with 32% (w/v) of H_2SO_4 with a 1:25 g/mL ratio of cellulose to the dilute acid at room temperature for 24 h under constant stirring. This reaction was then quenched by addition of 10 fold deionized water to the reaction mixture, followed by centrifugation at 3000rpm for 15 min three times to remove the acidic solution till neutral. The supernatant was discarded and the cellulosic precipitate re-dispersed in deionized water and filtered by suction. The colloidal suspension was then sonicated in an ice bath sonicator for 2 h to homogenize the generated cellulose nanocrystals. The generated nanocrystals were further centrifuged at 2000 rpm for 30 min. This was then allowed to settle for 24 h and centrifugated. The colloidal suspension formed is Cellulose nanocrystals(CNC).

2.4 SYNTHESIS OF ZINC SULFIDENANOPARTICLES IN ALKALINE MEDIUM

MATERIALS

- ☐ Zinc Chloride (0.1M)
- ☐ Sulphur Powder (0.1M)
- ☐ NaOH (5M)

METHOD

Zinc chloride (0.1M) and sulphur powder (0.1M) was separatelydissolved in distilled water and made upto 100 ml. It was thenmixed in a magnetic stirrer at 70°C. NaOH (5M) was slowly addedinto the mixture till an alkaline pH of 10.44 was attained. It wasthen kept for 5 hours under constant stirring. The precipitateobtained was washed several times with distilled water andcentrifuged at 6000 rpm for 10 minutes. The sample was then driedin the oven at 80°C for 2 hours and annealed at 300° C to obtainZnSnanoparticles[33].

2.5 SYNTHESIS OF ZnS-CNC COMPOSITE MATERIALS

- ☐ Zinc Chloride (0.1M)
- ☐ Sulphur Powder (0.1M)
- ☐ NaOH (5M)
- ☐ Cellulose Nanocrystals

METHOD

Zinc chloride (0.1M) and sulphur powder (0.1M) was separatelydissolved in distilled water and made upto 100 ml. It was thenmixed in a magnetic stirrer at 70°C. CNCs(1g) dissolved in100 ml water is added to this mixture. Then NaOH was added slowly into mixture till an alkaline pH of 10.44 was attained.Itwasthen kept for 5 hours under constant stirring. The precipitate obtained was washed and filtered. The sample was then dried at 50°C in oven.

2.6 ANTIMICROBIAL STUDIES

ANTIMICROBIAL ASSAY BY AGAR WELL DIFFUSION METHOD

The antibacterial activities of the CNC, ZnS and CNC-ZnS were assessed by agar well diffusion method. One ml of thefresh culture of E.Coli was inoculated in the sterile Petri dishesdistinctly. 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 wereCNC, ZnS and CNC-ZnS.Then, the plates were incubated at 37°C for 24 hours.Antimicrobial activity was detected by measuring the zone of inhibition (including thediameter of the wells) that appeared after the incubation period. Distilled water was employed as a negative control. Tetracycline, an antibiotic was used as a standard.

Chapter3

RESULTS AND DISCUSSION

3.1 CHARACTERIZATION OF CELLULOSE NANOCRYSTALS

3.1.1 PHYSICAL APPEARANCES

The physical appearances of the raw sugarcane bagasse that was collected from juice shop, the powdered bagasse, the chemically purified cellulose and its nanocrystals prepared by hydrolysis method are captured below in Fig.3.1.The color of the raw bagasse whitened as a result of the chemical modification.The raw sugarcane bagasse and its powdered form had a brown color. The treatment of the bagasse with HNO_3, NaOH and sodium hypochlorite changed the texture of the powder and also changed the color to white when the cellulose was formed.This degradation process was observed through the disappearance of some functional groups as evident from the FTIR data respectively.The hydrolysis of the extracted cellulose with H_2SO_4 acidproduced the nanocrystals that were whiter and finer by texture.

(a) (b) (c) (d)

Fig3.1. Physical appearance of collected sugarcane bagasse (a), crushed sugarcane baggase (b), extracted cellulose (c) and nanocrystals (d)

3.1.2 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY ANALYSIS

The structural change of the SCB was determined by FTIR before and after chemical treatment. The Infra-Red spectra of SCB, CPC and the CNCs are captured below in Fig. 3.2. The broad peak at 3100-3500 cm^{-1} indicates the O–H stretching bonds while the peak around 2800-2950 cm^{-1} indicates the C–H stretching. The peak around 1750 cm^{-1} corresponds to C–O bond which is normally found in the linkages of the esters in the hemicellulose and lignin. The peaks observed around 1600-1700 cm^{-1} indicates the aromatic ring found in the lignin. The peak between 1200-1300 cm^{-1} depicts an out of plane C–O stretching in the aryl group of the lignin linked to the SCB before chemical modification. The modification of SCB with sodium chloride and sodium hypochlorite lead to the disappearance the bands, FTIR spectrum of CPC and CNCs (Fig. 2). The outcome of this chemical treatment can be noted from the main spectral bands which must be emphasized between 1500-1600 cm^{-1} and around 1250 cm^{-1} . The two spectral bands in these positions are seen to disappear after the chemical treatments to form the CPC and CNCs. The FTIR spectrum of CNCs is similar to that of CPC but it has sharp bands. The peaks observed at 1600–1650 cm^{-1} for the CPC and CNCs are as a result of O–H bending due to adsorbed water,the bands between 1400-1450 cm^{-1} is attributed to CH_2 intertwined in the cellulosic material. The peak at 1050 cm^{-1}indicates the C–O–C pyranose ring stretching vibration. The peak observed around 900 cm^{-1} is reported to be associated with the cellulosic β-glycosidiclinkages.These results depicts the formation of cellulose nanocrystals.

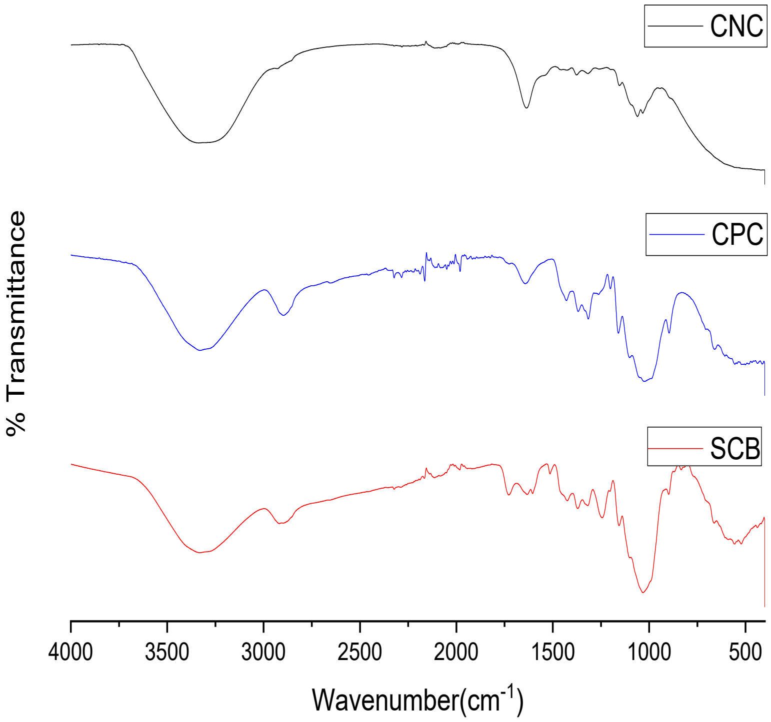

Fig.3.2. FTIR spectra for SCB, CPC and CNCs.

3.1.3 X-RAY DIFFRACTION (XRD) ANALYSIS

The X-ray diffraction patterns of the SCB, CPC and CNCs are shown below in Fig. 3.3.The crystallinity index of SCB, CPC and CNCs was calculated from the XRD. Crystallinity index (CrI) denotes the ratio of the crystalline constituents to the amorphous regions of a material. The SCB, CPC and CNCs exhibited three main characteristic peaks around a 2θ value of 16°, 22.1° and 34.7°. The peaks represents the characteristic patterns for the crystal form of cellulose I polymorph since there is no doublet peak around 2θ value of 22°. The peak at 2θ value 16° corresponds to (110) crystallographic plane while the one at 2θ value of 22.1° and 34.7° relate to the (002) and (004) crystallographic planes correspondingly.After the treatment, more intense crystalline peaks were observed with the main reflections at 2θ value of 22.1° and 34.7°. This was also a confirmation of the successful removal of the lignin from the bagasse. The samples presented high peak intensity around 2θ value of 22.1° which is correlated to the crystalline structure of cellulose. Also, from the XRD patterns, presence of a broad peak at 2θ around 16° is characteristic to the amorphous arrangement.

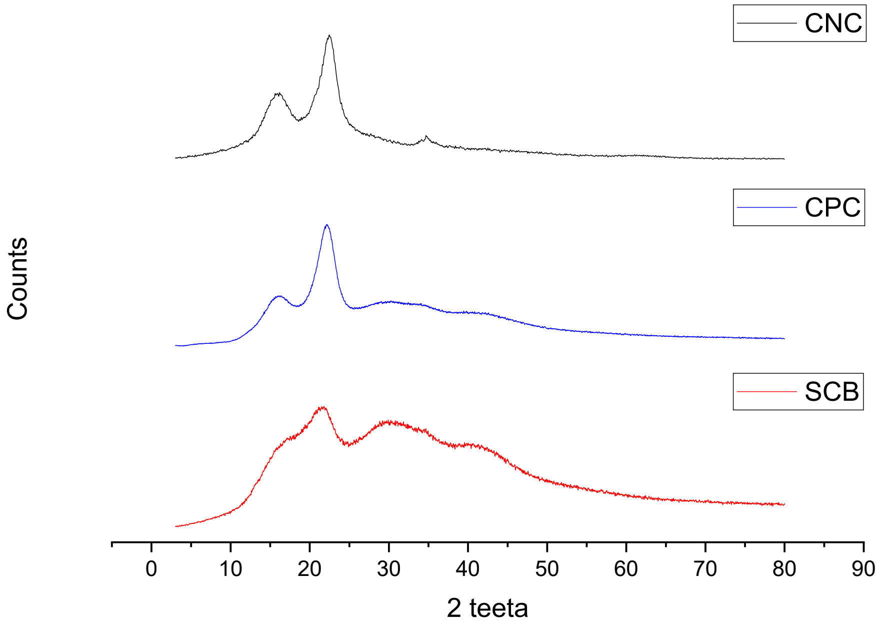

Fig. 3.3. X-ray diffraction patterns of SCB, CPC and CNCs.

3.1.4 TRANSMISSION ELECTRON MICROSCOPY

Transmission Electron microscopy was used to study the morphology and determine the particle sizes of the cellulosenanocrystals which is given in fig 3.4.The chemical treatment of the SCB with an alkali solution and further by acid hydrolysis was expected to remove the hemicelluloses and lignin which form part of the amorphous region of the cellulosic bagasse. This process is aimed at reducing the size of the cellulose to nanometer range while leaving the crystalline regions intact. The CNCs have a ‘rod like’ shape with average diameter of 10 nm and little agglomeration is observed when compared to other studies. This agglomeration is attributed to the surface ionic charge that made the crystallites get stacked together due to acid hydrolysis process.

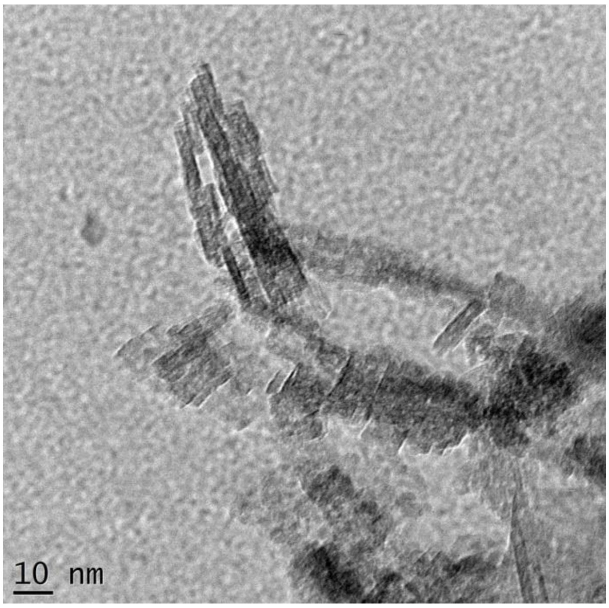

Fig 3.4 TEM image of Cellulose nanocrystals

3.2 CHARACTERIZATION OF ZINC SULFIDE NANOPARTICLES

3.2.1 X-RAY DIFFRACTION

The crystal size of ZnS nanoparticles was analyzed using XRD studies. Figure 3.10 shows the XRD spectrum of synthesized Zinc Sulphide nanoparticles. In the XRD spectrum of ZnS nanoparticles in alkaline medium three clear peaks were observed in the XRD spectrum of synthesized ZnS nanoparticles. The average crystal size was determined using Debye Scherrer equation,

$$D = 0.9\,\lambda / B\cos\theta$$

The average crystal size was found to be 41.2402nm. This confirms that the particles belongs to nanoparticle range.

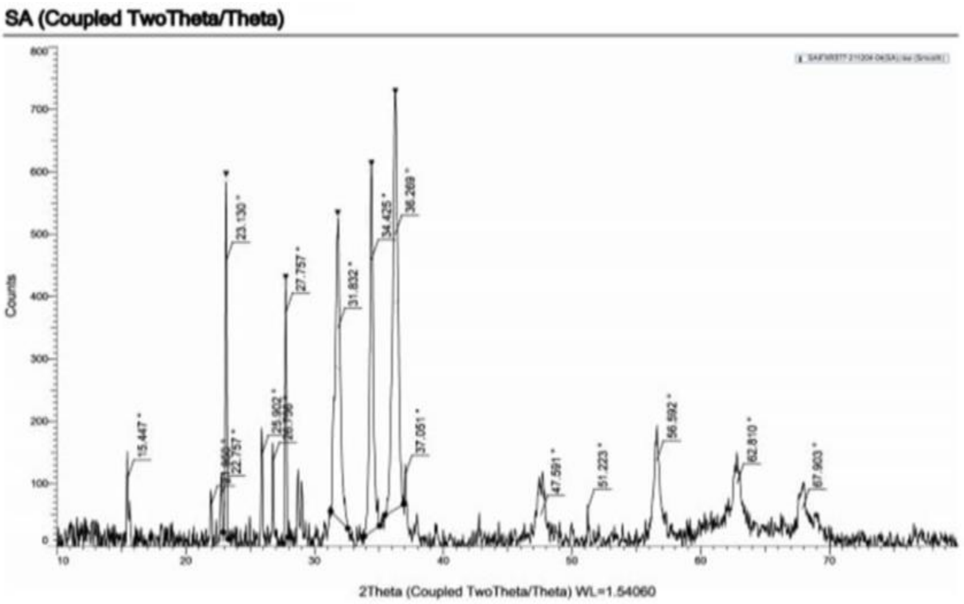

Fig 3.5 : XRD spectrum of synthesized Zinc Sulphide nanoparticles in Alkaline medium

Analysis of peaks:

Peak 1

$2\theta = 23.133°$

$\theta = 11.5665° = 0.2019$ radian

$B = 0.113 = 0.00197$ radian

$\lambda = 1.54060 \times 10^{-10}$

From Debye Scherrerequation ,

$D=0.9\ \lambda/B\cos\theta = 0.9\times 1.54060\times10^{-10}/0.00197\cos 0.2019 = 71.842$ nm

Peak 2

$2\theta = 27.771°$

$\theta = 13.8855° = 0.2423$ radian

$B = 0.133 = 0.0023$ radian

$\lambda = 1.54060\times10^{-10}$

From Debye Scherrerequation ,

$D=0.9\ \lambda/B\cos\theta = 0.9\times 1.54060\times10^{-10}/0.0023\cos 0.2423$

$= 61.563$ nm

Peak 3

$2\theta = 34.432°$

$\theta = 17.216° = 0.3005$ radian

$B = 0.257 = 0.0045$ radian

$\lambda = 1.54060\times10^{-10}$

From Debye Scherrerequation ,

$D=0.9\ \lambda/B\cos\theta = 0.9\times 1.54060\times10^{-10}/0.0045\cos 0.3005$

$= 32.401$ nm

Peak 4

$2\theta = 36.290°$

$\theta = 18.145° = 0.3167$ radian

$B = 0.417 = 0.0073$ radian

$\lambda = 1.54060\times10^{-10}$

From Debye Scherrerequation ,

$D = 0.9\ \lambda/B\cos\theta = 0.9\times 1.54060\times10^{-10}/0.0073\cos 0.3167$

$=19.960$ nm

Peak 5

$2\theta = 31.805°$

$\theta \;= 15.9025° \;= 0.2775$ radian

$B \;=0.403 \;= 0.0070$ radian

$\lambda \;= 1.54060\times10^{-10}$

From Debye Scherrerequation ,

$D=0.9\,\lambda\,/B\cos\theta = 0.9\times 1.54060\times10^{-10}/0.0070\cos 0.2775$

$= 20.508$ nm

Table 3.1 : Average particle size of Zinc Sulphide nanoparticles in Alkaline Medium

2θ of the intense peak (deg)	FWHM of intense peak	Size of particle, D,(nm)	Average particle size (nm)
23.133°	0.113	71.842	41.2402
27.771°	0.133	61.563	
34.432°	0.257	32.401	
36.290	0.419	19.960	
31.805	0.403	20.508	

3.2.2 FT-IR STUDIES

FT-IR studies was performed in order to study and determine the functional groups of synthesized ZnS nanoparticles in alkaline medium. Figure 3.6shows the FTIR spectrum of synthesized ZnS nanoparticles. FTIR spectral studies shown in figure give information regarding the chemical bonding between Zinc and Sulphur . The broad absorption bands at 3423.59 cm^{-1} and 1629.96 cm^{-1} are attributed to O-H stretching and bending vibrations of the absorbed water molecules on the surface of nanoparticles. The and methylene groups. The peak appearing at 432.95 cm^{-1} is associated to ZnS vibration and are characteristics of cubic ZnS. The FTIR analysis confirmed the metal to Sulphur bond formation peak at 1384.28 cm^{-1} region could be attributed to carboxyl(C=O) by showing the characteristics band at 1153.06 cm^{-1} [39].

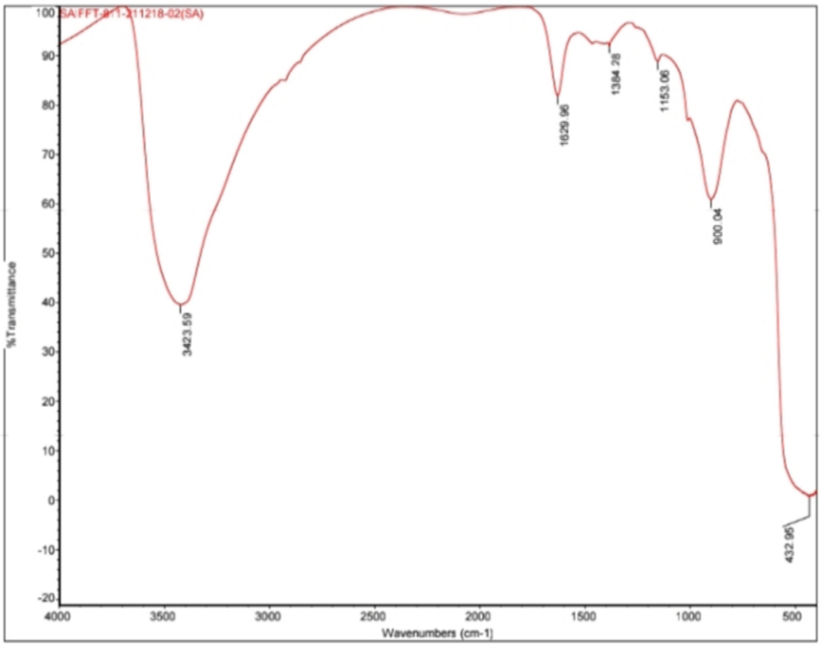

Fig 3.6: FT-IR spectrum of synthesized Zinc Sulphide nanoparticles in Alkaline medium

$3423.59cm^{-1}$	O-H stretching of H_2O
$1629.56cm^{-1}$	O-H stretching of H_2O
$1384.28cm^{-1}$	Carboxyl and methylene groups.
$432.95cm^{-1}$	Zn-S vibration
$1153.06cm^{-1}$	Metal to sulphur bond formation

Table 3.2. FRIR peak assignments of Zinc Sulphide nanoparticles in Alkaline medium

3.2.3 SEM

SEM studies were conducted in order to examine the morphology of the prepared ZnSNanopowder. The SEM images is shown in figure 3.7.

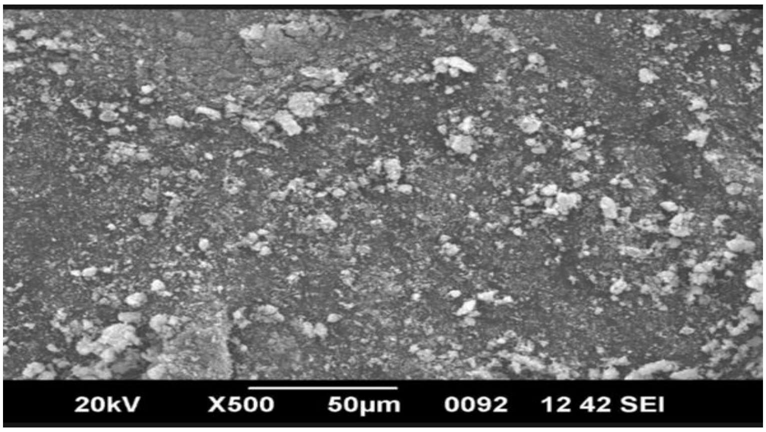

Fig 3.7: SEM images of synthesized Zinc Sulphide nanoparticles in Alkaline Medium

3.2.4 EDAX

EDAX was used in order to determine the element composition that present in the samples. Figure 3.8 shows the EDAX spectrum of synthesized ZnS nanoparticles. Spectrum shows the presenceof two elements which are Zinc andSulphur . The presence of oxygen is due to the oxidation of ZnS to ZnO while annealing at high temperatures. Thus, the EDAX result revealed that the synthesized ZnS nanoparticles contains high Zinc and Sulphur elemental composition[40].

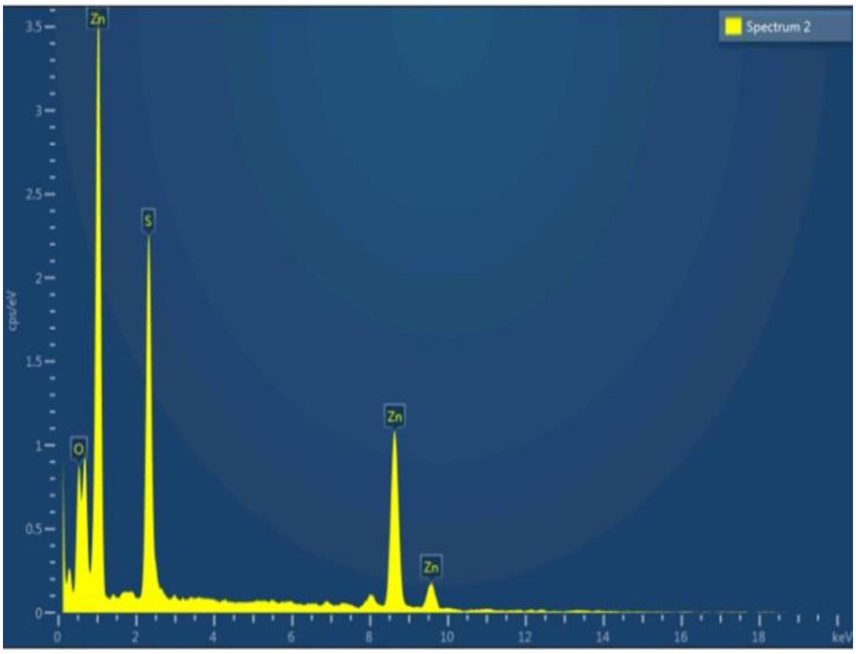

Fig 3.8: EDAX spectrum of synthesized Zinc Sulphide nanoparticles in Alkaline medium

3.3 ANTIMICROBIAL STUDIES

The antibacterial studies was observed against *Escherichia coli* (gram – negative) by agar well diffusion method.

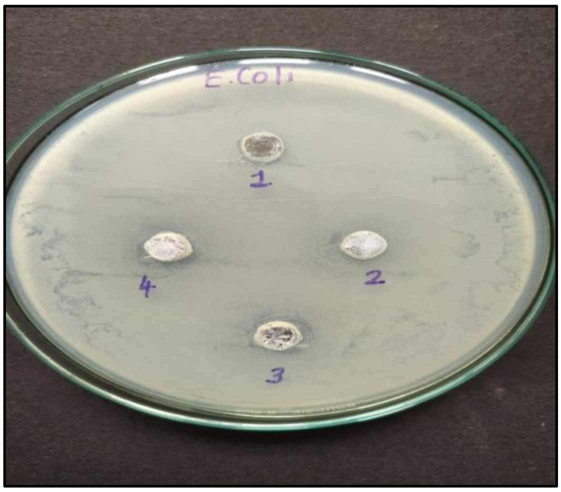

Fig 3.3Antibacterial activity of synthesized cellulose nanocrystals, ZnS and ZnS-NC composite against *Escherichia coli.*

After incubation of 24 hours,it has been found that CNC is bacteriostatic against *E.Coli*. CNCs is showing a zone of inhibition of diameter o.8cm against E.Coli bacterial strain. When Cellulose nanocrystals were incorporated with ZnS it showed an enhancement in antibacterial activity with an increase in zone of inhibition to 1.1cm.Therefore the isolated nanocellulose can be considered appropriate reinforcement to develop the polymeric composites and films for packaging applications[17].

SAMPLE	INHIBITION ZONE DIAMETER
	E.Coli
CNC	0.8cm
ZnS	1cm
ZnS-CNC	1.1cm

Chapter4

CONCLUSIONS

The aim of this study was to demonstrate the viability of the preparation of CNCs from sugarcane bagasse and to characterize the prepared CNCs using a wide range of analytical and imaging techniques. It is hoped that this study will drive further interest in the preparation of CNCs from renewable biomass sources and agro waste products.

The CNCs were prepared using the acid hydrolysis technique. Acid hydrolysis is the most popular and efficient method of isolating CNCs from biomass. The CNC solution was a milky white stable suspension of rod shaped nanocrystalline cellulose. The stability of the suspension was as 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 be performed in 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. Thorough rinses after each pre-treatment is strongly advised as traces of alkaline and bleaches interfere and neutralise the acid used during the hydrolysis procedure.

The removal of non-cellulosic compounds can be enhanced by the use of nitric acid during chemical treatment. An increase in crystallinity was observed for the nanocrystals in XRD result which indicated the exposure of the crystalline phase after successful elimination of the lignin and hemicelluloses.

The FTIR analysis indicate the presence of different functional groups in the prepared CNCs and also helped to determine the structural change after the chemical treatment.The two spectral bands in positions 1500-1600 cm^{-1} and around 1250 cm^{-1} are seen to disappear after the chemical treatments which confirms the formation of CNCs..

The TEM images show that CNCs have a rod like shape with average diameter of 10 nm. The particle size greatly reduced in diameter after the acid hydrolysis of cellulose as observed by TEM and this is an indicator in improved properties of the CNCs.

The results obtained from this work suggest that SCB which is a menace in all the sugar factories can be utilized effectively for several other aspects since they possess the advantages of being sustainable and biodegradable.

Zinc Sulphide nanoparticles were also synthesized. The synthesized nanoparticles were characterized using XRD, FT-IR, EDAX and SEM studies. The formation of the Nanoparticles was confirmed by obtaining the XRD and IR results. The average crystal size was found using XRD studies and it was found to be 41.2402 nm for Zinc Sulphide.

The antimicrobial activity of the CNCs, ZnSandZnS-CNC composite were evaluated against Escherichia coli.The study reveals an enhancement in antibacterial activity of CNCs when incorporated with ZnS nanoparticles.

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