

**Studies on Functionalization of Cellulose
Nanocrystals for Development of Polymeric
Bionanocomposites and Applications in
Sustainable Chemical Processes**

Thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

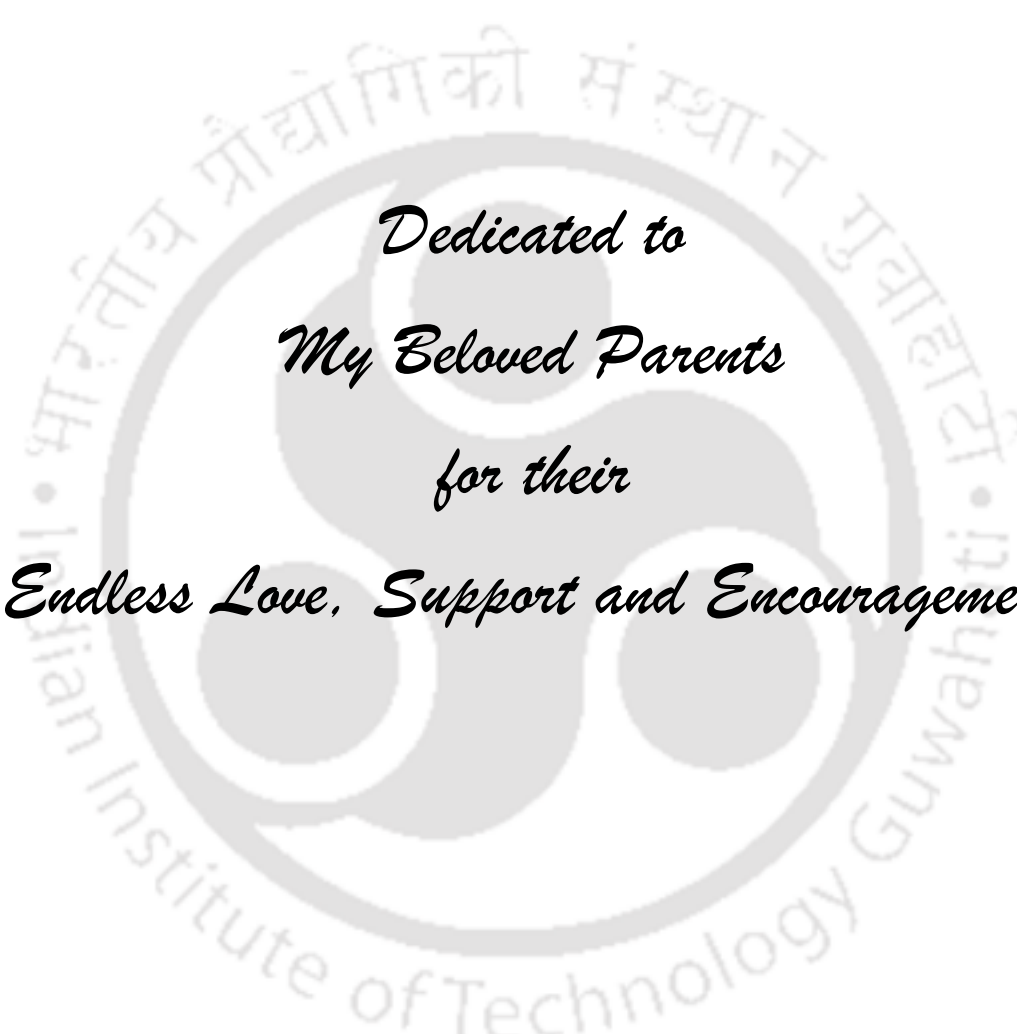
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(April 2017)**





*Dedicated to
My Beloved Parents
for their
Endless Love, Support and Encouragement*



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CERTIFICATE

This is to certify that the research work in the thesis entitled “**Studies on Functionalization of Cellulose Nanocrystals for Development of Polymeric Bionanocomposites and Applications in Sustainable Chemical Processes**”, is carried out by me at the Chemical Engineering Department, Indian Institute of Technology Guwahati, under the supervision of Dr. Vimal Katiyar and Dr. Amit Kumar. The results documented in this thesis are achieved by me and has not been submitted to any other University or Institute for the award of any degree or diploma.

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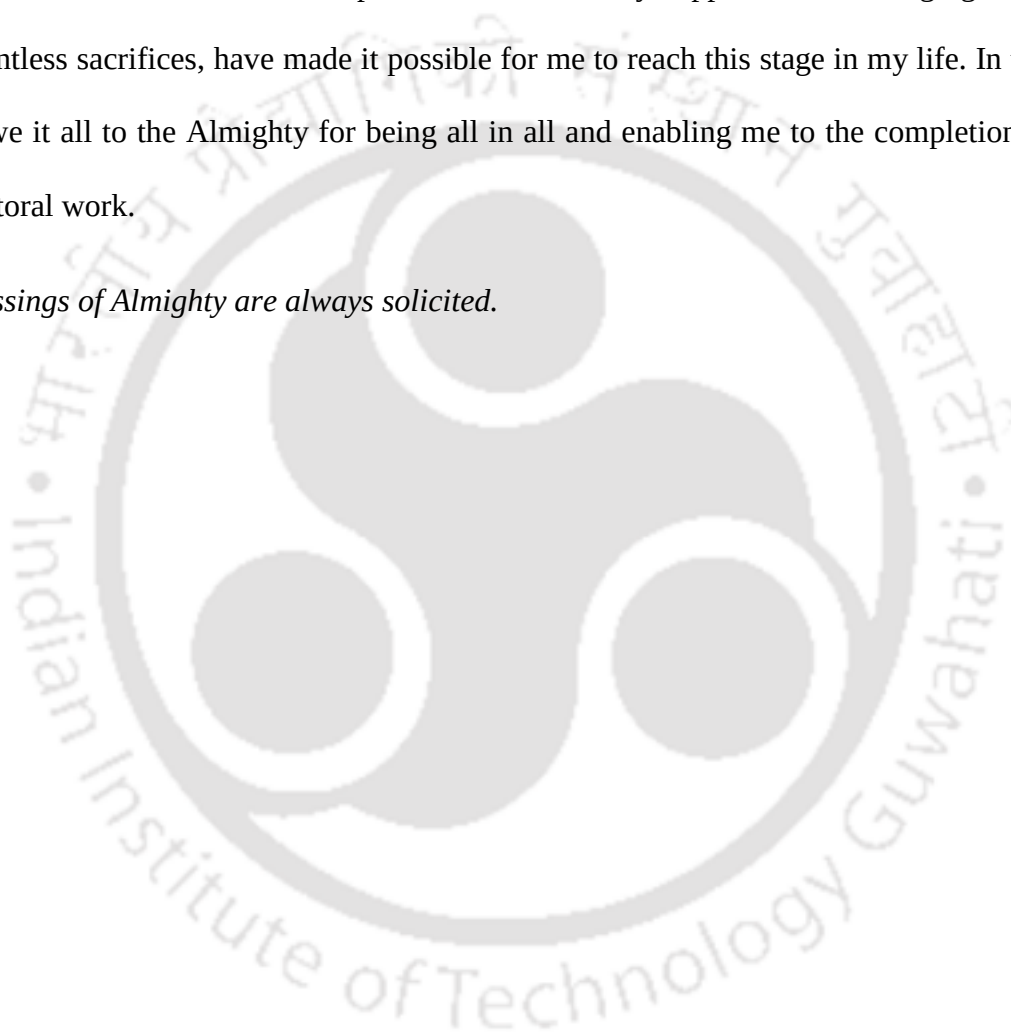
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Abstract

In recent decades, increased environmental concerns to reduce carbon foot print and rising prices of petrochemicals have attracted much attention towards developing green and sustainable biodegradable polymers and nanomaterials, which will help in securing the planet's ecological balance. The studies based on utilization of renewable resources for development of value-added products ensuring environmental sustainability have become essential to confront the unprecedented implications of petroleum-based products related to global warming. Nature-based nanomaterials such as cellulose nanocrystals (CNCs), are derived from the abundantly available cellulosic biomass with unique inherent characteristics that have led to increased scientific and industrial attention. CNCs are the crystalline domain of cellulose with rod-like morphology and interesting properties such as high surface area, tunable aspect-ratio, chemical functionality, anisotropic mechanical properties, self-ordering behaviour to form optically active materials along with improved biocompatibility, biodegradability and non-toxic nature, provides it an upper hand in comparison to other nanoparticle derivatives. CNCs due to its sustainable nature, bio-origin and anisotropic mechanical behaviour have promising applications as reinforcing agents in polymeric nanocomposites, as targeted drug delivery vehicles, in biocatalysis, and in paper-based electronics as sensors. However, production of CNCs through industrially viable approach is still a challenging task due to high processing time, uncertainty in initial cellulosic precursor and requirement of stringent acid hydrolysis conditions. Moreover, literature survey suggests that processing of CNC-based polymeric nanocomposites through scalable extrusion techniques results in agglomeration due to their hydrophilic nature alongwith severe degradation in the molecular weight of polymer. Therefore, the present doctoral thesis aims to address both the techno-economic challenges associated with the processing of CNCs or its polymeric composites and develop advanced functional materials with CNCs as building block for high performance applications.

In the present work, CNCs have been fabricated from renewable feedstock such bamboo stems, rice husk, commercial pulp and newspaper for their potential application as commercially viable value added products, with a strategy to convert “wastes to wealth”. The challenges associated with high production time was overcome with an indigenously developed CNC production unit coupled with continuous dialysis system which reduces the processing time to ~24-26 hours in comparison to ~7 days through traditional approach. Interestingly, CNCs with tunable physico-chemical, structural and morphological properties, are fabricated by simply varying the initial cellulose polymorphism or the type of acids used as hydrolyzing agents, explicitly during its processing step. Strategic modification of CNCs through this sustainable approach avoids the utilization of harmful and toxic chemicals which might affect the biocompatible and non-toxic properties of CNCs. Dispersion of surface-modified CNCs into polymeric matrix leads to the formation of percolation network which results in considerable improvement of mechanical and barrier properties of biodegradable polymers (such as polylactic acid (PLA) and polyhydroxybutyrate (PHB)), making them ideally suited for food packaging applications. An industrially viable approach for processing PLA/CNC films through reactive extrusion process have been developed which was successfully tested on a pilot-plant scale extruder in presence of wide range of compatibilizers. The reactively processed PLA/CNC cast films shows improved molecular weight characteristics, thermo-mechanical and barrier properties with dimensional stability and migration values within the standard limits, which is generally difficult to achieve through traditional extrusion process limiting their commercial viability. The developed films are promising for food packagings and result in improved self-life of ~5 months and ~2 weeks respectively for the storage of oil and dairy based products. In addition, these films have the potential to be produced at commercial scale through utilization of industrial scale extruders with improved dimensional stability, stable molecular weight and physico-chemical characteristics. Magneto-responsive CNCs,

are one of the versatile nanomaterial which have been developed in this work, with potential applications as self-propagating nanobots with autonomous motion as targeted drug delivery vehicles, biocatalysts with high activity and as templates for fabrication of graphene nanoscrolls with tunable geometry and encapsulated metallic nanoparticles as hydrogen storage devices. Magnetic field directed alignment of such nano-block units introduces anisotropic thermo-mechanical, electrical and magnetic properties alongwith the hyperthermia characteristics finds potential applications in development of bio-inspired materials with microstructural features comparable to biological materials for high performance engineering applications. Therefore, this doctoral thesis focusses on functionalization of CNCs for development of polymeric bionanocomposites as well as their advanced functional derivatives and studying their efficacy in sustainable chemical processes, which have been summarized in nine different chapters and explained in details in subsequent sections.



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Nomenclature

Abbreviations

INSCC	International Nanocellulose Standards Coordination Committee
ASTM	American society for testing and materials
DSC	Differential scanning calorimetry
CNC	Cellulose Nanocrystals
CNF	Cellulose Nanofibrils
CNWs	Cellulose Nanowhiskers
TEMPO	2, 2, 6, 6-tetramethyl-1-piperidinyloxy
APS	Ammonium persulfate
Hbr	Hydrobromic acid
Hcl	Hydrochloric acid
PHAs	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PLA	Poly(lactic acid)
PBS	Polybutylene succinate
PCL	Polycaprolactone
PHBV	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
DP	Degree of polymerization
LCA	Life Cycle Assessment
Lbl	Layer by Layer Assembly
TEM	Transmission electron microscopy
FESEM	Field Emission Scanning Electron Microscope
AFM	Atomic force microscopy
EDX	Energy dispersive X-ray analysis
POM	Polarizing optical microscope
FTIR	Fourier transform infrared spectroscopy
XRD	X-ray Diffraction
TGA	Thermogravimetric Analysis

DCP	Dicumyl peroxide
ZVI	Zerovalent Iron
MMT	Montmorillonite
CNFs	Carbon nanofibers
VSM	Vibrating sample magnetometer
GPC	Gel Permeation Chromatography
NMR	Nuclear Magnetic Resonance Spectroscopy
DSC	Differential Scanning Calorimetry
OP	Oxygen permeability
OTR	Oxygen transmission rate
UTM	Universal Testing Machine
DMA	Dynamic Mechanical Analyzer
CA	Contact Angle
WVTR	Water vapor transmission rate
WVP	Water vapor permeability
DLS	Dynamic light scattering

Notations

$E_{ }$	Elastic Modulus along axial direction
$E_{\perp}$	Elastic Modulus along transverse direction
E'_r	Storage modulus at rubbery state (85°C)
E'_g	Storage modulus at glassy state (46°C)
Da	Dalton
M_N	Number average molecular weight
M_W	Weight average molecular weight
F_{H-CO}	Hydrogen bond fraction
T_i	Initial decomposition temperature
T_f	Final decomposition temperature
T_p	Peak degradation temperature
E_a	Activation energies
T_s	Reference temperature
P	Permeability

P	Oxygen partial pressure
L	Film thickness
Z''	Imaginary impedance
Z'	Real impedance
Φ_c	Percolation concentration
E_m	Elastic moduli of the polymer
E_f	Elastic moduli of the fiber
E_c	Elastic moduli of the composite
G_m	Poisson ratio
W_{ad}	Work of adhesion
ΔH_m	Enthalpy of melting,
T_m	Melting temperature
ΔH_{cc}	Enthalpy of crystallization
T_{cc}	Crystallization temperature
Greek letters	
γ_s	Total surface energy
γ_s^d	Dispersive surface energy
γ_s^p	Polar surface energy
γ_i^{LW}	Surface energy components of Lifshitz-van der Waals
γ_i^+	Surface energy of electron acceptor
γ_i^-	Surface energy of electron-donor
α	Mass fraction

Chapter 1

Introduction and Literature Review

This chapter focuses on the emerging technologies developed for fabrication of cellulose nanocrystals (CNCs) based value added composites for high performance applications. CNCs due to its interesting inherent characteristics are promising smart materials which have found growing demands with wide-spread applications in various engineering sectors such as healthcare, biomedical, electronics, packaging etc. Alongwith the strong reinforcing capability, these bionanoparticles possess several attractive properties such as biodegradability, non-toxicity and bio-based origin which makes it a sustainable alternative to other known nanomaterials. Although, fabrication of CNC based films via industrially viable approaches are challenging tasks, however, novel strategies of surface modification and innovative fabrication techniques needs to be developed to disperse CNCs into polymeric matrix. CNCs, due to their bio-origin and renewability, can be used as replacement to petroleum derived products alongwith their strategic modifications to develop sustainable advanced functional nanomaterials, with the capability to address some of the major global challenges. This makes CNCs an emerging nanomaterial for the 21st century, whose expected demand will continue to grow in near future and will require its industrial scale production to meet the increased world demand.

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1.1 INTRODUCTION

Nanocellulose, are an important class of emerging bio-nanoparticles of 21st century, because of their numerous unique properties which makes them potentially applicable in diverse fields of scientific and technological advancements. Due to their interesting structural and physico-chemical properties, they have attracted researchers to develop nanocellulose-based technologies and led to an increase in demand among industries for the successful commercialization of the developed technologies. Some of the noteworthy exceptional properties of nanocellulose include tunable aspect ratio, controlled morphology, excellent structural (mechanical) properties, high specific surface area and hydroxyl functionality that can be chemically modified depending on targeted applications. In addition to, the abovementioned characteristics, these bionanoparticles possess several attractive properties such as improved biocompatibility, biodegradability, non-toxicity and bio-based origin which further enhances the research interest and widens their range of applications [1]. The abundant availability of initial cellulosic precursors in the form of renewable biomass resources, makes the manufacture of nanocellulosic products particularly attractive and environment-friendly, in comparison to petroleum-based polymers which are derived from the depleting fossil-fuel reserves. To meet the increasing demand worldwide, scalable technologies for production of CNCs have been developed and its commercialization through industrial processes are actively carried out by companies in parts of Canada, USA and Finland [2]. Nanocellulose, can be tailored and functionalized into different types of materials and structures such as films, nanofibers, nanofluids, foams, hydrogels, xerogels, aerogels, emulsions or as pastes which opens up a plethora of technological innovations and commercial opportunities [3]. Till date nanocellulose have been extensively used in high performance applications for the development of wide range of value-added products, and finds potential applications in

daily life such as: (i) in the development of polymeric nanocomposites as reinforcing agents for fabrication of high strength fibers, textiles or ‘smart’ packagings [4], (ii) as drug delivery vehicles [5], (iii) scaffolds for bone replacement/ tissue engineering [6], (iv) as rheological modifiers for paints, coatings, adhesives or inks [7], (v) as building blocks to strengthen the concrete used in construction [7] or in the manufacturing of paper and related products [8], (vi) in optically tunable liquid crystalline materials [9] and (vii) as paper based flexible electronics [10] forming portable batteries [11], substrate to solar cells [12] or fuel cell membranes [13]. The utilization of sustainable and renewable nanomaterials in the designing and manufacturing of myriad newer products with exciting applications, will help address some of the issues and global challenges related to environmental pollution and shortage of petroleum resources.

Although nanocellulose were discovered in the last century, its relevance in research for development of new technological advancements have only been recently explored. Nanocellulose, generally termed as ‘cellulose micelles’, in earlier literatures, was first obtained by Ranby in late 1950s through the sulfuric acid treatment of cellulosic fibers [14]. The distinct needle-shaped morphology of the dried nanocellulosic powder was first observed by Mukherjee et al. In 1953 through TEM-based morphological analysis [15]. However, it was during the late 1970s that the gel-like product was first obtained through wood pulp homogenization at high temperature and pressure at the ITT Rayonier Lab in Whippany, New Jersey, USA [16]. The term ‘nanocellulose’ was first coined by Turbak, Snyder and Sandberg for this product [16]. This was followed by the grant of several patents to ITT Rayonier which detail the preparation and application of the nanocellulosic products in the early 1980s. Recently in 2011, TAPPI’s International Nanocellulose Standards Coordination Committee (INSCC) was constituted to propose international standards for cellulose-based nanomaterials which could remove the trade barriers and harmonize

research and development in nanocellulose-based products [2]. The existence of such standards helps to categorize nanocellulose in various types depending upon their inherent characteristics, thus overcoming the problems related to multiple definitions and ambiguity. The physicochemical and structural properties of nanocellulose are found to be strongly dependent on the initial biomass type or microbial source [4], cellulose polymorphs [17], pretreatment process of cellulose extraction and acid hydrolysis [4] or enzymatic treatment [18] followed for nanocellulose fabrication. Nanocellulose consists of primarily crystalline domains of cellulose which are of three different types depending on the degree of crystallinity and morphology of such crystalline segments: (i) cellulose nanocrystals (CNCs) or cellulose nanowhiskers (CNWs) with high crystallinity generally have small crystalline fragments, are of low aspect ratio and are usually fabricated through controlled acid hydrolysis, (ii) cellulose nanofibrils (CNF) are of comparatively lower crystallinity with high aspect ratio and are generally fabricated through mechanical disintegration process using intense homogenization or ultra-sonication, and (iii) bacterial cellulose (BC) composed of thin bundles of randomly assembled microfibrils which are microbially produced through the fermentation process [19].

Nanocellulose such as CNCs or CNWs are usually fabricated through controlled, stringent acid hydrolysis process which depends on the type of biomass and the acid systems used. The initial biomass selected can be classified into either softwood or hardwood subject to the degree of adhesion between the microfibrils, its geometry and cell dimensions, composition of the cellulose, hemicellulose and lignin content which tend to vary drastically. CNCs fabricated from cotton (usually with higher cellulose content than higher plants) are generally of aspect ratio ~ 10 , whereas CNCs from tunicin or green algae (highly crystalline) have higher aspect ratio (~ 67) [20]. Similarly, CNCs extracted from coconut fibers (which have higher lignin content) were ultrathin with diameter of $\sim 2\text{--}3$ nm

consisting of 16 to 30 cellulosic units [21]. In addition to the biomass source, the pretreatment reaction conditions must be optimized in order to yield cellulose with higher purity, crystallinity and degree of polymerization which strongly affect the properties of fabricated CNCs [4]. The mechanism of cellulose hydrolysis usually includes dissociation of acids into protonated ions followed by diffusion into amorphous layers of microfibrils disintegrating the glycosidic bonds of cellulose chains. The diffusion of protonated ions is strongly affected by the selected biomass type, which have different degrees of cellulose micro-fibril packing density and presence of impurities such as hemicellulose or lignin. However, the degree of acid protonation depends on the selected acid type, ionic strength of acidic media and ratio of cellulose to acid used for hydrolysis. Therefore, depending on the targeted applications it is possible to tune the inherent characteristics of the nanocellulose which, however, depends on the type of extraction process, biomass and hydrolyzing agent selected.

CNCs usually consist of parallelly stacked crystalline segments of short cellulosic chains which induces anisotropic mechanical behavior with the elastic modulus $E_{||} \sim 110\text{--}220$ GPa along axial direction and $E_{\perp} \sim 2\text{--}50$ GPa along transverse directions [22]. The elastic modulus is comparable to most of the strongest materials such as carbon-nanofiber ($\sim 125\text{--}181$ GPa) and Kevlar (70-122 GPa). Moreover, the inexhaustible availability of precursor materials and easy processing methodologies makes CNCs comparatively cost-effective than other known nanomaterials. The aforementioned mechanical properties of the CNCs are known to be significantly depend on the source of biomass origin, pretreatment method followed for the extraction of cellulose, type of acid hydrolysis methodology selected, cellulose polymorphism and functionality [4]. Due to its distinct rod-like morphology, CNCs have the capability to act as a strong reinforcing agents, the degree of which depends on its aspect ratio and inter/intra-molecular hydrogen bonding.

Even though carbon nanotubes have higher elastic modulus than CNCs, however, their ability to act as reinforcing agents when dispersed into polymeric matrix is almost identical [23,24]. Similar to the other nanomaterials (such as graphene, nanoclays, metallic and inorganic nanoparticles), CNCs have unique nanoscale properties with high surface area, but due to their high hydroxyl functionality are capable to form hydrogen-bonded networks which makes them distinctive. In addition to tunable morphology and functionalities, CNCs have exceptional optical properties with the capability to self-assemble into various liquid- crystalline phases such as isotropic, chiral-nematic or cholesteric forms, which depends on the initial concentration and ionic strength of the dispersion media [25]. In addition, the helically-shaped anisotropic CNCs are electro/magneto-responsive in nature with diamagnetic susceptibility behavior which leads to perpendicular alignment in the presence of magnetic field [26]. Alignment of CNCs can be introduced through various other routes such as shear-induced deformation, drawing of film-cum-casting and during dry/wet/electro-spinning or through a combination of above-mentioned steps [27]. Further, modifications with hybrid nanomaterials, hydrophobic polymers or monomeric units, surfactants, multiple-stimuli responsive materials, end functionalization etc. Provides more versatility and flexibility to tune the physico-chemical, structural and self-assembly behaviour of CNCs. Introduction of surface charge gradient and modification with various anionic moieties provides an alternative tool to tune the surface behaviour of CNCs. Due to such vast diversity in the intrinsic characteristics of CNCs, along with its sustainable and renewable nature, the demand for nanocellulosic materials have dramatically increased which encourages a paradigm shift from bench-scale to industrial production.

Increased research on the utilization of waste renewable biomass as cellulosic source, greener routes of hydrolysis, purification techniques, optimization of its morphology and efficient characterization tools have led to easier routes of process intensification. CNCs

have found interest from both research community as well as from the industry recently which is evident from the boom in number of patents or publications in reputed journals and increased commissioning of new industries directed towards production of CNCs and its derivatives. According to the Scopus database, the statistic on the annual number of journal publications shows a leap from ~25 papers in 2006 to more than ~500 papers published in 2016, with a jump of about 25 times which shows the interest on CNCs among the scientific community [28]. Most of publications during the period of 2012–2016 are focused on functionalization of CNCs, developing next generation of value-added nanocellulosic composites for wide range of engineering applications. Majority of the publications are focused on CNC based functional nanocomposites with potential applications as barrier films [29], flexible electronic devices [30], catalyst support/templates [31], food additives [32], targeted drug delivery [5], protein adsorption [33], gas-separation membranes [34,35], hydrogel/aerogel based composites [36] and in electronics as supercapacitors/batteries [37]. Some of the highly cited and ‘hot’ papers on CNCs are focused on attractive research topics such as CNC-based polymeric nanocomposites with self-actuating behaviour through the introduction of mechanical gradients [38], artificial-nacre mimicking biological materials [39], substrates for recyclable organic solar cells [12], photonic hydrogels [40], catalyst [41] and membrane in fuel cells [42], scaffolds for the growth of neuronal networks [43], foldable CNC-based rechargeable batteries with high energy density [44], as size exclusion filters for removal of virus [45], quantum dots-modified CNCs with photoluminescent behaviour for potential application in opto-electronic devices [46] and targeted drug delivery [47]. The strategic modification of CNCs with magnetic nanoparticles leads to introduction of magneto- responsive behaviour which has potential applications as membranes for water purification or as loud-speakers in electronic devices [48], in data storage, as aerogels [49],

as agents for biomedical imaging, as biocatalyst with high turnover frequency [50], in selective protein adsorption or separation [51], enzyme immobilization [52,53] and in wound healing [54]. Moreover, most of the patents filed on nanocelluloses are focused on efficient routes of CNC production and its purification through commercially-viable and scalable approaches [55].

Improved processability of CNCs and potential market growth have led to significant increase in the number of industries participating in its production. CelluForce, commissioned by FPInnovations, Pointe Claire, QC, Canada is the world's largest CNC producer with a turnover of 1 ton/day, which utilizes the traditional sulphuric acid-based hydrolysis process and Kraft pulp as initial cellulosic precursor [2]. U.S. Department of Agriculture Forest Products laboratory (FPL), at Madison, WI has a production capability of 10kg/day of CNCs, which are supplied in freeze- or spray-dried forms. Alberta Innovates Technology Futures (AITF), Canada utilizes two different cellulosic source, cotton and wood for production of CNCs at a scale of 100 kg/week, through acid based-hydrolysis. Some of the interesting proprietary techniques of CNC production which have been recently developed at pilot-scale by industries are American Process Inc. (API) and Blue Goose Biorefineries Inc. (BGBI), respectively [2]. API utilizes a method named AVAP, which requires SO₂ and ethanol treatment followed by mechanical disintegration to separate out CNCs and CNFs, which are marketed with trade name of 'Bioplus Crystals'. BGBI utilizes a novel transition-metal-based catalysts which can produce a mixture of nano-fibrillar cellulosic gels through oxidative routes and are commercialized with the trade names BGB Natural, BGB Goose or BGB Ultra depending on their inherent properties.

However, with growing CNC community and manufacturers, there remain several challenges and ambiguity associated with the inherent characteristics of CNCs, which will

vary with the producers, basic characterizations tools or protocol followed and unexpected changes with inconsistency in cellulosic precursor. To overcome such challenges, Canadian Standards Association has effectively sketched out guidelines and methodology for nanocellulose preparation and its property evaluation in the form of current standards by ISO and TAPPI. The strict regulations and adherence to standards will help in selecting the appropriate type of CNCs with suitable inherent properties depending on the end-user applications.

Among the various class of nanomaterials reported in literature, CNCs are versatile in nature with the presence of manifold functionalities and physico-chemical characteristics (as shown in Figure 1.1) which could be synergistically utilized for numerous remarkable applications that surpasses the current expectations [56]. Moreover, this century has witnessed increased demand for green and sustainable materials, which makes CNC an ideal candidate with excellent biophysicochemical properties, such as enhanced biocompatibility, non-toxicity, biodegradability, bio-based origin alongwith economic viability compared to other nanomaterials (as shown in Figure 1.1). CNCs generally have adaptable surface behavior which can either be modified through covalent approaches such as esterification, acetylation, silylation, polymer grafting or by non-covalent interactions with ionic salts or surfactants. Furthermore, with the utilization of single nanocellulosic materials it is possible to incorporate various desired features to suit the targeted applications, manipulating the initial cellulosic precursor, hydrolysis process followed, and by making chemical alterations. Therefore, the potential research topics on CNCs have been classified into four distinct areas viz. Fabrication and strategic surface modifications, followed by evaluation of properties and potential applications in various sectors, as discussed in subsequent sections.

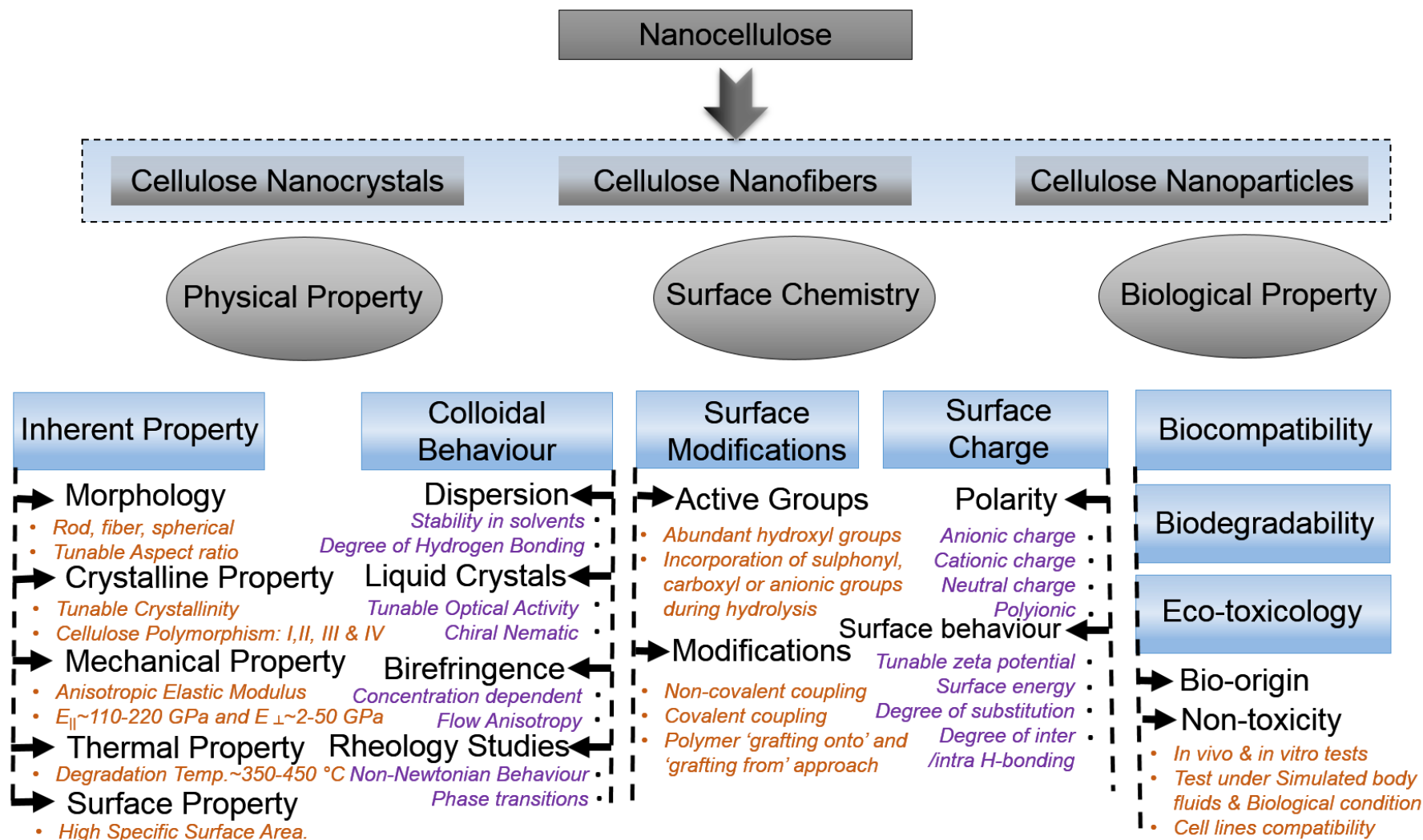


Figure 1.1: The multi-functional physicochemical, structural and biological properties of cellulose nanocrystals (CNCs).

1.2 SCOPE AND STRUCTURE OF THESIS

In the preceding section, a brief overview on fabrication of CNCs through utilization of various biomass resources as cellulosic precursor and different acid hydrolysis-based processing techniques have been presented. The traditional process of CNC fabrication reported in literature is generally carried out through stringent acid hydrolysis which is a time consuming approach requiring almost ~7–10 days. Moreover, fabrication of CNCs with distinct rod-like morphology, high purity and yield through the utilization of waste renewable biomass resources is a challenging task, due to variation in initial cellulosic precursor which requires stringent optimization at each processing steps.

Due to inferior properties of the biodegradable polymers, CNCs are generally used as reinforcing agents to improve their physicochemical, structural and barrier properties. As CNCs are hydrophilic in nature they tend to get agglomerate in hydrophobic polymers. Hence, strategic surface modification of CNCs needs to be carried out in order to improve their dispersion behaviour. However, most of the surface alteration techniques are based on chemical-based approaches, which increases concerns regarding their non-toxicity, biocompatibility and potential application in biological environment. Despite significant research on fabrication of wide variety of CNC-based polymeric nanocomposites through melt-extrusion process, fundamental problems such as reduction in polymer molecular weight, thermal or shear induced degradation and formation of blackish coloration in nanocomposite films with inferior properties are some of the major concerns which still need to be addressed.

As discussed previously, CNCs are strong reinforcement agents but their anisotropic nature with elastic modulus of $E_{\parallel} \sim 110\text{--}220$ GPa in parallel/axial direction and $E_{\perp} \sim 2\text{--}50$ GPa in perpendicular/transverse direction, have been seldom utilized for fabrication of directionally tunable nanocomposites. Most of the studies reported on

alignment of CNCs in polymeric matrix are based on application of very high magnetic/electric fields, shear induced deformation or through drawing process. However, these processes are technically difficult to scale-up and the alignment is possible only in hydrophilic polymers, which gets distorted once the external forces are removed. Incorporation of magneto-responsive behaviour in CNCs might provide an alternative approach to align them under low magnetic fields and introduce the multi-functional behaviour for potentially high performance applications.

Therefore, this PhD. Research work focuses on addressing and overcoming the above mentioned challenges and techno-economical issues related to processing of CNCs and its CNC-based polymer nanocomposites through industrially viable and scalable approaches. Strategic surface modification of CNCs with environment friendly nontoxic iron nanoparticles leads to incorporation of multi-functional properties such as magnetic hyperthermia and magnetotaxis, with applications as biocatalysts for conversion of chemical moieties and, degradation of dyes, in targeted drug delivery, and in the fabrication of graphene nanoscrolls for high electrochemical charge storage and hydrogen storage. The noteworthy results of research activities carried out are compiled in this thesis as six chapters (chapters 3-8). The organization of the thesis and the content of each chapter (excluding the present one) is briefly discussed next:

Chapter 2, provides a detailed description of the experimental protocol, materials and methodologies followed for fabrication of CNCs and its strategic modification to develop polymeric and magneto-responsive nanocomposites. The analytical instrumentation, mathematical models and the molecular simulations studies carried out in this study have been discussed in details.

Chapter 3, deals with the fabrication of CNCs from different biomass resources such as bamboo stems, rice husks, etc. which are generally waste agricultural feedstock, and from waste newspaper through a single-step decoloration process. We have developed a novel approach of tuning the physicochemical and structural properties of CNCs by simply altering the polymorphism and substitution of anionic moieties during the hydrolysis step itself. Moreover, the technical problems related to CNC processing have been overcome through an indigenously developed pilot-scale CNC production unit equipped with continuous dialysis system which has the capability to manufacture CNCs within 24-26 hours compared to 7-10 days through traditional approach.

Chapter 4, describes the fabrication of biopolymeric/CNC-based “green” nanocomposite films utilizing the microbially derived polyhydroxybutyrate (PHB) for potential food packaging applications. The formation of percolating network of CNCs in PHB matrix, corroborated through linear and non-linear rheological studies, leads to significant improvement in structural, crystallization and thermal properties. The processed films show high resistance to oxygen gas permeation and overall migration values within the limits as per standard legislations. These favorable properties along with their non-toxicity, sustainability and complete biodegradability makes the PHB/CNC nanocomposites suitable for food packaging applications.

Chapter 5, details the fabrication of bio-based polymer/CNC nanocomposites where, the bio-based polymer is polylactic acid (PLA). The nanocomposites are fabricated using two different types of CNCs: CNCs with different cellulose polymorphs and acid-derived CNCs with substituted anionic moieties. Due to the variation in inherent properties, incorporation of both the CNCs types leads to tuning of mechanical, crystallization, thermal and barrier properties of the PLA/CNC films. This study provides insight into rational selection of

appropriate type of CNCs for manufacturing high performance PLA/CNC nanocomposites with tailored properties

Chapter 6, presents a novel, industrially viable approach of processing PLA/CNC nanocomposite films through a single-step reactive extrusion approach with improved processability. The reactively grafted PLA/CNC films are tried and tested on a pilot-plant scale extruder, which shows improved barrier properties towards oxygen and water vapor alongwith significant improvement in shelf-life for storage of dairy and oil-based products. The processed films through this novel approach are recyclable, show dimensional stability, improved physico-chemical, structural, thermal and barrier properties, and are adequate for fabrication of biodegradable food packagings replacing the petroleum derived non-degradable plastics.

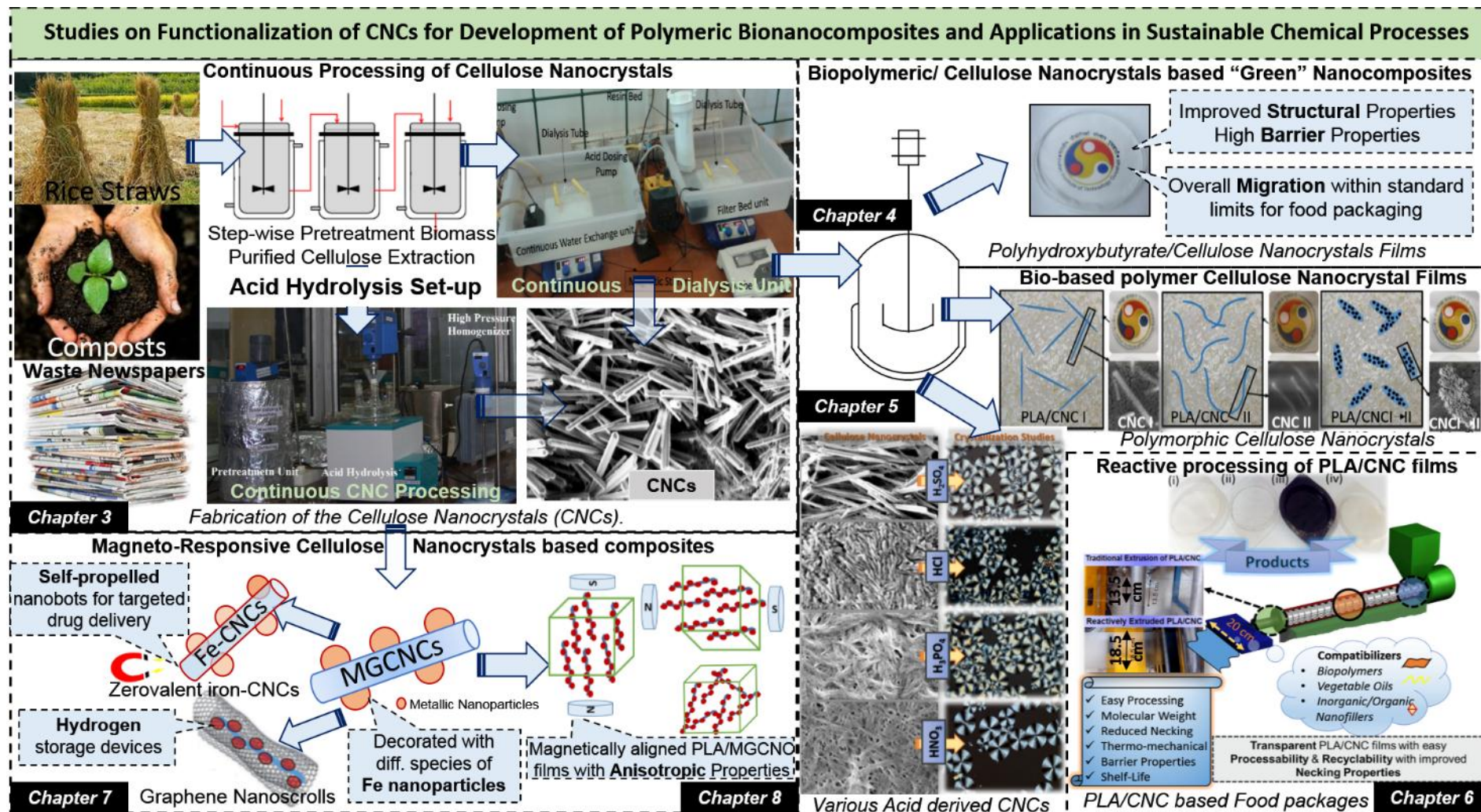
Chapter 7, details the fabrication of magnetic CNCs with different varieties of iron-based nanoparticles such as zerovalent iron (0), iron (II) oxide or iron (III) oxides which were chemically adsorbed on the surface of CNCs. CNCs with zerovalent iron nanoparticles show very high catalytic activity for dye degradation as well as conversion of chemical moieties, and autonomous motion under chemo-magnetic gradient. The Fe_2O_3 -grafted CNCs were used for fabrication of graphene nanoscrolls which encapsulated various types of metal nanoparticles to induce enhanced catalytic activity, high hydrogen adsorption capacity as well as electrochemical charge storage.

Chapter 8, utilizes the Fe_3O_4 -grafted CNCs for magnetically orienting them in the polymer matrix through directionally tunable magnetic field, which provides a novel noninvasive approach of aligning nontoxic bio-derived CNCs in the presence of low magnetic fields. This leads to the introduction of anisotropic structural, thermal, electrical and optical properties in prepared nanocomposites. The oriented nanocomposites can have which potential application in the manufacturing of three-dimensional composites with

microstructural features comparable to biological materials for high-performance engineering applications.

Chapter 9, summarizes the conclusions of all the chapters and highlights the important technical and fundamental issues addressed in each chapter. The effect of various acid- derived CNCs and polymorphic CNCs on their inherent properties as well as on the properties of prepared nanocomposites are compared and discussed. The industrially viable approach of processing CNCs with reduced processing time in comparison to traditional approach, as presented in the thesis, is highlighted. The proposed melt-extrusion approach for biodegradable polymer-based CNC nanocomposite films, which overcomes the technical challenges associated with traditional extrusion of biopolymers, is emphasized. The multi-functional applications of CNCs, developed and demonstrated in the thesis, are also highlighted including biocatalysis for conversion of chemical moieties and degradation of dyes, targeted drug delivery, magnetic hyperthermia and fabrication of graphene nanoscrolls for hydrogen storage. Based on the general conclusions of the work presented in the thesis, some suggestions for prospective future research work are made for the development of CNC-based advanced functional materials for high performance applications.

Graphical Abstract of the PhD. Thesis



1.3 LITERATURE REVIEW

Cellulose is an abundantly available biopolymer usually extracted from plant-based renewable resources. Due to high chemical functionality, its backbone can be modified through various routes depending upon the target applications. After its discovery by Anselme Payen in the year 1838, cellulose has been widely extracted from natural biomass resources worldwide with an annual production of 7.5×10^{10} tons per year [57]. Cellulose is composed of both amorphous and crystalline domains in varying ratios depending on their source of origin. The presence of anisotropic cellulose micelles was first confirmed by Nageli in 1858 [58], which are crystalline in nature and were observed through X-ray diffraction in 1917 [59]. The crystalline domains can be extracted through controlled acid hydrolysis by selectively removing the amorphous segments and the product isolated is cellulose nanocrystals (CNCs) [1]. The morphology and dimensions of such crystalline domains vary with the type of biomass selected, cellulose polymorph, type of pretreatment and hydrolysis process followed for its extraction, which ultimately contributes towards the mechanical properties [8]. CNCs usually have rod-like morphology with tunable aspect ratio and high hydroxyl functionality which makes them an ideal reinforcing agent when dispersed in biodegradable polymers such as poly(lactic acid) (PLA) [17], polyhydroxybutyrate (PHB) [60], polycaprolactone (PCL) [61], polybutylene succinate (PBS) [62] etc. In addition to the aforementioned properties, CNCs are non-toxic, biocompatible, possess high surface area and self-ordering behaviour which leads to unique properties. Therefore, the next sections provide a brief overview of the fabrication methodologies of CNCs, evaluation of their inherent properties, strategic surface modifications, and novel processing routes for development of CNC-based nanocomposites for high performance applications.

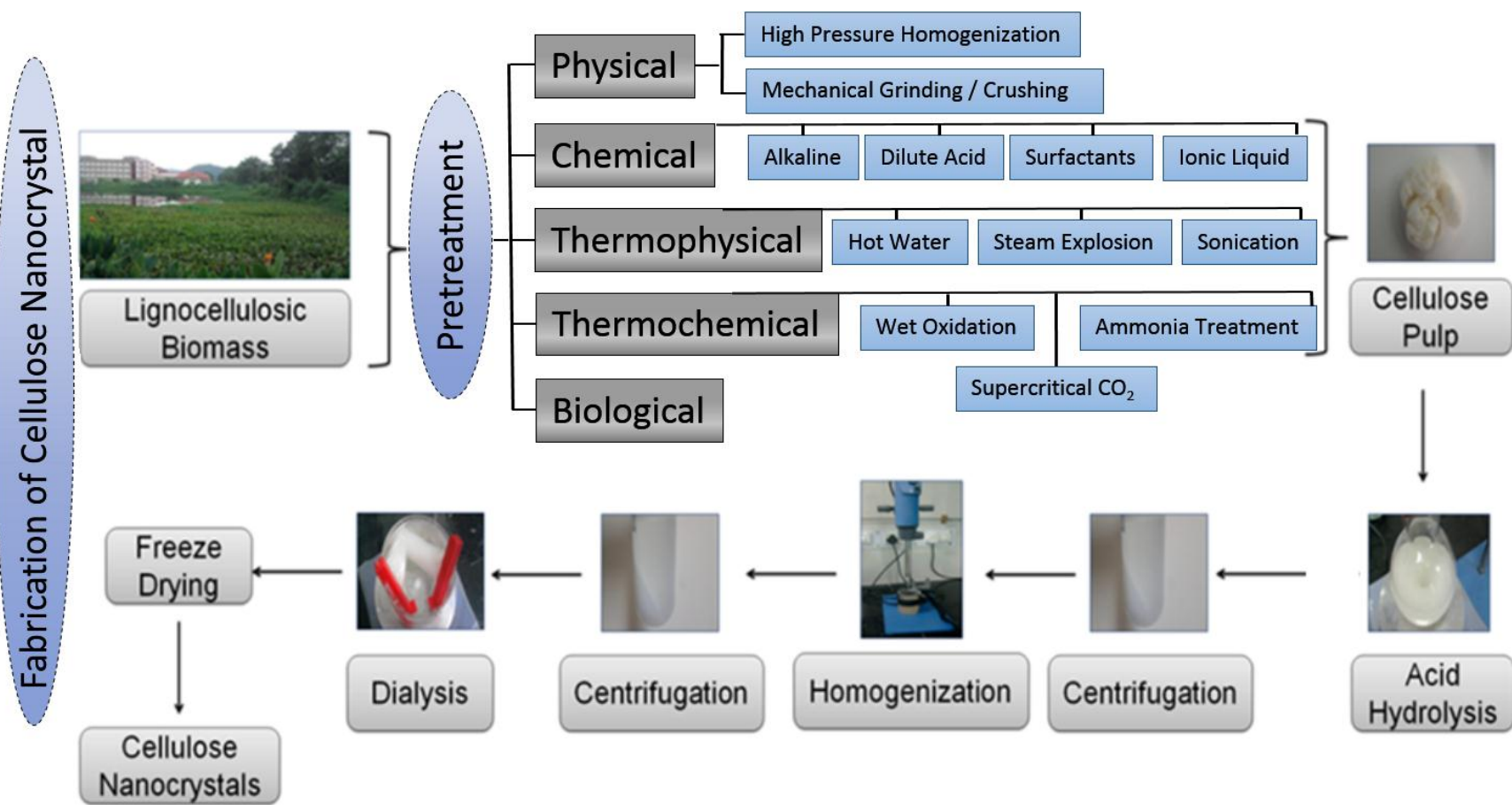


Figure 1.2: Schematic diagram for the production of cellulose nanocrystals through traditional acid hydrolysis approach with the cellulose pulp extracted through different pretreatments routes.

1.3.1 Cellulose Nanocrystals: Extraction and Fabrication Methodologies

CNCs are fabricated through stringent acid hydrolysis techniques, in which amorphous parts are selectively degraded, leaving behind only the crystalline section, which ranges in size from several hundred to thousands of nanometer in dimensions. CNCs fabricated from different biomass have different morphologies and yield. This is because of the different proportions of cellulose, hemicellulose and lignin content in biomass and interfacial binding between them, which makes it difficult to separate out pure cellulose during pretreatment process. There have been several pretreatment procedures reported in literature till date for extraction of cellulose such as mechanical crushing, chemical treatment, enzyme mediated hydrolysis, ultrasonication, high pressure homogenization, steam explosion etc. which have also been widely used at industrial scale. The pretreatment process yields relatively purified cellulose pulp with trace amounts of lignin and hemicellulose, due to which it requires multiple cycles or a combination of pretreatment procedures shown in Figure 1.2. The presence of trace amounts of these impurities significantly hinders the CNC fabrication process; therefore, appropriate pretreatment procedures should be selected depending on the biomass type. Figure 1.2 shows a detailed schematic diagram of CNC production through different pretreatments routes. Moreover, the different types of acids used for the fabrication of CNCs significantly alters the stability of the colloidal suspension and physical properties. Therefore, the fabrication of CNCs with different source of biomass utilizing various acids as hydrolyzing agents requires optimization of reaction conditions such as hydrolysis time, temperature, and cellulose pulp- to -acid ratio.

- **Effect of Bio-Feedstock on Cellulose Hydrolysis**

Production of CNC is a complex process which is influenced by several process parameters viz. acid concentration, biomass content, presence of lignin and hemicellulose,

biomass pretreatment process followed, temperature and reaction time. Hydrolysis of cellulose to CNCs and its yield significantly depends on the biomass type, impurities present as well as on the biological origin [20]. The degree of crystallinity of cellulose in the biomass/microorganism varies widely with species and natural location which is a critical parameter for the determination of the dimension and yield of nanocellulose. Cellulose, especially from algal and bamboo source, is highly crystalline in nature which hinders the penetration of acid to deep crystalline regions of cellulose leading to CNCs of higher aspect ratio [63]. Cellulose from cotton and wood, having lower crystallinity, yields much shorter dimensions of CNCs, with lower aspect ratio. Moreover, fabrication of CNCs from different biomass and waste products makes it a valuable nano-product with complete biodegradability and recyclability [64].

CNCs are generally produced at the laboratory scale using filter papers with sulfuric acid (64 wt. %) at room temperature with an yield of <50 %, making it a costly process [65]. Sulfuric acid has been used most frequently for CNC production as it gives high yield of CNCs with relatively improved physico-chemical properties, dispersion stability and distinct rod-like morphology in comparison to other hydrolyzing agents [66]. Several bio- feedstock sources for cellulose have been used till date for CNC synthesis ranging from microbial source, waste plant products, industrial wastes, bio-composts and other polysaccharides.

Brown et al. [67] reported the effect of biological origin of cellulose of green algae *Oocystis* by measuring the terminal complex (TC) cellulose synthase located at the cell membrane. It was observed that with change in origin there are significant differences in crystal geometries, due to variations in the arrangements of TC. Algal cell showed rectangular arrangement, whereas bacteria *Acetobacter* had linear array of ribbon like microfibrils [67]. Read et al. [64] proposed the rosette model based on microscopic

examinations which revealed that elementary crystalline cellulose units are composed of 36 chains, having 3–4 nm diameter, corresponding to the crystallite size found for wood microfibrils or particles from acid-hydrolyzed Avicel. The number of microfibrils laterally adhering to each other and the length of bound regions along the microfibrils may statistically vary in the cell wall, which after acid hydrolysis would result in a variety of particles with a wide distribution of width. A statistical study of cellulose hydrolysis was carried out taking several factors into consideration which showed that hydrolysis at harsh conditions leads to short crystallites with more charged sulfate half ester groups at the surface [20]. Moreover, they concluded that the energy of adhesion between two cellulose crystallites is proportional to the contact area of fibers; hence, less contact leads to easier separation of thinner and shorter particles into nanocrystals. Similarly, Candanedo et al. [68] characterized wood biomass into two types: softwood (length 3- 4 mm and width ~35 μm) and hardwood (length 0.5-1.5 mm and width ~20 μm) depending on the cell wall structure. They investigated the properties of the CNCs fabricated from softwood (black spruce) and a hardwood (eucalyptus) pulp taking into consideration the reaction time and acid-to-pulp ratio for hydrolysis. Atomic force microscopy (AFM) studies showed that microfibrils subjected to identical hydrolysis conditions produced nanocrystals of 5 nm for both wood species. The fabricated nanocrystals under different parametric conditions were identical, even when the initial microfibril microstructure was different, implying that the basic unit of wood cellulose organization is same for the two species [69].

Recent studies have focused on recycling the residue from industries or utilization of waste renewable resources or agricultural residues, which are available in bulk, as a primary source for CNC production to overcome the problems associated with their waste disposal. Oksman et al. [70] used bio-residue from wood bioethanol production plant as a raw

material for CNC production, as it contains almost 50% highly crystalline cellulose. The bioresidue was prehydrolyzed due to which CNC could be isolated by simple mechanical disintegration (high pressure homogenization or ultrasonication), leading to an economical process with yield of ~48%. Although the fabricated CNCs had higher thermal stability, the tendency for agglomeration was higher as well because of reduction in surface charge density. This was due to the increased inter- and intramolecular hydrogen bonding between CNCs which dominated the repulsive forces caused by lower sulfate groups. The study also led to the development of value-added products for bio-ethanol industrial process, where lignocellulosic residue is an excellent low cost source of raw material for CNC production. Teixeira et al. [71] used cassava bagasse, a by-product of the cassava starch industry, hydrolyzed under harsh acidic condition of 6.5 M sulfuric acid followed by ultrasonication for better dispersion. Colloidal suspension of nanocrystals thus obtained contained curved elongated particles (length ~360-1700 nm and diameter ~2-11 nm) where perfect rod-like nanoparticle morphology was absent [71]. The compost which consists of mostly agro-based biomass feedstock, was utilized for the fabrication of CNCs with different morphologies utilizing a two-step extraction process [72]. There have been various other sources of biomass reported in literature for the fabrication of CNCs such as pinecone, palm seed residues, mulberry [73], sugarcane bagasse [74], maize straw [75], sisal fibers [76], cotton linters [77], degraded fungi [78], corncob residues [79] etc.

Natural fibers derived from different plant products have crystalline structure, microfibril angle and cell dimension which differ drastically from one plant part to the other. Coconut fibers are agro-industrial byproducts, which are a large source of cellulosic wastes generated in bulk amount annually and remain an untapped potential resource. They have high toughness and are durable fibers compared to other natural fibers due to higher lignin content. A high lignin content also improves the dispersion of CNCs in hydrophobic

polymers due to the presence of aromatic carbons in lignin chains [80]. Prewashed coconut fibers are pretreated by the traditional alkaline treatment methods and further subjected to sulfuric acid hydrolysis with varying fiber-to-acid ratio (1:10) and time, under constant acid concentrations to obtain CNCs [81]. Fahma et al. [21] analyzed the effect of different pretreatment procedures on extraction of CNCs from coconut husks and found benzene-ethanol and sodium hypochlorite-potassium hydroxide extraction as best procedures to obtain cellulose with the highest crystallinity index, degree of polymerization and thermal stability. Increase in time and the number of runs for bleaching steps in pretreatment leads to a decrease in thermal stability of the synthesized CNCs after hydrolysis. It was found that trace residual lignin improves the thermal stability of CNCs as lignin decomposition starts from 200 °C and persists upto 700 °C; however, mechanical and other relevant properties might deteriorate considerably. Both studies showed that CNCs synthesized from coconut were ultrathin with diameters as low as ~5 nm, lengths ranging from 70 to 400 nm and an average aspect ratio of ~60 [81]. Kenaf (*Hibiscus cannabinus*), fibers from the Malvaceae family are single, straight, branchless stalks with inner woody core and fibrous outer bark. These fibers have a reasonably high cellulose content of 30–60% [82]. To obtain CNCs, the fibers were extensively prewashed and pretreated followed by hydrolysis with variation in hydrolysis time. Increased reaction times led to better disintegration and defibrillation of fibers but also led to decrease in thermal stability. This may be attributed to an increase in the amount of short fragments of cellulose chains, leading to larger number of chain ends which enhanced decomposition [83]. Thermal stability increased upon neutralization (pH~ 7) of sulfate groups with sodium hydroxide. The negatively charged sulfate groups present on CNCs act as active centers for cellulose chain degradation. CNC extraction from kenaf fibers was optimized at 65 wt.% sulfuric acid under a hydrolysis time of 40 min at 45°C [84]. Therefore, from the above discussions

it can be concluded that the morphological dimensions and physico-chemical characteristics of CNCs fabricated from the various biomass sources varies significantly depending on biological origin of cellulose.

- **Effect of Different Acids on Cellulose Hydrolysis**

Hydrolysis of cellulose using different acid leads to degradation of amorphous segments and yields highly crystalline rod-like colloidal suspension of nanometer dimensions. Stable suspensions of colloidal CNCs were first prepared by Ribi and Ranby in the year 1949 from wood and cotton cellulose [14]. Mukherjee et al. [85] studied the degradation of cellulose to CNCs through sulphuric acid treatment and captured the first electron microscopy images of CNCs which were approximately 50–60 nm long and 5–10 nm wide. Several types of acids have been used by researchers till date, ranging from strong acids to weak bio-based acids coupled with ultrasonication and homogenization to increase the activity of acids to degrade amorphous regions of cellulose. Sulfuric acid, the most commonly used acid for CNC extraction, provides highly stable aqueous suspensions, due to the esterification of surface hydroxyl groups to give charged sulfate groups, whereas hydrochloric acid leads to unstable CNC suspension, with minimal surface charge. One of the major drawback of sulfuric acid-based fabrication of CNCs is that sulphonyl functional groups catalyze the degradation of polymer at higher temperature [83]. Although hydrochloric acid-based CNCs lead to increased thermal stability of the polymer/CNC nanocomposite, the stability of dispersion is poor [86]. Hence, the application of the bio-based acids coupled with the mechanical disintegration processes for CNC extraction are being extensively studied extensively by researchers currently.

2, 2, 6, 6-tetramethyl-1-piperidinyloxy (TEMPO) has been extensively used as an oxidant for CNC fabrication. TEMPO acts as a free radical and selectively oxidizes the hydroxyl groups in cellulose chains. The oxidation process occurs at the surface of the

microfibrils only; hence, a combination of mechanical disintegration by ultrasonication and high pressure homogenization is required for high yield of CNCs (over 80%). However, till date the method has been seldomly used for the hydrolysis of non-wood fibers with high crystallinity and degree of polymerization (DP). TEMPO coupled with NaBr/NaClO oxidation carried out at pH~ 10 and room temperature leads to significant amount of sodium carboxylate group functionalization and small amount of aldehyde group formation at C6 primary hydroxyl groups of cellulose. Trace amounts of aldehyde can be further converted to carboxyl groups by overnight treatment with NaClO₂ [87]. Analysis of DP values with the Mark Houwink Sakurada (MHS) equation showed a decrease from 1200 to approximately 600 with increase in reaction time. Replacing the oxidation system from NaBr/NaClO to TEMPO/NaClO/NaClO₂ resulted in lower DP, with no aldehyde byproducts and lower carboxylate group content [88]. This is because in this process the NaClO oxidizes TEMPO to N-oxoammonium ion which converts the C6 primary hydroxyl groups of cellulose to aldehyde under acidic or neutral conditions forming hydroxylamine. The NaClO oxidizes hydroxylamine to N-oxoammonium ion which further converts aldehyde to carboxyl group. Thus the depolymerization process of cellulose chains due to the β -elimination is avoided. The NaBr / NaClO system had a maximum carboxylate content of 1.7 mmol/g- pulp whereas latter system had 0.8 mmol/g-pulp [65]. Here, sodium chlorite acts as a primary oxidant, which immediately oxidizes aldehyde groups, formed as intermediate structures, to carboxyl groups under weakly acidic or neutral conditions. Similar trends were observed with biomass having higher percentage of cellulose crystal structure such as cotton linters, bleached kraft and sulfite pulps, bacterial cellulose, ramie fibers, bamboo pulps and other fiber based bio resources. ¹³C solid state NMR study supports the finding that TEMPO based oxidants attack the glucosyl units in cellulose chains at C6 ends to convert them to anionic sodium carboxylate groups [87]. The

functionalized microfibrils are further converted to nanofibers by mechanical disintegration processes through application of ultrasonication and homogenization for short periods [87]. The whitish cellulose slurry obtained after the disintegration process is converted to transparent dispersion due to formation of nanofibers of cellulose. A recent study showed that transparency increased with increase in carboxylate content [89]. As expected, lower width of microfibrils leads to higher carboxylate content [90]. When treatment was carried out using different biomass, nanofiber widths were almost constant whereas length distribution varied significantly. Such variation in length of CNCs could be attributed to variations in oxidation and disintegration process conditions. The TEMPO-oxidized CNC, when dispersed in water, had a highly stable negative potential of -80 mV due to anionic charged carboxyl groups, without formation of bundles and aggregates [65]. This may be due to strong electrostatic repulsion between the negatively charged CNC rods. Moreover, zeta potential, which is related to the surface density of carboxylate groups on the CNC surface, showed very little variation on changing the biomass source [91]. However several properties such as transparency, viscosity and zeta-potential varied with the type of mechanical disintegration process such as magnetic stirring, homogenization and ultrasonic homogenization.

Recently, Espinosa et al. [92] fabricated CNCs by hydrolysis of cotton using phosphoric acid (85%), which was carefully added to cellulose slurry drop wise up to a predetermined concentration and further heated to 50 °C/100 °C. The maximum yield of CNC from phosphoric acid, determined to be ~80%, which was found to be strongly depended on the reaction temperature and acid concentration. The fabricated CNCs had a width of ~30 nm and a length of ~300 nm, obtained under optimized condition of 10.7 M phosphoric acid and 90 min reaction time at 100 °C. Cellulose phosphorylation has also found potential application in biomedical technologies such as bone tissue engineering. For example,

Ercain et al. [93] phosphorylated microcrystalline cellulose with *ortho*-phosphoric acid in presence of phosphorus pentoxide to regenerate cellulose hydrogels for biocompatibility and histological studies. Phosphorylated cellulose alongwith hydroxyapatite has also been used as an alternative to collagen fibers for biomimetic growth [94].

Ammonium persulfate (APS), an inexpensive oxidizing agent with high water solubility and long term low stability, has also been used for the fabrication of CNCs. APS based CNC fabrication is a sustainable process that is less hazardous than sulfuric acids and easy to scale up. Moreover, it has the capability of removing lignin, hemicellulose, pectin and other plant contents *in situ*, making CNC production a single step process. However, the yield is lower compared to acid-based systems and it requires a high purity of initial cellulose for better CNC yield. Leung et al. [95] fabricated CNCs from APS by heating the cellulose slurries at 60 °C with varied reaction times (in the range of 3–16 hours) depending on the complexity of the cellulosic biomass. The fabricated CNCs had average width of 3–7 nm with an yield of 28–36% from the complex flax and hemp fibers compared to the 81% yield from Whatmann filter paper which is pure cellulose source [95].

Hydrobromic acid coupled with ultrasonication was also used for fabrication of CNCs [96]. The optimized conditions for the CNC production, are found to be 3h of reaction time at 80 °C under 2.5 M HBr acid concentration, which resulted in a yield of ~80% with Whatman filter paper as cellulose source. HBr-modified CNCs showed complete absence of bromine in elemental analysis which makes it potentially applicable for site specific grafting reactions [96]. As HBr is a stronger acid than HCl or H₂SO₄, it offers appreciable savings when large scale production of CNC is considered. Figure 1.3, shows the plausible mechanism of cellulose hydrolysis with different acids in detail.

The use of harsh strong acids for CNC production makes the overall process environmentally harmful, causing significant pollution. Moreover the cost of CNC

production is increased due to the requirement of corrosion resistant equipment. Therefore, use of bio-based acids or other cation exchange resins have been explored for CNC hydrolysis to make the process less hazardous. Cation exchange resins are negatively charged solid catalysts which are easy and safe to handle, and can be readily recycled after reaction. In acidic medium the NKC-9 resin (styrene-divinyl benzene co-polymer) has an exchange capacity of 4.7 mmol/g $[H^+]$ [97]. CNCs fabricated using NKC-9 were 10-40 nm in diameter and 100-400 nm in length with yield ~50%. The main advantage of this process is that it doesn't require large amounts of water to stop the reaction; instead, direct removal of resin beads will stop the reaction [97].

Although bio-based acids have weak penetration power, when coupled with ultrasonication they can impregnate and break the glycosidic linkages of cellulose chains. Cellulosic slurry was esterified with acetic acid in presence of sulfuric acid which converted the hydroxyl groups to acetic anhydride[98]. The esterification was followed by high energy ultrasonication with CNC yield ~86% for a reaction time of 5 h at 70 °C [98]. Ultrasonication effectively disintegrates amorphous chains of cellulose making diffusion of weak acid molecules favorable [98]. Similarly, weak acids such as formic acid and tungstophosphoric acid coupled with oxidation and ultrasonication have been shown to be effective in the production of CNC [99]. Some new methodologies for CNCs fabrication have been recently reported through use of enzymes [100], ionic liquids [100] and sub- critical water-based hydrolysis [101] which provides green and sustainable routes for CNC fabrication, and are summarized in Table 1.1. Thus, significant variation in physico- chemical, yield and morphological properties of CNCs is observed when the hydrolyzing medium is varied by using different acids, enzymes or ionic liquids, which requires a stringent optimization of reaction conditions.

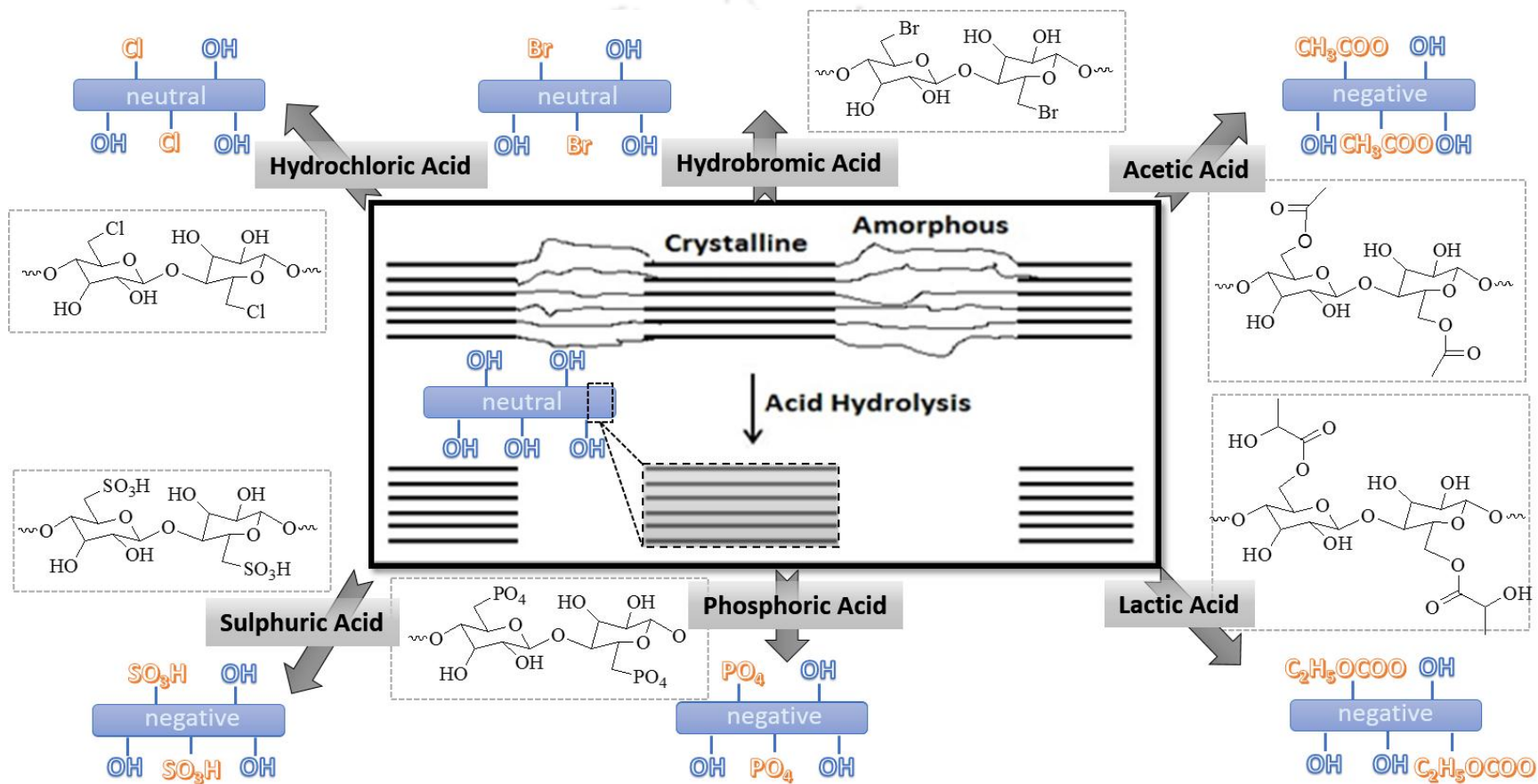


Figure 1.3: Fabrication of CNCs through hydrolysis by different acid sources with the substitution of its surface by various anionic moieties and relatively altered surface charges.

1.3.2 Production of Cellulose Nanocrystals: Scale-Up Strategies and Life Cycle Assessment

Nanocellulose has found wide applications in different areas starting from drug delivery in pharmaceutical industries to packaging industries. Hence, large-scale production of CNCs will become important to address the required product demand in the future. However, large-scale processing of CNCs has only recently started and was first carried out at CelluForce in Canada. Other manufacturers of CNCs are FPInnovation, Alberta Innovated Technology Futures, Biovision Technology, Blue Goose Biorefineries commissioned in Canada, US Forest Service Forest Products laboratory and American Process Inc. in USA, VTT Technical Research Centre in Finland and Melodea in Israel. The existing technologies for CNC production, which has been widely carried out with sulfuric acid-based hydrolysis process, is difficult to scale up due to its long processing time and harsh reaction conditions. A single batch of CNC production (with the lignocellulosic biomass as precursor) requires almost 7–10 days which includes the pretreatment step to extract the purified cellulose, followed by hydrolysis, centrifugation and dialysis to remove excess acids and finally freeze-drying to obtain the dried powdered form of CNCs. Moreover, the sulfuric acid based-hydrolysis requires acid resistant reactors and pipelines, which adds extra cost to the process. The huge amount of water required to stop the reaction during dilution process and proper disposal of acid, makes the bio-nanoparticle fabrication process environmentally unfriendly. Therefore, alternative technologies for CNC production with reduced processing time should be developed, to make the process a sustainable, environmental-friendly and industrially scalable approach. Further, the type of biomass and pretreatment procedures followed for cellulose extraction, significantly affects the reaction conditions for CNC fabrication as well as their inherent characteristics. Therefore, following proper pretreatment procedures for biomass is an

extremely difficult process, which needs to be optimized at regular intervals to achieve maximum yield. As discussed in previous section 1.3.1, alteration of the acid used for hydrolysis also have significant effect on the yield and morphology of the fabricated CNCs.

Table 1.2, shows a comparison of the energy consumption, technological drawbacks in scale-up, associated benefits, and potential applications of the CNCs fabricated through different methodologies and acids as hydrolyzing agents. A recent study by Reid et al. [2], provides a comprehensive summary of the variation of the inherent properties of CNCs produced in lab scale with industrial scale processes produced by different manufacturers through traditional and non-traditional hydrolysis approaches. It was observed that CNCs obtained from different manufactures have high degree of crystallinity and similar aspect ratio, but the surface charge, morphological dimensions, thermal and colloidal stability show variation. CNCs fabricated through alternative routes show significant variation in properties especially polydisperse morphology and dimensions alongwith the presence of microscopic/macrosopic impurities. Therefore, to obtain industrial scale quality and assurance certification in nanocellulosic properties, CNCs produced from the batch- to- batch process must always be characterized with further optimization in reaction conditions when the initial biomass feed stock and acid hydrolysis process is changed.

Life cycle assessment (LCA) is a tool to predict the environmental sustainability of a nanomaterial determining its impact to ecology, potential risk assessment to health and safety hazards during its product cycle. The life cycle of nanocellulose studied by Li et al. [107], is based on cradle-to-gate LCA approach which is compared and quantified for four chemo-mechanical processes, TEMPO-oxidation (first process) followed by sonication or homogenization (second process) and chloroacetic etherification (third process) followed by sonication or homogenization (fourth process).

Table 1.1: Comparison of yield and morphology of CNCs fabricated with different acids.

Acid	Optimized parameters	Morphology	Dimensions	Yield	Ref.
Sulphuric Acid	64% sulphuric acid at room temperature	Circular and rod shape	Length - 50–60 nm Width- 5–10 nm	<50%	[86]
Hydrochloric Acid	Hydro-thermal treatment	Rod Shape and Elliptical	Length - 250–300 nm Width- ~15 nm	~ 93%	[102]
2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO)	TEMPO coupled NaBr/NaClO at pH~10 at room temperature	Rod Shape	Length - 100–200 nm Width- 3–4 nm	~80%	[91]
Hydrobromic Acid	2.5 M Hydrobromic acid reaction temp 80-100°C & reaction time 3 hours	Rod Shape	Length - 100–200 nm Width- 7–8 nm	~80%	[96]
Phosphoric Acid	85% phosphoric acid reaction time 90 min at 100°C	Rod Shape	Length - ~300 nm Width- ~30 nm	~80%	[92]
Ammonium persulfate	Reaction time 3-16 hours and reaction temperature 60°C	Rod Shape	Length - ~300 nm Width- ~3–7nm	~28-36% (fibers) ~81% (filter paper)	[95]
Acetic Acid	Reaction temperature 70°C and reaction time 5 hours coupled with ultrasonication	Rod Shape	Length - ~200– 300 nm Width- ~10–100 nm	~86%	[98]
Cationic exchange resins	Capacity 4.7 mmol/g [H ⁺]	Rod Shape	Length - ~100–400 nm Width- ~10–40 nm	~50%	[97]
TEMPO (hydrolysis-cum-surface modification)	TEMPO based oxidation followed by surface initiated atom transfer radical polymerization	Fibrillar Shape	Width ~ 13–20 nm	~84%	[103]
Ionic Liquid	Hydrolysis for 2hours at room temperature with 1-ethyl-3-methylimidazolium acetate [EMIM][OAc]	Rod Shape	Length - ~75–125 nm Width- ~2–5 nm	~44%	[100]
Mixed Acid (Sulphuric and Hydrochloric acid)	3:1 acid ratio (at concentration of 55%) followed by ultrasonication	Axialitic Shape	Hydrodynamic radius ~89–205 nm	~68%	[104]
Tungstophosphoric Acid	8% Tungstophosphoric acid, reaction time ~30h at temperature 90 °C	Rod Shape	Length - >100 nm Width- ~15–35 nm	~85%	[99]
Formic Acid	98% Formic acid, Reaction time 6h and temperature 95°C	Fiber Shape	Length – 50-300 nm Width- ~2–4 nm	~70%	[105]
Enzymatic Hydrolysis	Endoglucanase (700 EGU/g), reaction temperature 50°C and time 60 min	Fibrous Shape	Length – 100-1800 nm Width- ~30–80 nm	~29% conventional ~38.2% microwave	[106]
Subcritical Water	Reaction temperature 120°C, pressure 20.3 MPa and time 60 min	Rod Shape	Length ~242±98 nm Width- ~ 55±20 nm	~22%	[101]

Based on Eco-indicator 99 impact assessment methodology with the Kraft pulp as lignocellulosic biomass, it was observed that TEMPO oxidation followed by homogenization is the best method with lower energy consumption and global warming potential compared to chloro-etherification [107]. Introduction of lignocellulosic fibers extracted from Kraft pulp have been shown to improve the physical, structural properties and durability of the recycled paper, thereby, providing a circular economy to paper industries [108]. Similarly, in another study Arvidsson et al. [109], assessed the environmental impact of CNFs production through three different routes namely (i) enzymatic hydrolysis, (ii) carboxymethylation, and (iii) direct homogenization without any chemical treatments. From the sensitivity analysis and cumulative energy demand (CED)/global warming potential (GWP) values it was found that chemical-based carboxymethylation route has severe environmental impact with the enzymatic process being more favorable. The effect of biomass on the behaviour of LCA cycles shows that precursors with higher cellulosic content (studied with waste carrot pomace [110] and coconut fiber [111] as cellulosic precursor) generally have higher CNC yield with lower polysaccharide byproducts and environmental impact. Due to their strong reinforcing capability, nanocelluloses are widely used in the development of composites; however, their sustainability, ecological impact and LCA are seldom studied. A recent study by Hervy et al. [112] compared the bacterial cellulose and cellulose nanofiber-based PLA composites with PLA and glass fiber-reinforced polypropylene composites in terms of abiotic depletion of fossil fuels (ADF) and GWP. It was observed that though the nanocellulose-based PLA composite have improved structural properties and was completely derived from bio-based materials, the measured environmental impact of bacterial cellulose was higher in comparison to cellulose nanofibers and PLA. An interesting conclusion drawn from the LCA study was that regarding the sustainability of

PLA/nanocellulosic composites will only be higher than PLA or other petroleum derived composites only when the nanocellulose content in it is higher than 60 vol. %. CED is an ideal tool to predict and compare the degree of sustainability and environmental impact of different nanomaterials reported in literature.

In a lab scale process, CNFs produced through TEMPO oxidation-cum-homogenization have a CED value of 1000 MJ/kg which is significantly lower than the chemical-based extraction process [107]. However, the carbon-based nanomaterials with similar reinforcing capability as nanocellulose have a CED value of 1000 to 1 million MJ/kg for carbon nanotube [113] and 72–1100 MJ/kg for graphene [114] respectively. It is observed that although nanocellulose and its derivatives are ideally eco-friendly in nature, more novel and greener routes for fabrication of nanocomposites need to be developed. Thus, it can be concluded that nanocellulosic materials are potentially much more environment- friendly and sustainable in nature and have improved physicochemical and structural properties in comparison to several other organic/inorganic nanomaterials reported in literature.

Table 1.2: Comparison of scale up strategies of cellulose nanocrystals with different methodologies and acid systems.

Acid	Sulphuric Acid	Hydrochloric Acid	TEMPO	High Pressure	Phosphoric Acid	Hydrobromic Acid	Acetic Acid	Ammonium persulfate
Methodology	Stringent hydrolysis at room temperature	Stringent hydrolysis at room temperature	TEMPO-mediated oxidation at room temperature	Repeated high pressure homogenization	Stringent hydrolysis at 100 °C for 90 min	Stringent hydrolysis at 80-100 °C for 3 hours	Ultrasonication assisted reaction time 5 hours	Directly using biomass reaction time 3-16 hours
Energy Consumption	<7 MJ/kg	~7-10 MJ/kg	<7 MJ/kg	700-1400 MJ/kg	~10-30 MJ/kg	~10-20 MJ/kg	700-1000MJ/kg	---
Application	Polymer nanocomposites	High Thermal stable CNC films	High gas barrier films, health care materials	Filter aid nano fiber	Better dispersed CNC films with good stability	Surface modification through click chemistry	Bio-material, Drug delivery	CNC from mixed biomass as starting material
Stability (in water)	+	-	+	-	+	-	+	+
Advantage	Stable and uniformly dispersed in wide range of solvents	Thermally stable CNCs at higher temperature.	No swelling and change in morphology of fibers, carboxyl end groups	Process requires no acid treatment less health hazardous	Highly thermal stable and uniformly dispersed in solvents.	Surface grafting & chemical modifications	Bio-based acid, favors fabricated CNCs for biomedical application	Requires no Biomass pretreatment.
Disadvantage	Presence of sulphonyl groups degrades polymer nanocomposites	Difficult to disperse in solvents	Partial dispersion of biomass difficult CNC synthesis	High electricity consumption, non-uniform CNC size distribution	Slow CNC fabrication process, requires concentrated acid and high temperature	Low stability	Requires long reaction time.	Extremely low yield and very high reaction time
Scale Up Problems & benefits	Health hazardous, Environmentally unsafe process, requires acid resistant process equipments which increases cost. High end applications of fabricated CNCs	Limited application restricts large scale production of CNCs through these process.	CNC production from inexpensive wood pulps. Radical based reaction so difficult to scale-up.	Costly production due to high electricity consumption, efficiency of homogenization decreases at large scale.	Fabricated CNC suitable for polymer nanocomposites, Time consuming compared to other process. Costly process.	HBr highly corrosive in nature. Health hazardous process.	Ultrasonication coupled process difficult to scale-up, energy intensification process.	Suitable for CNC fabrication from mixed biomass resources, low yield and long reaction time come as hurdle for scale-up.

1.3.3 Cellulose Nanocrystals: Surface Modification Strategies

The surface behaviour and interfacial interactions in CNCs significantly depends upon the abundant hydroxyl groups present on it and type of functional groups introduced during the hydrolysis process. CNCs are generally hydrophilic in nature with negatively charged surface, which imparts high ionic strength and electrostatic character, due to which they tends to agglomerate in hydrophobic media. However, in aqueous media or hydrophilic polymer solutions, CNCs undergo improved dispersion due to the formation of strong inter/intra-molecular hydrogen bonding. Dispersion of CNCs into polymer has been a challenging problem because the strong intermolecular hydrogen bonding between cellulosic chains hinders the interaction of CNCs with organic solvents or non-polar hydrophobic polymers. Therefore, surface modification of CNCs is usually carried out to increase the compatibilization of polymer matrix and nanocrystals. Surface modification is accomplished by altering the highly accessible polar hydroxyl groups present on the cellulose chains. This leads to reduction in surface energy of CNCs which thereby enhances the degree of interaction and dispersion in non-polar polymers or solvents. The surface properties of CNCs can be tuned through introduction of negative or positively charged ionic moieties during the hydrolysis step, counter ion polyelectrolyte solutions or chemical modification with functional groups. There have been several surface modification reactions carried out on CNCs till date depending on the desired application. They can be classified as physical modifications based on non-covalent interaction of surfactants or macromolecules and chemical modifications based on covalent coupling of CNCs with monomers, polymers and functional moieties (as shown in Figure 1.4). Some of the important chemical-based surface modification reactions include sulfonation, silylation, grafting with isocyanate, acid anhydride, acid chloride and surfactants [1]. The surface modification process of CNCs (through above mentioned approaches) should be

judiciously selected in such a way that the inherent characteristics such as the rod-like morphology, degree of crystallinity and cellulose polymorphism are preserved in the modified species.

- **Physical Modifications of Cellulose Nanocrystals: Non-covalent Coupling**

The physical absorption of chemical moieties such as surfactants or macromolecules on the negatively charged CNCs leads to improved dispersion in organic solvents. However, such CNCs form agglomerates when dispersed in the polymeric matrix due to the absence of covalent interactions. Surfactants are amphiphilic molecules which on simple mixing with CNCs leads to adsorption of the hydrophilic head of surfactants on the CNC surface, while the hydrophobic tail protrudes out. Formation of such micelles-like structure on CNC leads to reduced surface tensions with improved compability with organic media or hydrophobic polymeric solutions. However, the colloidal stability of the surfactant modified CNC dispersion depends upon the concentration of CNCs or surfactants, ionic strength, pH and temperature of the suspensions.

Different types of surfactants such as cationic, anionic and nonionic forms have been absorbed onto CNCs surface through electrostatic interactions. The CNCs absorbed with cationic surfactant Dioctadecyldimethylammonium bromide (DODA-Br) helps in development of monolayer CNC films through Langmuir–Schaeffer technique, required in coating technologies [115]. The processed films are highly stable with improved smoothness, the packing density of which can be tuned through relative concentrations of surfactants. Introduction of non-ionic surfactant such as sorbitan monostearate into CNC suspension (0.3-0.6 wt. % in tetrahydrofuran), have found to improve the dispersion stability in hydrophobic solvents as confirmed through turbidity tests [116]. At lower concentrations of surfactants, the processed nanocomposite films shows improved dispersion and thermo-mechanical properties, which deteriorates at higher loadings due to

self-aggregation of surfactants. Utilization of sorbitan monostearate also leads to formation of beadless nanofibers of polystyrene through the electrospinning approach [117]. Moreover, introduction of the small surfactant molecule such as tert-butanol into CNCs, shows improved structural stability of PLA nanocomposites alongwith the presence of liquid crystalline behaviour [118]. A recent study by Rehman et al. [119] shows that introduction of CNCs modified with anionic surfactant, sodium dodecylsulphate (SDS) and cationic surfactant hexadecyltrimethyl ammonium bromide (CTAB) leads to formation of bionanocomposite films with improved transparency and thermo-mechanical properties. An interesting study by Cranston et al. [120] shows that modification of CNCs with cationic CTAB and didecyltrimethylammonium bromide (DMAB), leads to formation of Pickering emulsion which can be PHase inverted. In another study, the commercially available anionic surfactant Beycostat NA (BNA) shows the formation of 15 Å thick coating layer adsorbed onto surface of CNCs which leads to perseverance of chiral nematic ordering in organic solvents such as cyclohexane [121]. BNA adsorbed CNCs, have improved dispersion in polymeric matrix such as PLA and PP with the presence of nucleation as well as reinforcing behaviour of CNCs [122].

The physical adsorption of the macromolecules such as polymers onto the surface of CNCs are significantly enhanced due to the capability of CNCs to form intra/inter- molecular hydrogen bonding which subsequently leads to improved dispersion in hydrophobic polymer matrix. Adsorption of polyethylene oxide (PEO) onto CNCs surface shielded the sulfate ester groups and hydroxyl groups present on its surface, thereby, reducing the thermal degradation during melt-extrusion with low density polyethylene (LLDPE) [123]. Adsorption of poly(ethylene glycol)-co-polyethylene (PEG- co-PE) leads to incorporation of CNCs at higher loadings ~23 wt.% into LLDPE matrix which undergoes homogenous dispersion with improved tensile modulus [124]. In

both the cases mentioned above, it was found that the polymeric chains adsorbed onto CNCs, have strong affinity between the hydroxyl groups of CNCs and PEO/PEG chains the degree of hydrogen bonding depends upon the molecular weight of adsorbed polymers [125]. Similarly, introduction of Plouronic family of triblock copolymers such as PEO– PPO–PEO adsorbed onto CNC surface (at 1:1 ratio) leads to limited thermal and shear induced degradation during melt extrusion alongwith enhancement in mechanical and thermal properties[126]. Recently Sakakibara et al. [127] adsorbed poly(lauryl methacrylate) (PLMA) onto CNCs which resulted in reduced interfacial surface energy, improving the dispersion of CNCs into high-density polyethylene (HDPE) and mechanical behaviour (higher Young's modulus by 140% and tensile strength by 84%). In another study, physical interaction of the CNC-poly(acrylamide) leads to formation of tough and highly stretchable hydrogels due to presence of reversible adsorption-desorption phenomenon of hydrogen bonded network which provides easy recovering of gels under periodic stress-strain conditions [128]. Interaction of the oligosaccharides with CNCs such as xyloglucan leads to formation of amphiphilic triblock copolymers which shows two type of adsorption kinetic behaviour depending upon the concentration of xyloglucan [129, 130]. Such studies provide an opportunity to design novel biomimetic glycopolymers which have potential applications as blood anticoagulants, in interaction with cells for targeted drug delivery etc.

- ***Chemical Modifications of Cellulose Nanocrystals: Covalent coupling***

The modification of CNCs through the formation of covalent bonds during the chemical conjugation process can be classified into two types depending on the type of substituted moieties (as shown in Figure 1.4). Surface modification of CNCs through introduction of small hydrophobic molecules which leads to incorporation of functional behaviour is termed as chemical derivatization/ functionalization. This process is effectively used to

introduce advanced functional behaviour into CNCs through click chemistry techniques such as tagging with fluorescent molecules, antibacterial/photo-bactericidal effect, light-responsive behaviour, bio-imaging etc. The second chemical modification approach is termed as polymer grafting, through which the hydrophobic polymeric fragments are covalently grafted on the CNC surface in order to improve the dispersion of hydrophilic CNCs in the hydrophobic polymers. Both chemical modification routes should be strategically carried out in such a way that the integrity of the crystalline nature of CNCs is preserved without destroying the substrate morphology and physicochemical properties.

Chemical Derivatization of Cellulose Nanocrystals

Surface silanization of CNCs is carried out with various alkyl dimethylchlorosilanes (alkyl moieties ranging from 3 to 12 carbons) through partial silylation process with a degree of substitution of 0.6 [131]. Functionalization with trialkoxysilanes ($R'Si(OR)_3$) leads to tunable dispersion of CNCs in various polymers by changing the organic functionality [132] (which can be alkyl, amine, vinyl, methacrylic or long hydrocarbon chains). The one step surface modification of CNCs with methacryloxypropyl triethoxysilane leads to a stabilized dispersion of CNCs in poly(styrene-co-2-ethyl hexylacrylate) copolymer during miniemulsion polymerization [133]. Similarly, surface modified CNCs, when melt blended with PLA using twin screw micro compounder, showed better dispersion and compatibility with the polymer matrix than the non-functionalized CNCs [134]. Improved dispersion of CNCs leads to enhancement in the barrier and thermo-mechanical properties of PLA, due to the presence of reinforcing effect from CNCs [135].

Acetylation of CNC surface improves the dispersion of CNCs in organic solvents and increases adhesion of CNC with synthetic polymer matrixes. Acetylation can be carried out with alkenyl succinic anhydride (ASA), which is widely used as sizing agent in paper

making. Nair et al. Studied the acylation of hydroxyl functional groups in chitin using ASA and other reagents [136]. CNC acylation using ASA was studied by Yuan et al. [137]. In this study, cellulose nanowhiskers slurry is mixed with ASA, freeze dried and further heated to 105°C, which introduces a degree of substitution of ~0.022. The acylated CNCs exhibited good dispersion in low polarity solvents such as 1, 4-dioxane, and also in polymers such as polystyrene [137]. Acylation has also been carried out through Fischer esterification reaction in which a mixture of organic acids (such as acetic acid and butyric acid) is used for hydrolysis. In this process a combination of 17.5 M acetic and 10.9 M butyric acid, alongwith a trace amount of hydrochloric acid, was optimized for CNC production resulting in nanoparticles of 10-20 nm diameter and 200 nm length [134]. The butyrate and acetylated CNCs show better dispersibility in non-polar toluene compared to chlorinated and sulphonated CNCs. Surface modification of CNCs with esterification process leads to improved dispersion in organic solvents and prevents the aggregation behavior of CNC due to hydrogen bonding. Introduction of these functionalities can lead to the synthesis of more hydrophobic CNCs by incorporating complex multiple chemical reactions [137].

Along with CNC fabrication as discussed in section 1.3.1, oxidation of CNCs is generally carried out through TEMPO-mediated reactions, which can be tuned in three different ways. Depending on the concentrations of TEMPO, it can be effectively used to tune the surface behaviour of CNCs or for conversion of hydroxyl groups into carboxyl groups. However, the degree of substitution is influenced by reaction conditions. In the third process, coupling of the TEMPO-oxidation with high pressure homogenization leads to the preparation of CNFs or CNCs. The other routes of oxidation of CNCs reported in literature are through utilization of strong oxidizing agents such as ammonium persulfate (APS) [138], sodium periodates [139] and ethanol solvothermal treatment in presence of

hydrogen peroxide [140] through which the carboxyl group functionalities are incorporated on CNCs surface.

Carboamination of CNCs is generally carried out through coupling of isocyno groups to the hydroxyl groups on the surface of CNCs in presence of different isocyanates and their derivatives. This approach comparatively has higher reaction rate, with the absence of secondary products as confirmed through high degree of substitution ($DS \sim 0.07$) of long n-octadecyl isocyanate chains [141]. The general route for amidation of CNCs is usually through reaction with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) followed by N-hydroxysuccinimide (NHS) which leads to high $DS \sim 0.12$ [142]. Application of diisopropylcarbodiimide (DIC) with NHS as coupling agents leads to amidation of CNCs in organic solvents with similar DS values [143]. Modification of CNCs with grafting of Jeffamines as studied by Azzam et al. [144] shows DS values as high as ~ 0.2 . The carbodiimide-activation coupling of CNCs provides an efficient route to conjugate lysozyme or proteins at higher concentration which shows improved activity (1500U/mg of substrate) [145]. In a recent study by Lavoine et al. [146] showed that the alkyl ammonium carboxylate ionic bonds in CNCs can be converted to amides through a simple and controlled heat treatment. It was found that the degree of amidation depends on molecular weight and molar ratio of ammonium-to-carboxylate ions, which interestingly improved the thermal degradation temperature of modified CNCs by 90°C [146].

The surface cationization is a process in which the anionic charges on CNCs are converted to cationic charges, which leads to change in surface polarity. The nucleophilic addition of 2,3-epoxypropyltrimethylammonium chloride to CNCs leads to reversal of zeta potential values from -30 to +30mV [147], with drop in surface charge density. The other routes of cationization includes adsorption of quaternary ammonium salts on carboxylated CNCs with different alkyl chain lengths which leads to tunable surface

hydrophobicity [148]. Several approaches such as electrostatic adsorption of glycidyltrimethylammonium chloride or chemical grafting of cationic poly(ethylene imine) (PEI) have shown to alter the surface behaviour of CNCs, which results in improved dispersion in organic solvents [149]. Therefore, the surface of CNCs can be efficiently modified with small functional moieties through the above-mentioned chemical derivatization techniques, depending on targeted applications.

Polymer grafted Cellulose Nanocrystals

The grafting of polymer chains on the surface of CNC rods is broadly classified into two categories, namely “grafting onto” and “grafting from”. “Grafting onto” approach refers to the functionalization of the hydroxyl end groups on cellulose surface with polymer chains in the presence of a coupling agent. Several polymers have been ‘grafted onto’ the CNC surface using this approach resulting in better dispersion and compatibility with the polymer matrix. Habibi et al. [150] grafted CNC surface with poly (ϵ -caprolactone) (PCL) using an isocyanate-mediated coupling agent. Nanocomposite films fabricated using PCL- grafted nanoparticles displayed high modulus and good ductility reflecting the reinforcement of polymer matrix by CNCs. Cao et al. [151] used a similar approach to graft waterborne polyurethane chains on CNC surface. Mangalam et al. [152] used TEMPO oxidized CNCs with -COOH functional groups to graft single stranded DNAs in the presence of carbodimide derivatives. Ljungberg et al. [153] grafted maleate functionalized atactic polypropylene of low molecular weight on CNCs extracted from tunicates. Films casted using grafted CNCs showed good compatibility with the polymer matrix leading to higher transparency and tensile modulus. Amine functionalized polyethylene glycol (PEG) was grafted on TEMPO oxidized CNC through carbodimide chemistry by Araki et al. [142] to obtain CNC suspensions of high stability. Surface modified CNCs have remarkable properties such as high colloidal stability, better

dispersion in polar and organic solvents and thermo-reversible aggregation [8]. Chemical grafting of CNCs with poly(ethyl ethylene phosphate) (PEEP) through click chemistry leads to incorporation of negative surface charge which has the capability to immobilize doxorubicin and release it at targeted tumor cell sites. The grafting of CNCs with amine-terminated Jeffamine polymers through peptide-coupling reaction leads to introduction of thermo-responsive behaviour [144]. The prepared Jeffamine-grafted CNCs shows temperature-controlled aggregations which finds potential applications in the development of stimuli-responsive polymers with tunable structural and physical properties.

In the “grafting from” approach, either the monomer molecules are grafted on the CNC surface (and further polymerized in the presence of a catalyst) or *in situ* surface initiated atom transfer radical polymerization is carried out. This process leads to nanocomposites having high molecular weight polymers along with well dispersed nanoparticles in the polymer matrix. PCL was grafted through ring opening polymerization in the presence of a catalyst and grafting agent stannous octoate [154]. PCL-grafted CNCs showed higher stability in toluene and improved thermo-mechanical properties such as Young's and storage modulus, because of better compatibility with the polymer matrix [154]. Similar studies, when carried out in the presence of microwave [61], lead to higher grafting density, which in turn shields the hydrophilic surface of CNCs thus enhancing its water resistance properties [155]. Pranger et al. [156] carried out *in situ* polymerization of furfuryl alcohol in the presence of CNCs using sulfonic acid as catalyst at high temperatures. Furfuryl alcohol served both as a dispersing agent and the matrix precursor for *in situ* polymerization. The resulting nanocomposite showed higher onset degradation temperature of ~80°C for 0.75 wt. % CNC. In a recent study, CNCs were modified with poly(methyl acrylate) (PMA) through radical polymerization in the presence of surface

initiated *photoinduced* Cu-mediated catalysts [157]. Through this route higher grafting efficiency of polymer (40–85 wt. %) is achieved within short reaction time span with improved thermal stability and dispersion in organic solvents. Similarly, in another study PMA and poly(methyl methacrylate) were successfully grafted from the macroalkoxyamine modified CNCs through nitroxide-mediated radical polymerization [158]. In a recent study Miao et al. [159], prepared a PLA-grafted CNC nanocomposite with molecular weight of ~35 kDa through insitu ring opening polymerization in the presence of lactide. The PLA-grafted CNCs when dispersed into PLA matrix at low loading fractions ~1 wt. %, leads to significant improvement in the water vapor and oxygen barrier properties by ~81% and 87% respectively, alongwith enhancement in thermo-mechanical behaviour. Moreover, grafting of CNCs with L/D-lactide using the above-mentioned approach leads to formation of stereocomplex crystals of PLLA/PDLA which results in improved modulus, superior heat distortion resistance and fast crystallization [160].

The comparison of the ‘grafting onto’ and ‘grafting from’ approach using poly (ϵ - caprolactone) (PCL) as model polymer system shows distinct variation in its grafting density, molecular weight of grafted polymers, dispersion stability and surface energy properties. Through “grafting from” approach it is possible to obtain comparatively higher grafting density in which the degree of polymerization i.e. the chain length of polymer grafted on CNC surface can be controlled. However, in the case of “grafting onto”, approach the grafting efficiency is significantly lower with the possibility of formation of CNC agglomerates in poorly dispersed nanocomposites. For the case of ring opening polymerization of PCL in the presence of CNCs fabricated from ramie fibers, it was observed that the grafting efficiency varied as ~30% in case of “grafting onto” which increased to ~85% in “grafting from” approach.

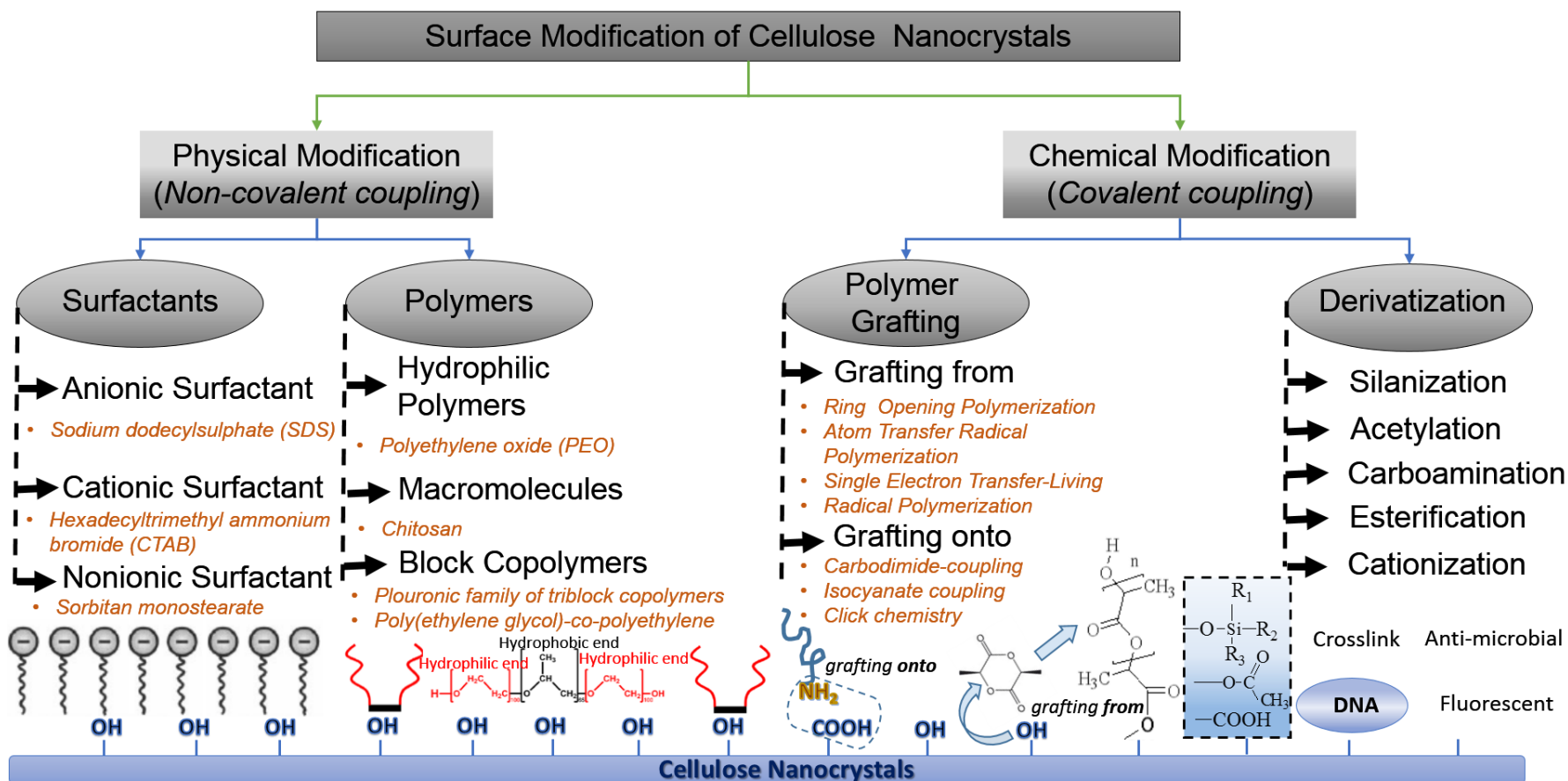


Figure 1.4: Strategic surface modification of CNCs through various routes and their related mechanisms.

However, the molecular weight of PCL covalently bonded to CNCs in “grafting onto” approach remains unchanged from the initial precursor selected due to which high molecular weight chains can be grafted. On the other hand, in the case of “grafting from” approach, the molecular weight of PCL can be tuned by changing reaction conditions, with the formation of high molecular weight grafted PCL chains only under ideal reaction conditions. Furthermore, when the “grafting from” polymerization is carried out in a microwave reactor, the reaction time decreases to several minutes and in presence of citric acid as catalyst shows substantial enhancement in yield of polymer-grafted CNCs. Therefore, it can be concluded that both the “grafting from” and “grafting onto” polymerization process have their respective pros and cons and the choice of one or the other needs to be made depending upon the desired properties of nanocomposites for end-user applications.

1.3.4 Cellulose Nanocrystals: Reinforcement Capability in Biodegradable Polymers

Cellulose Nanocrystals (CNCs), have the strong capability to form percolated network-like structure through the formation of inter- or intra-molecular hydrogen bonding when pooled with the polymeric matrix. Due to high elastic modulus of CNCs, improved dispersion and interfacial interaction of CNCs with the polymeric matrix leads to effective stress transfer. Moreover, with the variable aspect ratio, morphological dimensions and degree of inter/intra- molecular hydrogen bonding, the reinforcing capability of CNCs can be appropriately tuned. In literature, CNCs have been widely used as reinforcing nanofiller to a wide variety of thermoplastic and thermosets. However, in this section we will focus on the biodegradable polymers, especially biobased polymers such as polylactic acid (PLA), biopolymers such as chitosan and bioplastics such as polyhydroxyalkanoates (PHA) and their derivatives. PLA and PHAs have attracted considerable interest among the wide class of

biodegradable polymers because of their easy processability and comparable physicochemical and thermal properties with conventional synthetic polymers. However, the brittle nature, poor mechanical behaviour, inferior oxygen or water vapor barrier properties and low heat distortion temperature ($\sim 55^{\circ}\text{C}$ for PLA) limits their application for manufacturing commodity products [161]. Incorporation of CNCs through different approaches leads to both reinforcing behaviour as well as provides a tortuous path to permeation of gas molecules, which results in improvement of both barrier and structural properties of the nanocomposites. Therefore, modifications of PLA or PHA matrix through physical approaches like direct dispersion of nanofillers [162,163] or through chemical approaches like polymerization using different routes such as grafting onto and from approaches [164,165] in the presence of CNCs as nanofillers have been extensively researched and are discussed next.

- **Polylactic Acid-based Cellulose Nanocrystal Nanocomposites**

PLA, a completely biodegradable thermoplastic polyester, when reinforced with CNCs forms “green bio-composites” having a percolated nanofiber network in the polymer matrix which leads to improvement in its physicochemical and structural properties. However, there are several technical problems associated with the dispersion of hydrophilic CNCs into hydrophobic PLA matrix which have been overcome through different chemical modification and processing routes. CNCs should be handled in partially hydrated state during processing with polymers as complete freeze drying of the material creates the problem of redispersion due to the strong hydrogen bonding between cellulosic chains and tendency of CNCs to self-assemble [166]. However, excessive hydration leads to incompatibility and poor dispersion of CNCs in hydrophobic polymers. Production of PLA-based nanocomposites through industrial based processing techniques such as melt compounding or extrusion makes the situation much

more complicated. In the melt state, polymer-nanoparticle interactions are extremely weak which leads to agglomeration of the filler nanoparticles. Moreover, the presence of abundant hydroxyl groups and sulfate ester groups act as initiators for thermal degradation of polymers. Surface modification of CNCs, carried out using complex chemical routes, have promoted dispersion to some extent. The surfactants used for dispersing nanoparticles have been observed to adversely affect molecular weight of PLA [167]. Surface modifications may create complications related to deterioration of biodegradability/biocompatibility characteristics of CNCs, with potential increase in toxicity due to migration of additives to packaged foodstuff in contact causing health-related problems.

Solution casting of CNCs into PLA matrix at relatively low weight fraction has shown good dispersion, with improved mechanical properties. However solution casting has several drawbacks related to solvent entrapment and difficulty in industrial scale up. Alternatively, melt extrusion, an industrially viable approach of processing PLA/CNC films, can be employed. However, in the presence of high shear and temperature conditions, the nanocomposites undergoes drastic reduction in molecular weight which results in inferior properties. Several studies on effectively dispersing CNCs in polymer matrices have been carried out till date, which address the techno- economical difficulties associated with both the processing methods to some extent. Bondeson and Oksman [167] investigated the effect of adding polyvinyl alcohol (PVA) to improve the dispersion of CNCs in PLA. It was observed that immiscibility of PLA and PVA led to phase separation. CNCs got primarily localized in the PVA phase due to more favorable interactions, with negligibly small amount present in the PLA phase. Due to phase separation and inadequate dispersion, there was no significant improvement in thermal and mechanical properties of PLA/CNC nanocomposite. However, in

a recent study by Haque et al. [168], the miscibility of the two phases was improved through reactive functionalization in the presence of glycidyl methacrylate, resulting in slight improvement in elastic modulus. Surface modification of CNFs through silylation reaction with 3-aminopropyltriethoxysilane resulted in better dispersion into PLA matrix through melt compounding [169]. The improvement in thermo-mechanical properties of the composites could be attributed to better interfacial adhesion between cellulose and the polymer matrix. CNCs exhibit good dispersion in ethylene-vinyl alcohol copolymer (EVOH) when preincorporation methods (such as electrospinning) are used before melt compounding, leading to improvement in mechanical properties over neat EVOH [170]. Further studies on incorporation of bacterial CNCs into PLA by electrospinning and solution precipitation with polar EVOH revealed significant improvement in water and oxygen barrier properties over neat PLA [171]. Recently, a novel approach of coating the CNCs with biobased polysaccharides such as lignin resulted in improved compatibility with hydrophobic PLA, even at low loading of CNCs (~0.3- 0.5 wt. %). The developed nanocomposites show improved rheological behaviour due to which they can be efficiently processed through blown film process leading to enhancement in storage modulus by ~60% [172].

Alternative routes of chemically grafting of PLA on the surface hydroxyl groups of CNC through *in situ* polymerization of lactide as reported by Braun et al. [173] shows improved compatibility. The hydroxyl groups initiated lactide ring opening polymerization, which subsequently led to the grafting of PLA chains onto the CNC surface. Acetylation of a fraction of surface hydroxyl groups of CNCs using Fischer esterification process resulted in grafting of high molecular weight PLA chains on the CNC surface. The nanocomposite thus obtained displayed significant improvement in several properties over neat PLA such as heat distortion

temperature, rate of crystallization and shear modulus. In fact, the shear modulus obtained for the nanocomposite was two orders of magnitude larger than that obtained by Capadona et al. [174] for PLA nanocomposites prepared using unmodified CNC. The property enhancement is probably due to the reinforcement effect of percolating network formed by the CNC nanofillers. Melt pressed films of the PLA-grafted CNC nanocomposite material retained transparency indicating good dispersion of the filler in the polymer matrix. Similarly, grafting of poly (butylene succinate) (PBS) onto CNCs was carried out through insitu polymerization technique to improve their dispersion in PLA matrix [175]. It was observed that the PBS- grafted CNCs forms droplets in PLA matrix which leads to improvement in crystallinity and significant increment in impact strength from 105 to 726 J/m [175]. Recently, a new approach of nanocomposite formation through semi-interpenetrating polymer networks (SIPN) has been developed in which two entangled network of polymers are formed through chemical cross-linkages that are subsequently dispersed in the continuous polymer phase. A current study by Sethi et al. [176] shows that formation of such entangled network of CNCs reinforces both polyurethane and PLA matrix improving the thermo- mechanical behaviour, i.e., elastic modulus by ~54%. Through the use of similar approach, PLA can be cross-linked to amide- modified CNCs, which results in increased tensile strength and elongation alongwith reduced water absorption that can be tuned through variation in cross-linking density of SIPN network [177]. Therefore, from the above discussion it can be interpreted that through novel surface modification strategies it is possible to induce improved dispersion of hydrophilic CNCs into hydrophobic PLA, which leads to enhanced physicochemical, structural and barrier properties of nanocomposites. However, several technical challenges remain, associated with the processing of PLA/CNC nanocomposites, particularly the issue of reduction in molecular

weight, and dimensional instabilities during processing through industrially viable extrusion which limits their commercial viability due to deterioration of properties.

- **Polyhydroxybutyrate-based Cellulose Nanocrystals Nanocomposites**

Polyhydroxyalkanoates (PHAs) are class of biodegradable polyesters produced through microbial fermentation routes utilizing renewable biomass resources, where the specific raw materials are processed into fermentable broths through chemical treatments. Interestingly, the PHAs produced consists of different fractions of about 11 types of hydroxyalkanoates based monomeric units, with some of the major fractions being 3-hydroxybutyrate, 3- hydroxyvalerate, 3-hydroxyhexanoate, 3-hydroxyoctanoate and 4-hydroxybutyrate with R configuration of the stereo-specificity [178]. These heterogeneous units undergo polymerization to form homopolymers as well as co- and heteropolymers with different molecular weights in range of 200,000 to 3,000,000 Da [179]. Compared to other biodegradable polymers, PHB are readily degradable with improved crystallization and thermal properties; however, the structural and barrier properties need to be improved through introduction of nanoparticles or biobased nanofillers, for PHB to be competitive with the conventional petroleum-derived polymers [180].

It is well known that the incorporation of CNCs in polyhydroxyalkanoate (PHA) matrix leads to improvement in the physical and mechanical properties. Nanocomposites of cellulose and poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) leads to introduction of reinforcing behaviour which improves the structural properties. PHBV/CNC nanocomposites fabricated by Jiang et al. [181] using solution casting showed improved tensile strength and modulus alongwith elevated glass transition temperature compared to neat PHBV. However, melt processed PHBV/CNC nanocomposites shows reduction in mechanical properties due to

agglomeration, moisture sensitivity of PHB and degradation in molecular weight during processing in presence of nanofillers. Srithep et al. [182] however, reported improvement in the mechanical properties and crystallization kinetics of PHBV/CNC nanocomposites prepared through melt compounding. They utilized CO₂ as foaming agent to develop biodegradable PHB/CNC foams in which the degree of foamability, porosity and void fraction decreased with increase in CNC concentrations. Electrospun fiber of the PHB/CNCs showed improved biocompatibility towards L929 cell line with improved adhesion and cell proliferation, which suggests the non-toxic characteristics of the PHB/CNC films [183]. Therefore, completely green, sustainable and biodegradable PHB/CNC nanocomposites can be fabricated by combining hydrophobic PHB obtained from microbial source and hydrophilic CNCs derived from plant source, with potential applications in food packaging industry.

PHB/CNC nanocomposites have been blended with other biodegradable polymers such as PLA to form ternary composites which are extensively studied in literature due to their improved compatibilization when processed through different techniques. CNCs are known to act as a reinforcing agent between the PLA and PHB matrix thereby increasing the interfacial adhesion between the two polymeric matrixes at a ratio of 70:30 (PLA/PHB), which in turn improves the flexural modulus by ~104% and reduces oxygen permeability by ~27 % [184]. At PLA/PHB ratio of 71.25:23.75 wt. % with 5 wt. % of incorporated CNCs, the elongation at break significantly increased to ~175 %, with enhanced disintegration rate of ~10 days under composting conditions [185]. Introduction of the high aspect ratio cellulose nanofibers into the PLA matrix with PHB as results in significant improvement in the tensile and flexural modulus, and shear thinning behaviour as confirmed from rheological studies [186]. Electrospinning of PLA/PHB nanomats at a ratio of 60:20, with the addition of 5 wt. % CNC

and 15 wt. % acetyl (tributyl citrate) as plasticizer leads to the formation of uniform morphology and bead-less fibers with high flexibility and thermal properties alongwith enhanced composting rate of ~10 days [187]. In another study by Kenny and co-workers [188] showed that melt blending of the PLA/PHB blends at a ratio of 71.25/23.75 wt. % with 5 wt. % CNCs f results in the CNCs forming as bridge between the two polymeric matrix to improve their interfacial adhesion. The fabricated ternary nanocomposites show improved processability and subsequently incorporate synergistic improvement in the crystallinity and thermal properties. PHAs tends to have poor film forming ability and extrudability due to enhanced degradation in molecular weight during processing, which makes it very difficult to fabricate PHA-based nanocomposites for packaging applications at commercial scale. Formation of the ternary blends of PHB with PLA and CNCs not only improves the processability but also provides structural stability, reduced oxygen and water vapor permeabilities and most importantly maintains the biodegradability. Thus, CNCs not only act as strong reinforcing agent but also as a compatibilizer between the two PHB and PLA in the fabricated ternary nanocomposites, whose properties can be finely tuned through alteration in PLA/PHB ratio depending on the targeted end-user applications.

- **Chitosan-based Cellulose Nanocrystals Nanocomposites**

Chitosan is a biodegradable and biocompatible natural biopolymer which possesses antimicrobial properties making it a suitable material for food packaging applications. Both chitosan and CNCs are hydrophilic in nature, with high moisture sensitivity and poor film forming ability which have restricted their widespread applications in packaging sectors. However, the functionalized chitosan-CNC nanocomposites finds potential applications in wide range of sectors such as in biomedical engineering for development of wound dressing,

high barrier coatings, polyelectrolyte membranes, targeted drug delivery, in water remediation for removal of toxic dyes and encapsulation of antioxidants. Chitosan has amine functional groups, which makes it an ideal candidate for grafting on to the hydroxyl and carboxyl groups of polymers. Chitosan/CNC composites are easy to fabricate because both components are hydrophilic and highly compatible. In the presence of acidic medium the amine groups of chitosan gets protonated and can associate with the negatively charged sulphonyl groups on CNCs through electrostatic attraction. Grafting of both biopolymers can easily be carried out by amide linkages between the carboxyl functional groups of CNC with terminal amide groups of chitosan. De Mesquita et al. [189] fabricated chitosan/CNC nanocomposites by first functionalizing the CNCs with methyl adipoyl chloride (MAC) and reacting the surface functional groups of CNCs with amino groups of chitosan. The nanocomposite showed considerable improvement in mechanical properties and a remarkable reduction in hydrophilicity as suggested by water uptake results. Such property improvements make these completely bio-based nanocomposites promising materials for food packaging applications. The negatively charged CNCs and positively charged chitosan self-assembled into polyelectrolyte-macroion complex (PMC), the morphological dimensions of which depend upon the concentration and surface charge density of CNC/chitosan. Due to the tunable surface polarity of pmcs, with negative surface charge at amino/sulfate ratio <1 and positive charge at amino/sulfate ratio >1 , they find potential application in drug delivery for their capability to adsorb both the cationic/anionic type of drugs [190,191]. Interestingly, coating the 5 wt. % reinforced CNC/chitosan on *Pyrus communis* L. And *Pyrus communis* L. Pears shows significant improvement in their shelf-life due to reduced degradation of chlorophyll or senescence scalding, preventing browning and retaining freshness under ambient conditions

[192]. The coatings are found to be quite stable even under cold storage conditions with high adhesion to fruits surface, which suggests that the nontoxic, biobased and sustainable CNC/chitosan based coating could be used to delay ripening of pears increasing their shelf-life and storability postharvest. It was found that incorporation of CNCs into chitosan membrane as functional entities followed by cross-linking with glutaraldehyde leads to formation of membranes with higher structural stability. The negatively charged membranes with pore sizes in the range of 10–13 nm could selectively adhere positively charged dyes such as Victoria Blue 2B, Methyl Violet 2B and Rhodamine 6G with removal efficiency of 98%, 84% and 75% respectively [193]. In another study by Naseri et al. [194], electrospun nanocomposites of chitosan/CNC nanofibers with comparatively high structural properties (tensile modulus ~ 3.1 GPa) and water vapor/oxygen/carbon dioxide permeability and biocompatibility required for wound dressing applications. Akhlaghi et al. [195] substituted CNCs with oligomers of chitosan (degree of substitution ~ 0.26) through carbodimide reaction to fabricated CNC- chitosan nanoparticles which showed high binding efficiency of $\sim 21\%$ and drug loading capacity $\sim 14\%$ w/w. The CNC-chitosan nanoparticles, show fast triggered release of procaine hydrochloride within 1 hour, which is promising for the development of fast stimuli-responsive drug carriers. A recent study by Wang et al. [196] shows the formation of macroion complex of CNCs cross-linked with chitosan-sodium tripolyphosphate which assembles into spherical microcapsules. It was observed that blueberry anthocyanin extract could be encapsulated in microcapsules during crosslinking which can be recovered upto $\sim 94\%$ through release in optimum pH conditions. Therefore, both the biopolymers chitosan and CNCs could be self- assembled into complex structures through simple tuning of concentrations and surface

charge which results in the development of novel materials with high performance applications.

1.3.5 Cellulose Nanocrystals-based Polymeric Composites: Processing Techniques

CNC is an ideal material for fabrication of nanocomposite because of its high structural strength 130-145 GPa [197], low density ~ 1.5 g/cc (hence strong but lightweight), ease of functionalization with the ability to improve the properties of polymers at lower loading fractions. CNC-based films can be fabricated through solvent casting, vacuum filtration followed by air-drying under compression etc. The films prepared from CNFs through TEMPO oxidation of cotton are usually transparent with crystallinity in the range of 75–90%, which leads to significantly high oxygen barrier properties ($\sim 17 \pm 1$ ml.m⁻²day⁻¹) which interestingly, are comparable with the petroleum-derived polymers such as ethylene vinyl alcohol and polyvinylidene chloride. Surface modification of bacterial cellulose membranes through esterification process leads to significant reduction in the permeation of oxygen, carbon dioxide and nitrogen gases by $\sim 50\%$ [198]. However, their potential applications is limited due to two major problems. Firstly, the CNC based films cannot be processed through industrially-viable extrusion due to its brittle nature with weak dimensional stability. Secondly, due to the hydrophilic nature of CNCs they tend to lose their stability under moist environmental conditions. To overcome such challenges, CNCs are generally pooled into the biodegradable polymers for improvement of their structural, physicochemical and barrier properties which depends on the degree of dispersion and interfacial intra/inter-molecular hydrogen bondings. Therefore, selection of proper fabrication and processing techniques for uniform dispersion of CNCs into the polymer matrix is an important factor, which governs the percolating network structure and consequently, the mechanical and barrier properties of the

nanocomposites. CNC-based film preparation using different processing techniques, such as solution casting, electrospinning, extrusion and layer- by- layer approach in different hydrophilic or hydrophobic polymeric systems are discussed next.

- **Solution Casting-cum-Evaporation Technique**

Solution casting-cum-evaporation is a process in which nanoparticles are dispersed into the polymer solution and film is cast by subsequent solvent evaporation. It has been the most feasible technique of dispersing aqueous CNC solution into hydrophilic polymers, due to favorable interactions between the nanoparticle and the polymer. Due to its hydrophilicity, CNC can be dispersed either in a hydrated or a freeze dried state into the aqueous hydrophilic polymer solution, because of enhanced inter- and intra-molecular hydrogen bonding in the system. However, the applications of hydrophilic polymers are limited in presence of high moisture and humid conditions, which have diverted attention towards the synthetic hydrophobic polymers. Therefore, current studies are focused on dispersing cellulose nanocrystals into polar or dipolar organic solvents, so that it could get well intercalated in the hydrophobic polymer solutions for creation of better reinforced polymer nanocomposites. Freeze-dried CNCs prepared from sulfuric acid got well dispersed in organic solvents DMSO and DMF directly forming a stable suspension [167]. This was because of the negative charges imposed by sulfate groups which increases the repulsion between the CNCs, limiting the formation of hydrogen bonds leading to a stable dispersion. The trace amounts of water was found to be critical towards stability of final solution, since aggregation would take place due to hydrogen bonding [199]. However, the type of acid used for CNC production greatly affects the stability. HCl based CNCs show very poor dispersion in N,N-dimethyl formamide (DMF), N-methyl pyrrolidone (NMP) and dimethyl sulfoxide (DMSO), which can be inferred from the

zetapotential values that fall within the unstable region [200]. This was because of the absence of surface charge on HCl-derived CNCs, the effect of intermolecular hydrogen bonding dominated which led to agglomerations even in water [201]. Similar approach of dispersing CNCs filler into the biopolymer PHB through melt compounding lead to subsequent reduction in molecular weight [202]. Formic acid and m-cresol showed good dispersion of CNCs fabricated from both sulfuric acid and hydrochloric acid. These could be due to the esterification of the hydroxyl ends during the esterification process, which was indicated by the FTIR studies [203]. Therefore, these polar aprotic solvents have been extensively used for the fabrication of polymer nanocomposites, which helped in dispersion of CNCs to large extent. Solution casting technique has been used for film preparation with several biopolymers especially PHB, PLA, chitosan etc. But the threshold limit of dispersion is maximum 5 wt. % of CNCs. The strategy of selecting the appropriate solvents for dispersing CNCs depends upon the extent of miscibility of solvent-CNC suspension with the polymer solution, which in turn depends on the concentration and ionic strength of dispersion media. Poly (methyl methacrylate) (PMMA), being completely soluble in DMF, was used for preparation of CNC composite through solution casting technique by heating the solvent at 75°C for 10 hours [204]. PHAs are completely soluble in chlorinated solvents like chloroform or in DMF; hence, CNCs are dispersed into polymer matrix through solvent exchange with these solvents. Cellulose acetate butyrate was solubilized in acetone, and was cast into films by dispersing CNCs in acetone through solution casting method [205]. Although, the fabrication of CNC-based polymeric nanocomposites through solution-casting approach is an effective way of dispersing CNCs into polymers, it suffers from two major limitations. Firstly, the CNCs can be dispersed only at low loadings fractions and secondly, the entrapment of toxic

solvents severely affects the structural, physicochemical and biological properties of fabricated nanocomposites. Moreover, if the solvent evaporation occurs at uneven and uncontrolled conditions there is a possibility for formation of small pores which leads to poor gas barrier properties and sites for stress-induced propagation of cracks leading to failure. Another major drawback related to solution casting method is its inability to process large polymeric nanocomposites films required for industrial scale production. Even though solution casting has several drawbacks it is still extensively studied and reported in literature to understand the interfacial interaction between the polymer and the modified CNCs.

- **Extrusion or Melt Compounding**

Extrusion is an industrially viable technique for processing of CNC-based nanocomposite films, which is a sustainable approach that does not require any toxic solvents. In melt extrusion, polymer is forced to flow under shear along the helical screw direction which is divided into various temperature zones. The polymer is processed at various specified zones such as feeding section, mixing and melting section, compression section and finally takes the shape of the die at the other end. [206]. The stage of introduction and type of chemical modifications during polymer processing through extrusion is an important factor, for improvement of the dispersion of CNCs in polymeric matrix and formation of the percolation network. During the processing of nanocomposites, CNCs can be either directly mixed with polymer beads/granules, modified with the polymers to form a master batch or injected in the form of CNC suspension at an intermediate step of extrusion. However, these different techniques have several challenges associated with proper dispersion of CNCs into polymer matrix and preventions of agglomerations.

The extrusion of CNC-based polymeric films is widely carried out through direct mixing followed by extrusion at high temperature and shear conditions. Due to poor thermal stability of sulphuric acid derived CNCs, the processed films undergo drastic reduction in molecular weight with degradation of both polymer and CNCs which leads to black coloration in the films. To overcome such challenges, hydrophilic polymers are adsorbed onto the surface of CNCs followed by its extrusion, which shields the sulfate groups present on the surface responsible for degradation. Surface-modified CNCs with adsorbed PVA and PEO chains could be successfully dispersed into the polymer through extrusion [123], which prevents the black color formation as discussed earlier. However, due to enhanced hydrogen bonding in CNCs problems related to agglomeration of the hydrophilic phases in hydrophobic polymers still exists which leads to inferior properties. As discussed in earlier section, Oksman et al. [207] fabricated the PLA/CNC nanocomposites through the incorporation of CNC suspension dispersed in N,N- dimethylacetamide (DMAc) and polyethylene glycol (PEG) as processing aid during extrusion. However, the developed nanocomposites displayed brownish coloration, suggesting the degradation of PLA backbone and agglomeration of CNCs which resulted in poor mechanical behaviour. The effects of additives, compatibilizers, processing parameters such as extrusion temperature, screw speed, recycle time etc. play a significant role on the properties of the prepared nanocomposites which requires stringent optimization. However, the most important drawback of extrusion with CNC as nanofillers is the polymer degradation, which is caused due to sulphonyl groups that act as an initiation site for polymer decomposition. Hydrochloric acid-fabricated CNCs, prevents the degradation of polymer during processing; however, problems related to agglomerations in polymer remains. Therefore, proper functionalization of CNCs or fabrication of CNCs with

different acids should be optimized, which could simultaneously lead to better dispersion stability in polymeric matrix and prevent its degradation while processing.

- **Electrospinning**

Electrospinning is a versatile process in which the polymer/polymer nanocomposite solutions can be continuously processed into nanofibers through application of electrostatic field in form of high-voltage between two electrodes. During electrospinning process, voltage is applied to polymer solution to overcome its surface tension which causes the formation of nanofibers, whose dimensions can be finely tuned depending on the spinning parameters and conditions. The electrospun nanofibers possess some interesting properties such as high porosity, presence of wide range of nano/micro-pores, large specific surface area, improved gas/water vapor permeation etc. With physicochemical and structural behaviour mimicking the natural extracellular matrix (ECM) [208]. Due to absence of molecular chain orientations, the electrospun fibers are flexible in nature but possess relatively poor mechanical properties. Therefore, incorporation of CNCs into the electrospun fibers helps in improving the structural properties of the nanofibers by providing reinforcement. Further, biopolymer-based CNC nanofibers developed through electrospinning process are nontoxic, sustainable with improved biocompatibility which can have potential applications in development of scaffolds or wound dressings for tissue engineering [209], as filter membranes for water purifications, as food packaging based coatings, as substrate for catalyst support and in optoelectronic devices [210].

In the literature, CNCs have been widely used to reinforce variety of biodegradable polymer-based electrospun nanofibers such as poly(ϵ -caprolactone) (PCL), poly(vinyl alcohol) (PVA), polyethylene oxide (PEO), poly(lactic acid) (PLA) etc. For biomedical applications, it is desired to fabricate biodegradable electrospun nanofibers

utilizing hydrophilic polymers dissolved in aqueous solvents, otherwise, the toxic organic solvents are known to hamper the biocompatibility of fibers. The PEO/CNC electrospun fibers show improved mechanical and thermal behaviour due to the enhanced inter-molecular hydrogen bonding and undergo a transition from homogenous to heterogeneous microstructures on increasing the CNC concentration [211]. Moreover, the alignment of cellulose fibers with CNCs incorporated in it during the electrospinning results in significant improvement in tensile strength and elastic modulus (by more than ~100% and 170% respectively) [212]. Interestingly, the aligned fibers further helped in guiding the cell growth and proliferation in the specific aligned directions, which finds potential application in fabrication of artificial scaffolds for regeneration of nerve cells and blood vessels. Furthermore, Liu et al. [213] biomimicked the molecular orientation of silk fibers produced by the natural silkworms through the introduction of CNCs into silk-fibroin matrix by electrospinning process. CNCs act as ordered β -sheets crystalline structure which led to significantly improved Young's modulus of 28.8 ± 2.6 GPa, comparable to that of natural silk materials. Therefore, the electrospinning provides an ideal approach to uniformly disperse CNCs into polymeric matrix, to develop flexible nanocomposite fibers with improved structural and physicochemical properties adequate for biomedical engineering applications.

- **Layer-by-Layer Assembly**

Layer-by-layer (LbL) technique is a cost effective, robust and efficient film fabrication technique, where oppositely charged polymers or nanoparticles are deposited through electrostatic interactions. By controlling the conditions of film preparation it is possible to tune the architecture of the nanoparticle dispersion and its thickness on substrate. Moreover, one can tune several other properties such as optical and barrier by proper calculation of multilayer

deposition on the polymer substrate [214]. The LbL technique used for fabrication of CNC based polymeric films possesses an advantage over CNC films prepared by solvent casting. During solvent casting of films, uneven evaporation of water leaves behind small pores/void space which increases the oxygen permeability of films. In contrast, in polyelectrolyte LbL approach the cationic polymer gets impregnated into anionic void of nanofibrils thereby decreasing the void space and oxygen permeability. Since in LbL approach drying is related to nanolayers of nanoparticle coatings, very less probability of defect formation arises.

Generally, LbL approach is used for development of multilayer CNC on bio-based PLA, by tailoring the number of layers deposited on polymer substrate, to obtain the desired gas barrier and transparency, so that films could be fabricated for different applications. In a recent study, PLA films were fabricated through LbL approach with CNC by dip coating the PLA films with cationic polyethylenimine (PEI) and anionic carboxylate CNC to make 20- 50 bilayers of CNCs on its surface [29]. Due the presence of electrostatic interaction of CNCs with polymeric substrate through H-bonding and van der Waals interaction it leads to lowering of free volume in polymers resulting in high barrier properties [29]. Poly (diallyldimethylammonium chloride) with high density of positive charges per unit length, rendered the binding site for the negatively charged CNCs prepared by sulfuric acid hydrolysis [215]. With a 10 min interval of adsorption time, LbL assembly led to the formation of 11 nm thick bilayers, which was densely packed and uniformly covered with CNCs [215]. In recent studies to understand the complex hydrolysis process of lignocellulosic biomass, different multilayered model films of CNCs and xyloglucan were prepared by spin assisted LbL approach [216]. Fabrication of poly (allylamine hydrochloride) and CNC based multilayered thin films was studied by varying different process parameters such as the drying

step between each layer of adsorption, ionic strength of polymer solution, dipping time and CNC concentration. Drying process and ionic strength are found to be the two important parameters for film preparation by LbL approach, whereas the thickness was monitored by dipping time and CNC concentration [217]. Drying step has been the crucial point for the film construction and architecture, because water molecules are ejected from the film, leading to the formation of dense polymer layers with higher cohesion of films. The cationic polymers fabricated by LbL approach with anionic CNCs leads to the fabrication of molecularly dense structured films, with polymers getting adsorbed onto the CNC surface thereby decreasing the free volume fraction to a large extent. The biopolymer chitosan and CNC based “Green” composite films were fabricated by LbL approach through alternating layer deposition [218]. The driving forces for the growth of these multilayered films was hydrogen bonding and electrostatic interaction between the positively charged amine groups of chitosan and negatively charged CNCs [218]. Through multilayer approach, TEMPO-oxidized anionic CNCs were deposited on chitin nanofibrils, which showed uniform layer deposition [219]. The hydrophilic nature of the films was revealed from contact angle studies as opposed to earlier studies where hydrophilicity decreased. The cast films showed antireflective properties which suggests, that these can be used as thin nanolayer coatings on glass and window panes of vehicles [219]. Aulin and Ström [220] deposited renewable multi-layered alkyd-resins through continuous roll-to-roll process over CNCs and subjected the layers to auto-oxidation. This led to formation of moisture sealant layers with composites showing significant moisture barrier properties. The water vapor permeability decreased from 1707 to 5.2 g.mm/m² day.atm with 6 g/m² of CNC coating [220]. The improvement was possibly due to two reasons: firstly, decrease in pore size as CNCs act as sealants and secondly, increase in the smoothness of the

surface. Moreover, recently CNCs have been directly used as coating material on different substrates such as paper or other plastics through layer by layer approach. The CNC coatings on paper reduces permeability by ~91% alongwith drastic improvement in burst index and bending stiffness values [221]. This shows that CNCs can either be used a nano-filler in the fabrication of biodegradable films or as coatings through LbL approach on perishable food products directly which will improve the shelf-life of stored items.

1.3.6 Cellulose Nanocrystals-based Advanced Functional Materials.

Based on the diverse functionalities and interesting physico-chemical properties, CNCs have found potential applications as nano-reinforcing agents, optically active materials, as chiral inducers, templates for inorganic nanoparticles, as biocatalysts with high turnover frequency, permselective membranes for water decontamination, as scaffolds for cell adhesion or proliferation, self-healable materials and can be architected into aerogel, hydrogel and foam based value-added products. In addition to the improved structural properties, tunable morphologies, chemical modifications of hydroxyl functionalities, CNCs are liquid crystalline nanomaterials with the ability to form chiral inducers which have been extensively utilized to develop nanocellulose based advanced functional materials.

The reactive hydroxyl functionalities in CNCs can be labeled with various fluorescent molecules through covalent interactions such as fluorescein isothiocyanate (FITC), rhodamine B isothiocyanate (RBITC) [222,223], pyrene derivatives [224], peptides [225] and soluble photosensitizers [226] for potential applications as bio-probe for sensing, bio-imaging, photodynamic therapy and flow cytometry. Moreover, due to their improved biocompatibility and non-toxicity, CNCs modified with fluorescent moieties can be directly used as probes under biological conditions, after the *in vivo* studies or successful trials through clinical

research. The negatively charged sulfate ester groups incorporated on CNCs during hydrolysis provides sites for both electrostatic adsorption and chemical conjugation of enzyme and proteins [227]. Immobilization of lysozymes [145], cyclodextrin glycosyl transferase, peroxidases, alcohol oxidase [228] etc. Through carbodiimide-activation coupling reaction provides enhanced stability to proteins alongwith high catalytic activity and increased turnover frequency and recyclability. A recent study, shows that the combination of cellulose with fungi results in its hydrolysis due to improved fungal enzyme activity which leads to the formation of CNFs/CNCs with the simultaneous production of second generation fuels [229]. Some of the other interesting functional modifications carried out on CNCs are duplexing of oligonucleotides of DNA [152], ferrocene- [230] and porphyrin-decorated CNCs [231] for fabrication of biosensor, photo-bactericidal and bio-electrochemical assemblies.

The naturally-derived CNCs with abundant hydroxyl groups act as strong reducing agents due to their enhanced electrostatic interaction with metal salts, and as structure directing agent with rod-like morphologies. Different inorganic nanoparticles such as gold [228], silver [232], nickel [233], selenium [234], palladium, titania [235], Au-Ag / Ag-Pd alloys [236] etc. Have been reported in the literature. The growth of titania on CNCs has been extensively studied in literature which leads to formation of nanotubes [235], nanocube [237], porous nanoclusters with various shapes and flower-like TiO_2 morphologies which depends on the concentration of metal salts, surfactant type and its concentration, reaction time, temperature and pH of reaction media [238]. The developed CNC-based metal precursor finds potential applications in dye degradation for wastewater remediation, as catalysts/photocatalyst for conversion of chemical moieties, and as catalytically active membranes in fuel cell applications.

Due to the bio-based origin and improved biocompatibility, CNCs have been extensively used to study the interaction, growth and proliferation of the cells especially under simulated biological conditions. The cytotoxicity of CNCs has been studied through their interaction with the cells derived from various microorganisms such as insect cells SF9 and Hamster lung fibroblast V79, murine myoblasts cells, C2C12 human fibroblasts, human embryonic kidney cells HEK-293 etc [239, 240]. Interestingly, results show that CNCs provide sites for improved cell adhesion, cellular uptake, cell proliferations and guidance for growth of skeletal muscles which can be directed by changing the orientation of CNCs [241,242].

CNCs have abundant hydroxyl groups present on their surface due to which they are able to form a network of inter/intra-molecular hydrogen bonding entrapping the aqueous solvents in the form of a hydrogel matrix. CNC-based composite hydrogels can be divided into two types: firstly, physical hydrogels formed through physical interactions or self-assembly of CNCs due to presence of strong hydrogen bondings and secondly, chemical gels formed through chemical cross-linking of polymers. Removal/replacement of the trapped solvents in the hydrogels with gas or other molecules through controlled freeze-drying leads to formation of porous ultralight aerogels, which are high performance materials with diverse applications. Till date most of the CNC-based hydrogels reported in literature utilizes hydrophilic polymers such as poly(ethylene glycol) [243], poly(vinyl alcohol) [244], starch[245], agarose[246], poly(NIPAM-co-BMA) [247] etc. Which are based on the formation of physical linkages through strong hydrogen bonding. Chemical cross-linking of the CNCs with polymers such as poly(acrylic acid) (PAA) [248], gelatin [249], poly(oligoethylene glycol methacrylate) [250] etc. Have been carried out to fabricate chemically cross-linked hydrogels. Fabrication of anisotropic hydrogels, covalently grafted aerogels [251] and aerogels through gas esterification

reactions [252] provides a novel approach of preparing hydrogels/aerogels for high performance applications. A current study shows that incorporation of various types of metal organic frameworks (MOFs) into the CNC aerogels leads to potential applications in waste water treatment which can be extended to air-filters and supported catalysis [253]. In another study by Yang et al. [254], processing of chemically cross-linked aerogels leads to formation of porous aerogels with excellent shape recovery and superabsorbent capacity of water and dodecane. The same research group further extended the study and incorporated capacitive nanoparticles on the aerogel substrates for development of supercapacitors with excellent charging and discharging capabilities for electronic applications [255]. The fabricated hydrogel/aerogels generally have high specific surface area and porosity which is of great interest for stimuli-responsive targeted drug delivery, in the preparation of scaffolds for tissue engineering and bioseparation or filtration processes (for water purifications).

Some of the interesting and noteworthy applications of CNCs reported in last few years have opened up new avenues of research on the potential utilization of bio-based renewable materials, which suggests their promising and futuristic applications as discussed in this section. CNCs due to their strong reinforcing capability and tunable hydrogen bonding have been utilized to develop various high performance polymeric nanocomposites with chemo-responsive behaviour, self-healing capability and shape memory characteristics, controlled mechanical gradient, artificial-nacre with bio-inspired properties mimicking the biological materials. Incorporation of the chemo-responsive behaviour in nanocomposites provides an opportunity to chemically trigger the percolation behaviour of CNCs in the presence of some external stimulus due to which the structural properties can be remotely controlled [256]. Introduction of CNCs to PVA/clay solution leads synergistic toughening due to the presence

of hydrogen bonding which induces modulus ~ 20 GPa and toughness of ~ 4 MJ/m³ in nanocomposites, which is comparable to natural nacre [257]. Similarly, processing of such ternary mixtures into aerogels shows significantly high mechanical performance (elastic modulus ~ 20.6 GPa), with light weight and fire retardant behaviour [258]. In a recent study, replacement of the clay nanoplatelets with graphene sheets showed the formation of electrically conductive artificial nacre with tensile strength of ~ 320 MPa and comparatively higher toughness of ~ 10 MJ/m³ [259]. A recent study by Wang et al. [260], shows that introduction of CNCs into ultrahigh molecular weight polyethylene through compression molding, leads to improved tribological properties with reduced wear and improved biocompatibility. Processing of the CNFs into continuous filaments through wet-spinning provides an industrially viable approach of orienting CNFs in fibers which leads to improved structural properties with increased tensile strength and elastic modulus [261]. Surface modification of the CNCs with mechanophore leads to formation of self-healable polymer which can recombine through formation of covalent linkages [262]. A new innovative way of processing CNC-based polyether urethane acrylate composite was developed through 3D-printing technology, crosslinking the composite in presence of photoinitiators 2-hydroxyethyl methacrylate monomer by exposing to UV light [263]. CNCs processed through such novel techniques provides an opportunity to develop complex 3D structures. However, there remain several challenges associated with industrially viable extrusion processes which needs to be addressed. In this section as well as in the previous sections, an overview of the research activities carried out so far in areas related to CNCs has been presented, reviewing the critical issues and highlighting the techno-economic problems which need to be addressed. Some of these critical issues have been identified and addressed in the present thesis.

Chapter 2

Materials and Method

This chapter provides details of the materials, reagents and protocols followed for performing various experiments, modelling and simulations. Fabrication of cellulose nanocrystals (CNCs) from different renewable resources, different cellulose polymorphs and various acid sources as hydrolyzing agents have been discussed. Surface modification of CNCs with different iron nanoparticles to fabricate Fe-CNC, Fe₂O₃-CNC and Fe₃O₄-CNCs, followed by the precipitation of catalytically active platinum and palladium nanoparticles to fabricate Pd/Pt-Fe₂O₃-CNCs has been detailed. Preparation of graphene nanoscrolls from Fe₂O₃-CNCs and Pd/Pt-Fe₂O₃-CNCs, and fabrication of anisotropic nanocomposites of polylactic acid (PLA) and magneto-responsive CNCs has been described. Processing of the CNC-based polymeric nanocomposites through different approaches such as solution casting, melt and reactive extrusion has been reported. The analytical instrumentation used and experimental procedure followed for the characterization of aforementioned nanocomposites have also been discussed in this chapter.

2.1 MATERIALS

Poly L-lactic Acid (PLA) granules (grade: 4032D, with weight average (M_w) and number average molecular weight (M_n) ~200,000 and ~150,000 Da respectively and content of L- lactic acid/D-lactic acid: 98.6/1.4) were purchased from Nature Works® LLC., USA. Sodium hydroxide (>97%), sodium hypochlorite (4%), hydrogen peroxide (30%) and sodium sulfite (>99%) (Analytical grade) used for the extraction and purification of cellulose were purchased from Merck, India. Fabrication of CNCs using different acids such as sulphuric acid (>99%), phosphoric acid (>85%), hydrochloric acid (>35%) and nitric acid (>72%) as hydrolyzing agents were purchased from SISCO Research laboratories (Analytical Grade,

SRL Chemicals, India). The radical initiator dicumyl peroxide (DCP) and different compatibilizers such as carbon nanofibers (CarbFiber), nanosilica, alumina, montmorillonite (MMT) and cellulose used during reactive extrusion of PLA with CNCs were purchased from Sigma Aldrich, India. Rest of the compatibilizers such as potato starch (SRL, India), coconut oil and olive oil (Adani Oil, India) were used as received. For GPC and NMR analysis the reagents chloroform (Merck, HPLC grade) and deuterated chloroform (Merck, India) were used. For the fabrication of magneto-responsive CNCs, chemical required such as ammonium hydroxide (NH_4OH) (>99% purity), ferric (III) chloride (FeCl_3) (96%), ferrous (II) chloride (FeCl_2) (98%) (analytical grade) were received from SISCO Research laboratories (SRL Chemicals, India). For the fabrication of catalytically active CNCs, platinum (PtCl_2) and palladium chloride (PdCl_2) salts were purchased from Sigma Aldrich, India. Diiodomethane (Sigma Aldrich, India), and glycerol (Merck, India) were used for contact angle measurements. Ultrapure water synthesized from Milli- Q synthesis unit, Millipore[®] (TOC<10ppb; particles<0.22 $\mu\text{m}/\text{ml}$; resistivity: $\sim 18.2\text{M}\Omega\cdot\text{cm}$ at 25°C) was used as solvent for all the experiments reported in the thesis.

2.2 METHODS

2.2.1 *Fabrication of the Cellulose Nanocrystals through Different Routes and Approaches*

- **Fabrication of CNCs through Sulphuric Acid as Hydrolysing agents**

CNCs are fabricated through the traditional route of sulphuric acid based hydrolysis with the different biomass sources such as bamboo stems, rice husks and newspaper as the initial cellulosic precursor. The biomass resources contains cellulose as main ingredients in which trace quantity of hemicellulose and lignin were present as impurities. The extraction of purified cellulose was carried out through soda-pulping route followed by bleaching techniques which

remains same for all the biomass with certain modifications as mentioned below. Firstly, biomass are chopped into small pieces and washed several times with water and acetone to remove the dirt and foreign impurities. Pretreatment was carried out with 2 wt. % NaOH at 85°C for 2 hours, followed by bleaching with H₂O₂ (2 wt. %) and NaOCl (2 wt. %) at 90°C for 2 hours. The pulp was filtered out, washed with copious amount of Milli-Q water at each step three times to remove trace amount of hemicellulose and lignin and subsequently the cellulose was dried in oven at ~80°C. For the case of filter paper, as the initial cellulosic precursor, CNCs are fabricated directly through acid hydrolysis routes without requiring any pretreatments. The reaction conditions for the fabrication of CNCs through sulphuric acid based hydrolysis utilizing the purified cellulose from various sources remains unchanged as discussed below.

The purified cellulose from biomass source (~2g) and filter paper (~2g chopped) was acid hydrolyzed with 64 wt. % sulphuric acid for 2 hours under vigorous magnetic stirring (~500 rpm) at room temperature (~25°C). Subsequently, equal volume of chilled deionized water was added to stop the reaction, followed by centrifuged (Remi, India) at 10,000 rpm for 20 min and the precipitate was transferred to cellulose acetate dialysis membrane with a cutoff molecular weight 12-14 kDa (Sigma Aldrich, India). Further, the neutralized pH suspension was precipitated (after centrifugation) followed by freeze drying (at ~ -80°C) (Scanvac, Denmark) into powdered form.

- **Fabrication of CNCs through Phosphoric Acid as Hydrolysing agents**

Phosphoric acid-based CNC, represented as CNC-PO₄, was produced by hydrolyzing the chopped filter papers (~2 g) (Whatmann® filter paper (grade 1)) with phosphoric acid solution (11M, 100 ml) at a temperature of 100°C under vigorous stirring (~500 rpm) for 5 hours. The

product after hydrolysis shows a pale yellow coloration which was centrifuged and dialyzed to remove the excess acid. Finally, the CNC suspension was lyophilized into dried powder form.

- **Fabrication of CNCs through Hydrochloric Acid as Hydrolysing Agents**

Hydrochloric acid (6M, 120 ml) was used to hydrolyze the chopped filter paper (~2g) at a temperature of 120°C under magnetic stirring (~500 rpm) for 4 hours. After the reaction, excess of deionized water was added followed by centrifugation and dialysis to remove the acid till the pH of CNC suspension reached ~7. The neutralized CNC suspension was further freeze dried to obtain the dried form of CNCs.

- **Fabrication of CNCs through Nitric Acid as Hydrolysing agents**

Chopped filter paper (~2g) was reacted with nitric acid (6M, 50 ml) at a temperature of 40°C for 4 hours under magnetic stirring speed of ~500 rpm. The suspension thus obtained was centrifuged and dialyzed to remove the excess acid followed by freeze drying (as per the protocol discussed in the above section).

- **Fabrication of CNCs with different Polymorphism and Sulphuric Acid as Hydrolysing Agents.**

Fabrication of Cellulose Nanocrystal with Cellulose I polymorphism (CNC I).

The fabrication of CNC I with cellulose I polymorphism was carried out with bamboo stems as the initial cellulosic precursor following the similar pretreatment as well as acid hydrolysis route mentioned in earlier section. The purified 2g of cellulose I was hydrolyzed with sulphuric acid (64 wt. %, 100ml) under vigorous magnetic stirring for 2 hours, at room temperature (~25°C). Excess of chilled deionized water was added to stop the reaction followed by centrifugation and dialysis (until pH reached ~7) using cellulose acetate membrane (Sigma Aldrich, cut of molecular weight ~14 kDa) to remove the acid. The final CNC suspension was

lyophilized using Labconco Bench Top 2K XL freeze dryer and dried CNC I was stored in the inert atmosphere.

Fabrication of Cellulose Nanocrystal with Cellulose II polymorphism (CNC II).

The conversion of cellulose I to cellulose II was carried out in the presence of NaOH, under reflux conditions at 150 °C. Optimization studies for cellulose II was carried out by varying NaOH concentration from of 5–20 wt. %. Sequentially, the aforementioned pretreatment protocol was followed to remove residual chemicals. Purified 2g of optimized cellulose II (treated with 20% NaOH) was hydrolyzed with sulphuric acid (64 wt. %, 100ml) and subsequent steps were performed as similar to that for CNC I to achieve CNC II (mentioned in Section 2.2.1.I).

Fabrication of Cellulose Nanocrystal II from Cellulose I (CNC: I→II).

Fabrication of CNC:I→II, was carried out according to a method reported in literature [264] however, we have found a different reaction conditions where the CNC II morphology appeared entirely different than regular CNC II. In order to achieve above conditions, the pretreated pulp was fragmented into small pieces and uniformly added into 64 wt. % sulphuric acid over a time period of ~30 min. Thereafter, the reaction was carried out at 40°C for 2 hours, which led to the conversion of cellulose I to CNC II (CNC: I→II). The other processing steps after hydrolysis were the same as those used for fabrication of CNC I.

2.2.2 Fabrication of Cellulose Nanocrystals through Continuous Dialysis System

An indigenously developed CNC production unit coupled with continuous dialysis system have been fabricated to reduce the CNC processing time (as shown in Figure 2.1). The CNC suspension after the hydrolysis is directly purged into the long coiled dialysis tubing (~6 feet used in this study) to determine its efficiency in processing large volume of CNCs. The dialysis

tubing with CNC suspension in it was placed in a tub containing continuous flow of water using a submersible pump such that a constant gradient for removal the acidic protons is created. To determine robustness of developed system, CNCs are produced with different batch sizes varying from 2-40 grams, various acids as hydrolysing agents, different initial cellulosic biomass precursor (such as newspaper, bamboo pulp, filter paper and microcrystalline cellulose). The preparation methodology of CNCs from different biomass, batch sizes and acid as hydrolysing agents for this study, remains same as mentioned in Section 2.2.1.

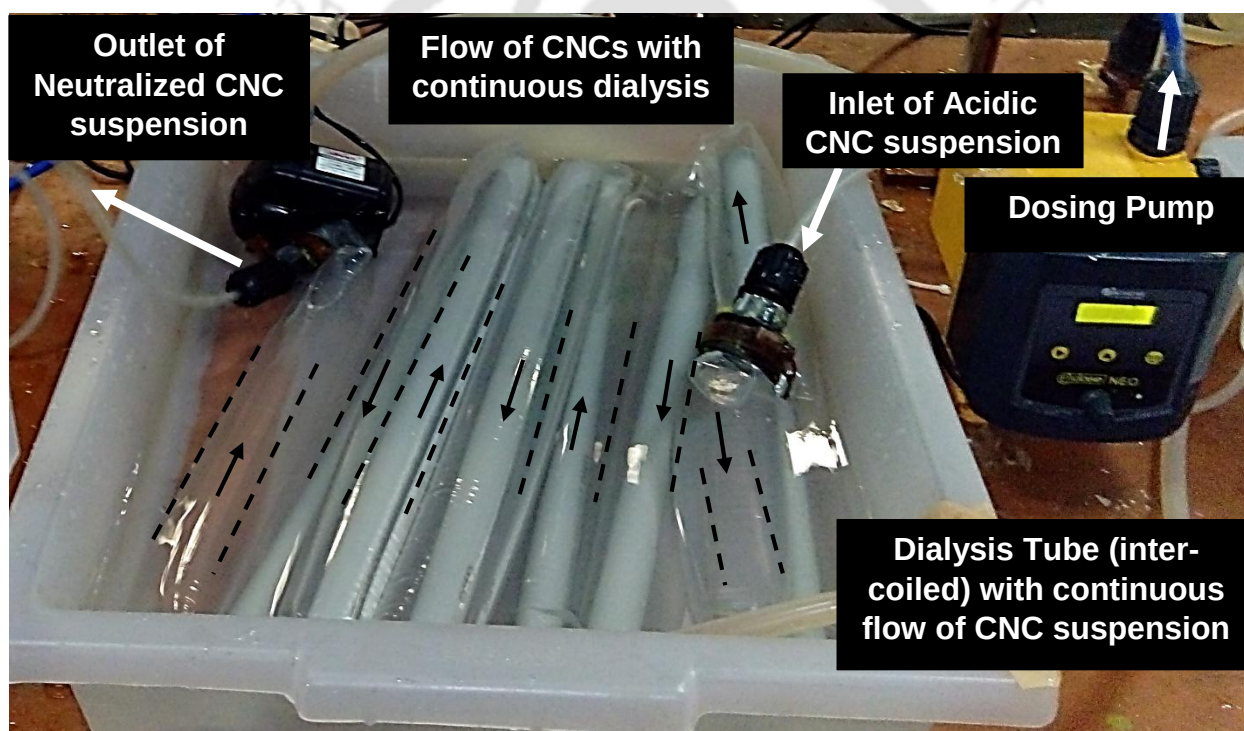


Figure 2.1: Indigenously developed CNC production unit with continuous dialysis system attached to it, showing the continuous flow of white colored CNC suspensions (marked with black arrows) along the inter-coiled dialysis tubes. The CNC suspension is pumped through the acid resistant dosing pump directly from the reaction vessel in which the acid hydrolysis have been carried out.

2.2.3 Surface modification of Cellulose Nanocrystals with Different Iron precursors

- **Fabrication of Zerovalent Iron supported Cellulose Nanocrystal (Fe-CNC)**

Synthesis of CNCs supported Zerovalent Iron (ZVI) nanoparticles (Fe-CNC) was achieved through the reduction of precursor of iron salts with CNCs. Ferric chloride (1.6g) was dissolved in 50 ml of water (Milli-Q[®]) to which 0.5 ml of NH₄OH (1%) was added to make the pH basic under inert atmosphere (by continuously purging with argon gas). 1 wt. % NaOH solution (previously purged with argon gas for 15 min) containing uniformly dispersed CNCs (30 and 70 wt. %) was immediately added dropwise to the iron solution. The system was vigorously stirred (1000 rpm) and kept at a temperature of 90°C for 4 hours wherein full reduction of the iron salt occurred. The black precipitate of Fe-CNC nanoparticles was washed with ethanol several times and further dried under vacuum overnight at 50°C.

- **Fabrication of Fe₂O₃ supported Cellulose Nanocrystal (Fe₂O₃-CNC)**

Cellulose nanocrystals were magnetized by precipitating iron nanoparticles onto its surface hydroxyl groups. FeCl₃ (2.0 g), was selected as initial iron precursor and dispersed into CNC suspension (1.0 g in 50 ml of ~1 wt. % NaOH solution) through sonication at amplitude ~30 % for 5min. The dispersed iron salt-CNC suspension was stirred for ~3 hours at 80°C purged under inert nitrogen gas flow for ~3 hours to remove the trace amount of oxygen. The precipitation of iron nanoparticles was carried out by dropwise addition of the reducing agent, sodium borohydride (~50 mg/ml) under vigorous stirring (~500 rpm) at a temperature of ~90°C. After ~6 hours, the iron salt-CNC suspension turned black suggesting the completion of reaction and was separated using permanent magnets followed by washing it with ethanol (for three times) to remove the unreacted impurities. The Fe₂O₃-CNC suspension was finally

precipitated through centrifugation and followed by its drying (at 50°C in vacuum oven) to obtain the powdered form of Fe₂O₃-CNC.

- **Fabrication of Fe₃O₄ supported Cellulose Nanocrystal (Fe₃O₄-CNC)**

The iron oxide (Fe₃O₄) nanoparticles were adsorbed onto the CNC surface (MGCNC) using co-precipitation method in presence of two different iron salts. CNC (1.80 g) was uniformly dispersed into 1 wt. % NaOH solution followed by sonication for 2 min (amplitude ~30%, bench top sonicator). Subsequently, both FeCl₂ (0.82 g) and FeCl₃ (1.90 g) salts (at a constant molar ratio of [Fe³⁺]/[Fe²⁺]=2) were dissolved into the CNC suspension, followed by vigorous stirring (~1000 rpm) at a temperature of 90°C for 4 hours. For fabrication of Fe₃O₄ nanoparticle-adsorbed CNCs (MGCNC), 7.5 ml of the NH₄OH solution was added dropwise into the iron salt-CNC solution at 85°C and stirred for 4 hours, during which the suspension turned black. The MGCNC were separated from the solution using permanent magnets and washed three times with ethanol and water to remove the impurities. Finally, the precipitated MGCNC were centrifuged out and dried in an oven at 50°C and crushed into powder using mortar.

2.2.4 Surface Modification of Magnetic Cellulose Nanocrystals with Different Catalytically Active Metallic Nanoparticles

Platinum and Palladium nanoparticles modified Fe₂O₃-CNC were fabricated through precipitation of the platinum and palladium chloride salts using sodium borohydride as reducing agent in presence of inert conditions. A homogenous dispersion of CNCs was formed by dissolving PtCl₂ and PdCl₂ (~50 mg) salts (in slightly acidic water), through sonication (for ~5 min) followed by stirring at ~500 rpm for 3 hours at 80°C which led to formation of light yellowish/ brownish coloration. The impregnated salts on the surface of Fe₂O₃-CNC were

precipitated through dropwise addition of NaBH_4 solution under vigorous stirring (~ 500 rpm) at a temperature of $\sim 90^\circ\text{C}$ for 4 hours which subsequently led to formation of grey-black coloration.

2.2.5 Fabrication of Graphene Nanoscrolls with Encapsulated Metallic Nanoparticles

- **Fabrication of Graphene and its oxidation (GRO)**

Graphene was fabricated through thermal exfoliation of the expandable graphite with the optimized conditions mentioned in our earlier reported study. Expandable graphite (~ 500 mg) was placed in a silica crucible and subjected to a temperature of $\sim 750^\circ\text{C}$ (preheated) for a period of 2 min. Due to the rapid heating and thermal shock, the intercalated compounds present in the inner layer of graphite creates a pressure which leads to exfoliation of graphite into graphene. The obtained graphene (~ 500 mg) was dispersed into methanol followed by its sonication (at an amplitude of $\sim 30\%$) for a duration of ~ 30 mins in presence of the hydrogen peroxide and ammonium peroxidosulfate. Due to the presence of strong oxidizing agents, the epoxy and hydroxyl groups are introduced onto the surface of the graphene.

- **Fabrication of Graphene nanoscrolls using Fe_2O_3 -CNCs as Templates (Fe_2O_3 -NS)**

Epoxidation of the Fe_2O_3 -CNCs was carried out through reaction with epichlorohydrin as per the earlier reported studies. The Fe_2O_3 -CNCs (~ 50 mg dispersed in ~ 20 ml of water through sonication at an amplitude of $\sim 30\%$ for 5 min) was reacted with excess epichlorohydrin (~ 5 ml), at a temperature of $\sim 85^\circ\text{C}$ for ~ 4 hours. The epichlorohydrin modified CNCs, was washed with water and ethanol (for ~ 3 times) to remove the unreacted components, followed by drying (at 50°C in vacuum oven).

Fabrication of the graphene nanoscrolls using Fe_2O_3 -CNCs as templates was carried out through reaction of epoxidized Fe_2O_3 -CNCs with partially oxidized GRO, which leads to

covalent coupling of the Fe_2O_3 -CNCs with GRO. After the fabrication of nanoscrolls samples were washed with ethanol (~ 3 times) and then subjected to calcination in muffle furnace (placed in a silica crucible) at a temperature program of: 200°C for 2 hours, 400°C for 2 hours and 600°C for 15 mins. The fabricated samples are designated as Fe_2O_3 -NS, with the incorporated iron nanoparticles in the graphene nanoscrolls.

- **Fabrication of Graphene Nanoscrolls using Pt- Fe_2O_3 -CNCs and Pd- Fe_2O_3 -CNCs as Templates**

Pt- Fe_2O_3 -CNCs and Pd- Fe_2O_3 -CNCs ($\sim 20\text{mg}$ each in 20ml of water) were dispersed into water through sonication as mentioned earlier followed by its epoxidation through reaction with epichlorohydrin (as mentioned in case of Fe_2O_3 -CNCs) for ~ 6 hours at a temperature of $\sim 85^\circ\text{C}$. The modified Pt- Fe_2O_3 -CNCs and Pd- Fe_2O_3 -CNCs were washed with water and ethanol through centrifugation for several times to eliminate the trace quantities of unreacted components.

The epoxidized Pt- Fe_2O_3 -CNCs and Pd- Fe_2O_3 -CNCs ($\sim 50\text{ mg}$ dispersed in methanol) was reacted with partially oxidized GRO ($\sim 50\text{ mg}$ dispersed in methanol) at a temperature of 80°C under reflux conditions for about ~ 6 hours. After the reaction for the nanoscrolls formation is complete, the samples were centrifuged to obtain the precipitate followed by its washing (with ethanol) and drying. The obtained dried samples were placed on a silica crucible and calcined in a muffle furnace to remove the CNCs from its inner core following the temperature program: 200°C for 2 hours, 400°C for 2 hours and 600°C for 15 mins. The fabricated samples are designated as Pt- Fe_2O_3 -NS and Pd- Fe_2O_3 - NS, with the incorporated Pt and Pd nanoparticles in the graphene.

2.2.6 Fabrication of Poly (3-hydroxybutyrate)/CNC Nanobiocomposites

PHB (2g) was dissolved in chloroform (50ml) at 90°C and stirred magnetically at ~500 rpm under reflux conditions for 2 hrs. CNCs (1–5 wt. %) fabricated from bamboo pulp were solvent exchanged from water to acetone and subsequently to chloroform by centrifugation at each step at 10000 rpm. The resultant CNC suspension in chloroform was finally added to PHB solution dissolved in chloroform under strong mechanical stirring at 50°C for 20 min. PHB/CNC bio- composite films (1–5 wt. %) having thickness of $\sim 50 \pm 5 \mu\text{m}$ were fabricated on Teflon petri dishes by using solution casting method.

2.2.7 Fabrication of Poly (L-lactic Acid)/CNC Nanobiocomposites Films

- **Processing of PLA/CNC Films through Solution Casting**

All the three CNC polymorphs were solvent exchanged in a stepwise manner from water to acetone and subsequently to chloroform solution. Dispersion of CNC in water was centrifuged at 10000 rpm for 10 min to remove the excess water and obtain the CNC precipitate. Similarly, following the same protocol, the precipitate was dispersed in acetone, centrifuged and finally dispersed in chloroform. The milky white dispersion of CNCs in chloroform was sonicated (Bath Sonicator) for 5 min, to obtain a disperse suspension. Thereafter, CNC dispersed in chloroform was added into 2 gm PLA solution (50 ml) in chloroform. The films were solution cast on circular Teflon petri plates and dried under fume hood for 1 day. All the films were further dried in hot air oven at 40°C under vacuum (to remove the trapped solvents) for 24 hours and stored in desiccator. The average film thickness (l) was measured at 25 positions on the film using a digital thickness meter (Model- 6156, Indi, India). PLA films with different polymorphs and weight fractions of CNCs were

prepared and designated as PLA/CNC I (1 wt. %, 2 wt. % and 3 wt. %), PLA/CNC II (1 wt. %, 2 wt. % and 3 wt. %) and PLA/ CNC: I→II (1 wt. %, 2 wt. % and 3 wt. %).

- **Processing of PLA/CNC Films through Melt Extrusion**

The PLA granules (~10 g) and freeze dried CNCs from four different acids were pre-mixed at three different weight ratios 1, 2 and 3 wt. % followed by vacuum drying at 40°C (for ~24 hours) to remove the adsorbed moisture. Nanocomposites of PLA with different acid-hydrolyzed CNCs were extruded into strips (dimensions ~ 5 mm (width) × 0.5 mm (thickness)) in a twin screw extruder (Haake Rheomix) at a processing temperature of 180°C, screw speed of ~50 rpm and recycle time of ~2 minutes. Hereafter, the extruded strips with the CNCs produced from sulphuric, phosphoric, nitric and hydrochloric acid over the loading range of 1–3 wt. % are represented as PLA/CNC-SO₄1p-3p, PLA/CNC-PO₄1p-3p, PLA/CNC- NO₃1p- 3p and PLA/CNC-Cl1p-3p respectively.

- **Processing of PLA/CNC Films and its Recycling through Reactive Extrusion in Lab Scale Extruder**

PLA granules and freeze dried CNCs were vacuum dried at 40°C overnight to remove the adsorbed moisture. DCP, the radical initiator (1.2 wt. %) used in this study, was dissolved in acetone (10 ml) and sprayed on PLA beads. The PLA beads were further dried in vacuum at 40°C to remove the excess acetone before processing. The DCP coated PLA granules (10 g) were mixed with CNC (at different ratios 1–3wt. %) in a beaker and extruded in a twin screw extruder (Haake Rheomix). The extrusion parameters, i.e. screw speed and processing temperature were maintained at 50 rpm and 180°C respectively with a recycle time of 5minutes. The PLA grafted CNC (PLA- *g*- CNC) nanocomposites were extruded into strips of dimension ~5mm (width) × 0.5mm (thickness). Hereafter, the PLA-*g*-CNC nanocomposites

extruded in the presence of DCP and with various loadings of CNCs (1–3wt. %) are represented as PLAD, PLADCNC1, PLADCNC2 and PLADCNC3, respectively.

The vacuum dried PLA-g-CNC gels were chopped into small strips and were further reprocessed through the extruder for all CNC weight fractions. The parameters for extrusion were kept similar to the first processing, i.e. Screw speed at 50 rpm, extrusion temperature of 180°C and recycle time of 5 min. The dimension and drawing speed of the extruded strips were also kept constant. Hereafter, the reprocessed PLA-g-CNC samples for all weight percent of CNCs (1– 3wt. %) are represented as rPLA, rPLACNC1(1.35), rPLACNC2(3.33) and rPLACNC3(5.15) with the numbers in bracket representing the probable CNC wt. % present in the samples after the ungrafted PLA have been removed (calculated using eq. (2.14)).

- **Processing of PLA/CNC films through reactive extrusion in pilot-plant scale extruder**

Different acid derived CNCs as compatibilizers

The granules of PLA and freeze dried CNCs (both CNC-SO₄ and CNC-Cl) were placed in a vacuum oven for 24 hours at a temperature of 40°C. DCP (1wt. % dissolved in acetone) was sprayed over the PLA granules and dried in vacuum oven to remove the solvent. The PLA granules coated with DCP (~5 kg) were mixed with 1 wt.% and 2 wt. % loadings of each types of CNCs, derived from sulphuric and hydrochloric acid (CNC-SO₄ and CNC-Cl respectively). Thereafter, the PLA/CNC nanocomposite films were fabricated in a pilot plant twin screw extruder-cum-sheet casting unit (Boolani Engineering, India) (with length/diameter (L/D) ratio =48) at ~200rpm which has five temperature zones maintained at T₁=185°C, T₂=185°C, T₃=190°C, T₄=195°C and T₅=200°C respectively (with slit die width~20 cm) and residence time of ~3minutes. Reactively extruded PLA grafted CNCs (PLA-g-CNC) based nanocomposite films fabricated in presence of DCP with various acid derived CNCs are

represented as PLA/DCNC-S-1p (with 1wt. % loadings of CNC-SO₄), PLA/DCNC-S-2p (with 2wt. % loadings of CNC-SO₄), PLA/DCNC-Cl-1p (with 1wt. % loadings of CNC-Cl) and PLA/DCNC-Cl-2p (with 2wt. % loadings of CNC-Cl).

Biopolymers as compatibilizers

The extrusion process and parameters were kept fixed, as mentioned in the previous section, where the extrusion of the DCP coated PLA beads was carried out with CNC-SO₄ (~ 1wt. % loading) but with two different biopolymers namely cellulose and starch added as compatibilizer (~1wt. % loading) in this case. The fabricated nanocomposites with the biopolymer compatibilizer are represented as PLA/DCNC-S/Cellulose (with ~1wt. % cellulose) and PLA/DCNC-S/Starch (with ~1wt. % starch).

Inorganic/Organic Nanofillers as compatibilizers

Different inorganic/organic nanofillers such as MMT, nanosilica, alumina and CNFs were used as compatibilizers at 1 wt. % loading and extruded with the DCP coated PLA pellets and CNC- SO₄ (1wt. %) at conditions mentioned in previous section. The nanocomposites with inorganic/organic compatibilizers were represented as PLA/DCNC-S/MMT (with ~1wt. % MMT), PLA/DCNC-S/Nanosilica (with ~1wt. % nanosilica), PLA/DCNC-S/Alumina (with ~1wt. % Alumina) and PLA/DCNC-S/CarbFiber (with~1wt. % CarbFiber loadings).

Vegetable Oils as compatibilizers

Different saturated and unsaturated vegetable oils such as coconut oil (~90% saturated) and olive oil (~86% unsaturated) were mixed with the DCP coated PLA beads and CNC-SO₄ at 1 wt. % loadings, followed by extrusion under similar conditions specified for different acid CNCs. Hereafter the nanocomposites with the coconut oil are marked as PLA/DCNC-S/Coco and with olive oil as PLA/DCNC-S/Olive respectively.

2.2.8 Fabrication of Polylactic Acid/Magnetic Cellulose Nanocrystals Composite Films

- **Anisotropic Films through Directional Orientation of MGCNCs by Magnetic Field**

The fabricated MGCNC dried powder was dispersed into the chloroform solution (~10 ml) at different loading percentages of 1, 2 and 3 wt. % using bath sonicator for ~10 min. PLA granules (2g) were dissolved in chloroform solution at ambient conditions, further mixed with the MGCNC- chloroform suspension and stirred for 2 hours to obtain a uniform dispersion. The magnetic alignment of the MGCNCs in the PLA matrix was obtained through directionally tunable electromagnets (EMU-75, SES Instrument, India). The N and S cylindrical poles of the electromagnet were fixed at a distance 10 cm apart (both in the parallel and perpendicular conditions), the center of which contains the Teflon plate for film fabrication. The PLA/MGCNC suspensions of different compositions (1, 2 and 3 wt.%) in chloroform were poured on the teflon plates in between the magnetic poles and allowed to dry under ambient conditions (~12 hours) in the presence of continuous magnetic field (~60 mT). The films were peeled off and dried in vacuum oven (to remove the trapped solvents) for 24 hours at 40°C and stored in the desiccators. The fabricated PLA films with different compositions were designated as per their directional alignment in the presence of magnetic field: PMGCNC1p $\parallel$, PMGCNC2p $\parallel$ and PMGCNC3p $\parallel$ in the case of parallel alignment, PMGCNC1p $\perp$, PMGCNC2p $\perp$ and PMGCNC3p $\perp$ in the case of perpendicular alignment and PMGCNC1p uno, PMGCNC2p uno and PMGCNC3p uno in the absence of any magnetic field.

- **Lamination of Anisotropic PLA/MGCNC Nanocomposites**

Two anisotropic PLA/MGCNC nanocomposites films were laminated under a shear force of two rotating cylinders moving at a speed of 1mm/s and maintained at a temperature of

~140°C. The films were passed through three rolling cycles to obtain uniform stretched laminated films. For fabrication of laminated films, a white piece of paper was sandwiched in between two PMGCNC || films and was processed under same conditions as specified above.

2.3 ANALYTICAL INSTRUMENTATION AND CHARACTERIZATION

2.3.1 X-ray Diffraction (XRD)

XRD studies were carried out using D8 Advance diffractometer (Bruker, Germany) equipped with Cu-K α radiation ($\lambda = 0.1541$ nm) as X-ray source operating (40 kV, 40 mA) at a scan rate of 0.05° per 0.5 s in the 2 θ range 10–80°. The crystallinity index (C.I.) and degree of crystallinity were determined using the Reflex module in the Materials Studio software. The program deconvoluted the XRD data to remove the background noise and calculated the degree of crystallinity from the difference in area of crystalline peak to amorphous fractions.

2.3.2 Fourier Transform Infrared Spectroscopy (FTIR)

The powdered samples (pelletized with KBr) were investigated in diffusive reflectance spectroscopy (DRS) mode using dried KBr as background. The polymeric films were scanned in attenuated total reflectance (ATR) mode using Perkin Elmer IR spectrometer in the spectral range of 400-4000cm⁻¹ and at a scan rate of 4 scans/sec. The hydrogen bond fraction (F_{H-CO}) [265], which represents the degree of the hydrogen bonding between the PLA matrix and the CNCs, was calculated using the following equation:

$$F_{H-CO} = \frac{A_H/r_{H/a}}{(\frac{A_H}{r_H} + A_a)} \quad (2.1)$$

Where A_a And A_H Are the areas of the peaks at ~1746 and 1712cm⁻¹ corresponding to the free and hydrogen bonded carbonyl groups of PLA respectively, which are deconvoluted using

Gauss/Lorentz functions. The specific absorption ratio, $r_{H/a}$, calculated from the absorption ratio of the crystalline to amorphous peaks of polymers, was found to be 1.32 for PLA [266].

2.3.3 Gel Permeation Chromatography (GPC)

The molecular weight of the polymer nanocomposites samples were determined using high performance liquid chromatography (HPLC), Shimadzu LC-20A system (Shimadzu, Japan) equipped with two PL gel 5 μ m mixed D columns (Agilent, UK) in series which were calibrated using polystyrene standards in chloroform. The extruded samples (~20mg) were dissolved in HPLC grade chloroform for 3 days and filtered with 0.2 μ m filter paper before analysis. For the case of the reactively extruded PLA/CNC nanocomposites the measured molecular weight distribution (MWD) for the cross-linked/ branched PLA is not exact because of the change in the hydrodynamic volume; however, significant amount of information could be obtained from this study.

2.3.4 Nuclear Magnetic Resonance (NMR) Spectroscopy

The chemical structures of PLA and reactively grafted PLA/CNC films were studied with 600MHz nuclear magnetic resonance (NMR) spectrometer (Bruker, Germany). The samples were dissolved in deuterated chloroform (CDCl₃) for 3 days and filtered with 0.25 μ m filter (3 times) before analysis.

2.3.5 Raman Spectroscopy

Raman spectra were measured using Horiba Jobin Vyon (labram HR, Japan) equipped with a 1W, 1064 nm Nd:YAG diode-pumped laser at an excitation wavelength of 514 nm, and 1024 scans were accumulated with the use of a double-sided forward-backward scanning mode. The finely grinded powdered sample were placed on a glass slide and spectra were collected at different locations viewed through an attached microscope.

2.3.6 Ultraviolet–visible spectroscopy

The transparency of the polymeric nanocomposites, dye degradation and catalytic activity studies were carried out with UV-Vis spectrophotometer (Perkin Elmer, USA) in the wavelength range of 200-800 nm. For the case of dye degradation and catalytic activity studies, the calibration curve was prepared ($R^2 \sim 0.999$) to measure the analytes concentrations and transparency measurements were carried out with film of dimensions 30 mm x 10 mm.

2.3.7 Differential Scanning Calorimetry (DSC)

The thermal properties of the films were characterized with (Mettler Toledo, Switzerland) under inert nitrogen gas flow (~ 60 ml/min). DSC studies were carried out at a scanning rate of $10^\circ\text{C}/\text{min}$ with temperature varied from 25°C to 190°C for both heating and cooling cycles. The calibration was carried out using Indium standard beforehand and samples weighing ~ 5 - 6 mg were placed on a platinum crucible for analysis.

For the isothermal crystallization study, the samples were heated from 25 to 200°C at a heating rate of $50^\circ\text{C}/\text{min}$ and kept for 2 min to remove the thermal history completely (Netzsch DSC (Germany)). Thereafter, the samples were rapidly cooled down to the respective crystallization temperatures of 110 , 120 and 130°C at a scan rate of $50^\circ\text{C}/\text{min}$ and kept under isothermal conditions for 60 min.

2.3.8 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) (STA449F3A00, Netzsch) for the nanocomposites (5 mg sample weight) was performed under nitrogen atmosphere (at a flow rate of 250 ml/min) from 25°C to 600°C at a heating rate of $10^\circ\text{C}/\text{min}$.

Thermal properties of CNCs fabricated from different acids and their PLA based nanocomposites (~ 2 - 3 mg) were measured with thermogravimetric analyzer (TGA) (Perkin

Elmer STA) in the temperature range of 25–600°C at a heating rate of 10°C/min (under an inert atmosphere of nitrogen gas). The activation energy (E_a) for thermal degradation of the four different acid-derived CNCs have been measured from the differential thermogravimetric plots from the following equation[267]:

$$\ln(1 - \alpha)^{-1} = \frac{E_a}{RT_s^2} \frac{100}{T_f - T_i} \Theta + C \quad (2.2)$$

where, T_i , T_f And α represent the initial, final decomposition temperature and mass fraction of CNCs degraded at a particular temperature T (from DTG curves) respectively. Θ symbolizes the difference of the decomposition temperature (T) and reference temperature (T_s) respectively. The activation energies (E_a) of the CNC samples were measured from the slope of the plot between the reciprocal of $\ln(\ln(1 - \alpha)^{-1})$ and the temperature difference ($T - T_s$).

2.3.9 Universal Testing Machine (UTM)

The mechanical studies of the extruded samples (specimen size: 50mm x 5 mm (length x width)) were carried out following the standard protocol ASTM D 882, with the universal testing machine (UTM) (Kalpak Instruments, India) equipped with a load cell of 500N at a constant speed of 10mm/min.

Mechanical tests for the polyhydroxybutyrate-cellulose nanocrystal (PHB-CNC) films were carried out with the Micro Tensile Test Analyzer (Perkin Elmer, Germany), equipped with a load cell of 5kN at a constant speed of 1mm/min under standard conditions following ASTM D 882 for thin films.

2.3.10 Dynamic Mechanical Analyzer (DMA)

The thermo-mechanical analysis of the extruded strips was carried out with DMA242 (Netzsch, Germany) in tensile mode under a dynamic force of 2N, amplitude of 20 μ m at a frequency of 1 Hz. Samples were cut into 5 mm x 5 mm x 0.5 mm strips and measurements were carried out in the temperature range of 25-90°C at a heating rate of 2°C/min.

2.3.11 Rheological Analysis

Rheometer (Anton Paar, model: Physica MCR 301) with parallel plate geometry (50 mm diameter) was used to study the rheological behavior of the nanocomposite films at 180 °C. The gap between the plates was fixed at 0.1 mm and a constant temperature of 180°C was maintained for 10 min, to remove the thermal history of polymer melts before the start of the analysis. Care was taken to ensure uniform dispersion of polymer melt between the plates without any trapped air bubbles. Dynamic strain sweep measurements were carried out first to determine the linear and non-linear viscoelastic region of polymer melt. Subsequently, small amplitude oscillatory shear (SAOS) measurements in the linear region were carried out at a strain of 1% and large amplitude oscillatory shear (LAOS) studies in the non-linear region were carried at 50% strain with angular frequency variation from 0.01 to 10 rad/s. For the measurement of rheological properties such as normal stress differences (ΔN) were carried out using the MCR 101 Anton Paar Rheometer attached with the 25mm parallel plate geometry and data processed with the software Anton Paar Rheocompass 1.16.

2.3.12 Optical Polarimetry

The specific and optical rotation of samples (20 mg dissolved in 20ml chloroform) were measured with AUTOPOL II (Rudolph Research Laboratory, USA) at a wavelength of 589 nm using self-calibrated mechanism.

2.3.13 Contact Angle (CA)

The contact angle of the pelletized CNCs fabricated from different acids were measured using Kruss GMBH DSA25 (Germany). The freeze dried powder (~200 mg) of the different acid-derived CNCs was pressed into pellets under the application of ~20 MPa pressure inside an KBr press followed by vacuum drying of pellets at 50°C for 6 hours. Three different liquids namely water (Millipore®), diiodomethane and glycerol were used for contact angle measurements during which a sessile drop of liquid (~2 µl) was placed on the pellets and the geometry of the droplet was evaluated with CCD camera (measurements were taken after the droplet had stabilized and no significant changes in contact angle values were observed in consecutive images) at a temperature of 25°C. The contact angle measurements are repeated at least three times (for each measurement a new spot was selected) and the average values are reported.

2.3.14 Polarizing Optical Microscopy (POM)

The growth of the PLA spherulites at the respective crystallization temperatures (T_{cc} ~110, 120 and 130°C) was visually observed using Nikon microscope (fitted with cross-polarizers and CCD camera). The samples were placed in between the glass slides and rapidly heated to 200°C at a heating rate of 50°C/min (using Linkam Hot stage, UK) followed by a holding time of ~2 min to remove the thermal history. Thereafter, the samples were rapidly cooled down to the crystallization temperature at a cooling rate of 50°C/min (using liquid nitrogen) and maintained under isothermal conditions until the crystallization process was complete. The dimensions of the PLA spherulites at different time intervals were measured from the captured POM images using the Image J software.

2.3.15 Atomic Force Microscopy (AFM)

The surface topography and morphology of CNCs were assessed using AFM (Agilent, Model 5500 series) with silicon cantilever having a spring constant of 42N/m at a resonance frequency of 320 kHz. The samples were prepared by drop casting CNC suspension (0.01 wt. %) on cleaned microscopic slides (1 cm × 1 cm) and dried under vacuum. AFM images and 3D morphologies were analyzed with the WSxM software (version 12.0).

2.3.16 Field Emission Scanning Electron Microscope-Energy Dispersive X-Ray Spectroscopy (FESEM-EDX)

To examine the topography, samples were initially dispersed in water through sonication followed by drop casting on carbon tape covered with aluminum foil and drying under vacuum overnight. For studies on the fractured samples of polymeric nanocomposites, tensile samples after mechanical tests were dipped in liquid nitrogen immediately and placed on a stub with carbon tape. The samples were further coated in gold sputtering unit for 30s and characterized using FESEM (Sigma, Zeiss) at accelerating voltage of 2-5 kV.

The composition analysis and mapping of the nanocomposites was carried out using energy dispersive X-ray analysis (EDX) (Oxford Instruments, UK) at an accelerating voltage of 20 kV and analyzed using Aztec software. For the anisotropically oriented PLA/MGCNC samples, the order parameter (S) was determined from the FESEM micrographs of the PLA/MGCNC nanocomposites which were transformed using two-dimensional Fourier transform (2D-FFT) algorithm using Image J software. Thereafter, the power spectral density (PSD) function was used on a wedge shaped region (at ~1°) of the 2D-FFT images (using Gwyddion software) to predict 'S', similar protocol was reported elsewhere [268].

2.3.17 Transmission Electron Microscopy (TEM)

Morphological investigation and characterization of the samples were carried out using TEM and selected area electron diffraction (SAED). The samples were uniformly dispersed through sonication (~4-5 hours) in water (at a concentration of ~0.01 wt. %) followed by drop casting onto the carbon-coated grids (Tedpell, USA) and vacuum dried overnight at 60°C. Images were acquired using JEOL JEM 2100 TEM at an accelerating voltage of 200 kV, and dimensions of the synthesized nanoparticles were determined (using Image J software) and reported as average mean values.

2.3.18 Oxygen and Water Vapor Barrier Properties

The oxygen gas transmission rate (OTR) for the nanocomposite films were recorded using an OX2/231 oxygen permeability tester (Labthink International Inc., Medford, Massachusetts, U.S.A.). OTR measurements were carried out at 23 °C on circular films having an area of 50 cm² following the ASTM D3985 standard at 0% relative humidity using oxygen gas of high purity (>99.999%). The samples were initially conditioned in diffusion cell by purging with nitrogen gas (~99.999% purity) for a minimum of 24 h under equilibrium humidity. During test, pure oxygen at pressure 0.5 bar and a flow rate of 20ml/min was introduced into the upper half of the sample chamber while nitrogen gas was injected into the lower half of the chamber. The test was performed for at least 4h so as to reach steady state. The temperature of diffusion chamber was varied from 15 °C to 45 °C to study effect of operating temperature on oxygen permeability of PHB/CNC nanocomposites. Half time ($t_{1/2}$), measured as time at which OTR values reached 50% of steady state value, was used to estimate diffusion coefficient (D)[269]. The relationship between diffusion coefficient (D) and halftime ($t_{1/2}$) was derived by normalizing Fick's second law,

$$OTR(t) = \frac{Pp}{l} \left[1 + 2 \sum_{n=1}^{\infty} (-1)^n \exp\left(-\frac{D\pi^2 n^2 t}{l^2}\right) \right] \quad (2.3)$$

$$D = \frac{l^2}{7.199 t_{1/2}} \quad (2.4)$$

P is permeability, p is oxygen partial pressure and l is film thickness. The solubility coefficient (S) is calculated from following equation:

$$P = D \times S \quad (2.5)$$

Water vapor transmission rate (WVTR) measurements were performed using a PERMATRAN-W[®] Model 1/50 (Mocon, USA) following ASTM standard E398-03. The relative humidity (RH) was set to 100% in the wet chamber and 5% in the dry chamber, yielding a driving force of 95% RH. The circular film having an area of 50 cm² was tested in the chamber at atmospheric pressure and at a temperature of 37.8 ± 0.1 °C. Tests were carried out until ten successive readings deviated less than 5% from the average value for each sample, which was then taken as the WVTR value. The oxygen and water vapor permeability coefficients (P) were calculated as,

$$P = \frac{OTR/WVTR(t)}{\Delta p} \times l \quad (2.6)$$

where P is the permeability coefficient for oxygen or water vapor, $OTR/WVTR(t)$ is the oxygen gas transmission rate or water vapor transmission rate, l is the average thickness of the film and Δp is the difference in partial pressure of the oxygen gas or water vapor across the film in the test chamber.

2.3.19 Shelf-Life Predictions and Migration Studies

The shelf-life (θ) of reactively extruded PLA/CNC films were determined for storage of edible oils and dairy based beverage products from the oxygen permeability (OP) values and

by correlating it with the rate of lipid oxidation and changes in propanal or hexanol concentrations respectively using the following mathematical models:

$$\theta = (O_2(max)/k_{O_2}p_{O_2}) \quad (2.7)$$

where $O_2(max)$ Is the maximum concentration of O_2 which will react with food items that will lead to deterioration in its quality and k_{O_2} Is the oxidation rate constant for the dairy based beverages and edible oil products, which are simulated from the models present in literatures [270,271]. The p_{O_2} Is calculated using the following equation:

$$p_{O_2} = \left(0.21 * \left(\frac{OP}{d}\right) * A/k_{O_2} + \left(\frac{OP}{d}\right) A\right) \quad (2.8)$$

where OP is the oxygen permeability of the film, d and A are the thickness and area of the films respectively.

Overall migration tests were performed on the fabricated CNC based polymeric nanocomposites films (area equivalent to 1 dm² contact area per 100ml of simulant) in the presence of three liquid food simulants: ethanol 15% (v/v) (simulant A) and acetic acid 3% (v/v) (simulant B) and water (simulant C) according to the Commission Regulation EU No 10/2011 [272]. Rectangular strips (~10cm²) immersed in different food simulants were kept in a controlled chamber at 40 °C for 10 days, which were taken out at time intervals of 6, 12, 24 hours, 2 days , 3 days and 10 days, respectively according to EN-1186 standard. After the incubation period, the films were removed and simulants were evaporated to dryness. Further, the residue was weighed with a Kern[®] analytical balance with ± 0.01 mg precision in order to determine the overall migration values in mg/dm² of simulant. For each sample two

measurements were performed and the final migration value reported was the average of two measurements.

2.3.20 Vibrating Sample Magnetometer

The magnetic properties of the samples were characterized at room temperature with vibrating sample magnetometer (VSM) (Lakeshore, Model: 7410 series). The powdered samples weighing ~10 mg were wrapped with Teflon tape and the PLA/MGCNC films were cut in dimensions of 10mm × 5mm strips and placed onto the VSM sample holder before analysis.

2.3.21 Surface Area Measurements.

The Brunauer–Emmett–Teller (BET) surface area of the samples was estimated from N₂ isotherms at 77 K using a Beckman Coulter surface area analyzer (Coulter SA 3100, USA). Prior to nitrogen physisorption, all samples were degassed at 393 K for 2 hours.

2.3.22 Impedance Analyzer

The electrical conductivity of the PLA/MGCNC films (50mm × 20mm × 0.1mm) was measured using two-probe electrochemical impedance analyzer (Autolab, Netherlands) in the frequency range of 0.1Hz–1MHz, under an AC voltage of 0.1 V. The magnitude of AC conductivity (σ) was calculated from the measured real (Z') and imaginary (Z'') impedance values using the following equation:

$$\sigma = \left(1/\sqrt{Z'^2 + Z''^2}\right) * \left(\frac{L}{A}\right) \quad (2.9)$$

where L is the path length of the film along the measurement direction and A is the electrode cross-sectional area.

2.3.23 Hydrogen Adsorption Study

Hydrogen uptake by Pd-Fe₂O₃-NS and Pd-Fe₂O₃-NS with encapsulated Pd or Pt nanoparticles is measured over a wide range of pressures using temperature programmed volumetric sorption apparatus (Model: Isorb-HP, Make: Quantachrome). Prior to analysis the samples were initially outgassed at 150°C for 30 min in the presence of inert gas flow. Subsequently, the hydrogen was allowed to adsorb on the samples at a constant temperature of ~273 K with increasing pressure from 0 to 20 bar. Similarly, desorption of absorbed hydrogen was allowed to occur with declining pressure at a constant temperature.

2.3.23 Zetapotential Studies

The zeta potential of CNC suspension (dispersed in various solvents through sonication approach ~10 min) was measured with Delsa[™] Nano C (Beckman Coulter).

2.4 FILMS PROPERTY MEASUREMENT AND CALCULATIONS

2.4.1 Calculation of the Grafting Parameters

The extruded strips (processed in the presence of DCP and different compatibilizers) were washed in excess chloroform for 24 hours to remove the unreacted DCP and non-grafted PLA. The suspension was vacuum filtered (filter paper of pore size ~ 450µm) and the grafted PLA/CNC gel was washed with excess chloroform several times to remove the ungrafted PLA several times and the gel left over after filtration was collected. The gel was dried in vacuum (at ~50°C) to remove the trapped chloroform and the gel yield (%) was calculated using the following equation,

$$gel \% = \frac{W_{gel}}{W_i} \times 100 \quad (2.10)$$

where W_{gel} And W_i Are the mass of dry gel after vacuum drying and initial mass of extruded strips before dissolving in chloroform, respectively.

Several other grafting parameters such as graft percentage (%GP), grafting efficiency (%GE) and weight conversion (%WC) were also calculated as reported in the literature [273]. Graft percentage represents the mass of the grafted PLA with respect to initial mass of CNCs, as defined in equation,

$$\%GP = \frac{(W_{gel}-W_{CNC})}{W_{CNC}} \times 100 \text{ or } \%GP = \frac{[W_{gel}-(W_{CNC}+W_{Comp})]}{(W_{CNC}+W_{Comp})} \times 100 \quad (2.11)$$

Graft efficiency (GE), measures the mass of PLA grafted onto the CNCs and is represented as in equation,

$$\%GE = \frac{(W_{gel}-W_{CNC})}{W_{PLA}} \times 100 \text{ or } \%GE = \frac{[W_{gel}-(W_{CNC}+W_{Comp})]}{W_{PLA}} \times 100 \quad (2.12)$$

Weight conversion (WC) measures the percentage of the CNC grafted and is defined in following equation,

$$\%WC = \frac{W_{gel}}{W_{PLA}} \times 100 \quad (2.13)$$

where W_{gel} , W_{PLA} , W_{CNC} And W_{Comp} Represent the mass of grafted PLA/CNC gels, PLA, CNC and the compatibilizers respectively.

After washing the PLA-g-CNC nanocomposites with chloroform to remove the ungrafted PLA chains, the change in the wt. % of the CNC in the reprocessed PLA-g-CNC (rPLACNC) nanocomposites are calculated using the following equation,

$$\%CNC \text{ in rPLACNC nanocomposites} = \frac{W_{CNC}}{W_{gel}} \times 100 = \frac{GE}{GP} \times \frac{100}{WC} \times 100 \quad (2.14)$$

2.4.2 Film Property Measurement

The micro-structural properties such as necking and inhomogeneity index (%) of the films extruded through reactive extrusion using different compatibilizers and traditional approach

were calculated to understand the effect of the branching and cross-linking, using following equations,

$$\text{Necking ratio} = \frac{\text{Width of the film after extrusion}}{\text{Width of the die head of extruder}} \quad (2.15)$$

$$\text{Inhomogeneity Index} = \frac{\text{Standard deviation of film thickness}}{\text{Average of film thickness}} \times 100 \quad (2.16)$$

The width and thickness (Indi 6156, India) of the films were measured at approximately ~25 different locations of the extruded films and averaged for the calculation of necking and inhomogeneity index values.

2.5 MODELLING AND SIMULATION STUDIES.

2.5.1 Mechanical Modelling

CNCs have an exceptional reinforcing efficiency due to the formation of percolated network when they are pooled with the polymeric matrix. The volume fraction of CNCs responsible for the formation of percolating network under which it actively transfers its stress to the polymer matrix is predicted from Ouali model [274]. It is a modified version of the series-parallel model proposed by Takayanagi [275], which calculates the moduli of the polymer composites considering three phases: matrix, percolating network and non-percolating filler phases. The critical fraction of the CNCs responsible for formation of such percolation network (ϕ_c) in the polymeric matrix, is calculated theoretically as,

$$\phi_c = \frac{0.7}{\left(\frac{L}{w}\right)} \quad (2.17)$$

The elastic modulus of the composite (E_c) is calculated by

$$E_c = \frac{(1 - 2\phi\psi)E_m E_f + (1 - \phi)\psi E_f^2}{(1 - \phi)E_f + (\psi - \phi)E_m} \quad (2.18)$$

$$\psi = 0 \quad \phi \leq \phi_c$$

$$\psi = \phi \left(\frac{\phi - \phi_c}{1 - \phi_c} \right)^b \quad \phi > \phi_c$$

where the subscripts f , m and c refer to the filler, polymer matrix PHases and composite respectively. Φ is the fiber volume fraction, E_m , E_f and E_c Are the moduli of the polymer, fiber and the composite and ψ is the volume fraction of the percolating filler network and $P = \frac{L}{w}$ is the aspect ratio of CNCs.

Micro-mechanical model ‘Halpin-Kardos’ model [276] based on the mean field theory and ‘Cox– Krenchel’ model [277] based on classical shear lag theory were used for prediction of the elastic moduli of the composites. Halpin Kardos is a semi empirical model for short-fiber and particulate-reinforced composites, where it is assumed that each fiber is engulfed by the polymer matrix,

$$E_{\parallel} = E_m \frac{1 + \eta_{\parallel} \phi_3}{1 - \eta_{\parallel} \phi} \quad (2.19)$$

$$E_{\perp} = E_m \frac{1 + 2\eta_{\perp} \phi_3}{1 - \eta_{\perp} \phi} \quad (2.20)$$

where

$$\eta_{\parallel} = \frac{\frac{E_f}{E_m} - 1}{\frac{E_f}{E_m} + 3} \quad (2.21)$$

$$\eta_{\perp} = \frac{\frac{E_f}{E_m} - 1}{\frac{E_f}{E_m} + 2} \quad (2.22)$$

where $E_{\parallel}$ And $E_{\perp}$ Are the longitudinal and transverse Young's moduli of the unidirectional composite, ζ is the shape factor which depends on the fiber geometry and orientation, ϕ is the fiber volume fraction, E_m And E_f Are the moduli of the polymer matrix and CNC fibers. For short fibers like CNCs, especially with low aspect ratio, shape factor can be determined by the equation, $\zeta = 2L/w$ where L/w is the measure of aspect ratio [174]. The overall composite modulus (E_c) for the randomly oriented fibers, uniformly distributed in the polymeric matrix is determined by the laminate theory,

$$E_c = 0.184E_{\parallel} + 0.816E_{\perp} \quad (2.23)$$

Cox–Krenchel model has been developed based on three assumptions (i) fiber and matrix response elastically, (ii) no axial load on the fiber ends and (iii) perfect fiber–matrix interface.

$$E_c = \eta_o \eta_L v_f E_f + (1 - v_f) E_m \quad (2.24)$$

where,

$$\eta_L = 1 - \frac{\text{Tanh}(\frac{\beta L}{2})}{(\frac{\beta L}{2})},$$

$$\beta = \frac{2}{d} \left[\frac{2 \times G_m}{E_f \ln \left(\sqrt{\frac{\pi}{X_i \times v_f}} \right)} \right]^{0.5}$$

$$G_m = \frac{E_m}{2 \times (1 + V)}$$

where, $\eta_o, L, d, G_m, v_f, V$ represents the fibre orientation factor, length, fibre diameter, shear modulus of the matrix, volume fraction of fibres in the composites and Poisson ratio of the matrix respectively. X_i , The packing of CNC in the PLA matrix was considered to be $\sqrt{3}/2$, as per literature [277]. The modulus and density for the polymer matrix (PLA) were taken to be $E_m = 0.545$ GPa and 1.21 g/cc as per the reported literature [278]. For the case of PHB/CNC nanocomposites the modulus of neat PHB is measured experimentally to be 1321 MPa and the density was considered to be 1.25 g/cc. A wide range of elastic moduli values for the CNCs have been reported in literature depending upon the source, methodology for measurement, type of cellulose and its composition [279][280]. In this study, elastic moduli for the crystalline cellulose I and cellulose II (E_f) were taken to be 138 and 88 GPa as per values reported in literature from theoretical modelling [281]. The true densities of CNC I and CNC II were considered to be 1.57 and 1.54 g/cc [282]. The elastic modulus and density of CNC: I→II have not been reported in literature till date and hence are assumed to be similar to those of CNC II.

For the case of the anisotropically oriented PLA/MGCNC films the micro-mechanical model proposed by Cox [277], which takes into consideration the effect of alignment of nanofillers, was used to predict the composite modulus at different volume fractions. However, the model also assumes that the fillers are continuous in the polymeric matrix with perfect adhesion between the two systems. Kelly and Tyson [283] incorporated the effect of the aspect ratio, especially for low aspect ratio fillers which have low stress transfer efficiency in terms of the length efficiency factor (η_L). Therefore, the modified Cox models becomes [283],

$$Y_c = \eta \eta_L Y_f + (1 - V_f) Y_m \quad (2.25)$$

$$\text{Where } \eta_L = 1 - \frac{\tanh\left(a \cdot \frac{l}{d}\right)}{a \cdot \frac{l}{d}}$$

$$\text{and } a = \sqrt{\frac{-3Y_m}{2Y_f \ln V_f}}$$

where Y_c is the Young's modulus of the composite, Y_f is the filler modulus in the axial direction, Y_m is the modulus of the neat PLA matrix and V_f is the volume fraction of the MGCNCs. L/D is the aspect ratio of the nanofillers and η is the orientation efficiency factor which takes into consideration the degree of alignment of nano-fillers in the polymer matrix. The orientation efficiency factor is considered to be $\eta = 1$ for fully aligned parallel arrangement of the MGCNCs, $\eta = 3/8$ for the nanofillers in plane/perpendicular alignment and $\eta = 1/5$ for randomly aligned nanofillers in the polymer matrix.

2.5.2 Surface Energy and Work of Adhesion

Young's equation [284] is used to estimate the surface energy of the different acid-derived CNCs by measuring the series of contact angles with at least two different liquids (polar and non-polar solvents) over the solid surface as follows,

$$\gamma_s = \gamma_{sl} + \gamma_l \cos \Theta \quad (2.26)$$

where γ_{sl} , γ_l and Θ represent the solid/liquid interfacial surface energy, surface tension of liquid and the contact angle between the liquid and solid surface (CNC pellets) respectively.

The total surface energy (γ_s) is composed of two components, the polar surface energy (γ_s^p) and the dispersive surface energy (γ_s^d) which are calculated using the Owens and Wendt approach [285],

$$\gamma_s = \gamma_s^p + \gamma_s^d \quad (2.27)$$

From eq. (2.26) and (2.27), by measuring the contact angle between the cellulose/liquid interfaces experimentally the surface free energy for the CNCs is determined from following equation,

$$\gamma_l \cos \theta = 2 \left(\sqrt{\gamma_l^d \gamma_s^d} + \sqrt{\gamma_l^p \gamma_s^p} \right) \quad (2.28)$$

When two components are mixed and a new surface is formed it leads to change in surface free energy that depends on the interfacial tension between the two components, input energy and the morphology/size of the new surface formed. In our study, the polymer nanocomposite is formed by mixing polylactic acid and the different acid derived CNCs in an extruder for which the interfacial surface tension is calculated using the equation as follows,

$$\gamma_{AB} = \gamma_A + \gamma_B - 2 \left(\sqrt{\gamma_A^d \gamma_B^d} + \sqrt{\gamma_A^p \gamma_B^p} \right) \quad (2.29)$$

where γ_{AB} , γ_A And γ_B Represent the interfacial surface tension, surface tension of the A and B solid surfaces in contact with their dispersed and polar components respectively.

Lifshitz-van der Waals (dispersion)/ acid-base approach [286] takes into consideration the participation/removal of the electron pairs from the solid surface on interactions with liquids and hence introduces more components in the surface energy calculation,

$$(1 + \cos \theta) \gamma_l = 2 \left(\sqrt{\gamma_s^{LW} \gamma_l^{LW}} + \sqrt{\gamma_s^+ \gamma_l^-} + \sqrt{\gamma_s^- \gamma_l^+} \right) \quad (2.30)$$

where γ_l^{LW} , γ_l^+ And γ_l^- Are the surface energy components of Lifshitz-van der Waals, electron acceptor (considered as Lewis acid) and electron-donor (considered as Lewis base). This approach requires three liquids (two polar liquids and one non-polar liquid) and the total surface energy is calculated as:

$$\gamma_s = \gamma_s^{LW} + 2 \sqrt{\gamma_s^+ \gamma_l^-} = \gamma_s^{LW} + \gamma_s^{AB} \quad (2.31)$$

where γ_s^{AB} Represents the surface tension of the polar component (AB in superscript represents the acid-base).

The work of adhesion (W_{ad}) is a thermodynamic parameter which represents the magnitude of interfacial interaction between the two components (polymer and nanofiller CNCs) present in the polymeric nanocomposites using Dupre's relation [287], given as,

$$W_{ad} = \gamma_s + \gamma_l - \gamma_{sl} \quad (2.32)$$

The surface energy parameters calculated from Owens and Wendt approach (eq. (2.27)) and Lifshitz-van der Waals (dispersion)/ acid-base approach (eq. (2.30)) for the two solid surfaces A and B in contact could be evaluated as,

$$W_{ad} = 2 \left(\sqrt{\gamma_A^d \gamma_B^d} + \sqrt{\gamma_A^p \gamma_B^p} \right) \quad (2.33)$$

$$W_{ad} = 2 \left(\sqrt{\gamma_s^{LW} \gamma_l^{LW}} + \sqrt{\gamma_s^+ \gamma_l^-} + \sqrt{\gamma_s^- \gamma_l^+} \right) \quad (2.34)$$

2.5.3 Molecular Dynamics Simulations of Graphene Nanoscrolls

The molecular dynamics (MD) simulation of the scrolling of graphene sheets onto Fe_2O_3 -CNC as substrate was carried out using the Discover module of the Materials Studio 7.0 software package. In this work, we have used universal force field (UFF) to describe the interatomic interactions between the graphene sheet and Fe_2O_3 -CNC. The MD simulation was performed in the NVT ensemble at 298 K for a total simulation time of ~500 ps with a time step of 1 fs. Data were recorded every 1 ps to determine the changes in the trajectory of atoms. The Nosé- Hoover thermostat was used to maintain the temperature constant. Further, the van der Waals interactions between the graphene sheets and Fe_2O_3 -CNC were calculated with a cut-off distance of 9.5Å. The electrostatic interactions were calculated using the Ewald summation method with the same cut-off distance of 9.5Å. It should be noted that all the prepared structures namely the graphene sheet, Fe_2O_3 -CNC, and Fe_2O_3 -CNC/graphene system were energy minimized before starting the MD simulation.



Fabrication of Cellulose Nanocrystals: A Lab Scale Approach to Continuous Processing

Motivation

Cellulose Nanocrystals (CNCs) are one of the smart bio-nanomaterials of this century, derived from most abundant and inexhaustible renewable cellulosic biomass resources, which have received significant interest due to their unique mechanical, optical, physico-chemical, and rheological properties. Utilization of waste renewable feedstock, such as agricultural residues or waste newspapers, for production of CNCs provides a novel strategy for upconversion of 'waste to wealth'. Innovative processes to tune the inherent properties of CNCs, such as surface behaviour, structural and morphological characteristics, during the processing step itself increases the versatility of CNCs for their potential application in various sectors. Traditional processing of CNC is carried out under stringent acidic conditions and requires almost 7–10 days, which is both energy inefficient and environmentally challenging. We have developed a continuous CNC processing-cum-dialysis unit which reduces the production time to 24–26 hours. Such advanced and cost effective technologies for high volume industrial scale production of CNCs are required to meet their growing demand which will continue to grow in near future.

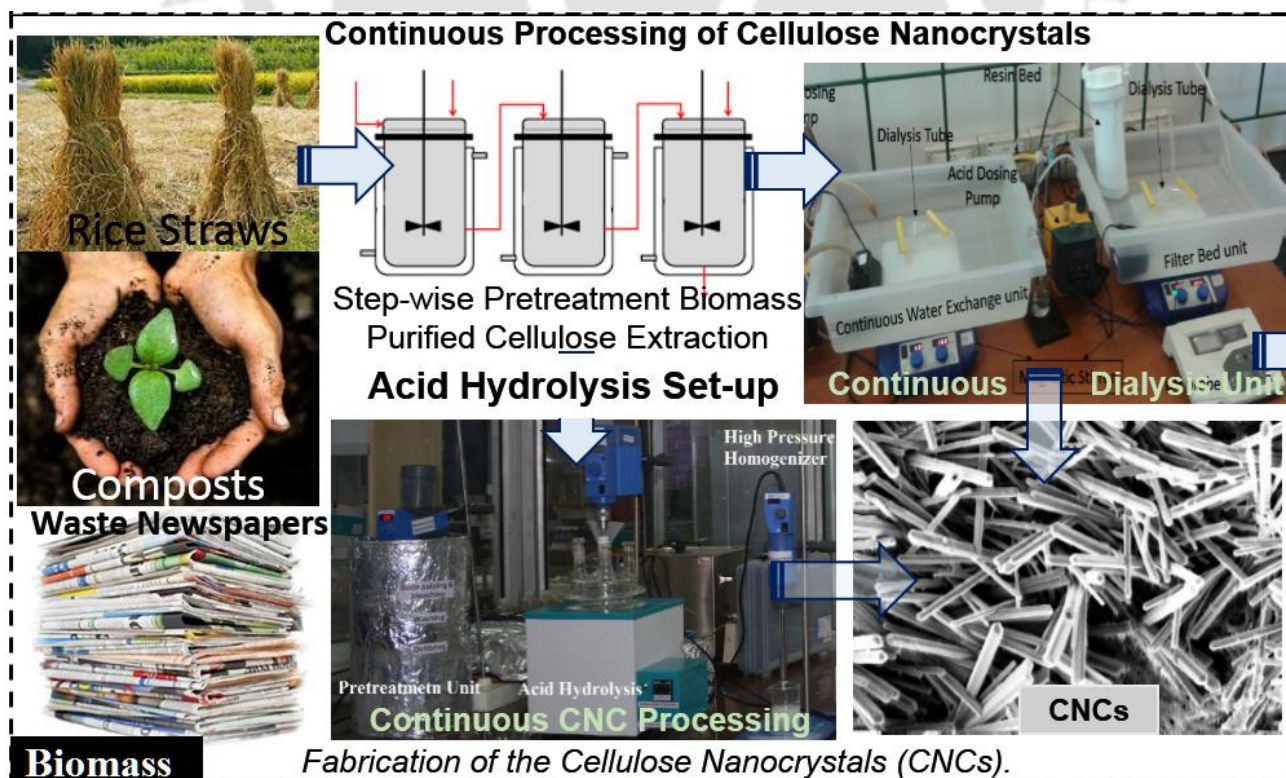
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- Dhar, P., Tarafder, D., Kumar, A., & Katiyar, V. (2015). Effect of cellulose nanocrystal polymorphs on mechanical, barrier and thermal properties of poly (lactic acid) based bionanocomposites. *RSC Advances*, 5(74), 60426-60440.
- Dhar, P., Bhasney, S. M., Kumar, A., & Katiyar, V. (2016). Acid functionalized cellulose nanocrystals and its effect on mechanical, thermal, crystallization and surfaces properties of poly (lactic acid) bionanocomposites films: A comprehensive study. *Polymer*, 101, 75-92.
- Katiyar, V., Kumar, A., Dhar, P., Soundarajan, N., Gaur, S., Tarafder, D., Continuous production of Cellulose Nanocrystals. (Patent application in preparation)

Abstract

This chapter focusses on the fabrication and characterization of CNCs from renewable resources through novel preprocessing-cum-acid hydrolysis routes and studying their effect on physico- chemical, structural and morphological properties of the CNCs obtained. It is found that the inherent properties of CNCs can be strategically tuned through variation in their polymorphism as well as substitution of hydroxyl groups with anionic moieties during acid hydrolysis step, depending upon the targeted applications. CNCs have been produced from various alternative feedstocks such as agricultural residues and waste newspaper through single step decoloration-cum-hydrolysis approach. Subsequently, we have indigenously developed a continuous CNC production-cum-dialysis unit which could efficiently process high volume of CNC suspensions after its acid hydrolysis within a period of 24–26 hours, which should potentially be of interest for industrial scale production of CNCs.

Graphical Abstract



3.1 INTRODUCTION

Nanocellulose such as CNFs, CNCs or CNWs are usually fabricated through stringent acid hydrolysis process or high pressure mechanical disintegration which depends on the type of biomass, acid systems and processing methodology followed. It has been observed that CNCs fabricated from softwood with lower microfibril density such as in cotton (usually with higher cellulose content than higher plants) are generally of aspect ratio ~ 10 . However, CNCs fabricated from hardwood such as in tunicin or green algae (highly crystalline) have higher aspect ratio (~ 67) [20]. Moreover, it has been observed that, the composition of cellulose and presence of impurities such as hemicellulose and lignin content in biomass tends to vary drastically which severely affects the morphological properties of the fabricated CNCs. Coconut fibers usually have higher lignin content due to which the extracted CNCs are ultrathin with diameter of $\sim 2\text{-}3$ nm consisting of 16 to 30 cellulosic units [21]. In addition to the biomass source, pretreatment reaction conditions must be optimized in order to yield cellulose with higher purity, crystallinity and degree of polymerization which strongly affect the properties of fabricated CNCs [4]. In literature, the fabrication of CNCs have been reported through utilization of several strong or weak acids such as sulphuric acid [15], hydrochloric acid [102], acetic acid [288], lactic acid [289], phosphoric acid [92], ammonium persulfate [95], hydrobromic acid [96] and 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) [290]. It has been observed that fabricated CNCs using different acids have tunable morphology (rod-shaped, circular or elliptical), surface functionality, different degrees of crystallinity, inter/intra-chain hydrogen bonding and dispersion quality in polar/non-polar solvents. Most of the reported literature for fabrication of CNCs, utilizes acid based hydrolysis approach which requires a lot of processing time for dialysis to remove the excess acids due to which they are

difficult to scale-up. Moreover, fabrication of CNCs with almost negligible concentrations of the acid content in it, are required for their potential applications especially in the areas related to food packaging, biomedical and healthcare products. The time frame for the production of acid free neutralized CNC suspensions reported in literature is approximately~7-10 days after the hydrolysis reactions of about~2 hours, which is principally due to the intermediate dialysis step that is relatively time consuming process. Therefore, industrially scalable innovative technologies for CNC production needs to be developed which could effectively reduce the processing time as well as address the related technical issues, which have been addressed in this chapter through an indigenously developed CNC production unit with continuous dialysis system.

Crystalline cellulose is known to exist in four different polymorphs, namely cellulose I, II, III and IV, which is due to parallel/anti-parallel stacking of the cellulosic chains and altered degree of inter/intra-molecular hydrogen bonding [281]. Cellulose I is obtained naturally in two different structures, cellulose I_α from microbes and cellulose I_β from higher plants. The difference between cellulose I_α and I_β is in the relative displacement of cellulose chain sheets along the axis, i.e., the packing density of the hydrogen bonded sheets differ significantly [291]. Cellulose II is another polymorph that is obtained from cellulose I through two possible chemical transformation routes: regeneration and mercerization. Regeneration is the process of solubilizing cellulose I in a solvent, followed by reprecipitation of glucose units to give cellulose II [91]. This process leads to rearrangement of inter- and intramolecular hydrogen bonding, which results in much lower crystallinity and thermal stability of cellulose [292]. However, in mercerization process, interdigitation of the cellulose microfibrils occurs which leads to the formation of ‘antiparallel’ cellulose II from ‘parallel’ cellulose I. Cellulose II is a

repeating structure of two parallel chains stacked with one antiparallel chain in between. Lattice planes (010) and (020) represent parallel chains and (110) represents the antiparallel chain [293]. Cellulose III consists of parallel chain packing structure of same polarity and is obtained by treatment of cellulose I_β with liquid ammonia which is a reversible process [294]. Structurally, cellulose III has two dimensional intermolecular hydrogen bonding, and two types of corrugated cellulosic chain sheets representing either (100) or (1 $\bar{1}$ 0) lattice planes [295]. The basic structural difference in polymorphism of cellulose is the relative position of oxygen atoms and rotation of the hydroxymethyl groups which form the intermolecular hydrogen bonding [296]. Cellulose IV is usually derived from cellulose II and III through thermal treatment, which have different polarity of cellulosic chains [291]. These differences in the inter- and intramolecular hydrogen bonding results in drastic variation in the elastic modulus of the cellulose polymorphs. Elastic moduli of cellulose I, II, III_I, III_{II} and IV are 138, 88, 87, 58 and 75 GPa respectively [281]. Interestingly, mechanical property investigations of CNCs fabricated from diverse biomass sources, different acids used for hydrolysis reactions and different cellulose polymorphs have revealed anisotropic mechanical behavior [22] with tailored transverse and longitudinal elastic modulus in the range of ~2–50 GPa and 140–220 GPa [297] respectively. Therefore, the intrinsic properties of CNCs can be finely tailored depending on the end-product applications by appropriately selecting the biomass, pretreatment methods for cellulose extraction, polymorphs of cellulose and the type of acids used for hydrolysis reaction, which have been studied and discussed in the subsequent section of this chapter.

3.2 RESULTS AND DISCUSSIONS

3.2.1 Fabrication of CNCs from Different Cellulose Polymorphs

Three different varieties of CNCs are fabricated with cellulose I and cellulose II polymorphism, namely CNC I (with cellulose I polymorph), CNC II (with cellulose II polymorph) and CNC:I→II (CNC II from cellulose I). The raw bamboo pulp is selected as an initial biomass sources for extraction of the purified cellulose through different pretreatment routes. The fabrication of CNC II is carried out through transformation of cellulose I to cellulose II by alkali treatment followed by its acid hydrolysis. Interestingly, for the case of CNC:I→II, transformation of the cellulose I to cellulose II occurred during the acid hydrolysis step itself. The basic difference between the two states of cellulose polymorphism is the orientation of cellobiose chains which exists in ‘parallel’ state for CNC I and in ‘antiparallel’ state for CNC II. This leads to structural difference in the relative position of oxygen atoms and rotation of the hydroxymethyl groups which alter the position as well as degree of intermolecular hydrogen bonding. CNCs with different cellulose polymorphs show distinct variation in morphology, physicochemical, structural, thermal properties and degree of hydrogen bonding, which have been investigated in this chapter, and are discussed in the subsequent section.

- **Physico-chemical and Structural Properties of CNCs with Different Cellulose Polymorphs**

X-ray diffractograms for the raw bamboo pulp and cellulose pulp after treatment with NaOH indicate the conversion of cellulose I into cellulose II. However, upto 10 wt. % addition of NaOH does not change the cellulose I crystal structure and peaks appear at $2\theta = 14.6^\circ$ (101), 16.6° ($10\bar{1}$) and 22.6° (002), as shown in Figure 3.1(a). On increasing the alkali

concentration (~20 wt. % NaOH) during pretreatment, the cellulose I peak at $2\theta = 22.6^\circ$ (200) splits into two peaks positioned at $2\theta = 19.9^\circ$ (110) and 22.0° (200), and a new peak also appears at $2\theta = 11.7^\circ$ ($1\bar{1}0$) which indicate the existence of cellulose II form [298]. This polymorphic transformation of cellulose I to cellulose II during the mercerization process is due to rearrangement of the cellobiose chains from 'parallel' state to 'antiparallel' state [299], as hydroxide ions impregnate and swell the cellulose molecular chains, resulting them to move apart [300]. Therefore, depending on alkali concentration from 5 to 25 wt. %, the crystallinity index (C.I.) decreased from ~50% to 46%, crystallite size and d-spacing of (200) planes increased from 5.0 to 9.5 nm and 0.394 to 0.413 nm, respectively. The crystallinity index (C.I.) of the raw bamboo pulp was calculated to be around ~41% (see Figure 3.1(a)). In the present case, a critical concentration of NaOH was found (~20wt. %) for conversion of cellulose I to cellulose II. However, NaOH concentration may need to be optimized depending on the chosen feedstock.

Further, the polymorphic transformation of cellulose pulp was corroborated by FTIR as shown in Figure 3.1(b). Cellulose I to cellulose II transformation is accompanied by small changes in the torsional angles of β -1,4-D-glycosidic linkages, which is evident from the shift in $-\text{CH}$ stretching from 2904 to 2881 cm^{-1} for 5 and 25 wt. % NaOH treated pulp respectively [301]. Mercerized cellulose shows distinct peaks at 3487 and 3442 cm^{-1} which is due to the intramolecular hydrogen bonding present in cellulose II. This result is consistent with the similar distinct peaks at 3487 and 3441 cm^{-1} reported by Oh.et al, [302]. The IR index at 3441 cm^{-1} (denoted as the ratio of IR intensity at a given wavenumber for cellulose II to that for pure cellulose I crystals) increased with increase in the concentration of NaOH used during the mercerization process [298]. This is due to the fact the cellulose II crystal structure has

higher density of hydroxyl groups compared to cellulose I, which enhances the hydrogen bonding interaction in them [293]. The XRD patterns for the fabricated CNC I, CNC II and CNC: I→II are shown in Figure 3.2(a). CNC I showed three distinct peaks at $2\theta = 14.3^\circ$ ($1\bar{1}0$), 16.2° (110) and 22.5° (200), similar to cellulose I (see Figure 3.1(a)). CNC II shows distinctive peaks at $2\theta = 11.9^\circ$ ($1\bar{1}0$), 19.7° (110) and 21.9° (200), almost similar to mercerized cellulose II. CNC:I→II interestingly shows similar cellulose II diffraction peaks at $2\theta = 11.8^\circ$ ($1\bar{1}0$), 19.8° (110) and 21.7° (200), as also reported in literature [264,303].

The C.I. values of CNC I, CNC II and CNC: I→II were found to be ~81%, ~77% and ~70 % respectively. The d-spacing of CNC:I→II (0.409 nm) was slightly higher than that of CNC II (0.403 nm) and CNC I (0.394 nm), consistent with observations by Yue et al. [298] and Sebe et al. [264] The subtle differences in C.I. and d-spacing of CNC:I→II are due to the acid-based mercerization phenomenon, which is not well understood. Two possible phenomena, namely acid-based mercerization and recently studied recrystallization of cellulose II structure during hydrolysis [304], might be responsible for fabrication of CNC: I→II. Both these phenomena might be responsible for the increase in intermolecular distance between the cellulose chains and disordered rearrangement in the crystalline structure of CNC: I→II. However, no significant change in the crystallite size corresponding to the (200) planes was observed among the three CNC polymorphs.

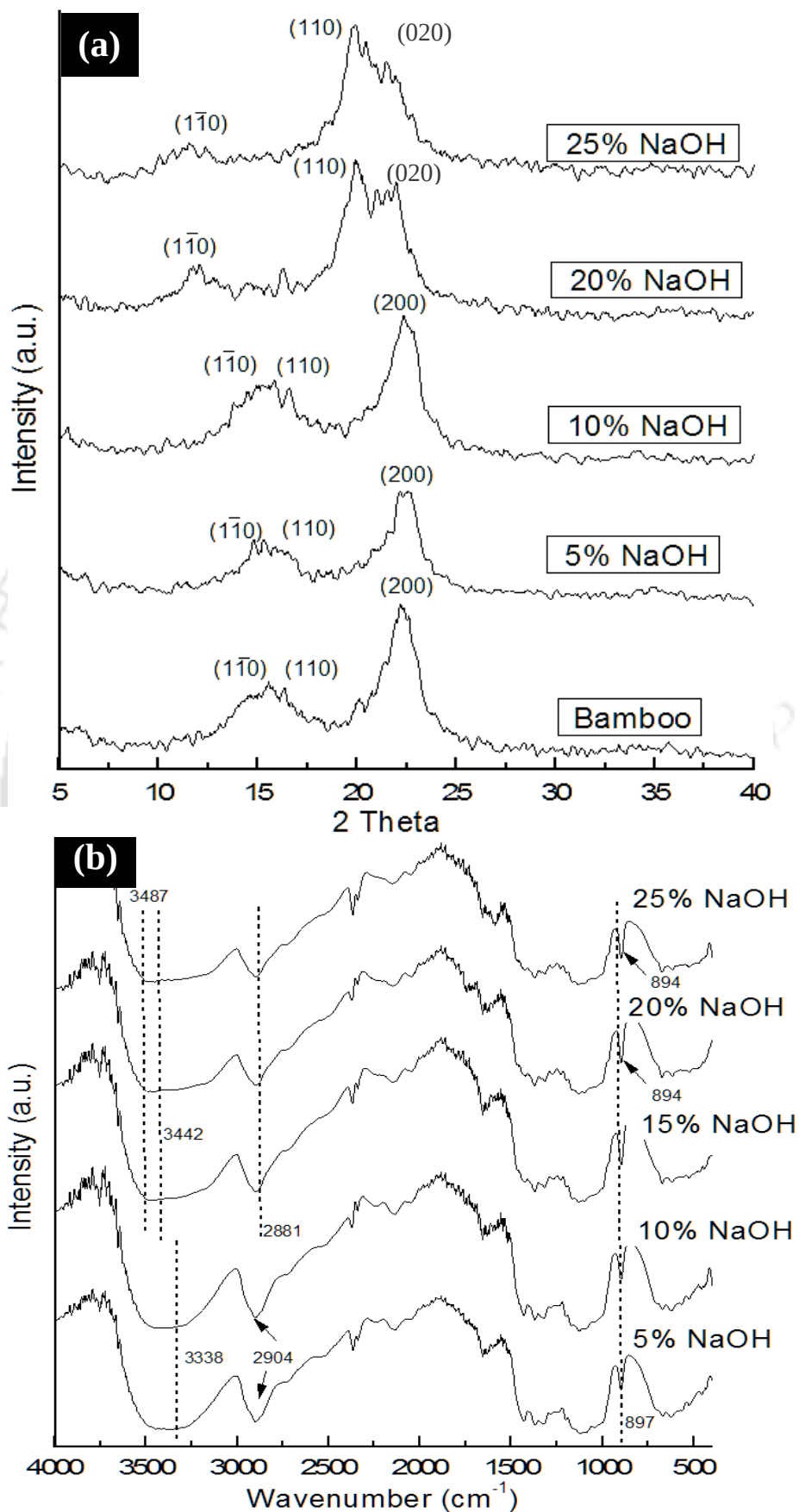


Figure 3.1: (a) XRD and (b) FTIR spectra of the NaOH pretreated bamboo pulp.

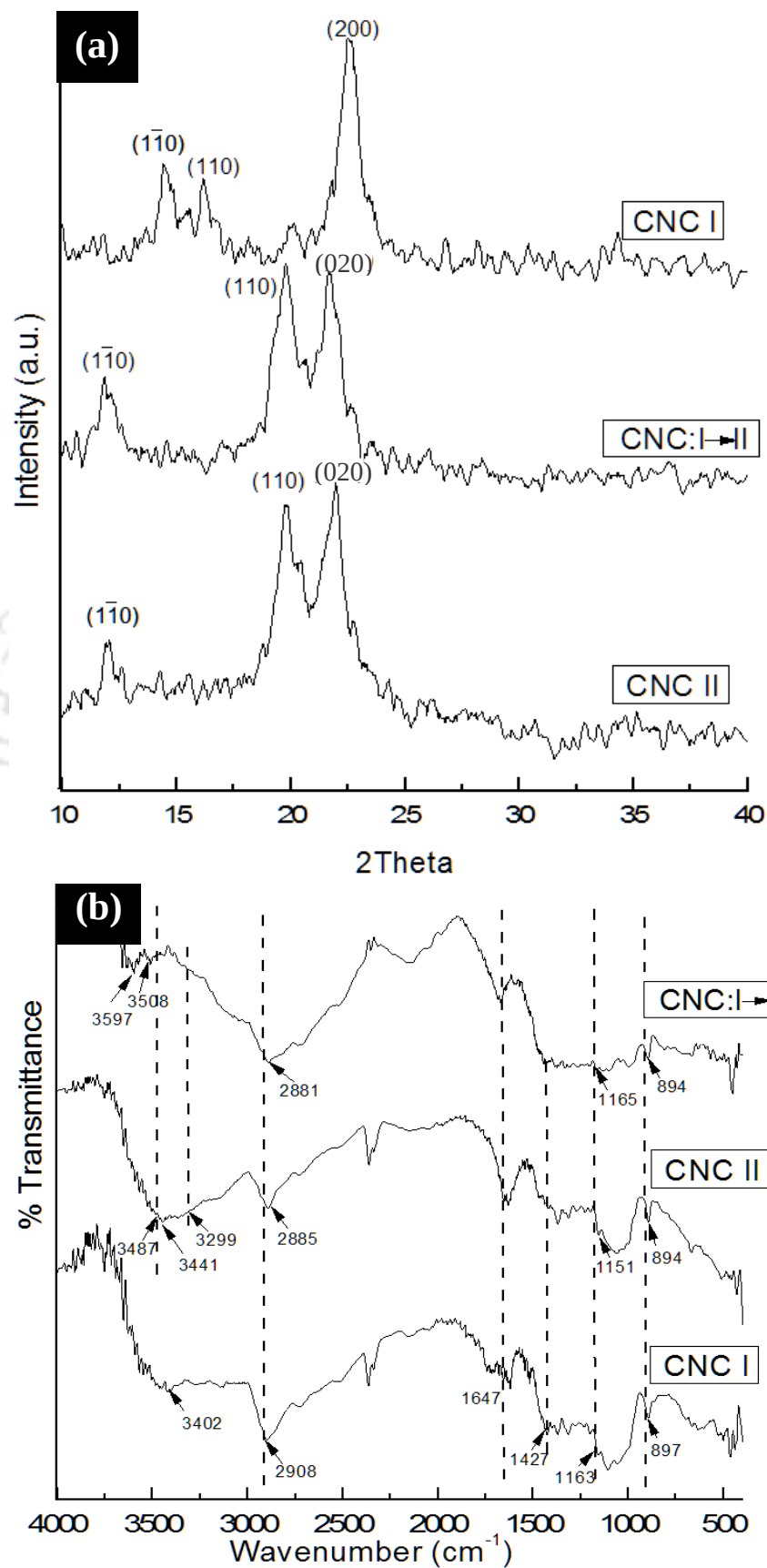


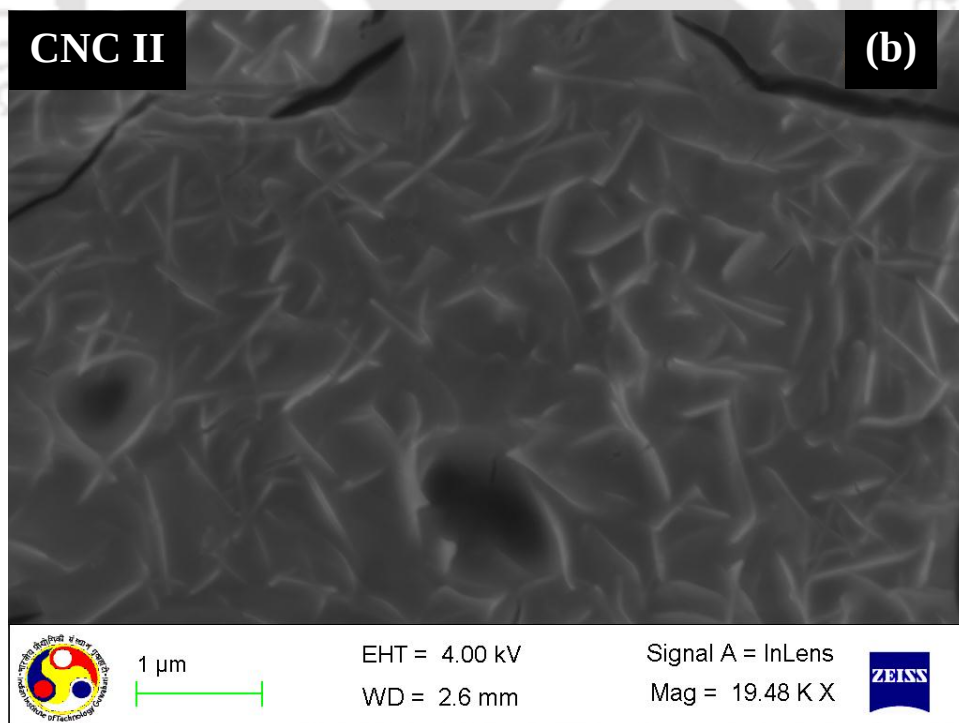
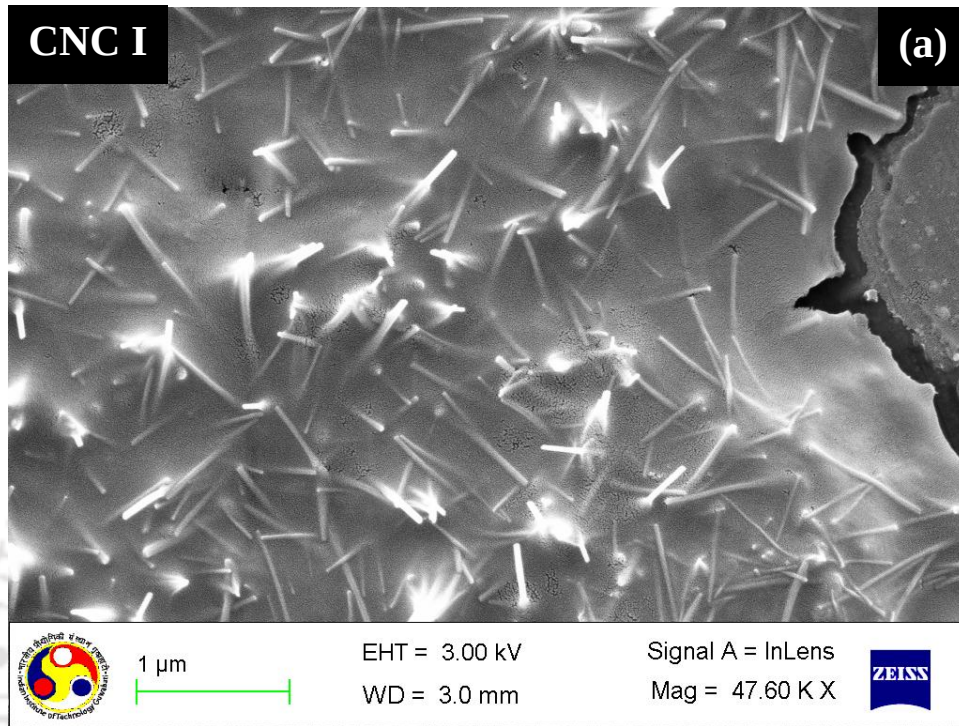
Figure 3.2: (a) XRD diffractograms and (b) FTIR spectra of different CNC polymorphs.

FTIR analysis was carried out to understand the structural differences in the three CNC polymorphs, arising out of the cellulose chain rearrangements during alkali and acid based mercerization. Figure 3.2(b) shows the FTIR spectra of the three CNCs. The broad band for the —OH stretching in the range of 3700-3000 cm^{-1} showed distinctive differences in the three CNCs. On comparison of the —OH stretching spectra of CNC I and CNC II, three new distinctive peaks at 3441, 3487 and 3299 cm^{-1} , corresponding to the intramolecular hydrogen bonding of cellulose II, were observed. However, in the CNC:I→II the —OH stretching spectra was found to be slightly different with the presence of new shifted peaks at 3508 and 3597 cm^{-1} alongwith the absence of 3299 cm^{-1} peak. The absorbance band of —CH stretching for CNC I was positioned at 2908 cm^{-1} , for CNC II at 2885 cm^{-1} and for CNC: I→II at 2881 cm^{-1} . Similarly, the peak corresponding to the C—O—C stretching in β - 1, 4- D- glycosidic linkages was located at 1163 cm^{-1} for CNC I and shifted to 1151 and 1165 cm^{-1} for CNC II and CNC: I→II respectively. It is noteworthy to mention that cellulose polymorphs may have different torsional angles of β -1, 4-D-glycosidic linkages, due to the presence of the new intra and intermolecular hydrogen bonds which could be responsible for the peak shifts. The intensity of the symmetric —CH₂ bending peak at 1427 cm^{-1} decreased in CNC II compared to CNC I and disappeared in CNC: I→II, which represents the change in conformation from *tg* to *gt* form of the CH₂OH. The rotation of the glucose residue around the β - glucosidic linkages led to shift of peak at 897 cm^{-1} in CNC I to 894 cm^{-1} for CNC II and CNC I→II. Interestingly, CNC:I→II has slightly different cellulose II crystal structure, which has also been observed by Sebe et al. [264] In summary, the three fabricated CNC polymorphs show distinctly different d-spacing and hydrogen bonding behavior due to varying molecular arrangement of the cellulose chains.

- **Morphological analysis of CNCs with Different Cellulose Polymorphs**

The morphology of the polymorphs of CNCs are investigated using FESEM and AFM as shown in Figures 3.3 and 3.4. The average widths (i.e., diameters) of CNCs are analyzed from the height profile of AFM micrographs, and average length distributions of CNCs are measured using FESEM micrographs. CNC I displays needle-like morphology with an average length of 610 ± 55 nm and average diameter of 19 ± 5 nm. CNC II displays ribbon-like morphology with an average length of 824 ± 60 nm and average diameter of 34 ± 8 nm. The nanocrystals in CNC II are found to be soft rods with an entangled structure whereas those in CNC I appear distinct, thinner and more uniform, with needle-like morphology. CNC: I→II displays rice grain like morphology with typical average length of 583 ± 40 nm and average diameter of 100 ± 30 nm. Closer examination of the FESEM micrographs at higher magnification ($>350,00\times$) (Figure 3.3(c) inset), showed presence of spherical aggregates, which self-assemble into a fibrillar structure. Similar morphology of cellulose II has also been reported by Notley et al. [305] Such morphology is probably due to the irreversible formation of hydrogen bonds in the crystalline regions and removal of more mobile amorphous cellulose regions upon drying. The possible phenomenon governing such process is hornification of the recrystallized CNC:I→II. The 3D AFM images with 20 times height enlargement (z-axis), showed some interesting differences in the cross-section of the three CNCs (Figures 3.4 (a), (b) and (c)). CNC I has sharp ‘mountain’-like peaks, suggesting an ellipsoidal cross-section of CNCs as also reported in literature [306]. CNC II has similar ‘mountain’-like peaks as well but with lower peak height, suggesting an ellipsoidal cross-section. CNC: I→II has ‘plateau’ peaks with ‘blunt wavy’ ends, which indicates a fibrillar cross-section of CNCs. The ‘blunt wavy’ ends suggest that the fibrillar structure is non-uniform and is composed of several

spherical aggregates. Interestingly, the three CNC polymorphs showed different aspect ratio of ~33, ~25 and ~6 for CNC I, CNC II and CNC:I→II respectively.



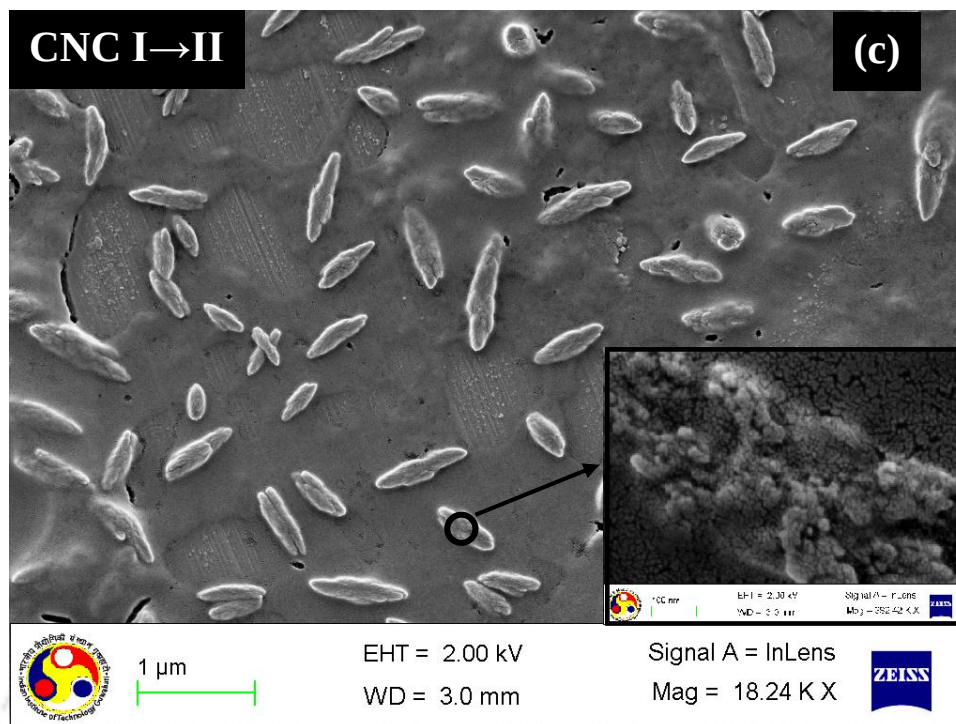
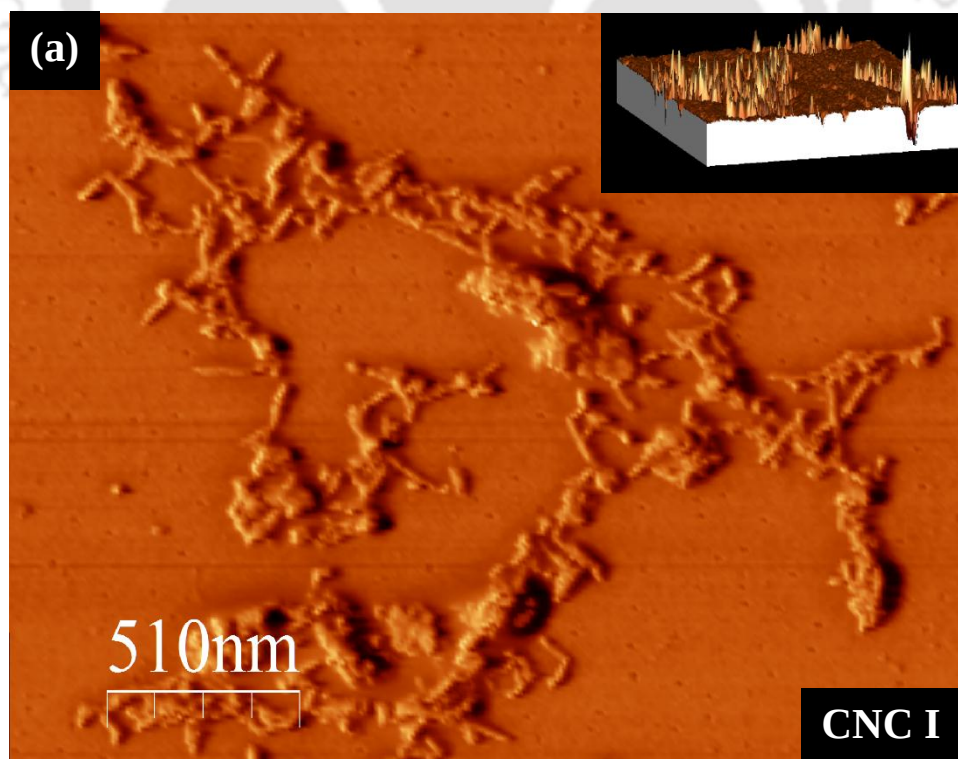


Figure 3.3: FESEM micrographs of the three different CNC representing (a) CNC I, (b) CNC II and (c) CNC: I→II with the inset showing the high resolution micrograph.



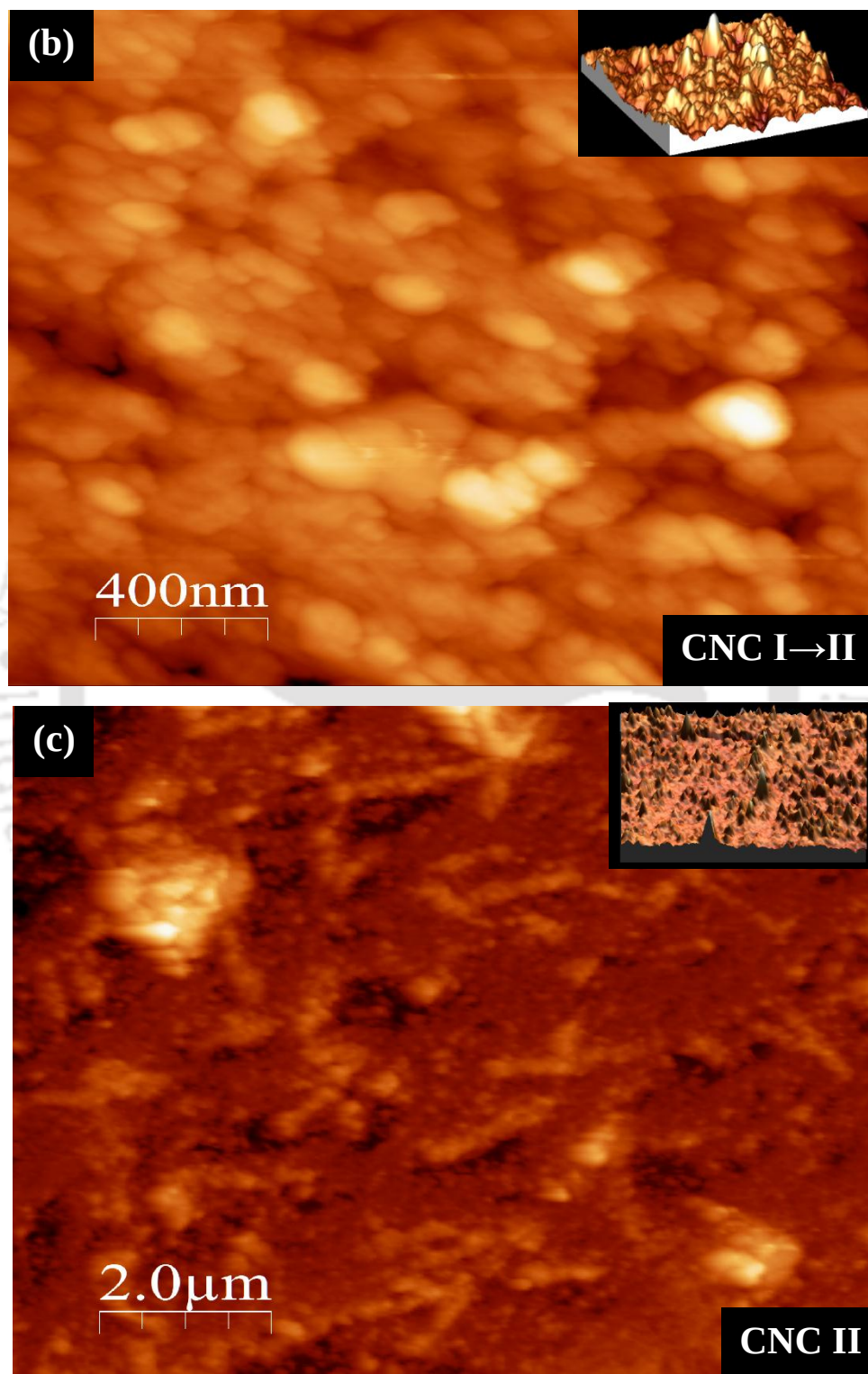


Figure 3.4: AFM micrographs for (a) CNC I, (b) CNC: I→II, and (c) CNC II with the inset showing the 3D-AFM height profile along the z-axis (enlarged about x 25 times).

- **Thermal Behaviour of CNCs with Different Cellulose Polymorphs**

TGA studies were carried out for the three CNC polymorphs to understand the differences in thermal properties of the CNCs. The water content in CNC I, CNC II and CNC: I→II was found to be 2.3%, 2.1% and 6.1 wt% (corresponding to mass loss at 100 °C). In case of CNC:I→II, the higher water content is probably due to the hornification process where the shrinking of amorphous regions occurred while drying; similar observation is reported by others in case of cellulose drying [305]. Hence, CNC II is most stable with only ~ 3.3 wt. % loss up to 200 °C. CNC I and CNC:I→II comparatively underwent a higher weight loss of ~15% and ~13% respectively (see Figure 3.5). The degradation of CNCs usually consists of two steps: removal of the sulfate groups on CNC surface (from ~150 °C), followed by the degradation of cellulose in the range of 250–500 °C. The reduction in the weight of CNC I, CNC II and CNC:I→II up to 150 °C was 5.6%, 3.1% and 7.95% respectively. The onset degradation temperature associated with the second step of cellulose degradation varied significantly at 176, 226 and 191 °C for CNC I, CNC II and CNC:I→II respectively. CNC: I→II showed slightly higher stability in the temperature range of 300–500°C, compared to the other CNCs. The amount of ash content estimated at 500°C varies for CNC I, CNC II and CNC:I→II as 1.9%, 4.7% and 2.5 wt. % respectively.

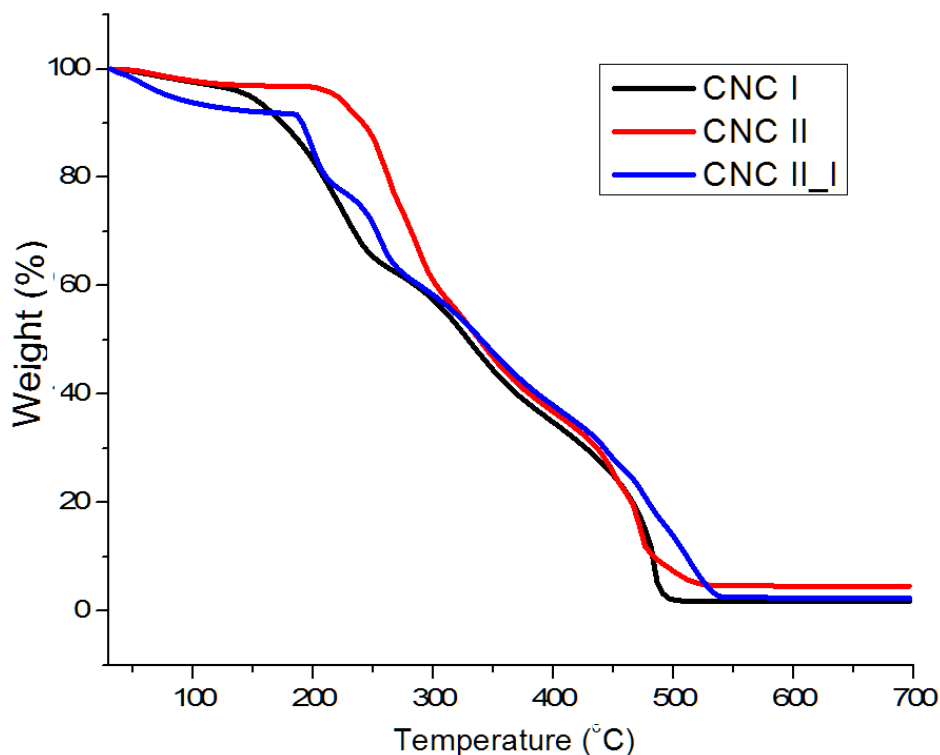


Figure 3.5: Comparison of the thermo-gravimetric (TGA) profiles for different CNC polymorphs measured at 10 °C/min heating rate.

3.2.2 Fabrication of CNCs with Different Acids as Hydrolysing Agents

Four different varieties of acid-derived CNCs have been fabricated utilizing sulphuric, hydrochloric, phosphoric and nitric acids as hydrolysing agents, keeping the initial cellulosic precursor constant. During the hydrolysis of the cellulose the hydroxyl groups present on it are substituted with the anionic moieties from acids with varying degree of substitution. Moreover, the degree of protonation of the various acids varied drastically, due to which their ability to explicitly degrade the amorphous sections differed. Hydrochloric acid (HCl) and nitric acid (HNO₃) are comparatively strong acids with the capability to produce abundant form of dissociated H⁺ protons which could effectively degrade the amorphous segments of cellulose especially through disintegration of the glycosidic bonds. However, sulphuric acid (H₂SO₄) and phosphoric acid (H₃PO₄) are comparatively weaker acids, and are less effective in completely degrading the amorphous segments. Therefore, the influence of the four different

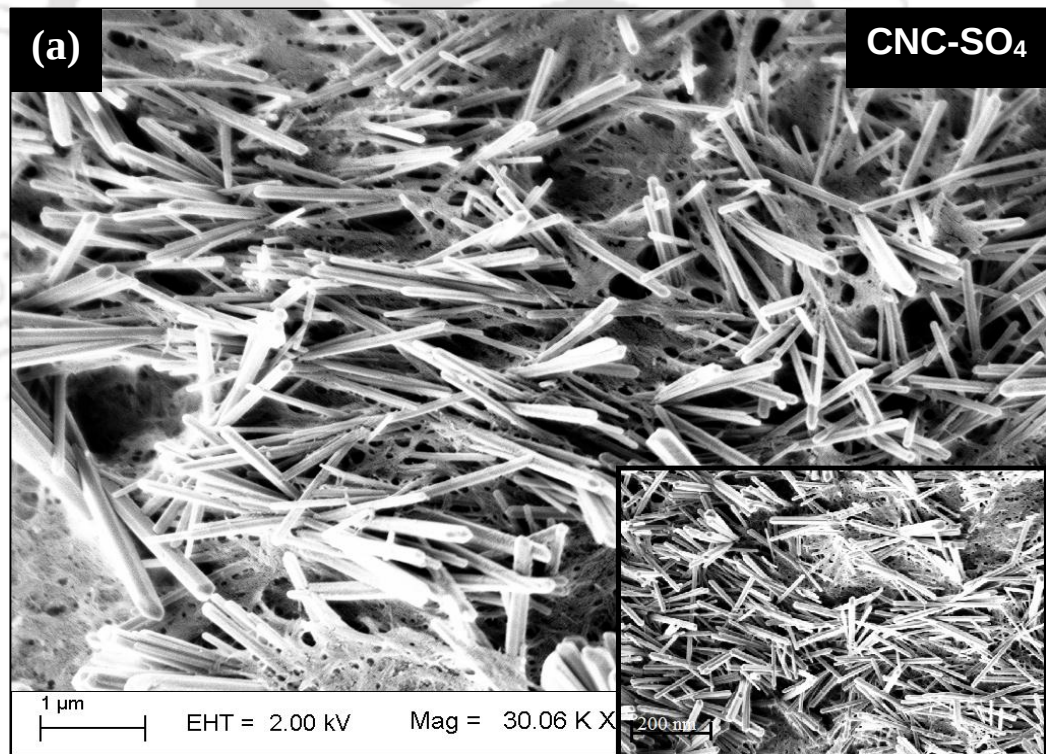
acids on the morphology, degree of substitution of hydroxyl groups, crystallinity, surface characteristics (especially the energy/charge) and thermal stability was investigated in detail.

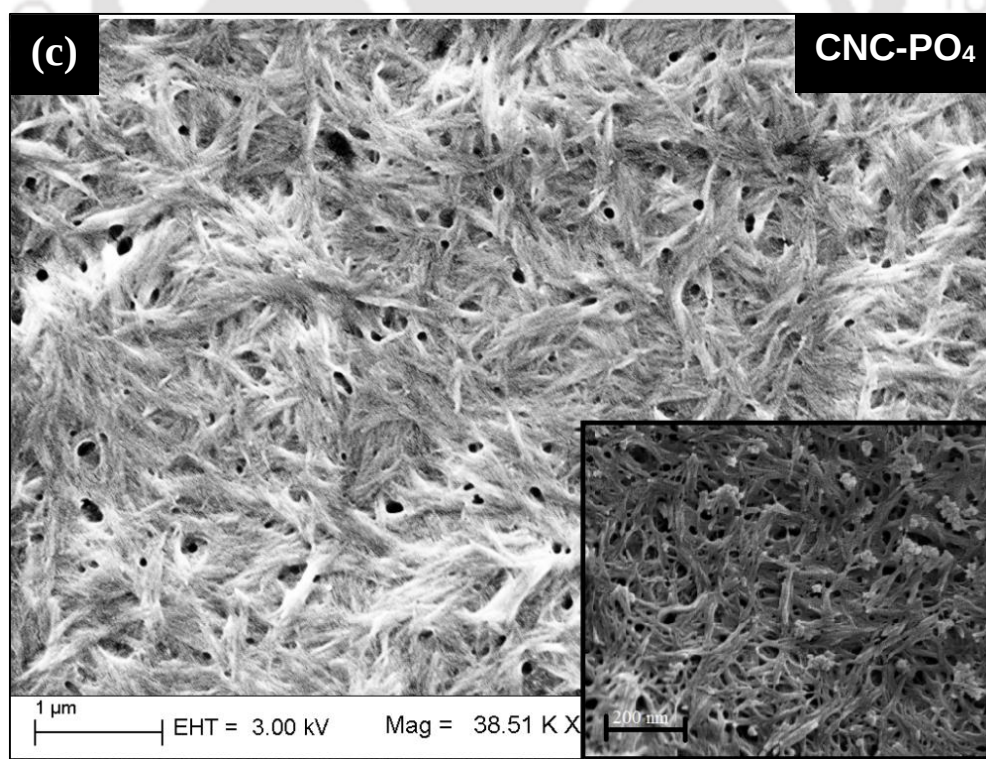
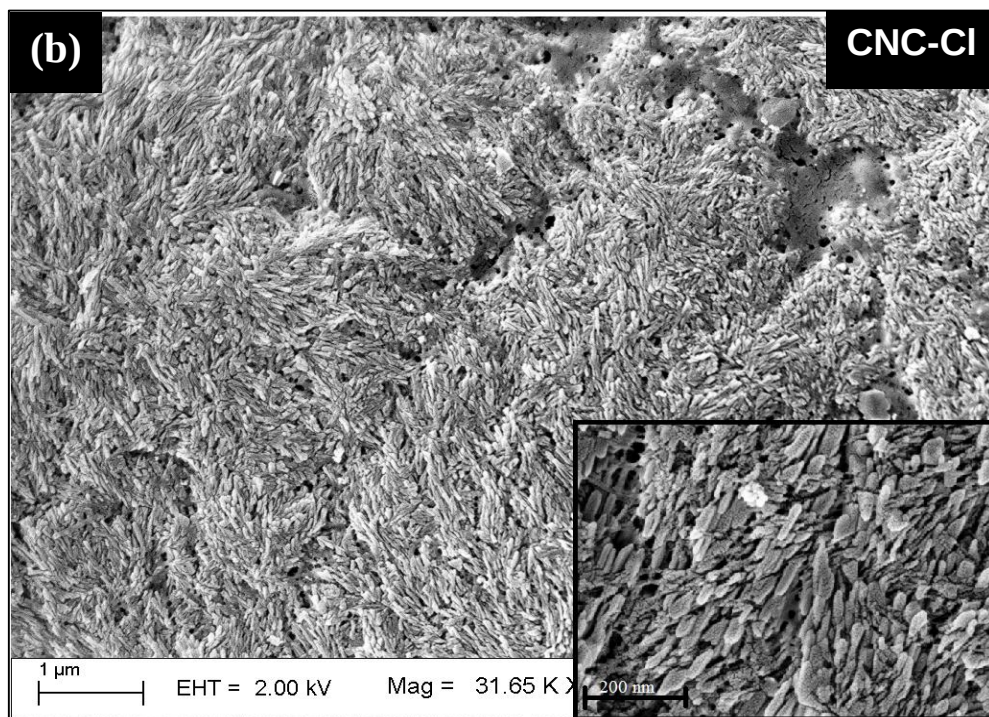
- **Morphological Analysis of Various Acid-derived CNCs**

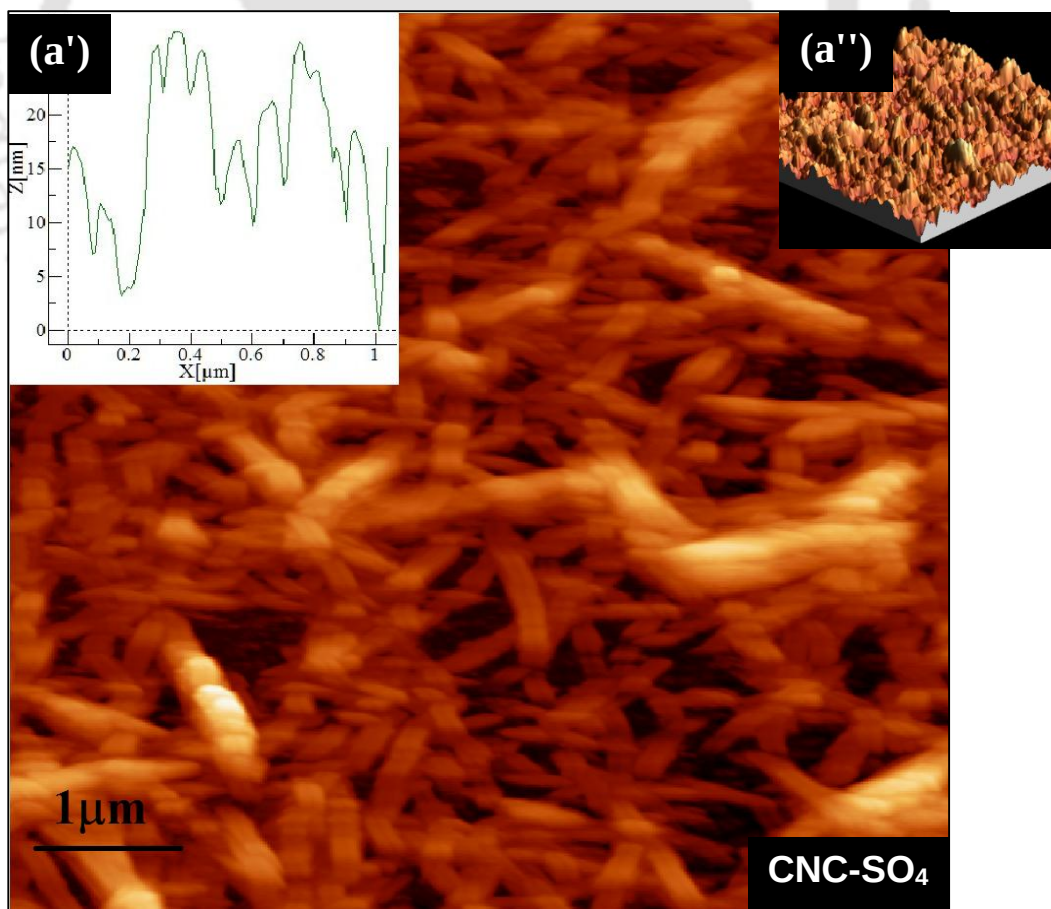
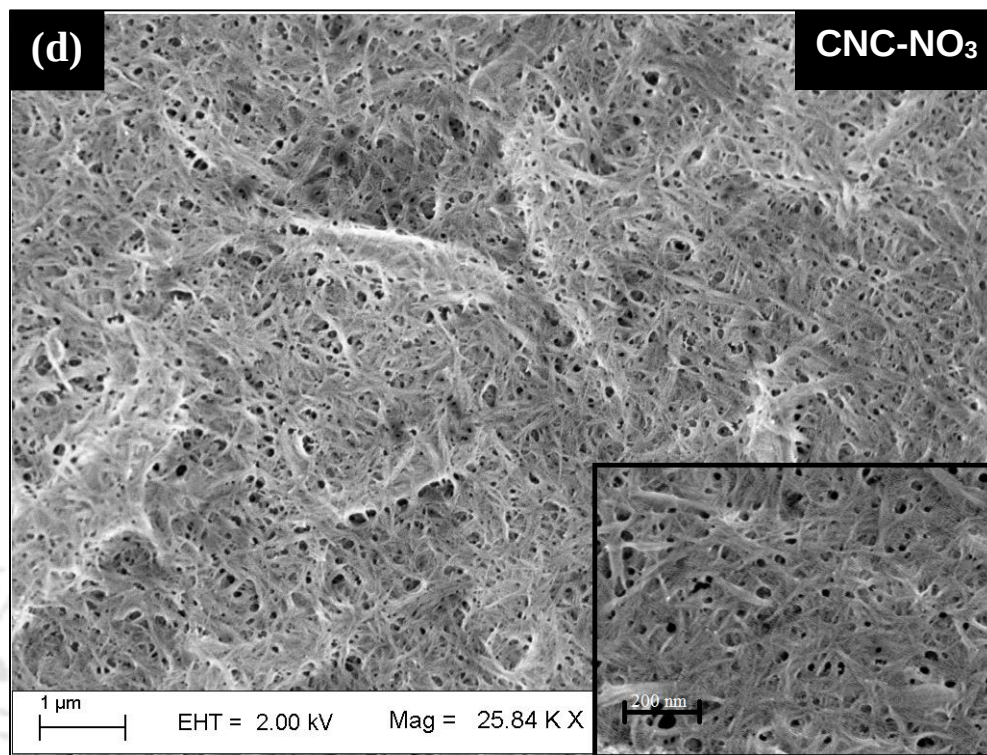
The morphological analysis of the four different CNCs in terms of variation in length, diameter and aspect ratio was conducted through FESEM and AFM micrographs. The dimensions of CNCs, i.e., the average length distribution, were calculated from FESEM analysis (Figure 3.6) and average width/diameter were measured from the height profile images of AFM (inset). CNCs hydrolyzed with sulphuric acid (CNC-SO₄) show needle-like shape with an average length of 1290±110 nm and diameter of 26±16 nm (Figure 3.6(a)). Hydrochloric acid-hydrolyzed CNCs (CNC-Cl) displayed small rod-like morphology with an average length of 198±30 nm and width of 12±5 nm (Figure 3.6(b)). CNCs fabricated using phosphoric acid (CNC-PO₄) have rice grain-like morphology with an average length of 983±55 nm and diameter of 17±10 nm (Figure 3.6(c)). Nitric acid based CNCs (CNC-NO₃) have entangled thin fibrillar structure with an average length of 506±40 nm and width of 21±8 nm (Figure 3.6(d)). Interestingly, the four acid-derivatized CNCs though fabricated from same cellulosic precursor show different morphology and dimensions which led to variable aspect ratio of ~50, ~17, ~57 and ~24 for CNC-SO₄, CNC-Cl, CNC-PO₄ and CNC-NO₃ respectively.

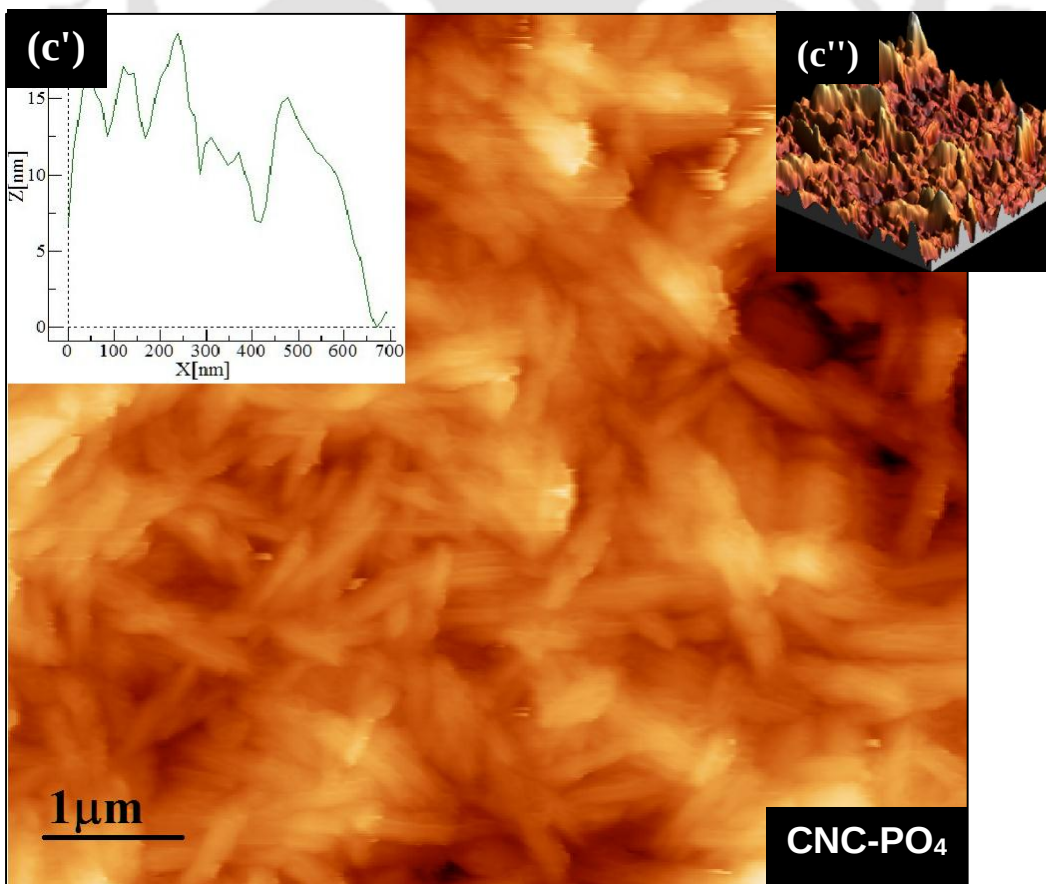
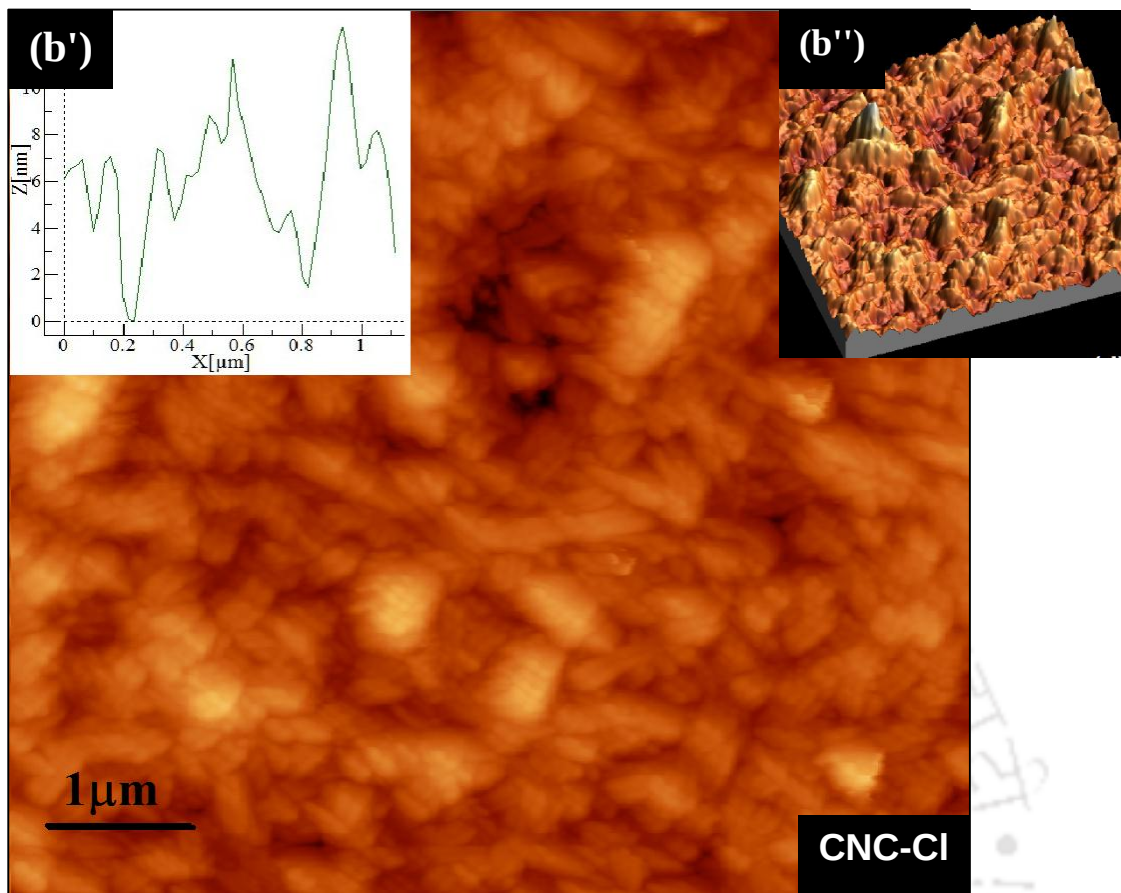
The variation in morphology of CNCs is probably due to the different degree of dissociation for various acids which thereby alters the extent of degradation of the amorphous segments present in the cellulosic chains. Further, the cross-sectional morphology of the CNCs, obtained using elevated 3D-AFM images with 25 times height enlargement along z-axis (Figure 3.6(a'')-(d'')). All the CNCs exhibited 'mountain'-like peaks, suggesting that they have ellipsoidal cross sectional area but with certain variations due to the change in morphology

[306]. CNC-SO₄ has intense pointed peaks suggesting needle-like morphology, whereas CNC-Cl showed small peaks which have merged to form small plateaus suggesting presence of agglomerates of low aspect ratio CNCs. The rice-grain morphology of CNC-PO₄ led to the formation of 'mountain'-like peaks without sharp ends, and the blunt wavy ends of CNC-NO₃ suggest the formation of fiber-like morphology. Therefore, the FESEM and 3D-AFM analysis show that the fabricated CNCs have significant variation in dimensions both in length, diameter and cross-section, which in turn suggests that the morphology of the CNCs could be tailored using different acids as hydrolyzing media.









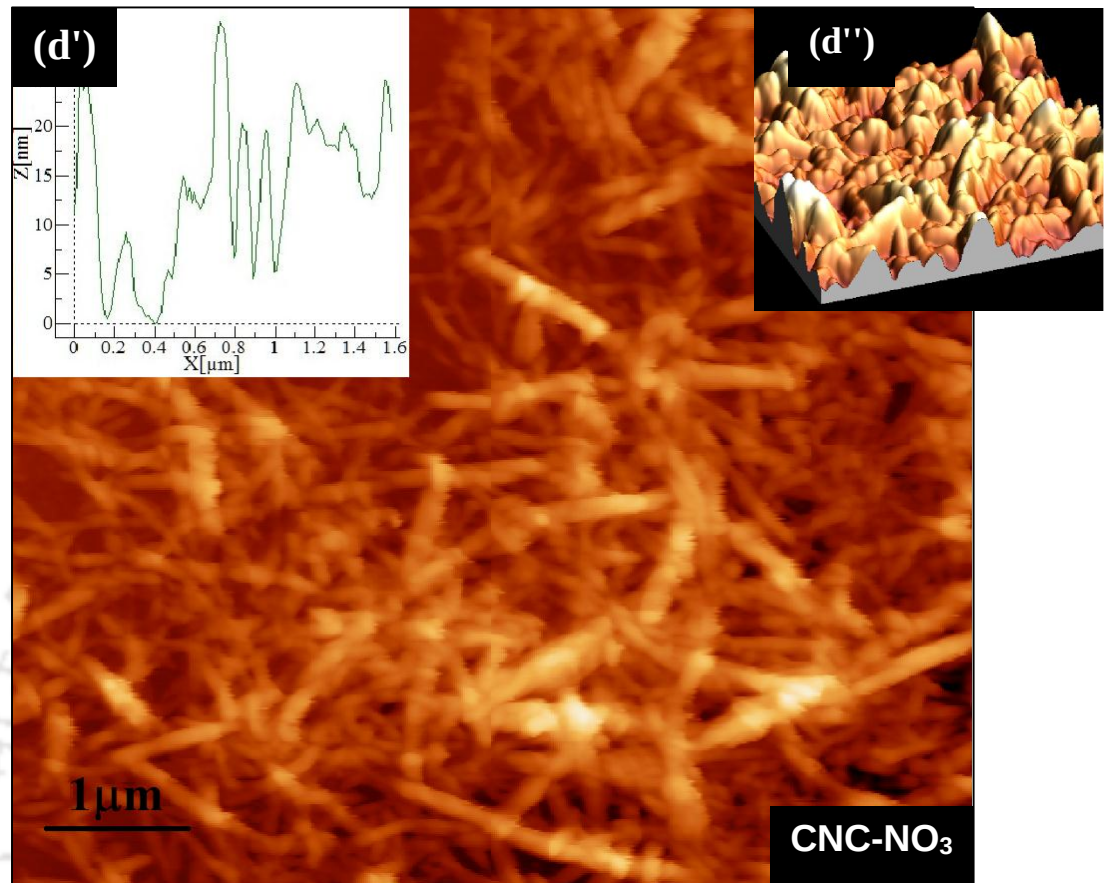


Figure 3.6: FESEM micrographs (with inset showing the high magnification images), AFM micrographs (with their respective height profiles in the inset) and 3D-elevated AFM images with height enlargements by 25 times showing the cross-sectional view of the CNCs fabricated using different acids, namely (a,a',a'') sulphuric acid, (b,b',b'') hydrochloric acid, (c,c', c'') phosphoric acid and (d,d',d'') nitric acid.

- **Physico-chemical and Structural Properties of the Different Acid-derived CNCs**

XRD studies were carried out to understand the physical properties of the crystallites and investigate whether CNCs have undergone polymorphic or chemical transformations during acid treatments. XRD diffractograms show the presence of three distinct peaks at 14.8° , 16.4° and 22.5° (with slight shift to 22.7° in case of CNC-Cl and CNC-SO₄) (see Figure 3.7(a)) representing the cellulose I crystallite structure with $(\bar{1}\bar{1}0)$, (110) and (200) lattice planes

respectively [307]. The diffraction patterns for all the acid-derivatized CNCs show the presence of cellulose I crystallite structure similar to initial cellulosic precursor used (chopped filter papers) suggesting that hydrolysis using different acids did not alter the inherent cellulose structure. However, significant variation in the crystalline properties of CNCs such as crystallinity index (χ_c) (calculated by Segal method [307]) and crystallite dimensions of different planes ($B_{(110)}$, $B_{(110)}$ and $B_{(200)}$) (calculated by Scherrer equation [264]) were observed for different acids (as shown in Table 3.1). The CNC-Cl showed higher crystallinity values of ~85% followed by the CNC-NO₃ of ~81%, CNC-SO₄ of ~75% and CNC-PO₄ of ~70% respectively. The crystallite size dimensions along the peak at 22.5° (200) for the acid-hydrolyzed CNCs were found to be 10.5, 9.8, 10.6 and 8.7 nm for CNC-SO₄, CNC-Cl, CNC-PO₄ and CNC-NO₃ respectively. Assessment of the crystallite dimensions for all the three planes (as shown in Table 3.1) of the four acid-treated CNCs suggests the formation of parallelograms with probably monoclinic crystal structure. The variation in the percentage crystallinity and the crystallite size could be attributed to the dissociation constant (pK_a) of the acids which follows the order $pK_a(\text{HCl}) \approx pK_a(\text{HNO}_3) \geq pK_a(\text{H}_2\text{SO}_4) \gg pK_a(\text{H}_3\text{PO}_4)$ [308]. HCl and HNO₃ are comparatively strong acids due to which the H⁺ protons after dissociation could effectively diffuse into the amorphous layers of cellulose disintegrating the glycosidic bonds completely leaving behind higher fractions of crystalline segments with smaller crystallite sizes. However, H₂SO₄ and H₃PO₄ are comparatively weaker acids and are less effective in degrading the amorphous segments entirely resulting in lower percentage crystallinity and larger crystallite segments. The crystallinity and the crystallite size of the fabricated CNCs are also expected to depend upon the concentration of the given acids, temperature of hydrolysis, contact time, ratio of cellulosic biomass-to-acid used for hydrolysis

and the type of initial biomass selected. Therefore, on the basis of XRD studies, it could be confirmed that varying the type of hydrolyzing acids did not alter the initial cellulose crystal structure but influenced the crystal properties of fabricated CNCs significantly.

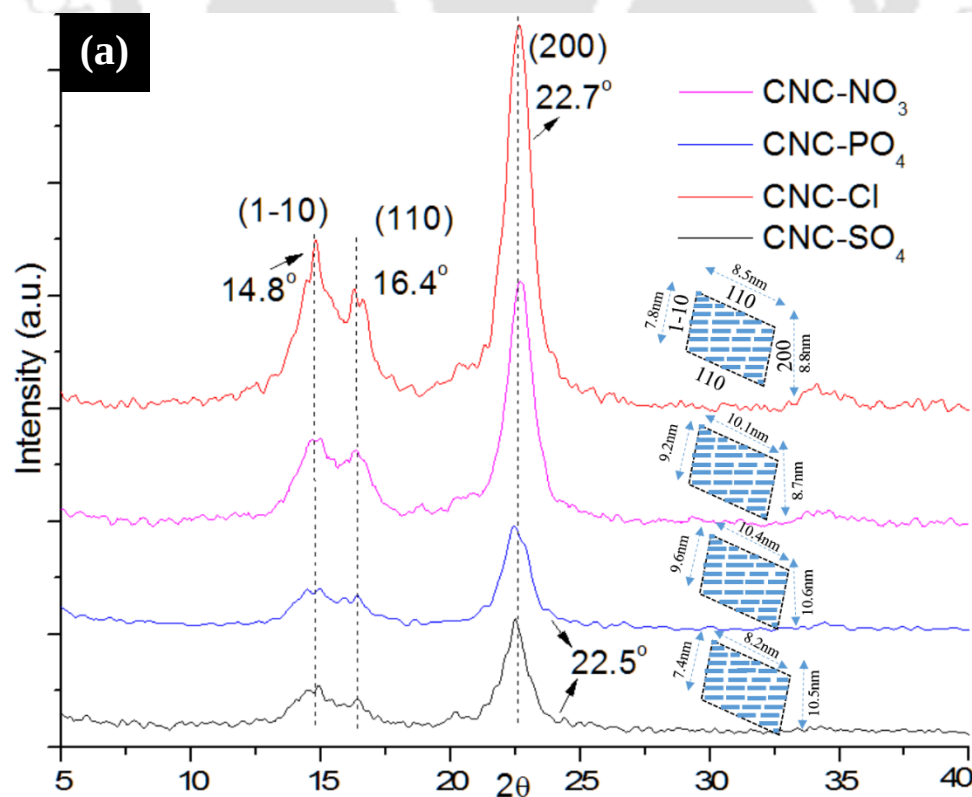
Table 3.1: Physico-chemical properties of the fabricated CNCs using different acids.

Sample	Dimensions (nm)		C.I. (%)	Crystallite Dimensions (nm)			Elemental Analysis (% wt. of functional group)	Aspect Ratio
	Length	Width		B ₍₂₀₀₎	B ₍₁₋₁₀₎	B ₍₁₁₀₎		
CNC-SO ₄	1290±110	26±16	75	10.5	7.4	8.2	1.2	~50
CNC-Cl	198±30	12±5	85	8.8	7.8	8.5	0.3	~17
CNC-PO ₄	983±55	17±10	70	10.6	9.6	10.4	2.8	~57
CNC-NO ₃	506±40	21±8	81	9.8	9.2	10.1	0.1	~24

(where C.I. represents the % crystallinity of CNCs)

FTIR spectroscopy analysis was carried out to understand the effect of acid types on the degree of hydrogen bonding prevailing in the intra/intermolecular cellulosic chains, influence on glycosidic linkages and the substitution of nascent hydroxyl groups of CNCs with chemical moieties (Figure 3.7(b)). The inherent FTIR peaks of CNCs at 2904, 1432, 1314, 1201, 1166, 1031 and 897 cm⁻¹ represent the -CH peak stretching, CH₂ bending peak, -CH₂ wagging, -C-OH in plane bending at C-6, asymmetric -C-O-C- stretching of glucosidic linkages, in-plane C-OH stretching at C-6 and -C-O-C asymmetric bending of glucosidic linkages respectively, which are found to be unaltered in case of all the acid-hydrolyzed CNC samples. The broad hump in the range of 3700–3500 cm⁻¹ represents the free hydroxyl groups and the sharp peak at ~1649 cm⁻¹ represents the free-OH bending of the cellulose which are present in all the CNCs except the CNC- PO₄. In case of CNC-PO₄, the intensity of the free -OH bending peak at ~1649 cm⁻¹ is significantly reduced alongwith the formation of narrow hump in range of 3700–3500 cm⁻¹, which suggests that the nascent hydroxyl groups of the CNCs are probably replaced by the phosphate moieties. Further, the phosphate groups on CNC-PO₄ are confirmed

through the presence of the distinct stretching vibration peaks at $\sim 1000\text{ cm}^{-1}$ and bending vibrations peaks at $\sim 609\text{ cm}^{-1}$. Simultaneously, the elemental analysis of CNC- PO_4 also confirmed higher degree of substitution of phosphate groups (by $\sim 2.8\text{ wt. \%}$) compared to the rest of the acid derived CNCs. CNC- SO_4 and CNC- Cl show the presence of sulphate and chloride peaks at 1033 and 808 cm^{-1} respectively [306]. The relative amount of the sulphate and chloride functional groups in the CNCs are found to be 1.2 and $0.5\text{ wt. \%}$ (from EDX analysis). The low intensity asymmetric stretching vibration peak at $\sim 1282\text{ cm}^{-1}$ indicates that the degree of substituted nitrate groups in CNC- NO_3 is lowest with only $\sim 0.1\text{ wt. \%}$ (as also confirmed by EDX analysis) [309]. From FTIR and elemental analysis it could probably be concluded that degree of substitution of hydroxyl functional groups on CNCs increases when acids with lower dissociation constant are used for hydrolysis. These acid-derived CNCs with different chemical functionalities can significantly affect surface characteristics as well as dispersion behavior of nanofillers in the polymeric matrix as discussed in the next sections.



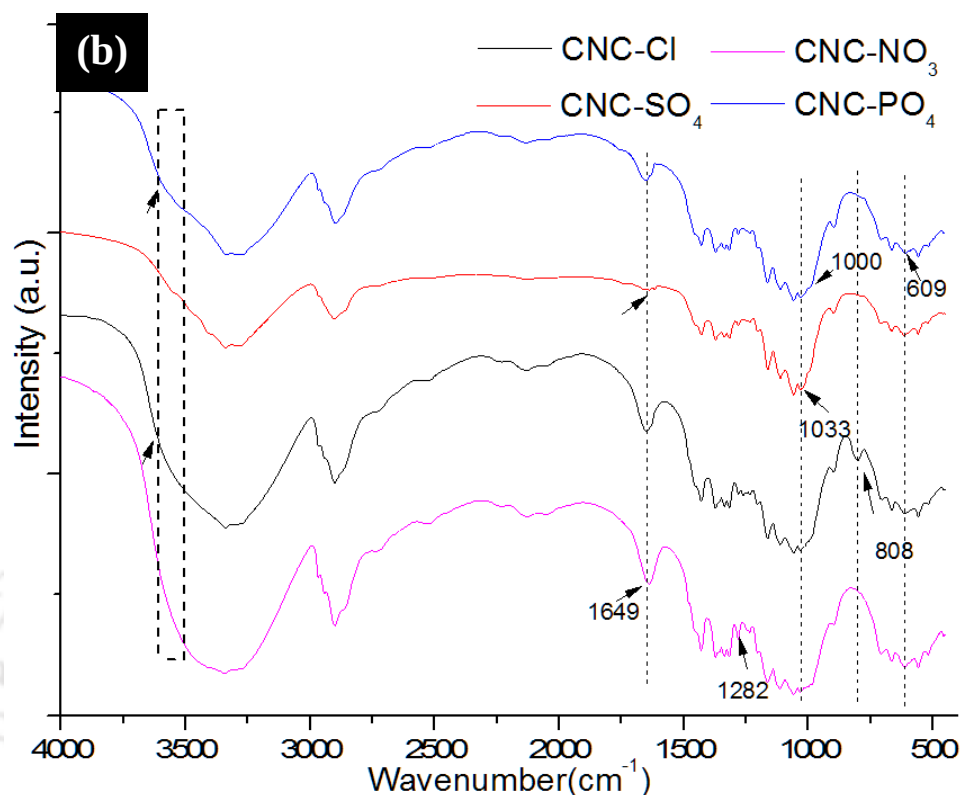


Figure 3.7: (a) XRD diffractograms of the CNCs fabricated through hydrolysis process using different acids (inset: shows the representative monoclinic crystal structure of the CNCs with their respective crystal parameters), and (b) FTIR spectroscopic studies of various acid-derived CNCs.

• Thermal Behaviour of the Different Acid-derived CNCs

The different chemical functionalities acquired by the CNCs during acid hydrolysis play a critical role in their thermal stability. From Figure 3.9(a), the water content in fabricated CNCs is found to vary as ~ 2.9%, 1.9%, 1.5% and 2.5 wt. % for CNC-SO₄, CNC-Cl, CNC-PO₄ and CNC- NO₃ respectively. Such variations in water content is probably due to different degrees of substituted hydroxyl functional groups present in various acid derived CNCs (as confirmed from the % elemental analysis). The peak degradation temperature (T_p) for the initial cellulosic precursor (whatmann filter paper Grade I) was ~357°C, which has lower ash content

(at ~400°C) compared to the rest of acid derived CNCs [310]. Potential application of the CNCs as nanofillers in fabricating polymeric nanocomposites through extrusion process are restricted due to the reduced thermal stability of CNCs at the processing temperature of biodegradable polymers such as PLA and PHB (~250°C). Over such temperature range (~150-250°C), CNC-SO₄ shows a significant weight loss of ~21 wt. % compared to 5.4%, 4.3% and 4.8 wt. % for the CNC-Cl, CNC-PO₄ and CNC-NO₃ respectively. The substantial weight loss for CNC-SO₄ is probably due to the removal of sulfate groups present on its surface (at ~150°C), which however, was found to be absent in rest of the acid-derived CNCs [124]. This confirms that the substituted functional moieties on the rest of the acid derived CNCs after hydrolysis are stable at high processing temperature for polymers. Also, CNC-SO₄ has significantly lower onset degradation temperature (T_{onset}) which is probably due to the presence of sulphate groups which act as catalyst in enhancing the decomposition rate of CNCs. Comparison of the T_{onset} for the different acid-derived CNCs shows a noteworthy variation with values of ~220, 271, 295 and 340 °C for the CNC-SO₄, CNC- Cl, CNC- PO₄ and CNC-NO₃ respectively. As the cellulosic backbone in all the CNCs are same, it is expected that the presence of anionic groups on the surface of acid-derived CNCs are responsible for variation in thermal stability. Improved thermal behavior for rest of the acid-derived CNCs could be attributed to higher thermal stability of the substituted anions on surface of CNCs (for e.g., thermal stability of $\text{Cl}^- > \text{SO}_4^{2-}$) or due to the formation of stable bond between the carbon and substituted anions (for e.g., the bond strength of $\text{C-N} > \text{C-P}$). Interestingly, thermographs of the four different CNCs display three similar degradation phenomenon: (i) initially, removal of trapped/absorbed water at ~100°C which usually accounts for ~5 wt. % loss, (ii) the main degradation step of cellulose which leads to cleavage of glycosidic units, the rate and onset temperature of which

depends on the acid type used for hydrolysis reaction and (iii) conversion of the residues into gaseous products like CO₂, CO and other byproducts. The peak degradation temperature (T_p) which accounts for degradation of the cellulosic backbone in CNCs is also found to vary significantly: ~238, 352, 365 and 371°C for CNC-SO₄, CNC-Cl, CNC-PO₄ and CNC-NO₃ respectively (Figure 3.8(a)). Interestingly, the CNC-NO₃ showed a significantly lower ash content of ~1.4 wt. % only, suggesting complete degradation at ~550°C compared to the CNC-SO₄, CNC-Cl and CNC-NO₃ with high ash contents of ~32.2, 27.8 and 24.3% respectively. Further, the energy of activation (E_a) of thermal degradation for the four different CNCs are calculated from the slope of the plot between $\ln(\ln(1 - \alpha)^{-1})$ and the temperature difference ($T - T_s$). The E_a values are found to be highest for the CNC-NO₃ (~174.4 kJ/mol) followed by the CNC-PO₄ (~75.6 kJ/mol), CNC-Cl (~42.0 kJ/mol) and CNC-SO₄ (~34.9 kJ/mol) respectively (Figure 3.8 (b)). Further from the E_a values, it can be confirmed that the thermal stability of CNCs fabricated with the different types of acids follows the order of CNC- NO₃>CNC- PO₄>CNC- Cl>CNC-SO₄. Therefore, thermal stability of CNCs could be finely tuned depending on end-user applications by simply changing acid used for hydrolysis.

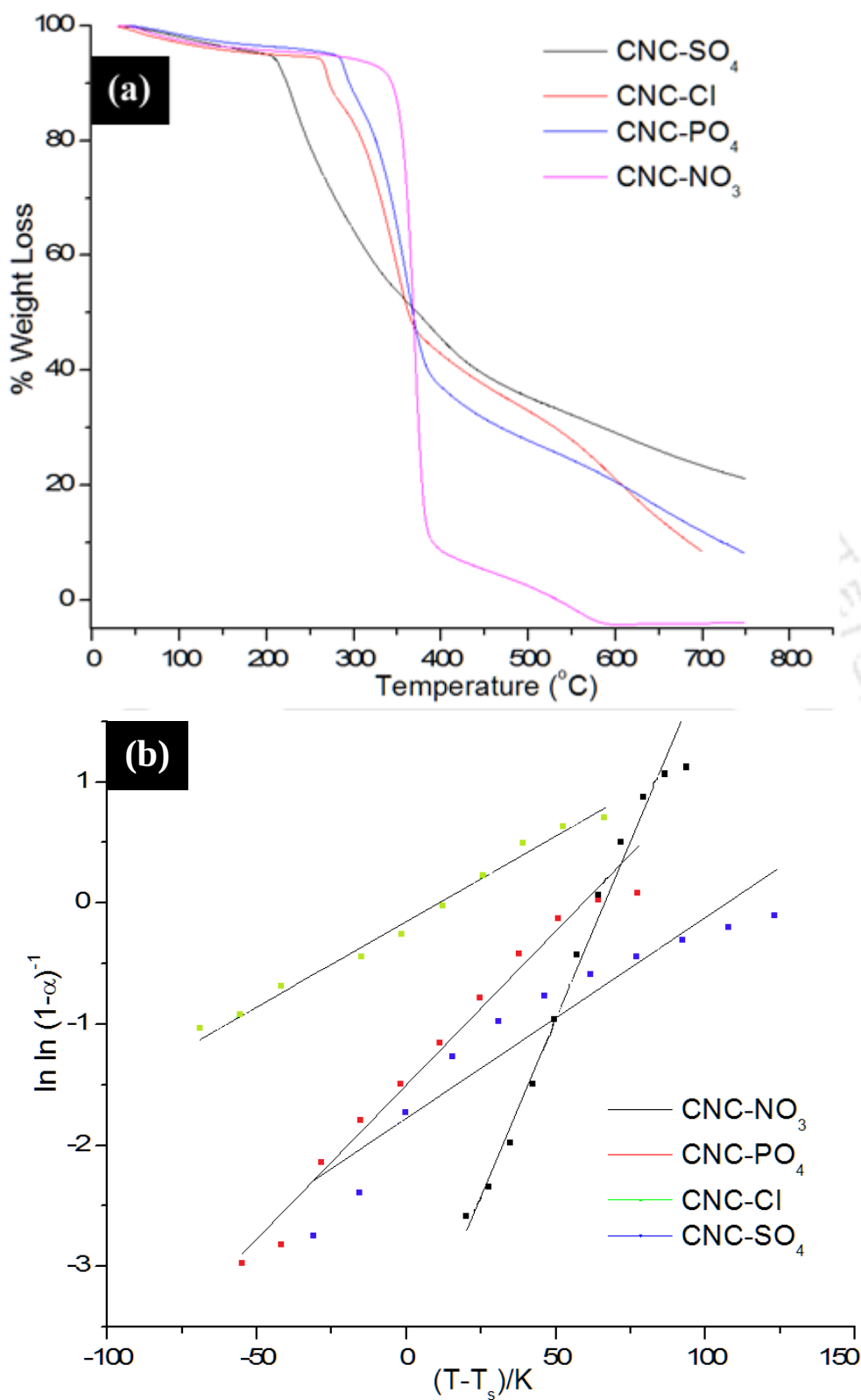


Figure 3.8: (a) Comparison of the TGA thermograms and activation energies plots of different acid derived CNCs.

- **Surface Energy Characteristics of Different Acid-hydrolyzed CNCs**

The effect of different hydrolyzing acids on the surface properties of the fabricated CNCs and therefore, on the interfacial interaction with the polymeric matrix were investigated through contact angle and surface energy (tension) measurements. It is well known that the contact angle values and the calculated surface energies depend on several factors such as chemical functionality, surface roughness, charge and crystallinity along with the sample preparation approaches used [311]. Therefore, the freeze dried powders of different acid-derived CNCs were carefully compacted into pellets under the application of uniform pressure so that the top and bottom surfaces of pellets are smooth to avoid errors in contact angle measurements. Due to high crystallinity and nanoscale sizes of CNCs, it is expected that the effect of water absorption capacity or wettability and surface roughness are negligible [311]. It was found that the liquid drops placed on CNC pellets were stable for few minutes only (especially in case of water) due to sorption/diffusion through the pores presents in between the nanoparticles. It is generally reported in the literature that when the surface roughness is in range of 0.15-0.1 μm , it tends to have no significant effect on the contact angle measurements [312]. AFM based topographic studies shows that different acid derived CNCs have surface roughness that varies within range of 30–80nm which falls well within the prescribed limits as stated above. The contact angles for three different liquids (water, diiodomethane and glycerol) and the effect on the total surface energy (and its dispersion and polar components) were calculated using Owens and Wendt approach and Lifshitz-van der Waals/acid-base approach (Table 3.2 and 3.3). For the two probe liquids, water and diiodomethane, several studies[312] have shown that the measured contact angles on the CNC pellets (sulphuric acid hydrolysis through traditional approach) are generally in the range of 30– 45° and 29–37° respectively.

The measured contact angle of CNC-SO₄ pellets using the three liquids are found to be almost in agreement with the reported literature values [312]. For the polymeric materials such as PLA, the reported contact angle values have been in the range of 65– 75° (for water as contact liquid) with total surface energy in the range of 35.9–43.9 mN/m which was found to depend upon the processing technique and parameters selected [313]. In this study, the total surface energy of PLA films was calculated as 39.9 mN/m with its disperse and polar components calculated as 36.8 and 3.1 mN/m respectively (Table 3.2).

Contact angle measurements of the acid-derived CNC pellets with different polar and non- polar liquids showed significant variation suggesting that the different acids used for hydrolysis led to modification of the functional groups in CNCs. Since acid hydrolysis is carried out under stringent conditions, the hydroxyl groups present on surface of CNCs are usually replaced by the anions of acids; however, the degree of substitution depends on the acid type and the reaction time of hydrolysis. CNCs are hydrophilic nanoparticles due to the abundant hydroxyl functional groups present on their surface, and hence, have lower contact angles with water as solvent. CNC- SO₄ and CNC-NO₃ show water contact angle of ~41.7° and 34.6° respectively, suggesting that CNC- NO₃ is more hydrophilic in nature. Introduction of nitrogen moieties have been known to increase the hydrophilicity of the nanomaterials [314]. Therefore, CNC-NO₃ has the lowest water contact angle due to the presence of nitrate groups on its surface, which are incorporated during hydrolysis reactions with nitric acid. Similarly, in case of glycerol which is a hydrophilic probe liquid, CNC- SO₄ and CNC-NO₃ have lower glycerol contact angle of ~21.2° and 34.3° respectively. This suggests increased hydrophilic nature of CNCs, which subsequently leads to higher contact angle values of ~41.9° and 36.3° for CNC-NO₃ and CNC-SO₄ respectively when diiodomethane is used as a probe

liquid. Introduction of the chlorine and phosphate moieties during acid hydrolysis with hydrochloric and phosphoric acids led to incorporation of hydrophobic behavior into CNCs. Thus, application of water and glycerol as probe liquids resulted in comparatively higher contact angles of 54.1° and 59.0° respectively for CNC-Cl and 51.5° and 36.3° for CNC-PO₄. As expected, introduction of non-polar liquid diiodomethane leads to lower contact angle of 38.4° and 39.3° for CNC-Cl and CNC-PO₄ respectively. This suggests that CNC- Cl and CNC- PO₄ are moderately hydrophobic in nature compared to CNC-NO₃ and CNC- SO₄, probably due to substitution of hydroxyl functional groups on CNCs with the anions of the acids. Furthermore, incorporation of such tunable surface properties into different acid- derived CNCs is confirmed from the surface energy (γ_s) values calculated using Owens and Wendt approach which shows that CNC-NO₃ and CNC-SO₄ have significantly higher total surface energy values of 65.9 and 63.4 mN/m respectively compared to 55.8 and 57.0 mN/m for the CNC- Cl and CNC-PO₄ respectively. Interestingly, it is observed that although the different acid- derived CNCs have quite similar values for the dispersive component of the surface energy (38.6–41.4 mN/m); the polar component varied significantly (from 15.4 to 27.3 mN/m). The variation in the polar component of the surface energy (γ_s^p) suggests change in surface properties of CNCs since it takes into account the dipole-dipole, dipole-induced dipole and hydrogen bonding-based interactions into consideration [315]. As γ_s^p contains information about the polar surface interactions, the γ_s^p values can be effectively used to predict the interaction of the nanofillers with the polymeric matrix. Lower values of γ_s^p lead to decreased hydrophilic behavior, and therefore enhanced the compatibility with hydrophobic polymeric systems [316]. CNC-PO₄ has lowest γ_s^p compared to rest of the acid-derived CNCs, and are

thus expected to compatibilize with the PLA matrix effectively during extrusion process leading to enhanced dispersion.

Table 3.2: Contact angle measurements and the dispersion, polar and total surface free energies (based on Owens and Wendt approach) for the acid derived CNCs using three different solvents: water, diiodomethane and glycerol at 25°C.

CNC	Water (°)	Diiodomethane (°)	Glycerol (°)	γ_s^d (mN/m)	γ_s^p (mN/m)	γ_s (mN/m)	R ²
CNC-SO ₄	41.7 ±2.5	36.3 ±1.2	21.2 ±1.1	41.4	22.0	63.4	0.98
CNC-Cl	54.1 ±1.6	38.4 ±3.1	59.0 ±2.9	40.3	15.4	55.8	0.97
CNC-PO ₄	51.5 ±1.5	39.3 ±2.2	36.3 ±1.4	39.9	17.1	57.0	0.99
CNC-NO ₃	34.6 ±2.1	41.9 ±1.0	34.3 ±1.7	38.6	27.3	65.9	0.99

To further understand the surface energy properties of the different acid-derived CNCs at the molecular level, detailed investigations are carried out with three different liquids (both polar and non-polar) using the Lifshitz-van der Waals/acid-base approach (Table 3.3). The dispersive component of all the acid-derived CNCs calculated using both Owens and Wendt approach and Lifshitz-van der Waals/acid-base approach showed comparable values in the range of ~38– 41 mN/m. In the agreement with previously reported studies [312], it is found that all the acid derived CNC pellets have significantly higher $\gamma_s^- \gg \gamma_s^+$ values suggesting electron donating tendency of CNCs. This is probably due to the probe liquids used for contact angle studies are acidic in nature ($\gamma_s^- \gg \gamma_s^+$) or due to the abundant oxygen moieties present in the hydroxyl functional groups of CNCs which could act as proton acceptors. Comparison of γ_s^- values for different acid-derived CNCs are expected to depend on the degree of ionization /electronegativity values of the substituted anionic moieties (during acid hydrolysis) present on CNC surface. As per the dissociation values of the acid used for hydrolysis (as discussed in

previous section 3.2.2), CNC-Cl and CNC-NO₃ have higher electron donating capacity (leading to higher γ_s^- values) compared to CNC-SO₄ with CNC-PO₄ being the lowest. All the acid-derived CNCs have almost similar γ_s^{ab} values except for CNC-Cl, which shows significant decrease in polar component of surface energy (Table 3.3). This is probably due to the presence of nascent chlorine moieties which have significantly reduced inter/intramolecular hydrogen bonding ability leading to agglomeration, as also evident from the previously reported studies [317]. A similar trend is observed in case of γ_s^+ values, for which CNC-Cl shows substantial reduction in the electron acceptor tendency due to the presence of highly electronegative chlorine moieties (Table 3.3). Therefore, the fabrication of CNCs using different acids leads to functionalization with different chemical moieties, thereby altering the surface properties significantly.

Table 3.3: Total and constituent surface free energies based on Lifshitz-van der Waals (dispersion)/acid-base approach for the acid derived CNC pellets using three different solvents: water, diiodomethane and glycerol at 25°C.

CNC Type	γ_s^{lw} (mN/m)	γ_s^+ (mN/m)	γ_s^- (mN/m)	γ_s^{ab} (mN/m)	γ_s (mN/m)
CNC-SO ₄	42.9	3.6	21.3	17.7	60.6
CNC-Cl	38.7	0.03	30.3	1.9	40.6
CNC-PO ₄	39.1	3.3	16.7	14.8	53.9
CNC-NO ₃	36.6	2.2	36.9	17.9	54.5

3.2.3 Fabrication of CNCs from Alternative Renewable and Waste Biomass Resources

Utilization of the renewable feedstock such as agricultural residue or waste newspaper for the fabrication of CNCs is a challenging task, due to the presence of wide variety of impurities and uncertainty in composition, which makes the pretreatment of biomass a complicated process. We have developed a novel process for simultaneous decoloration and acid hydrolysis

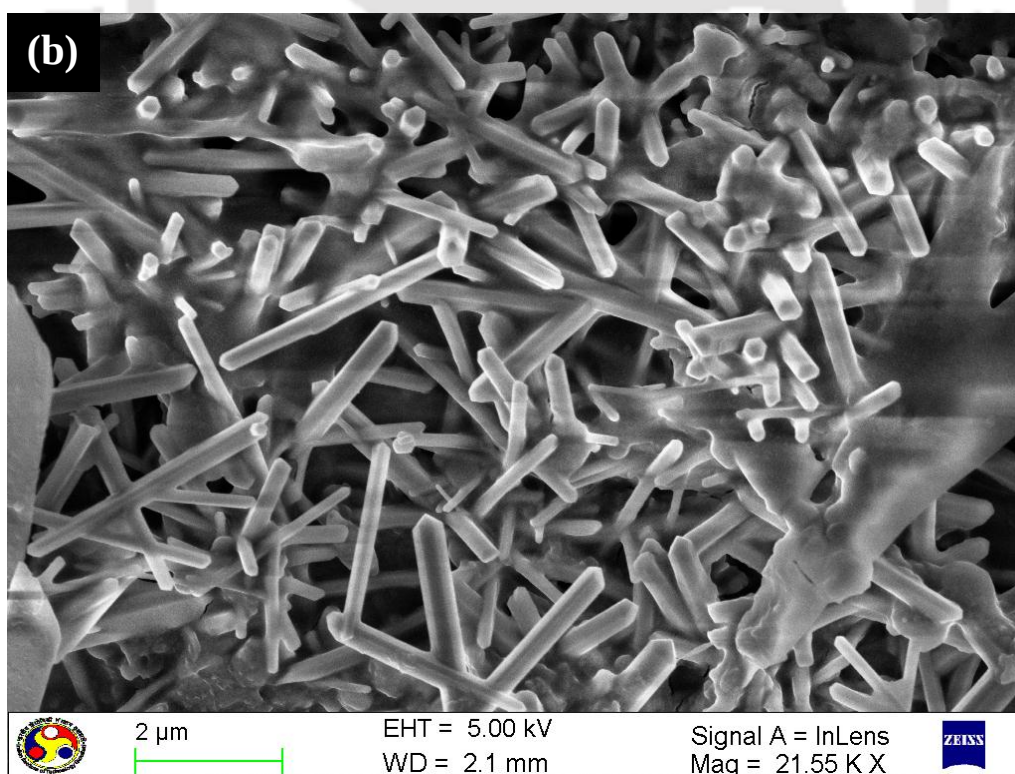
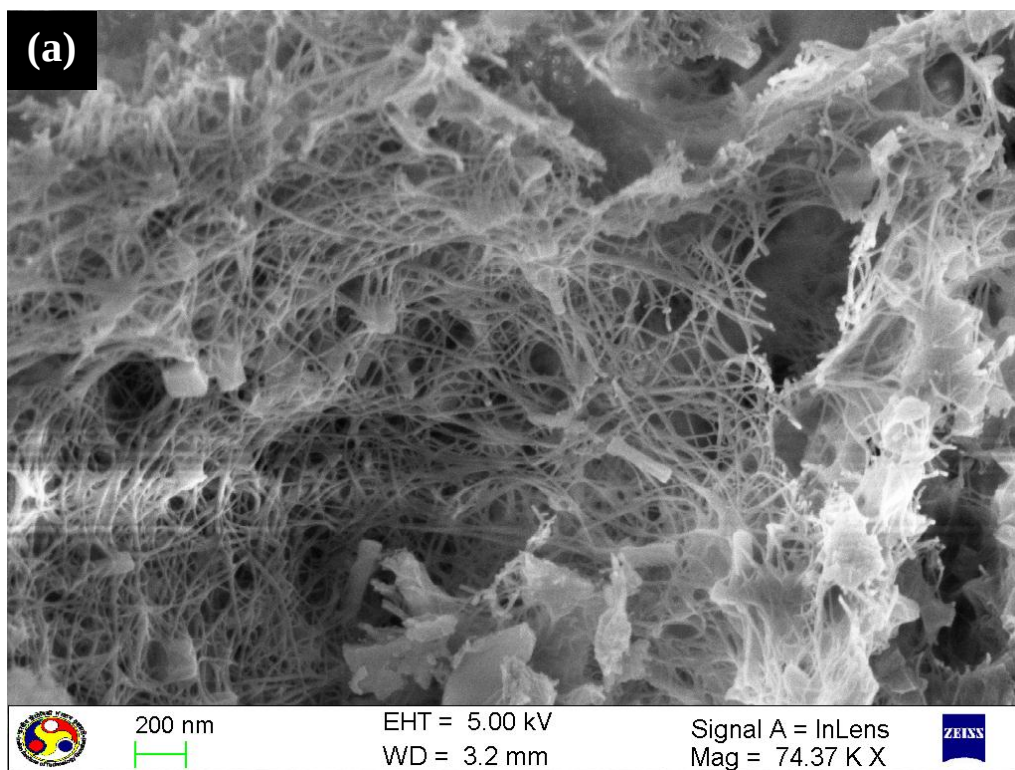
of the pretreated cellulose extracted from waste newspapers, for fabrication of CNCs, which provides us an opportunity to convert 'waste into wealth'. The waste newspaper are chopped into small pieces, and immersed in acetone overnight to remove the impurities such as color or ink. After, the removal of acetone, the obtained pulp was treated through soda-pulping followed by sodium sulfite treatment and bleaching to extract the purified cellulose. Introduction of the sodium sulfite treatment (2-5 wt. %), helps in removing the color and ink impurities present in the newspapers. Figure 3.9, shows the FESEM micrographs for the waste newspaper and the pretreated pulp which shows the presence of the fluffy bundled cellulosic fibers devoid of any other impurities. The acid hydrolysis (with 64 wt.% of sulphuric acid) of the pretreated cellulosic pulp from newspaper provides a single step approach for decoloration of pulp as well as CNC fabrication through the formation of biphasic system. After the hydrolysis is complete, the upper phase separates out in the form of black coloration which contains the trace amount of ink/color present in pulps. The lower phase, which is whitish in color, contains the CNC suspension which is separated out through simple decantation process. The fabricated CNCs through this approach are pure and devoid of any inorganic/organic impurities, with distinct rod-like morphology of length 851 ± 50 nm and diameter of 35 ± 8 nm respectively. Furthermore, we tried to fabricate different nanocellulosic structures such as cellulose nanofibers (CNFs) and spherical cellulose nanoparticles (SCNPs) by optimizing the acid concentrations and time of hydrolysis. Figure 3.10, shows the FESEM micrographs of thin and bundled CNFs with a diameter of 10 ± 5 nm formed through acid hydrolysis (with ~ 30 wt. % sulphuric acid), when carried out for a time period of ~ 4 hours. On further, increasing the acid concentration to ~ 80 wt.%, spherical CNPs are formed of dimensions 120 ± 40 nm which is probably due to enhanced degradation of cellulose at high acid concentrations. The

extraction of different morphologies of nanocellulosic materials from single waste biomass source provides us the opportunity to tune their inherent characteristics depending on the targeted applications.

The agricultural residues after the harvesting of crops are generally thrown as wastes or incinerated, either of which leads to severe environmental pollution. Therefore, in this study we have selected rice husks, which is abundantly available in India as a cellulosic waste biomass source for extraction of CNCs. The rice husks were crushed into fine powder ($\sim 20\text{--}25\text{ }\mu\text{m}$) through rotating ball mill, followed by washing with water and acetone several times to remove the impurities such as mud, dust or other foreign particles. The husks are subjected to soda-pulping followed by bleaching for the extraction of the purified cellulose fibers. The purified cellulosic fibers when acid hydrolyzed through traditional approach of ($\sim 64\text{wt. \%}$) leads to formation of CNCs with length of $650\pm 5\text{ nm}$ and diameter of $30\pm 2\text{ nm}$. Utilization of the waste bamboo stems (abundantly available in North-East part of India) for fabrication of CNCs through similar approach of soda pulping, bleaching cum acid hydrolysis leads to formation of distinct rod-like CNCs with high aspect ratio (length $743\pm 30\text{ nm}$ and diameter $21\pm 4\text{ nm}$) (see Figure 3.10(a)). Interestingly, the CNCs obtained from pretreated bamboo pulp are of high crystallinity ($\sim 92\%$) alongwith higher yield (50-60%) compared to other related biomass, due to which it have been selected for further study for fabrication of high performance composites as discussed in subsequent chapters 4, 5, 6, 7 and 8 in thesis. Therefore, the utilization of the waste renewable resources and their strategic conversion into value added products such as nanocellulose and its derivatives provides a great opportunity to convert 'wastes into wealth'.

3.2.4 Fabrication of CNCs through Indigenously Developed CNC Production-cum-Continuous Dialysis Unit

As shown in Figure 2.1, an indigenously developed CNC production unit coupled with continuous dialysis system have been developed to reduce the processing time of CNCs in comparison to the traditional approaches. Efficiency of the developed system have been evaluated through continuous monitoring of the pH of CNC suspension during the dialysis process. It is recorded in terms of the time period required by the CNC suspension to reach a pH of ~ 7 , which results in formation of neutralized CNCs. It is noteworthy to mention that the CNCs are viable for potential applications only when they are in neutralized state, which however requires atleast ~ 7 days when processed through traditional routes of dialysis. To overcome such challenges, the processing of CNCs through the developed set-up reduced the dialysis time significantly which have been tried and tested with various examples such as different batch sizes of the CNCs produced, different initial cellulosic precursor and various acids used as hydrolyzing agents as shown in Figure 3.11 (a)-(d).



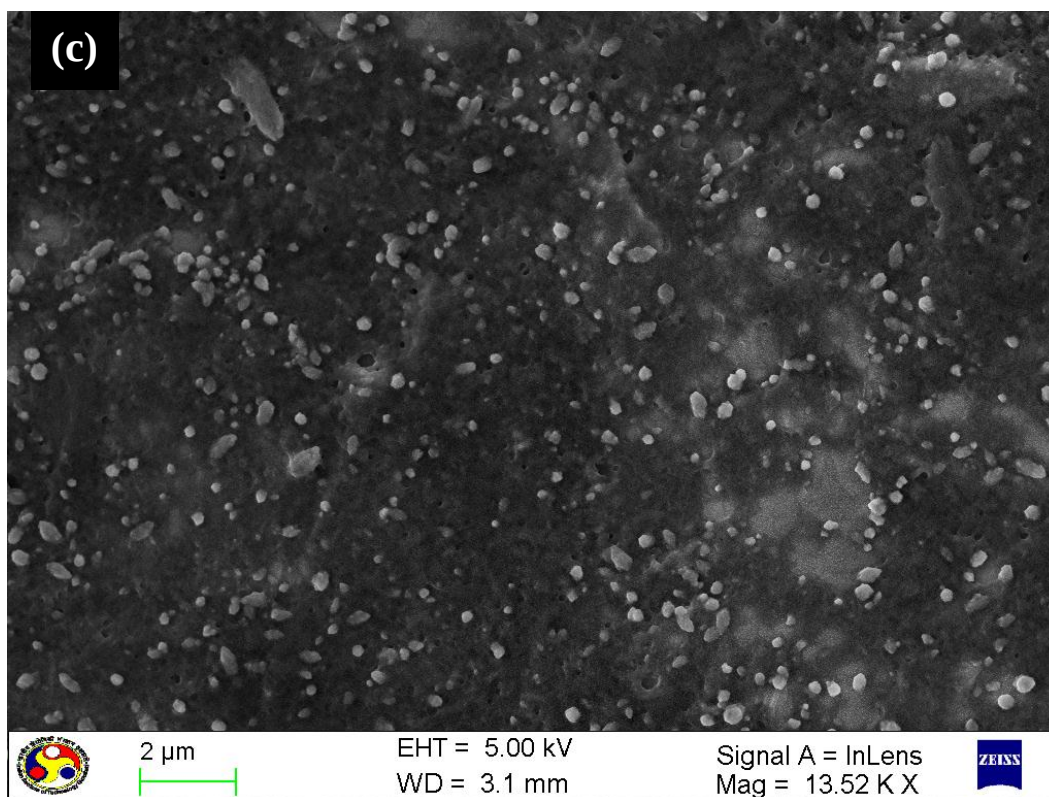
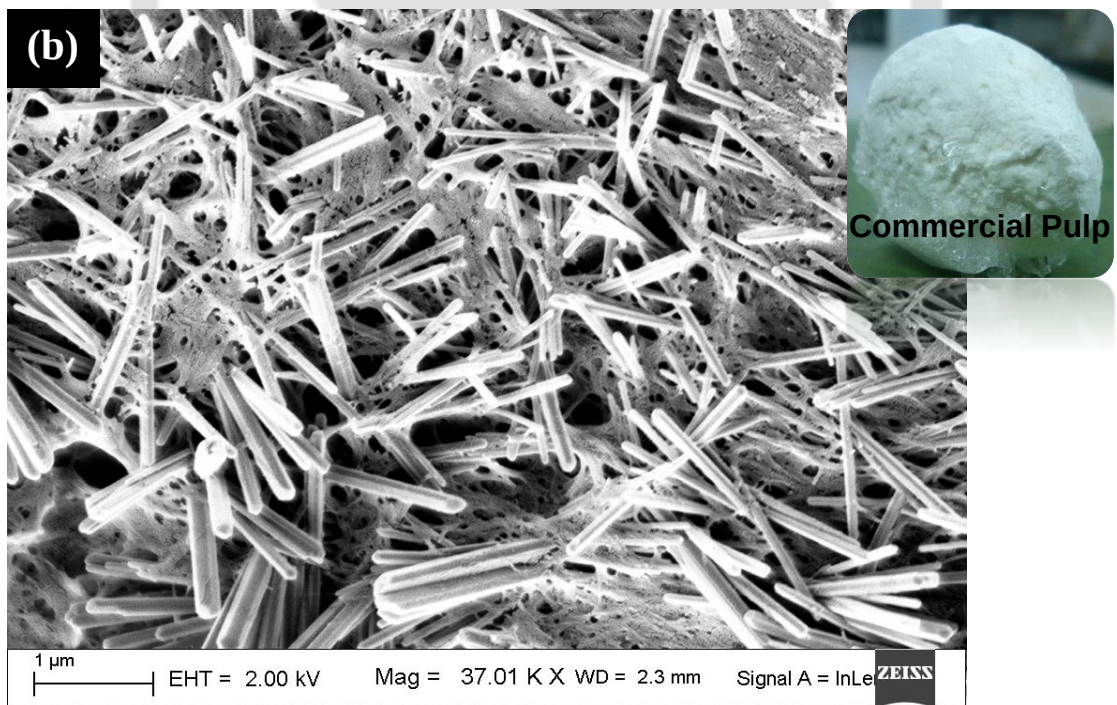
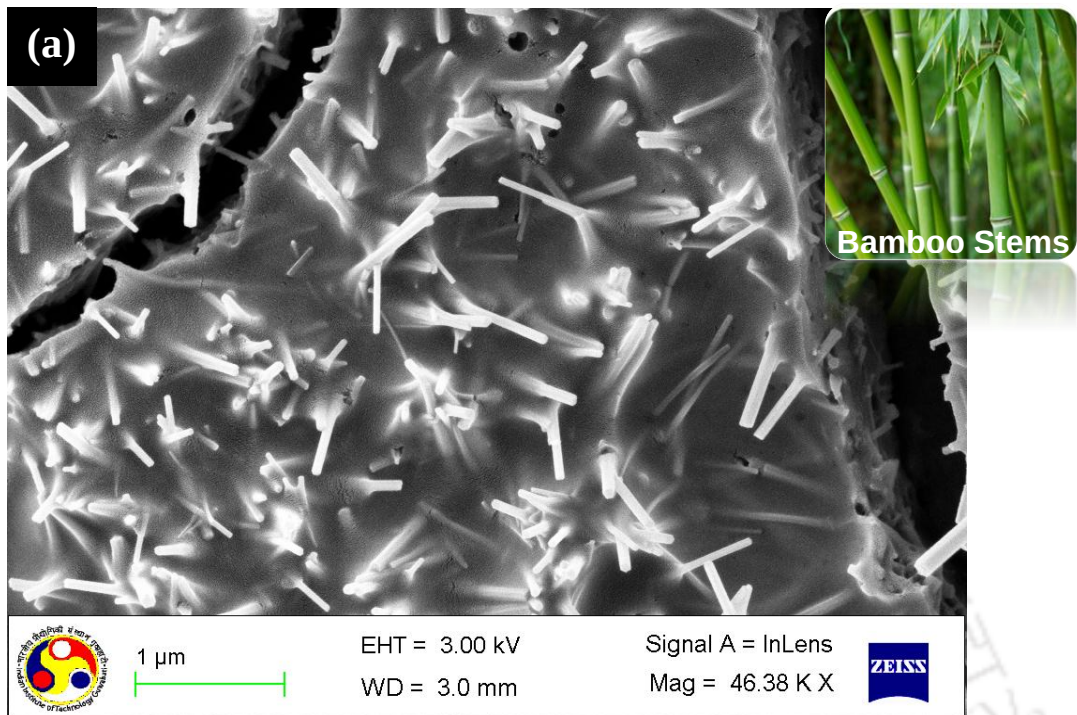


Figure 3.9: FESEM micrographs of different morphological dimensions of nanocellulose such as (a) cellulose nanofibers fabricated through 30 wt. % sulphuric acid hydrolysis, (b) cellulose nanocrystals fabricated through 64 wt. % sulphuric acid hydrolysis and (c) spherical cellulose nanoparticles fabricated through 80 wt. % sulphuric acid hydrolysis.



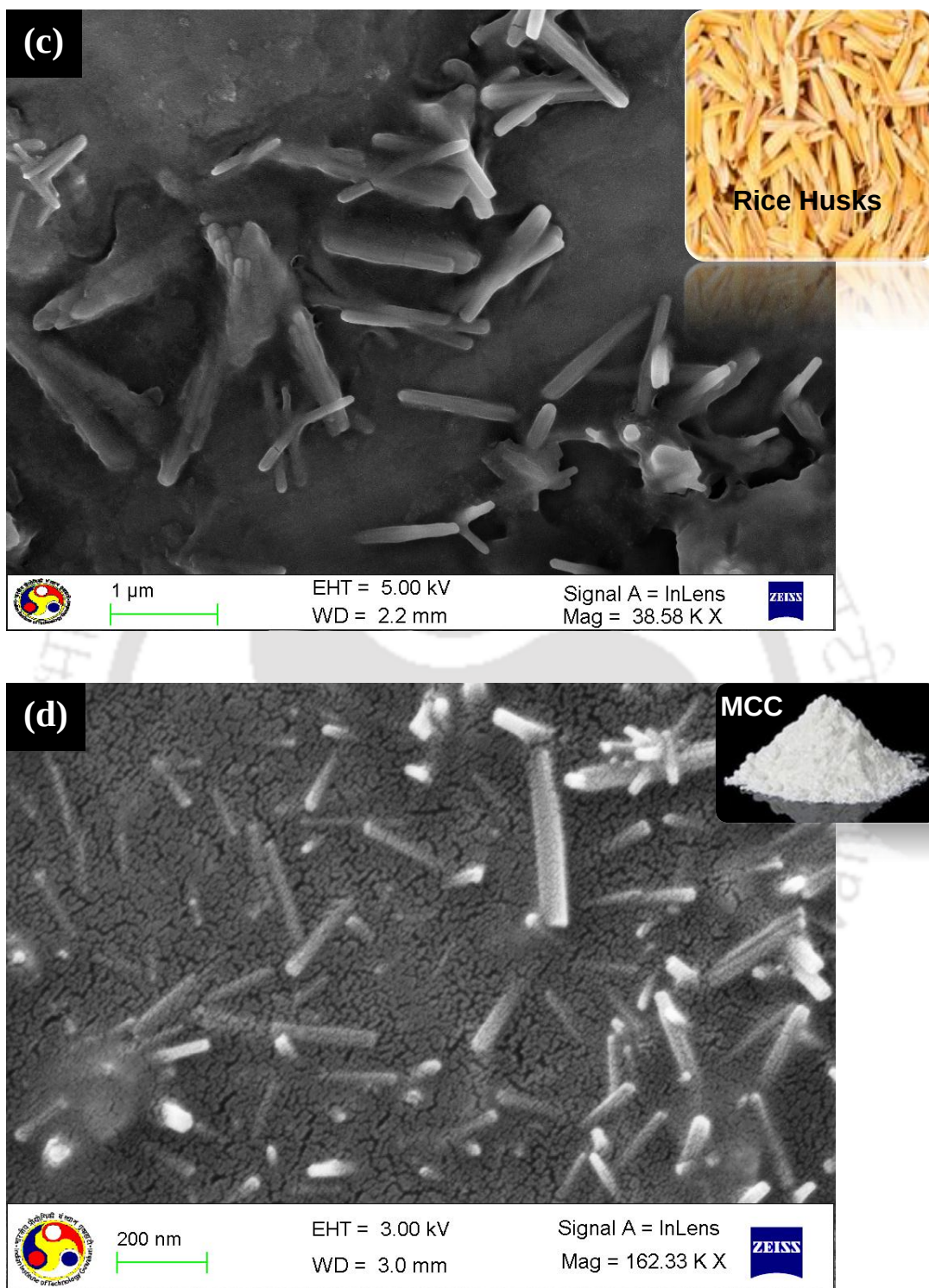
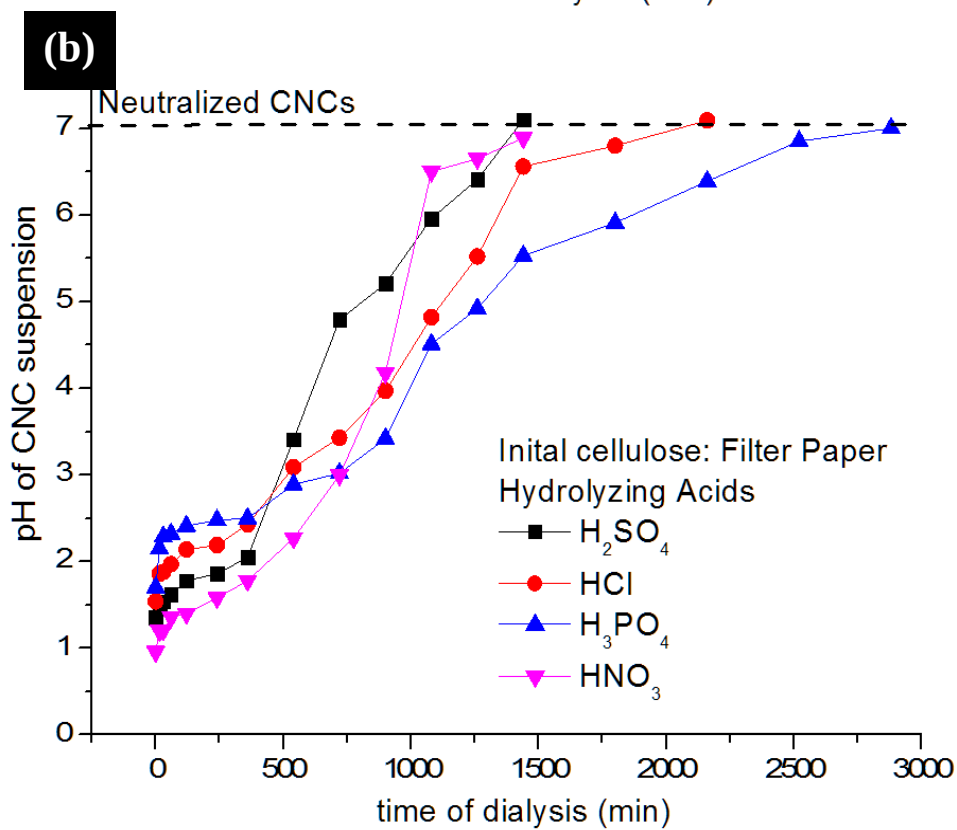
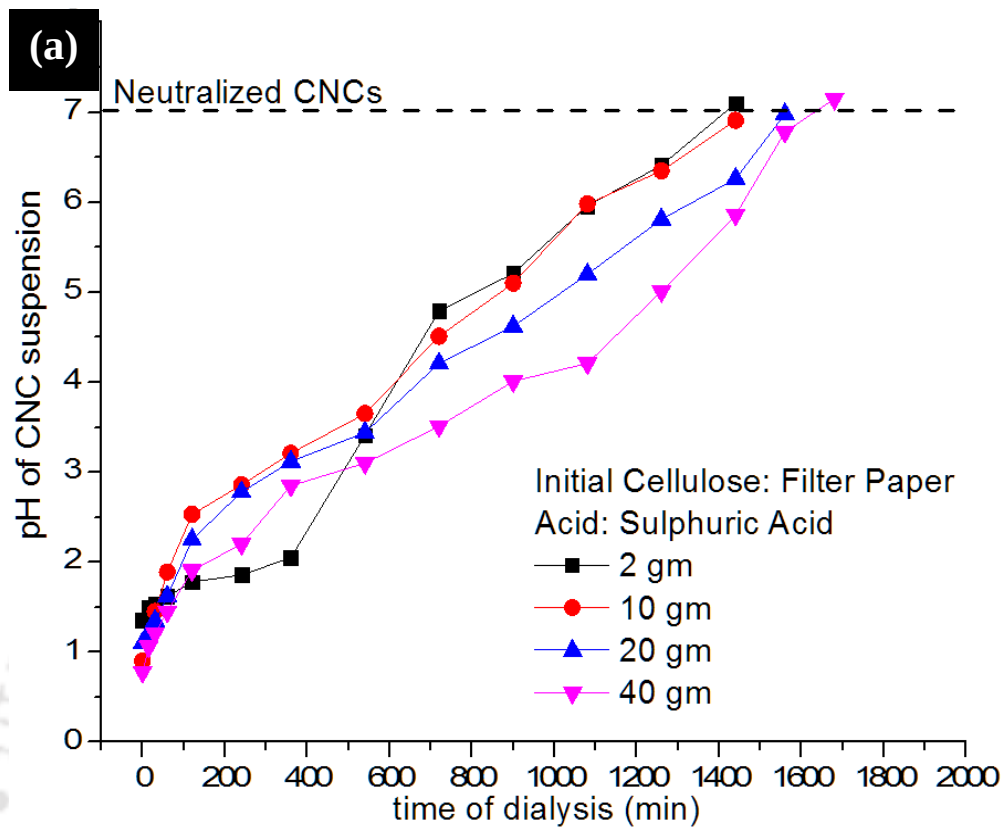


Figure 3.10: FESEM micrographs of CNCs fabricated from (a) bamboo stems, (b) commercial pulp, (c) agricultural residues of rice husks and (d) microcrystalline cellulose.

This is probably due to the variation in dissociation values of the acids as well as entrapment of protonated H^+ and anionic moieties in the cellulosic biomasses which becomes difficult to separate out in case of the HCl and H_3PO_4 derived CNCs. On the utilization of different cellulosic biomass as initial precursor through sulphuric acid hydrolysis it was observed that the dialysis time remains almost unchanged for case of the CNCs derived from bamboo pulp, filter paper and microcrystalline cellulose. Interestingly, CNCs derived from the newspaper shows reduced dialysis time of ~ 16 hours, which suggests that the developed set-up have the potential to be scale-up for processing of high volume of acidic CNC suspensions through a continuous dialysis approach. Moreover, dialysis is a diffusion driven phenomenon which is relatively slow process and depends upon the concentration gradient which gradually decreases with time in case of normal dialysis process. The developed dialysis set-up is a dynamic approach in which the CNC suspension remains in continuous recycling for multiple number of times coming in contact with the pure water at every cycle. These creates a high potential gradient between the acidic suspension and water which remains unaltered with time alongwith the recycling of the CNC suspension results in synergistic effect for removal of the acidic protons from the entrapped layers of CNC. Therefore, it could be concluded that the developed set-up for production of CNCs through continuous dialysis process have shown promising application in overcoming the techno-economical as well as environmental challenges associated with CNC processing with the potential to scale-up though industrially feasible process.



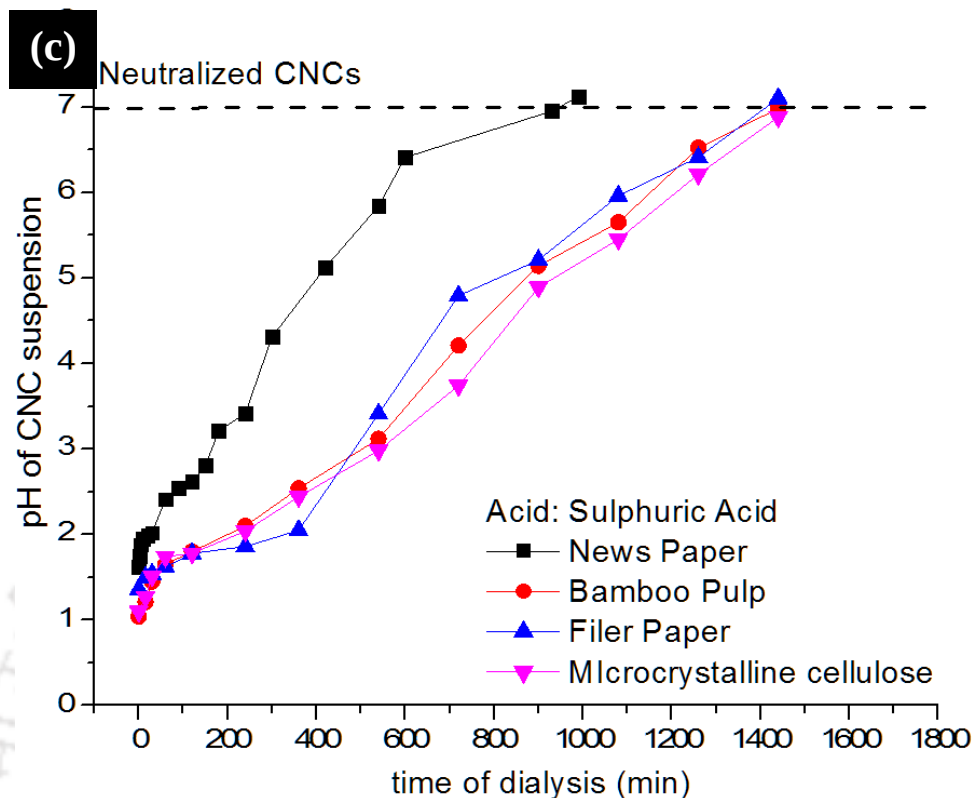


Figure 3.11: Continuous dialysis of CNCs which shows change in the pH of CNC suspension with respect to dialysis time for (a) different batch sizes of CNC suspensions, (b) various acids used as hydrolyzing agents and (c) different initial cellulosic precursor.

3.3 CONCLUSIONS

The renewable agricultural feedstocks such as bamboo stems or rice husks and waste resources such as newspapers can be effectively converted into nanocellulose and its derivatives, through strategic pretreatment and acid hydrolysis approach. The morphological dimensions, crystallinity and physico-chemical properties such as degree of hydrogen bonding significantly depends upon the type of the initial biomass selected and extent of hydrolysis. CNCs fabricated from bamboo stems shows distinct rod-like morphology with higher crystallinity and

productivity yield, hence, are selected for further detailed investigations. A novel indigenously developed CNC production unit coupled with continuous dialysis system was developed, which reduces the NC processing time from ~7 days to 24-26 hours. It was found the variation in polymorphism of cellulose and type of acids selected as hydrolyzing agents leads to tailored physical, structural, thermal and surface characteristics. Morphological analysis shows that CNCs fabricated with cellulose I and cellulose II polymorphism have distinct variation in morphology and dimensions (aspect ratio of ~50, ~17, ~57 and ~24 for CNC-SO₄, CNC-Cl, CNC-PO₄ and CNC-NO₃ respectively) in comparison to the different acid-derived CNCs (aspect ratio of ~33, ~25 and ~6 for CNC I, CNC II and CNC:I→II respectively). Due to variation in packing density in different polymorphs of cellulose, the percentage crystallinity of CNC I, CNC II and CNC: I→II is found to be ~81%, ~77% and ~70 % respectively. Similarly, due to variation in the dissociation constant of different acids the fabricated CNCs have crystallinity values of ~85% for CNC-Cl, ~81% for CNC-NO₃, ~75% for CNC-SO₄ and ~70% for CNC- PO₄. Comparison of the thermal stability shows that the onset degradation temperature for CNC I, CNC II, CNC:I→II , CNC-SO₄, CNC-Cl, CNC-PO₄ and CNC-NO₃ varies as 176, 226, 191, 238, 352, 365 and 371°C respectively. Moreover, the CNCs with different cellulose polymorphs as well as the different acid-derived CNCs, with altered hydrogen bonding and substituted anionic moieties, show tunable surface behaviour due to which their interfacial interaction with the polymeric matrix can be regulated depending upon targeted applications, which have been discussed and studied in subsequent chapter. Therefore, various waste, renewable biomass resources can be successfully utilized for fabrication of CNCs, followed by strategic modifications during the processing step itself, to tailor their inherent characteristics depending upon targeted applications.

Studies on Polyhydroxybutyrate/Cellulose Nanocrystal based ‘Green’ Nanocomposite for Food Packaging Applications

Motivation

Cellulose Nanocrystals (CNCs) derived from the renewable biomass resources, are smart nanomaterials with attractive anisotropic mechanical properties (comparable to steel with $E_{||}=110-220$ and $E_{\perp}=2-50$ GPa), high hydroxyl functionality, rod-like morphology with tunable aspect ratio, and non-toxicity, which makes it an ideal candidate for reinforcing agent. Polyhydroxyalkanoates (PHAs), are class of thermoplastic biopolymers produced through microbial fermentations also have interesting properties such as biodegradability and biocompatibility, but their potential applications as packaging based products have been limited due to poor physico-chemical, structural and barrier properties. Introduction of CNCs in PHB matrix forms percolated-network like structure through hydrogen-bonding, which leads to development of sustainable packages with improved structural stability, gas-barrier properties, biocompatibility and biodegradability required for stringent food packagings.

The work in this chapter are published as:

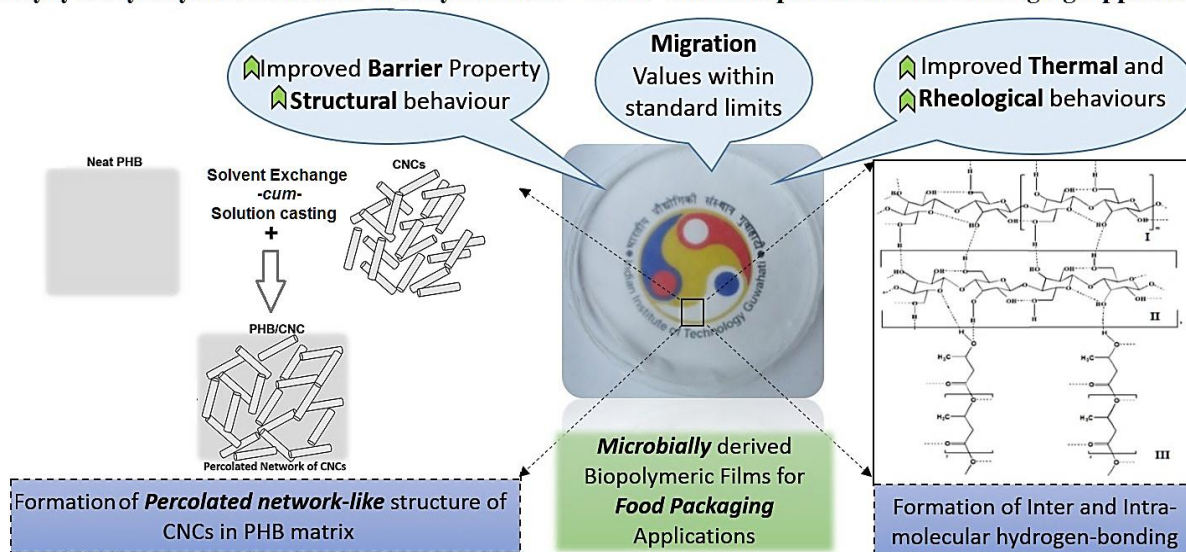
- Dhar, P., Bhardwaj, U., Kumar, A., & Katiyar, V. (2015). Poly (3- hydroxybutyrate)/cellulose nanocrystal films for food packaging applications: Barrier and migration studies. *Polymer Engineering & Science*, 55(10), 2388-2395.
- Dhar, P., Bhardwaj, U., Kumar, A., & Katiyar, V. (2015). Investigations on rheological and mechanical behavior of poly (3-Hydroxybutyrate)/cellulose nanocrystal based nanobiocomposites. *Polymer Composites*.

Abstract

The chapter deals with fabrication of green and sustainable polyhydroxybutyrate/cellulose nanocrystal based biodegradable films with its improved intrinsic characteristics such as thermal, mechanical, barrier, processing capabilities and rheological behaviour. Due to their high hydroxyl functionality, CNCs formed percolated network-like structure through intramolecular and intermolecular hydrogen bonding, which was fundamentally understood through linear/non-linear rheological studies and mechanical modelling. The stability of such networks was predicted from permeation activation energy and permeability parameters such as solubility and diffusion coefficients, which helps to mechanistically understand the transport properties of the gas molecules across the nanocomposite films. The fabricated PHB/CNC films have overall migration values within the standard limits as per the set legislations required for stringent food packaging applications. In addition, characteristics such as non-toxicity, biocompatibility and complete biodegradability make PHB/CNC nanocomposite-based packaging an ideal alternative to non-degradable petroleum-derived plastic packaging.

Graphical Abstract

Polyhydroxybutyrate/Cellulose Nanocrystal based ‘Green’ Nanocomposite for Food Packaging Application



4.1 INTRODUCTION

In recent decades, increased environmental concerns to reduce carbon footprint and rising prices of petrochemicals have led to increased attention on the development of green and sustainable biodegradable polymers, as an alternative to conventional petroleum-derived polymers. Among the several synthetic biodegradable polymers reported in literature, only a few such as poly (lactic acid) (PLA), poly (3-hydroxybutyrate) (PHB), poly (ϵ -caprolactone) (PCL), etc., have the potential to be manufactured at industrial-scale for development of commercially viable value-added products. However, the performance of biodegradable polymers in terms of their processability, structural stability, durability, reusability and recyclability are some of the major concerns which needs to be addressed. Biodegradable polymers have poor mechanical strength with brittle nature, low thermal and barrier properties which requires significant technological improvements through novel chemical modifications or polymerization approaches, incorporating different micro or nanofillers, plasticizers and blending with other compatible polymers.

Polyhydroxyalkanoates (PHAs) are a class of biodegradable polyesters produced through microbial fermentation routes utilizing renewable biomass resources, which need to be processed into fermentable broth through chemical treatments. The production process of PHAs usually consists of three steps: (i) preprocessing of the biomass into reduced sugars which are easily fermentable as nutrient broth, (ii) optimized fermentation process for accumulation of PHAs under nutrient stressed conditions and (iii) extraction and purification of the PHAs through cell lysis. PHAs are usually high molecular weight water-insoluble polymeric fragments present in the cytoplasm of microorganisms as intracellular granules of sizes in range of 0.2-0.5 μm , synthesized by the genetically engineered or cloned bacterial

strains [318]. Interestingly, the PHAs produced consist of various fractions of about 11 types of hydroxyalkanoates based monomeric units, some of the major fractions being 3- hydroxybutyrate, 3-hydroxyvalerate, 3-hydroxyhexanoate, 3-hydroxyoctanoate and 4- hydroxybutyrate in R configuration [178]. These heterogeneous units undergoes polymerization to form homopolymers as well as co-and heteropolymers with different compositions of molecular weights in the range of 200,000 to 3,000,000Da [179]. Interestingly, it was found that the fractions and types of PHAs produced apparently depends upon the synthesis route, bacterial strain used for fermentation and physiological stresses introduced by varying the carbon to nitrogen ratio during growth conditions, which, thereby alters the physico-chemical properties of the synthesized polymer [319]. In early 19th century, presence of the sudanophilic, lipid-like PHAs was first observed in *Azotobacter chroococcum* which was thought to contain 3-hydroxybutyrate (3-HB) units as the building blocks especially accumulated by the Gram-negative bacteria of genus *Bacillus* [320]. For last few decades, extensive research have been carried out in engineering the microbial strains, fermenter studies and optimization of the purification process, which successfully led to its industrial-scale productions by renowned companies such as Mitsubishi Gas Chemical Company Inc., BASF (Germany), P&G (USA), Jiangmen Biotech (China), etc. With a production capacity of 50-50000 tons/annum and commercialized under various trade names [321].

PHAs, have major fractions of the hydroxyalkanoates units as 3-hydroxybutyrate (3-HB), and exists in form of the homopolymeric units to form a brittle polymer, poly(3- hydroxybutyrate) (P(3HB) or PHB) which was first isolated and characterized by Maurice Lemoigne in year 1925 [321]. PHB the most popular type of PHAs, are reported to produce by more than 75 different genera of bacteria and are isotactic in structure with very

high crystallinity of ~55-85%. PHB, have typically molecular weight in range of ~10- 3,000 kDa with high melting temperature of (T_m) ~175-180°C, glass transition temperature (T_g) ~4°C and crystallization temperature (T_{cc})~102- 106°C. PHB have tensile strength of ~43MPa with Young's modulus of ~3.5 GPa, but are highly brittle with reduced elongation at break of ~5% due to its crystalline nature which limits its applications [322]. Compared to other biodegradable polymers, PHBs are readily degradable with good crystallization and thermal properties, but structural and barrier properties needs to be improved through introduction of nanoparticles or biobased nanofillers, to have a competitive market with conventional petroleum derived polymers [180]. As discussed earlier, the monomer units in PHB can be replaced with other hydroxyalkanoate units to form random copolymers of various chain lengths which can be fabricated through tuned microbial fermentation or chemosynthesis process. Depending upon the variation in HA units and their relative fractions, different species of PHAs are produced through alteration of carbon source and microbial strains during microbial fermentation process. The physico-chemical, thermal, mechanical, structural and barrier properties of PHAs can be remotely regulated by controlling the concentrations of hydroxyalkanoates monomeric units in homo, random or copolymeric segments, for development of value added products depending upon the targeted applications.

Polyhydroxyalkanoate-based derivatives produced through tuned microbial fermentations have been widely used for fabrication of nanocomposites for wide range of potential applications such as biodegradable food packagings, in tissue engineering as scaffolds due to their improved biocompatibility or as targeted drug delivery-based biomedical devices[323]. Two class of polyhydroxyalkanoate polymers, namely poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), have been widely studied in literature

for fabrication of the nanocomposites through several methods such as solution-casting and extrusion-based molding technologies. Processing of PHAs through industrially viable extrusion is a challenging task due to the reduction in their molecular weight under high shear and temperature conditions, which deteriorates the inherent characteristics of the polymer required for development of value-added products [324]. To overcome such deficiencies, micro/nano- fillers dispersed into the polymeric matrix at percolated threshold concentrations have been instrumental in improving the properties of polymeric nanocomposites. Inorganic nanofillers such as montmorillonite and its chemically modified forms such as neopentyl (diallyl) oxy tri(dioctyl) pyrophosphate titanate etc., have shown to improve the dispersion of nanoclays and acts as a strong reinforcement agents [325]. PHB/modified montmorillonite nanoclays based composites prepared through melt-extrusion leads to intercalation of polymer into clay galleries which enhances the Young's modulus and lowers the biodegradation rate [326]. Other clay derivatives such as kaolinite or saponite during melt processing leads to intercalation of the polymer into clay galleries which significantly enhances the thermal stability of prepared nanocomposites [327].

The effective dispersion of hydrophilic CNCs into hydrophobic PHB matrix is a challenging task through melt-compounding, which have known to cause degradation in molecular weight [328], thereby, deteriorating the inherent characteristics of the prepared nanocomposites. In the present study, CNCs are fabricated through acid hydrolysis approach using pretreated cellulose extracted from bamboo stems followed by their dispersion into PHB matrix through solvent exchange-*cum*-solution casting approach. The presence of abundant hydroxyl functional groups in CNCs helps in formation of percolating network-like structure through inter- or intramolecular hydrogen bonding with the PHB matrix. The stability of such

networks and the threshold concentration have been investigated through linear and non-linear rheological studies. Small and large amplitude oscillatory shear (SAOS and LAOS) studies were measured over a range of frequencies to understand the degree of dispersion and confinement effect of CNCs on the PHB chains. The barrier properties were studied, and transport properties of oxygen across the nanocomposite films are mechanistically understood through permeability parameters, namely solubility and diffusivity. To study the effect of CNCs on the polymer chain mobility and the stability of the percolating network, the change in permeation with temperature was modelled using the Arrhenius equation and the activation energy for permeation was calculated. Migration studies have been performed on films using two food simulants, one polar and one nonpolar, to measure the total amount of non-volatile substances that might migrate from PHB/CNC films when brought in contact with food. The thermal properties of films before and after being subjected to migration studies have been analyzed to understand the change in polymer chain dynamics resulting from its interaction with nanofillers and food simulants. Therefore, the work presented in this chapter mainly focuses on fabrication of completely biodegradable and sustainable PHB/CNC films using bio-nanofillers derived from renewable resources and evaluation of their properties for possible applications in food packaging as an alternative to the conventional petroleum-derived polymers.

4.2 RESULTS AND DISCUSSIONS

4.2.1 Cellulose Nanocrystals: Morphological Investigations and Dispersion in Solvents

The pretreatment of raw bamboo stems are carried out to extract the purified cellulose fibers removing the trace amounts of hemicellulose and lignin content in it, through soda-pulping followed by bleaching methods (as shown in Figure 4.1(a)). Hydrolysis of the purified bamboo pulp using sulphuric acid leads to formation of defect-free CNCs having typical dimensions ranging from ~400 to 600 nm in length and ~15 to 20 nm in width, with aspect ratio ~30, which has been confirmed by both FESEM (Figure 4.1(b)) and AFM micrographs and its height profile (Figure 4.1(c) and (d)).

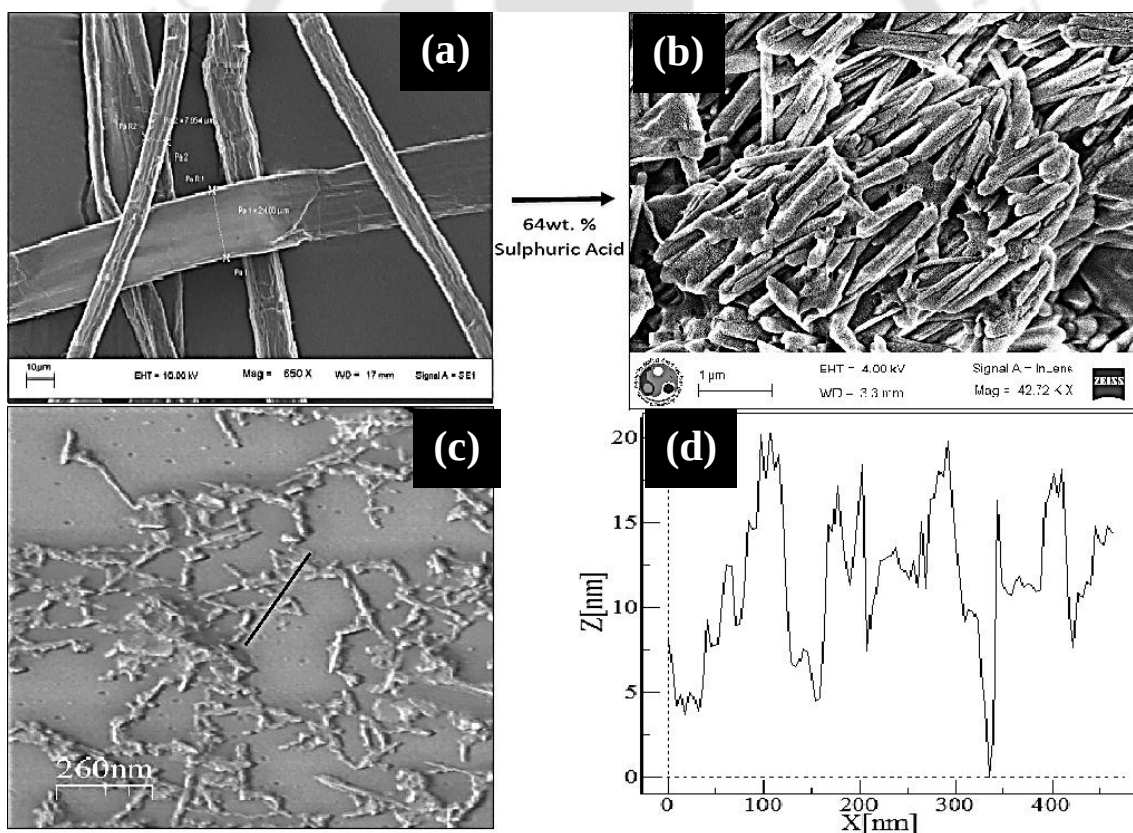


Figure 4.1: (a) Scanning electron micrographs of the purified cellulose fibers extracted from Bamboo stems and (b)FESEM and (c)AFM micrographs of fabricated CNCs with their height profile (of the selected region in black line).

The stability of CNCs suspension dispersed in different phases is analyzed with dynamic light scattering (DLS) by evaluation of its zeta potential values. Polymer nanoparticle solutions with high zeta potential (negative or positive) are considered to be electrically stabilized while those with low zeta potential tends to coagulate. Zeta potential measurements of solvent exchanged CNCs shows a transition from negative to positive (from water to chloroform), passing through a region of instability. CNC solution of 1 wt. % dispersed in water (through sonication) had a stable zeta potential value of -55 mV. Dispersion of CNCs in acetone after solvent exchanging, underwent a region of instability with a zeta potential value of 12 mV. On further exchange with chloroform, the zeta potential increased to +50 mV, thus showing improved dispersion of CNCs. CNCs mixed with PHB solution in chloroform, shows increase in zeta potential value to 170 mV indicating higher suspension stability. Such high zeta potential values suggest that CNCs are sufficiently charged in PHB solution due to presence of hydroxyl and sulphonyl groups which provide a stable state preventing aggregations [330]. Thus solvent exchange process helped in better dispersion of CNCs in polymer solution. However, it was observed that stability of PHB polymer solution decreased with increase in CNC loadings which could be due to formation of CNC agglomerates at high loadings.

4.2.2 Polyhydroxybutyrate/Cellulose Nanocrystal Nanocomposites: Morphological Investigations

PHB/CNC nanocomposites were casted into films after dispersing chloroform exchanged CNCs at various loadings (1–5 wt. %) in the PHB matrix and its effective dispersion were evaluated through analysing the transparency values over UV-Vis range. Compared with pristine PHB film, PHB/CNC composite films showed gradual reduction in transparency with increasing CNC loading as shown in Figure 4.2(a). The transparency decreased from 58% for

neat PHB to 49% for PHB/CNC (2 wt. %). However, at higher loadings, drastic decrease in percentage transparency is observed possibly due to agglomeration of CNCs as also suggested by zeta potential study. Surface morphology of PHB/CNC nanobiocomposites is studied by FESEM to understand the dispersion and interaction of hydrophilic nanofiller with hydrophobic polymer. Two forms of morphological orientation of rod-like CNCs are found in the polymer matrix from the micrographs. In first type, CNCs are vertically embedded in the polymeric matrix, as shown by the solid circles in Figure 4.2(b) and (c). Presence of small white dots represents the cross section of agglomerated CNCs on the film surface. In the second type of orientation, CNCs are randomly spread on the PHB surface as shown by the dotted circles in Figure 4.2(b) and (c). Figure 4.2(b) shows homogenous dispersion of CNCs (at 1 wt. % loading) on a smooth polymer surface, where a large fraction of the nanofiller is embedded in the polymer surface, providing more contact area of adhesion. Such orientation of CNCs in polymer matrix could be attributed to the improved interactions between PHB/CNC through adsorption of PHB chains on the hydroxyl groups of CNCs at lower loading fractions. However, at higher loadings (>2 wt. %), intermolecular hydrogen bonding forces become dominant, and the CNCs start aggregating in the form of clusters on the surface of the polymer. At further higher loadings incorporating 5 wt. % CNCs), morphology of nanocomposites, clearly shows small voids, which may be due to weak polymer– filler adhesion and phase separation [170]. The average cross-section of voids is estimated to be 132 ± 52 nm and 234 ± 34 nm, for loadings of 2 and 5 wt. % CNCs respectively. Presence of voids with such morphological dimensions suggests poor interfacial interaction between the polymer and nanofillers at higher loadings, which have been discussed subsequently through fractural behaviour studies in section 4.2.4.

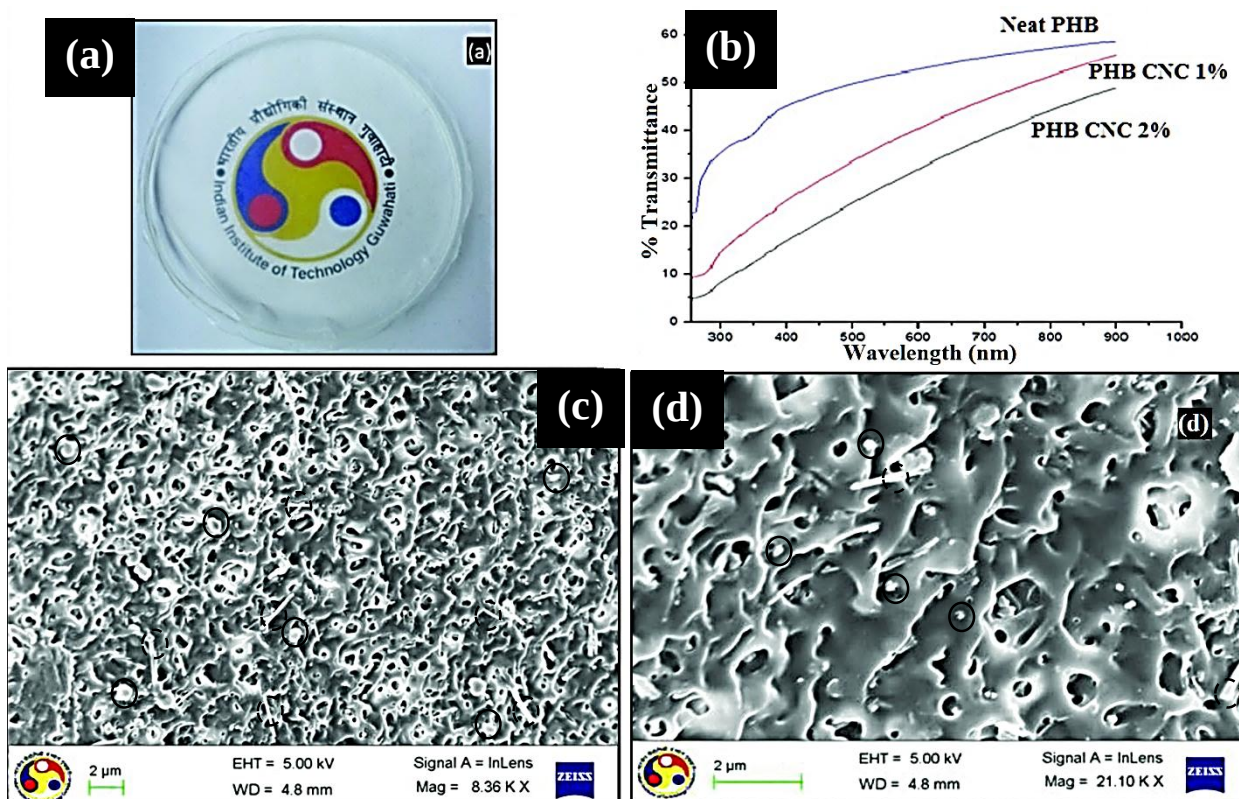


Figure 4.2: (a) Solvent exchange-cum-solution casted PHB/CNC nanocomposite films (with ~1wt. % CNC loading), (b) percentage transparency of the PHB/CNC films at different CNC loadings, FESEM micrographs of the PHB/CNC films with (c) 1wt. % and (d) 5 wt.% CNC loadings.

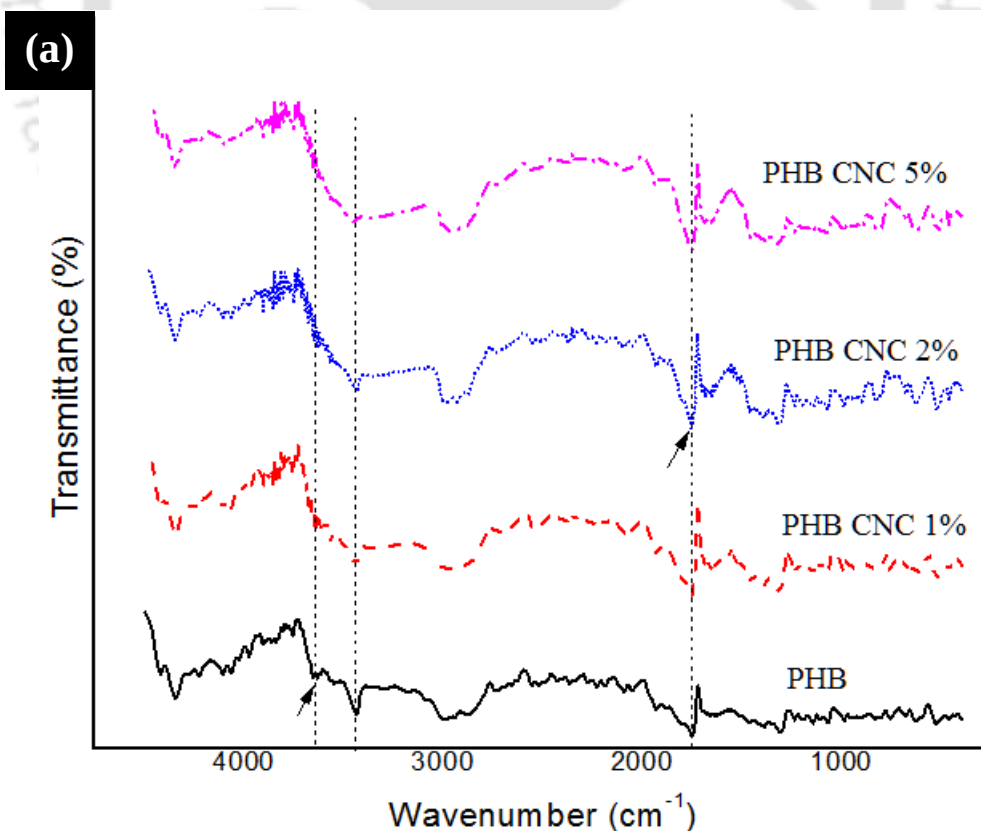
4.2.3 Dispersion of CNCs in PHB matrix: A Mechanistic Understanding

The improved dispersion of the hydrophilic CNCs into PHB matrix (at lower CNC loading ~2 wt%) is possibly due to formation of intra/intermolecular hydrogen bonding between the PHB matrix and CNCs, which is mechanically understood through FTIR and XRD studies. Figure 3.3(a) shows the comparison of FTIR spectra of CNCs, neat PHB film and PHB/CNC nanocomposites at different loading fractions of CNCs. Addition of CNC to PHB has an effect on C=O bond stretching at 1750 cm^{-1} . Maximum stretching has been observed for 1wt. % and

2wt. % CNC loading indicating better networking at these loadings than at 5 wt. %. Moreover, the hydroxyl peak corresponding to PHB at 3656 cm^{-1} is absent in the PHB/CNC nanocomposite. Stretching of C=O bond and absence of hydroxyl peak in the nanocomposites indicates the presence of hydrogen bonding between CNC and PHB. Presence of hydroxyl groups in cellulose and carboxyl and hydroxyl groups in PHB is expected to lead to strong intermolecular as well as intramolecular hydrogen bonding with various possible bonding schemes as illustrated in Figure 4.4(a). For example, hydroxyl (OH) at C6 and C3 of cellulose can bond with carbonyl (C=O) and C-O-C of PHB as shown in scheme I (Figure 4.4 (a)). However, OH at C6 also participates in intramolecular hydrogen bonding with OH at C2 within cellobiose. Scheme II illustrates the hydrogen bonding between PHB and cellulose nanocrystals end units, where C2 and C6 hydroxyl of cellulose bond with C=O and C-O-C of PHB. Another possibility of hydrogen bonding is shown in Scheme III where the end hydroxyl groups of PHB chains attach to C3 of cellulose. Moreover, C3 hydroxyl may participate in intramolecular (with cyclic C-O-C of cellulose) and intermolecular (with cellulose and PHB) hydrogen bonding or it may only have intermolecular bonding with PHB. The cyclic C-O-C of cellulose may also involve in intermolecular hydrogen bonding as suggested in Scheme III. Intramolecular hydrogen bonding in cellulose is observed primarily between hydroxyls at C6 and C2 whereas intermolecular hydrogen bonding possibly occurs between C6 and C3 hydroxyls.

XRD studies were carried out to further investigate the interactions between CNC nanoparticles and PHB polymer matrix. X-ray diffraction patterns for synthesized CNCs, pure PHB film, and PHB/CNC nanocomposites at different loadings are shown in Figure 4.3(b). XRD patterns of the fabricated CNCs show crystalline structure with diffraction peaks at

$2\theta = 14.8^\circ, 16.5^\circ, 22.4^\circ$, and 34.5° , corresponding to the cellulose crystal planes at ($\bar{1}10$), (110), (200), and (004), respectively (Figure 4.3(b)). These peaks indicate the existence of major fractions of cellulose I (peaks at $15.1^\circ, 17.5^\circ, 22.7^\circ$, and 34.3°) crystal structure in CNCs. The shift in theta angles is attributed to different alkali and bleaching pre-treatments, which lead to partial transformation of cellulose I to cellulose II (peaks at $12.5^\circ, 20.1^\circ, 22.7^\circ$, and 34.3°). Characteristic lattice planes of PHB are clearly observed in the diffractograms, with peaks at $13.5^\circ, 16.7^\circ, 21.2^\circ, 22.0^\circ, 22.7^\circ, 25.0^\circ, 27.5^\circ$, and 30.5° [331]. Lattice spacing and mean size of crystalline domains have been calculated using Scherrer equation and values are shown in Figure 4.3(b). XRD studies shows broadening of peak at 13.5° and shift to lower 2θ angles, suggesting possible intercalation of CNCs. Lower D_{hkl} values of $\sim 3.6 \text{ \AA}$ for 1% and 2 wt. % CNC loading as compared to ~ 8.4 and $9.2 \text{ \AA}$ for 5 wt. % CNC loading and neat PHB, respectively, indicates better intercalation of CNC into PHB matrix at lower loadings.



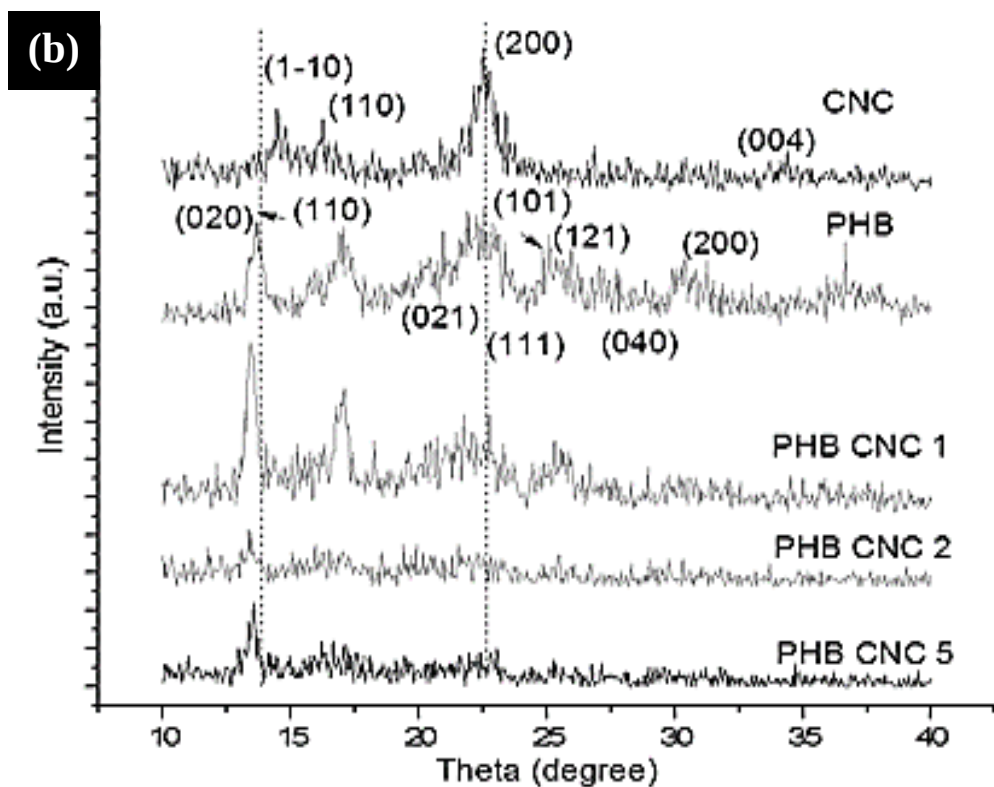


Figure 4.3: (a) FTIR spectrograms and (b) XRD diffractograms showing the different planes and the $d_{spacing} : D_{hkl}$ values for PHB, CNC and their nanocomposites at various CNC loadings.

The intercalation occurs due to better interfacial interaction between the PHB and CNC possibly through hydrogen bonding (as discussed in previous section). XRD curve for CNC peaks are absent at position $2\theta \sim 4.8^\circ$ and are overlapped by broader shoulder from PHB, whose intensity decreased with increasing CNC loadings. The peak at $2\theta \sim 16.5^\circ$ is most prominent indication of the presence of CNC in the PHB/CNC nanocomposites whose intensity decreased with increase in loading [332].

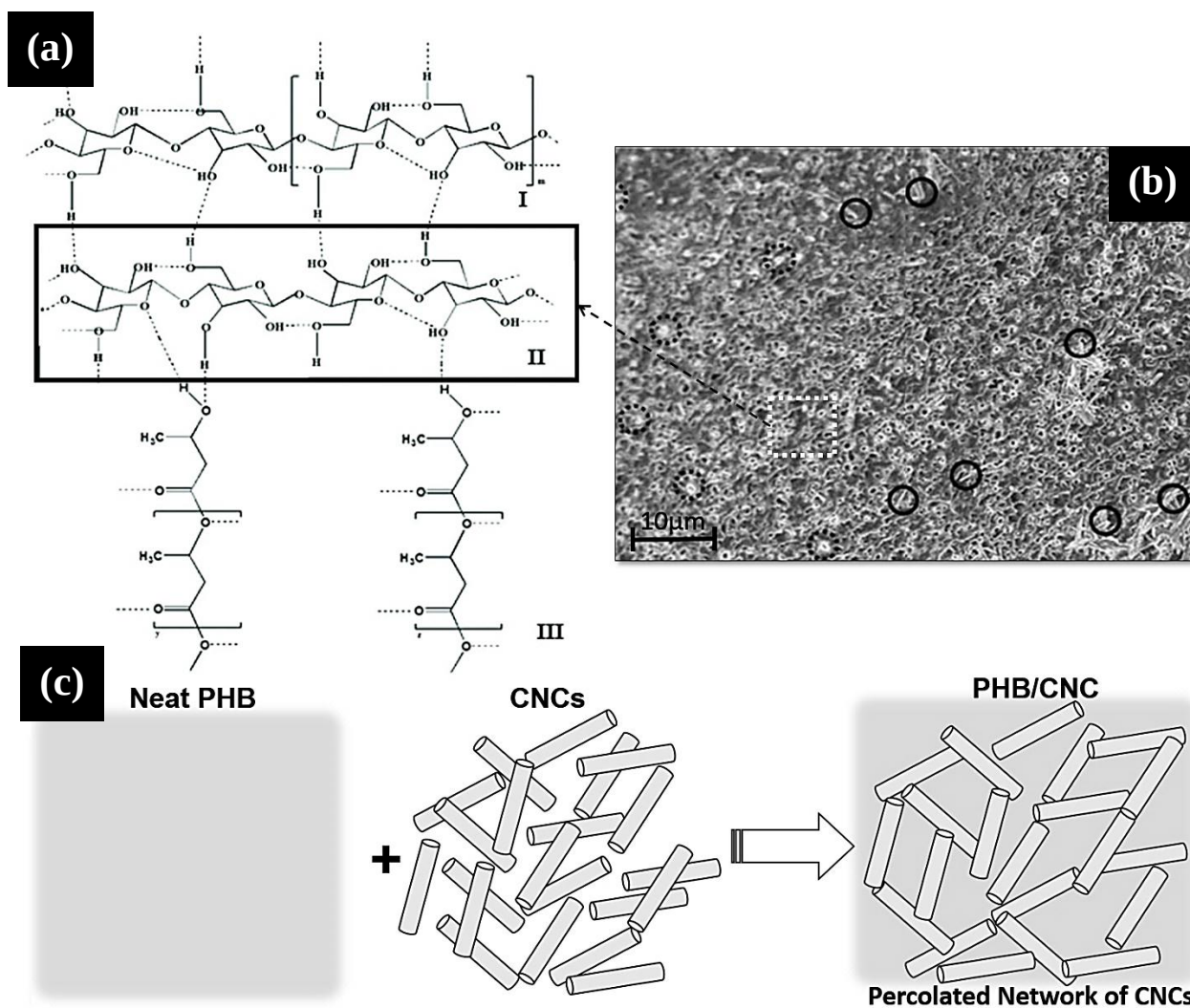


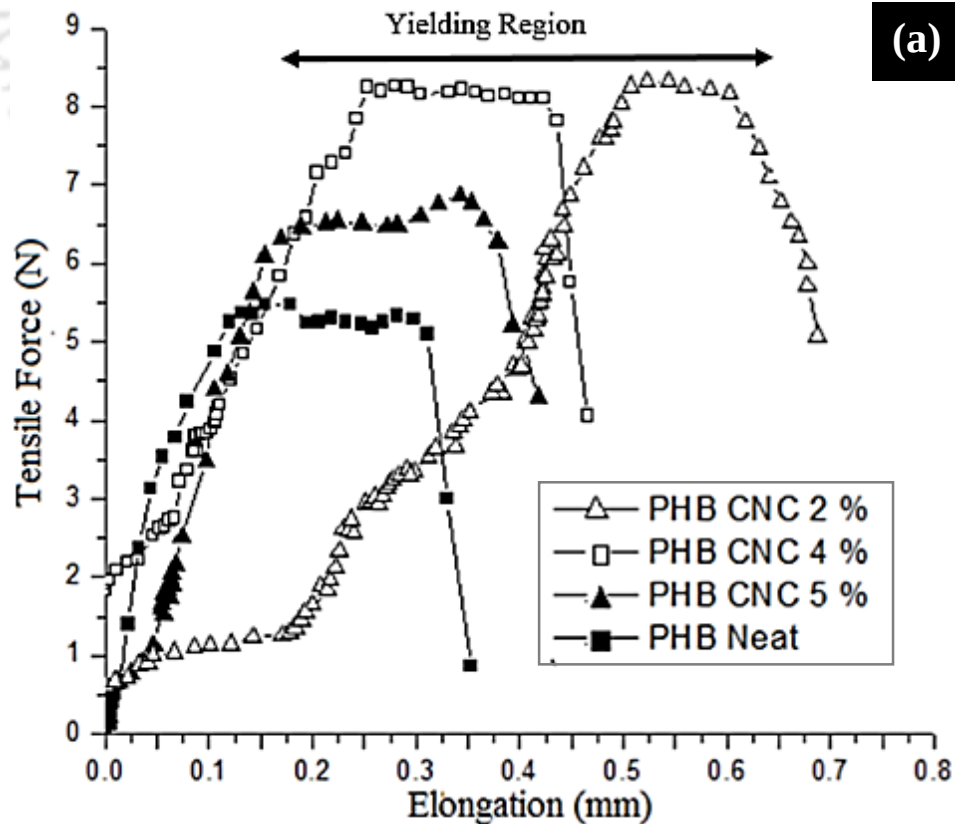
Figure 4.4: (a) Plausible mechanism for intermolecular and intramolecular hydrogen bonding between CNCs and PHB matrix. (b) FESEM micrographs of PHB/CNC films at threshold percolated concentration. (c) Plausible mechanism for formation of percolated network of CNCs in PHB matrix at threshold concentration of ~2wt. % CNCs.

4.2.4 Polyhydroxybutyrate/Cellulose Nanocrystal Nanocomposites: Mechanical Modelling and Fractural Behaviour Studies

CNCs is derived from natural biomass and possesses exceptional mechanical strength of 500 MPa and stiffness of 140–220 GPa [332,333]. The dispersion of hydrophilic CNCs into hydrophobic PHB, while inherently difficult, can lead to alteration of the mechanical properties of the nanocomposite films even at very low loadings. Although solvent exchange method helped in dispersion of CNCs up to a threshold loading of 2–3 wt. %, agglomeration of CNCs was observed at higher loadings of CNC and their effect on PHB's mechanical property diminished. At a CNC loading of 2 wt. %, the ultimate tensile strength increased significantly to 57.1 MPa from 36.0 MPa for pristine PHB (Table 4.1). At higher loadings, CNCs tend to get agglomerated in the PHB matrix resulting in reduction of tensile strength (45.2 MPa for 5 wt. % CNC loading) (Figure 4.5(a)). Further, the elongation at break, is higher at 2 wt. % CNC loading than at 5 wt. % (6.5% and 3.2%, respectively). Decrease in the value of Young's modulus of the PHB/CNC films at approximately 2 wt. % CNC loading indicates higher flexibility of the composite films at this loading. DSC analysis of the nanocomposite films, discussed in the subsequent section, shows that the crystallinity of PHB is lower at 2 wt. % CNC than at higher loadings. The higher flexibility and transparency of PHB composite films at 2 wt. % CNC loading can thus be attributed to the reduced crystallinity of PHB at this loading. The tensile strength of the PHB/CNC films is comparable to that of polyethylene terephthalate (PET); however, the Young's Modulus is lower compared with PET [334].

Figure 4.5(b), shows the comparison between the experimental and predicted moduli for the PHB/CNC nanocomposites using the Halpin-Kardos and Ouali models. Ouali model closely predicted the moduli of the nanocomposites, at lower CNC loadings but deviated

significantly at higher concentrations. Halpin-Kardos model showed good agreement with experimentally measured moduli at higher CNC concentrations. As Ouali model takes into account the filler-filler interactions, it predicted the PHB/CNC composite moduli with higher accuracy at low CNC loadings. The deviation of predicted values of Young's modulus from experimental values could be attributed to the inability of the models to incorporate certain molecular level interactions between the nanofillers and polymers. The model does not take into consideration the tendency of the CNCs to form intermolecular hydrogen bonding with PHB and intra- and intermolecular hydrogen bonding between themselves, which leads to reinforcement effect and aggregation phenomenon. Using the estimated aspect ratio of fabricated CNCs from Figure 4.1(b), critical threshold percolation concentration is calculated as 2.53 wt. %. The predicted percolation threshold is found to be consistent with rheological studies discussed in subsequent section 4.2.6.



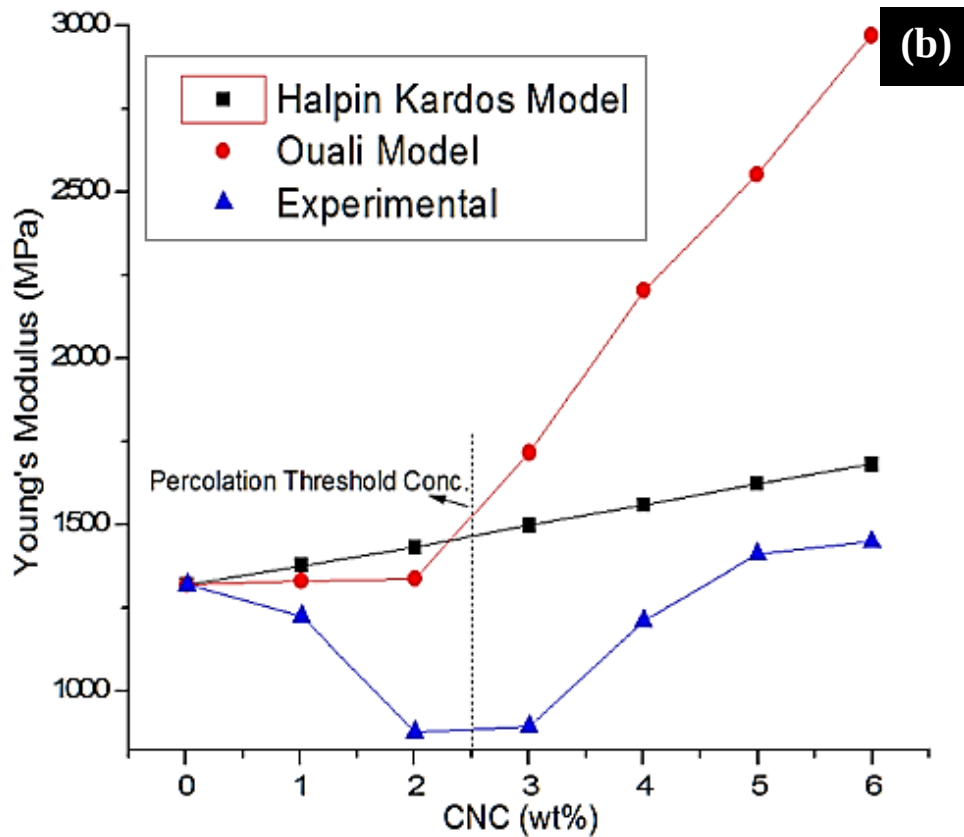


Figure 4.5: (a) Tensile force versus elongation of PHB/CNC samples; (b) Young's modulus as a function of CNC wt. % predicted using Ouali and Halpin–Kardos model and compared with experimental data for different PHB/CNC fractions.

At the percolation threshold ($\phi_c \sim 2$ wt. %), the Young's Modulus decreased steeply because of the yielding mechanism in PHB/CNC composites (as shown in Figure 4.5(a)). Such phenomenon of decrease in Young's Modulus with increase in CNC loading was also observed by S. De O. Patrício et al. [328] and Ten et al.[335]. CNCs tend to get aligned along the tensile direction during sample elongation and hence are able to withstand higher tensile force and elongation at break (Figure 4.5(a)). On the other hand, at low loading fractions, better interfacial adhesion exists between the PHB and CNCs through intermolecular hydrogen bonding. During tensile tests, such bonding results in the load being uniformly shifted or transferred by the fibers to the polymeric matrix, leading to alignment of CNCs along the

tensile direction and swift polymer matrix failure [336]. Such behavior is absent in pure PHB due to absence of reinforcement agents such as CNCs, and is observed only at low CNC loadings in the nanocomposites (Figure 4.4 (b) and (c)). At higher loadings (> 4 wt. %), the CNCs agglomerate in the PHB matrix resulting in poor fiber-polymer interfacial adhesion. The agglomerated CNCs act as nucleating sites for the initiation of fracture or material defects even under low tensile loads [333]. The mechanism explaining the two conditions of fiber and matrix failure is shown in Figure 4.6(a). After tensile analysis, the samples showed a transition from transparent films to whitish color, which is due nucleation under stress caused by interfacial debonding. The neat PHB films showed plastic deformation and brittle nature (Figure 4.5(a)), which is evident from the SEM micrograph in Figure 4.6(b) showing voids, rough and irregular surfaces with pullout fibrils. Analysis of the fractured surface for PHB/CNC nanocomposite films showed fiber pullouts and network bridge of CNCs (Figures 4.6(c) & (d)) which contributed to the increased elongation at break and tensile strength. Presence of network structure of CNCs at ~ 2 wt. % loadings in the composites, shows an increase in tensile strength and strain, which prevents the sudden failure or debonding of polymer surface during tensile analysis.

Table 4.1: Mechanical Properties of the PHB/CNC films at different CNC loadings.

Sample Name	Tensile Force (N)	Young's Modulus (GPa)	UTS (N/mm ²)(MPa)	Elongation at peak load (%)
PHB Neat	5.4±0.2	1.32±0.04	36.0±1.4	3.0±0.5
PHB/CNC 1wt. %	6.8±0.4	1.22±0.03	55.8±2.5	6.3±1.1
PHB/CNC 2wt. %	8.5±0.5	0.87±0.02	57.1±1.2	6.5±1.0
PHB/CNC 3wt. %	6.9±0.3	0.89±0.05	55.6±2.3	5.2±0.8
PHB/CNC 5wt. %	6.8±0.3	1.41±0.07	45.2±1.8	3.2±0.8

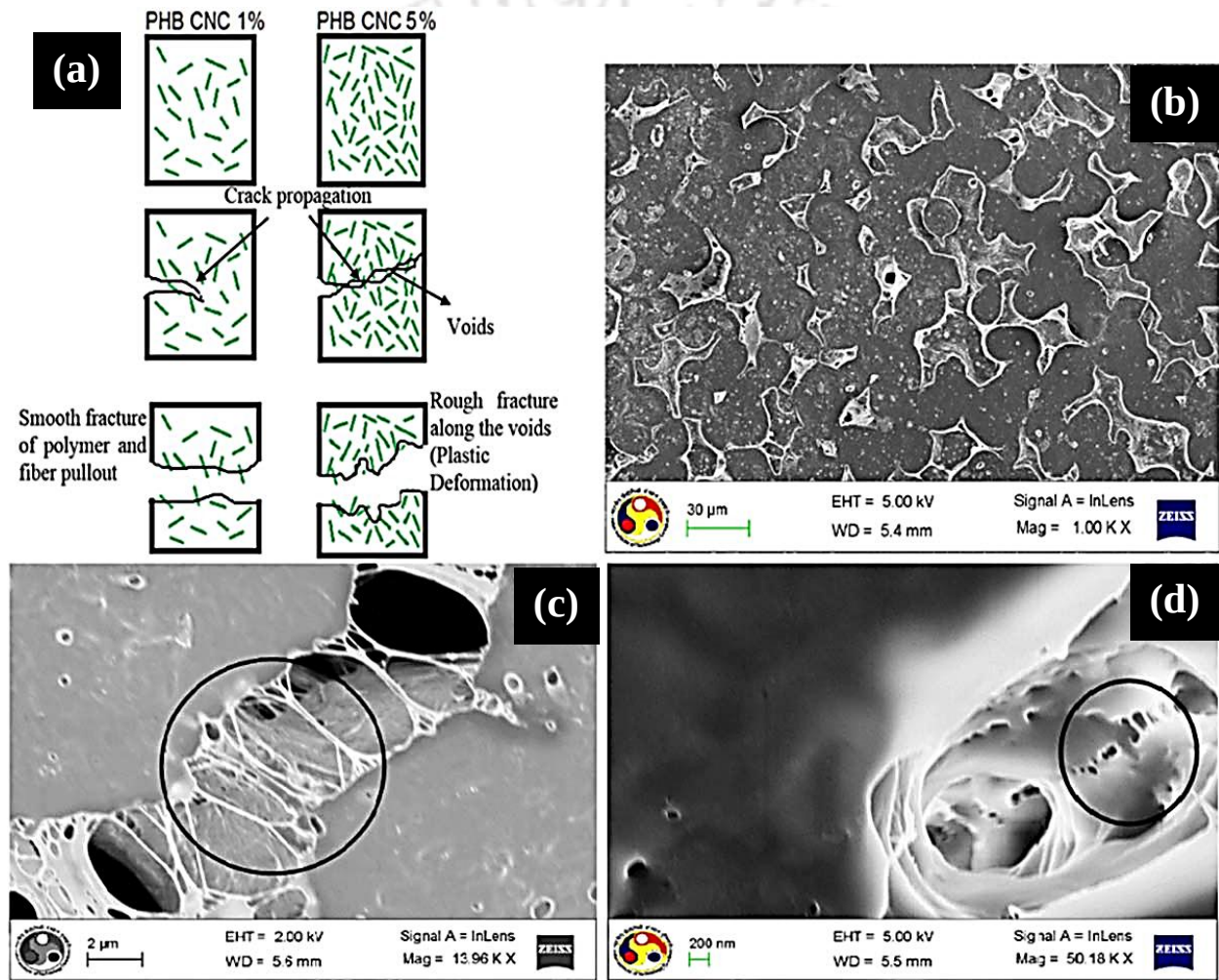
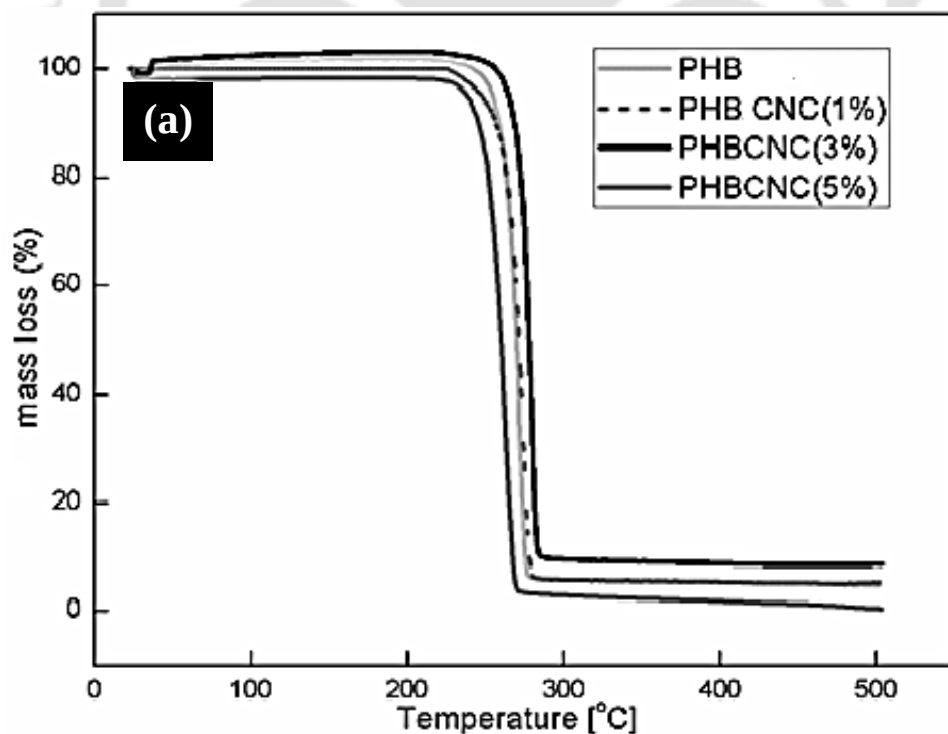


Figure 4.6: (a) Mechanism of fracture in PHB/CNC 1 wt. % and PHB/CNC 5 wt. %. (b) Fracture surface of neat PHB films, (c) and (d) Fracture surface showing the CNCs pull-outs, and formation of interlocking or network bridge between the polymer and CNCs (1 wt. %) (shown in dark circles).

4.2.5 Polyhydroxybutyrate/Cellulose Nanocrystal Nanocomposites: Thermal Degradation and Crystallization Studies

Thermal degradation behavior and thermal stability of pure PHB and PHB/CNC nanocomposites have been determined from single as well as multiple heating rates TGA data shown in Figure 4.7(a) and (b). It is observed from Figure 4.7(a) that the thermal degradation of PHB and its nanocomposites follows the single step mass loss indicating that degradation mechanism remains unchanged throughout the process. PHB nanocomposite with 3 wt. % CNC loading shows improved thermal stability compared to the pure PHB. However, with increased CNC loadings (~upto 5 wt. %), onset degradation temperature decreased from 246°C to 232°C, which shows that CNCs act have a catalytic effect on degradation of PHB polymer. The enhanced degradation might be due to the presence of pendant sulphate and hydroxyl groups on CNC surface which act as an active sites for the chain scission of PHB at higher temperature. Thus, bio-nanofillers such as CNCs helps in enhancing thermal degradation rate of the polymer which is advantageous from environmental point of view. However, rate of degradation (α_p) decreases with increase in CNC loadings upto ~3 wt. %, reaching a minimum α_p value of approximately 24.2%. This is due to the improved compatibility between the PHB/CNCs at lower CNC loadings which led to better hydrogen bonded interaction between nanofiller and polymer subsequently suppressing the polymer degradation rate. However, α_p increases at higher CNC loadings (~5wt. %) and the nanocomposite behaves as neat PHB possibly due to the formation of isolated CNC aggregates in PHB. The residual mass at 500 °C increased from 4.8% for the neat PHB to 8.6% (for 3wt. % CNC loadings) which increases at higher CNC loadings. Figure 4.7(b) shows multiple heating rate TGA curves for PHB/CNC (1 wt. %) nanocomposite at 5, 10, 15 and 20°C/min. Like pristine PHB, PHB/CNC (1 wt. %)

nanocomposite also follows the same degradation behavior with single stage mass loss. The onset (start and end) degradation temperatures increase with increasing heating rate for nanocomposites. Similar TGA curves are obtained for PHB/CNC (5wt. %) nanocomposite at various heating rates. Thermal degradation kinetics study of PHB/CNC nanobiocomposites revealed that thermal stability slightly improved upto CNC loading of ~3wt.%. Isoconversional analysis of thermal properties and evaluation of kinetic parameters using Ozawa–Flynn–Wall (OFW) and Kissinger-Akahira-Sunose (KAS) models didn't show significant change in activation energies. This could be attributed to the degradation mechanism which follows a single step degradation and remains unchanged for neat PHB and all other nanobiocomposites. On further evaluation of the kinetic parameters, it is concluded that PHB/CNC nanobiocomposites follows first order reaction and phase boundary controlled reaction models (contracting volume) with random chain scission mechanism.



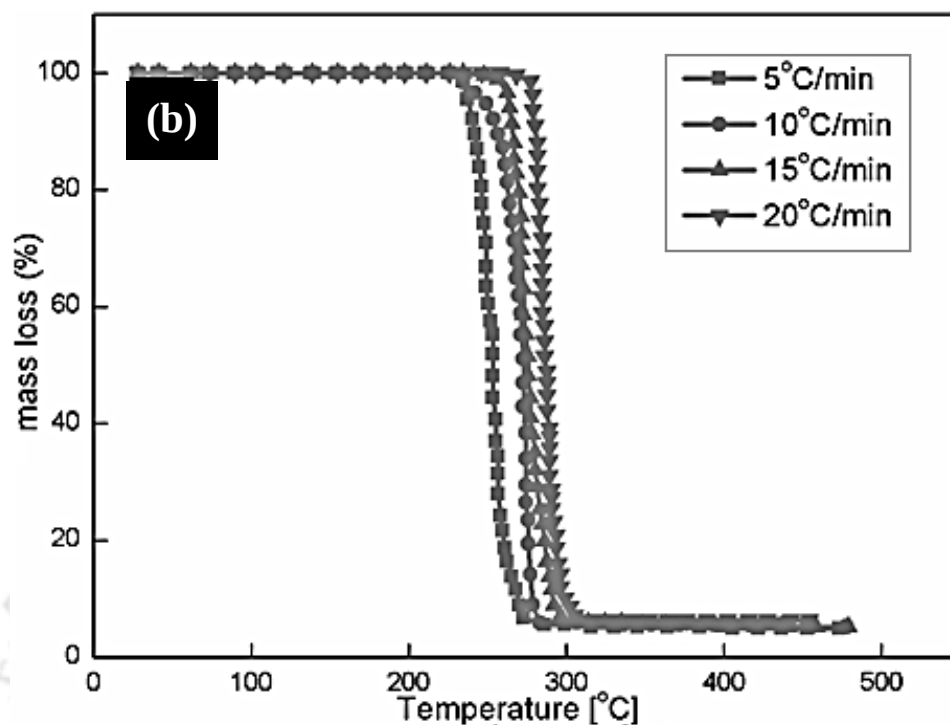


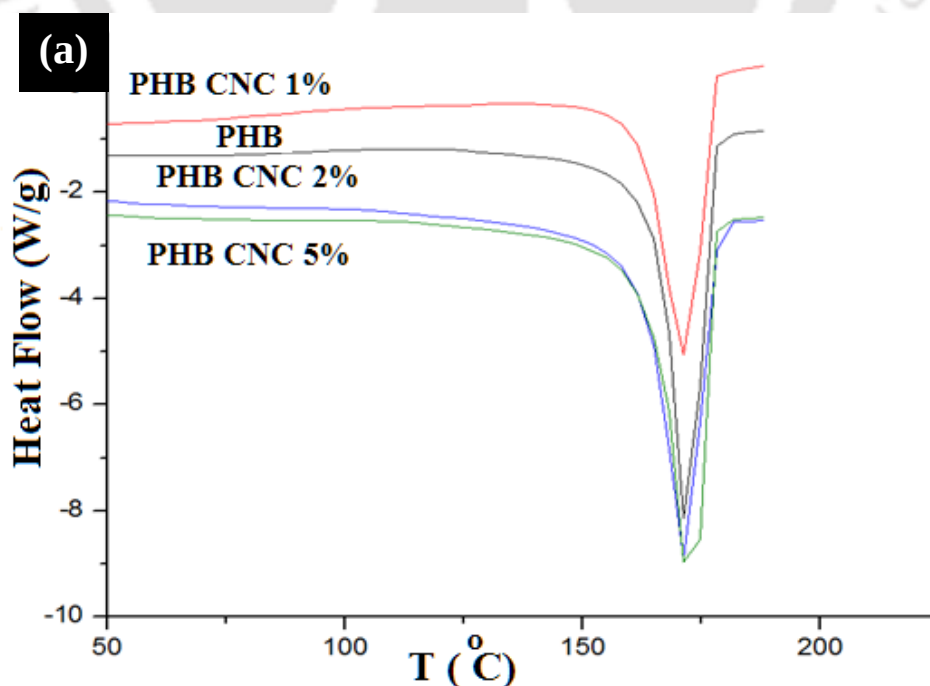
Figure 4.7: (a) TGA curves of pure PHB and PHB/CNC with various amounts of CNC at 10°C/min. (b) TGA curves of PHB/CNC (1wt. %) nanocomposites at multiple heating rates.

Figures 4.8(a) and (b), shows the DSC thermograms for both heating and cooling cycles of neat PHB and PHB/CNC nanocomposites films measured at a scan rate of 10°C/min. Melting peak of PHB during second heating is observed at 178°C, which remained almost unchanged with increase in CNC loadings. PHB with 1 wt. % and 2 wt. % CNC loadings have sharp melting peaks indicating uniform crystallites whereas 5 wt. % CNC sample exhibits a broad peak (Figure 4.8(a)). During cooling, a sharp crystallization peak is present (Figure 4.8(b)) and a trend in the decrease in crystallization temperature as well as the degree of crystallinity is observed. At lower CNC loadings (~1 and 2 wt. %), decrement in the crystallization temperature is observed whereas at 5 wt. % CNC loadings the crystallization temperature obtained is similar to PHB. Percentage crystallinity decreased marginally (from ~51% to 47%)

at low CNC loadings (up to 2 wt. %). As pure PHB is brittle and highly crystalline, decrease in crystallinity is important in fabrication of films from PHB [337]. The crystallization studies are further studied in details in subsequent section 4.2.7, to understand the phenomenon of migration of simulants on the thermal properties of PHB chains in the nanocomposites.

4.2.6 Rheological Investigations of PHB/CNC Nanocomposites: Evaluating Interaction of CNCs with PHB matrix

Melt rheology of the PHB/CNC nanocomposites was carried out under both SAOS and LAOS for evaluation of viscoelastic behavior of the nanocomposites. The region where the storage and loss modulus are found to be independent of the strain amplitude is selected as the linear viscoelastic (LVE) region, otherwise it is considered nonlinear viscoelastic (NLVE) region, as shown in Figure 4.9(a). The LVE and NLVE regions of the PHB/CNC nanocomposite were determined by dynamic strain sweep and was selected as approximately 1% strain and approximately 50% strain, respectively, for further rheological studies.



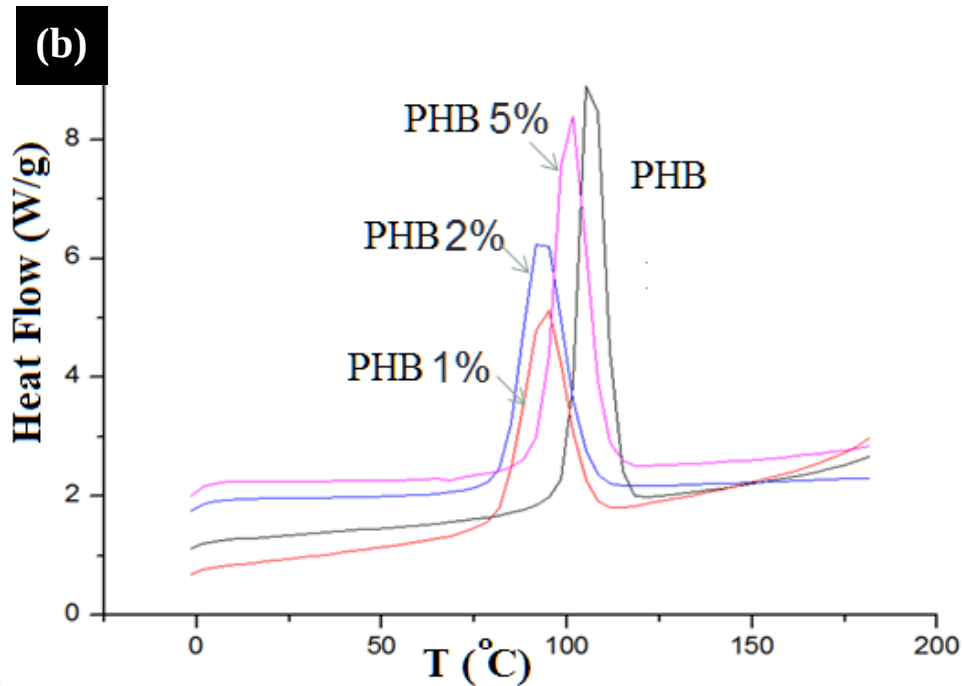


Figure 4.8: (a) Second heating cycle and (b) first cooling cycle representing the crystallization peaks for PHB/CNC films at different CNC loading fractions.

Storage modulus and complex viscosity of the PHB/CNC films were measured for various CNC loadings in the linear range of SAOS (at 1% strain) and non-linear range of LAOS (at 50% strain) conditions (Figure 4.9(b) and (c)). The effect of CNC loadings on the rheological behavior of PHB nanocomposites has been studied by measuring the complex viscosity (η) and the enhancement in the storage modulus (G') at low-frequency. As expected, for SAOS measurements, the magnitude of the storage modulus (G') of PHB/CNC films monotonically increased with CNC loading[124]. Compared with neat PHB matrix, the G' and η of PHB/CNC increases by about an order of magnitude at CNC loading of approximately 2 wt. %, as shown in Figure 4.9(d). This indicates that the viscoelastic properties of the nanocomposites at low CNC loadings (<2wt. %) are still dominated by the PHB matrix, and with increase in CNC loading, the PHB/CNC may experience a transition from viscous behavior to elastic

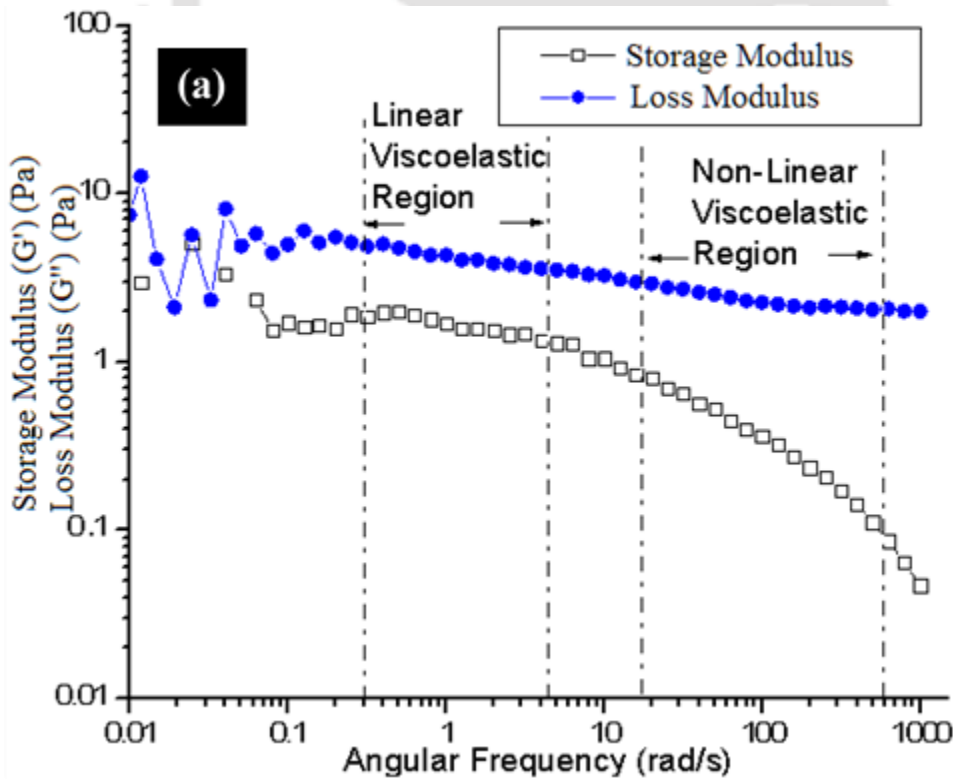
(solid- like). Therefore, the rheological percolation threshold is selected to be around 2 wt. % due to the appearance of such pseudo-solid like transition behavior [124, 338].

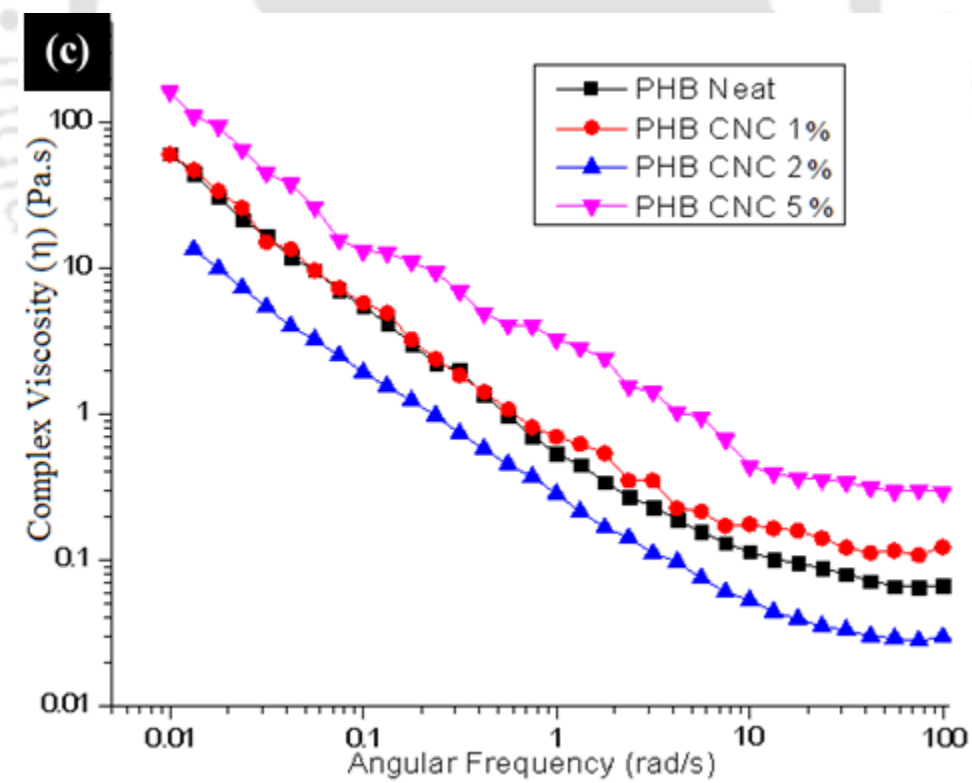
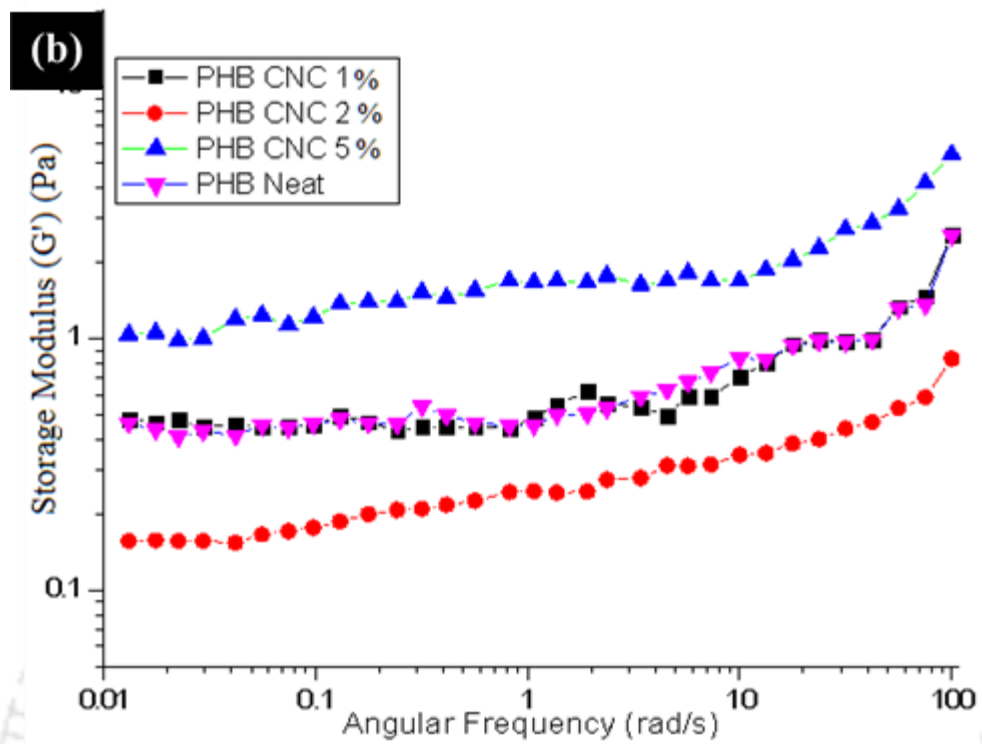
However, under large strain amplitude in the nonlinear region the PHB/CNC composites showed a different behavior. Compared with neat PHB, G' decreased significantly on the addition of 2 wt. % CNC. The complex viscosity (η) showed a similar trend over the entire frequency range (Figure 4.9(c)). The decrease in viscosity and storage modulus on application of large strain amplitude is probably because of the better adhesion of PHB chains on the surface of CNCs (Figure 4.9 (b–d)). Under SAOS conditions, nanocomposites with 1 and 2 wt. % CNC loading shows the formation of percolated network-like structure in the PHB matrix. As already explained, formation of such percolated network is due to the presence of intermolecular hydrogen bonding between the CNCs and PHB matrix. However, application of higher strain under similar loading conditions led to better adhesion/adsorption of the PHB chains onto the hydroxyl groups of CNCs. Presence of better interfacial interaction, probably leads to wrapping of individual CNCs with PHB matrix which is responsible for the decrease in the G' . Similar decrease in viscosity with increase in CNC loading has been reported in literature for polyethylene oxide (PEO)/CNC nanocomposites up to approximately 5 wt. % loading [124, 339]. Therefore, it could be concluded that at threshold loading, the application of large strain led to improved dispersion of the CNCs in the PHB matrix. Further, the linear and non-linear viscoelastic properties of PHB/CNC nanocomposites were evaluated at 1 and 2 wt. % CNC loadings (Figure 4.9(e) and (f)). At both loadings, the nanocomposites showed the interesting phenomenon of crossover of storage (G') and loss modulus (G'') when plotted as a function of frequency. The crossover point is referred to as the gelation point [338]. At SAOS conditions, increase in the percentage loading of CNCs resulted in the gelation point

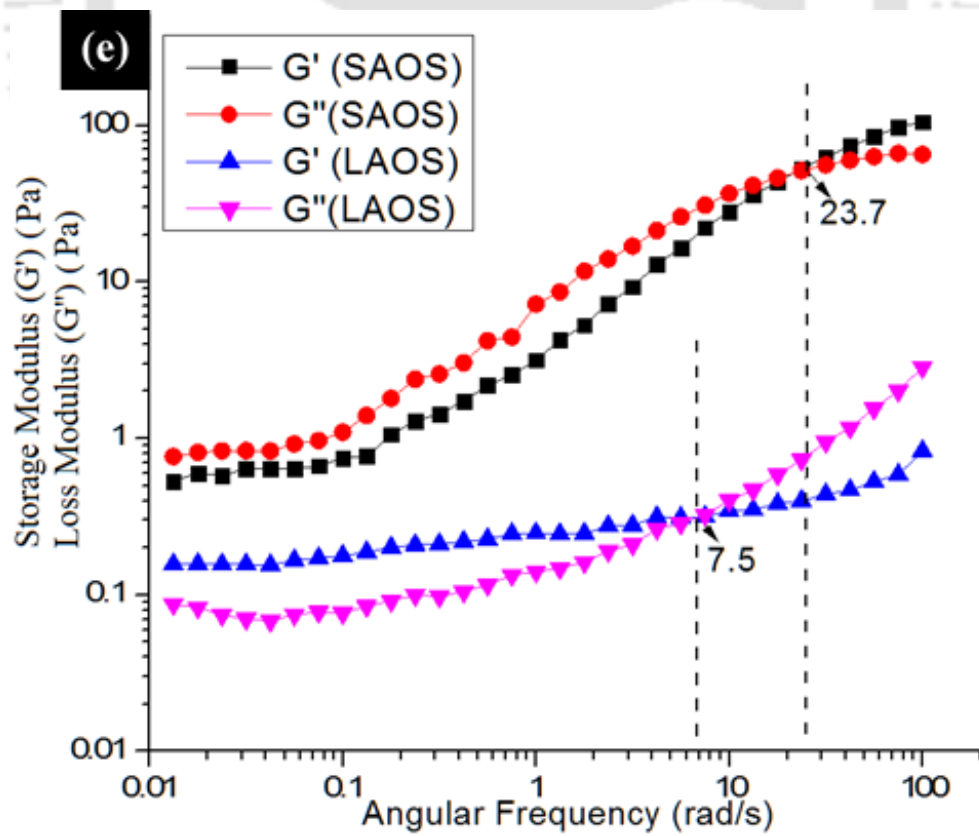
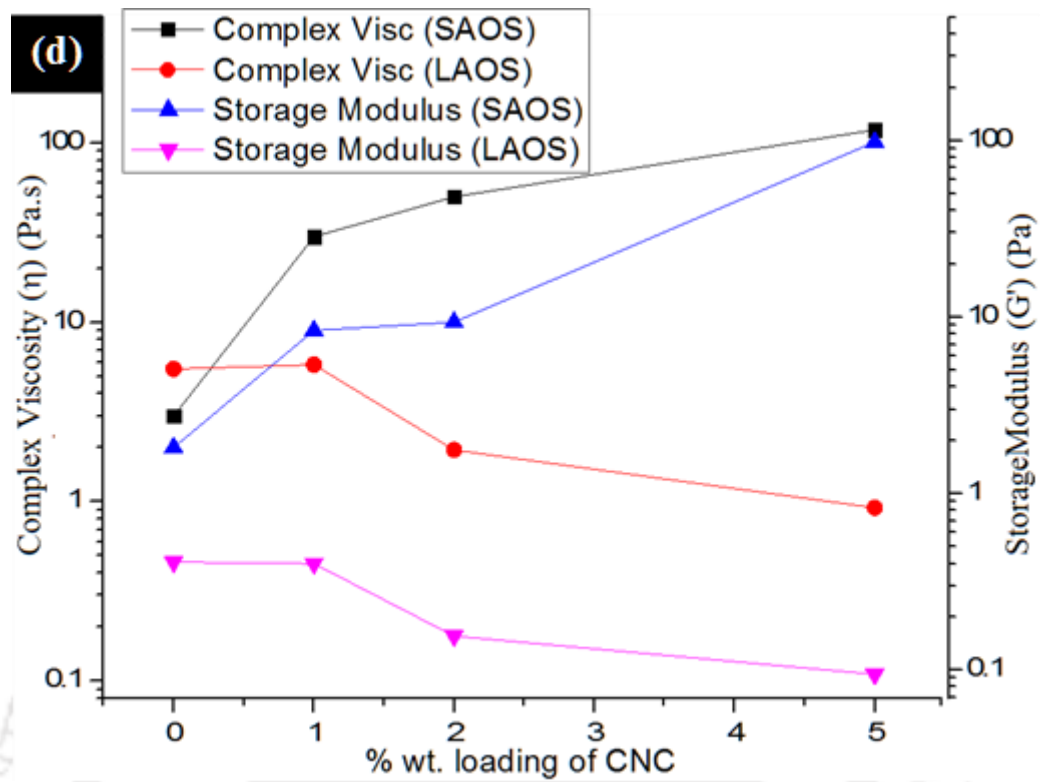
shifting from higher to lower frequency. In the lower frequency range, G' and G'' shows the formation of plateau-like region for 2 wt. % CNC loading, a feature absent in 1 wt. % nanocomposite. The formation of the low frequency plateau of G' is expected in nanofilled polymers, and is primarily attributed to the formation of a three dimensional percolating network that spans the whole sample. Thus, the percolation threshold for the PHB/CNC nanocomposite lies between 1 and 2 wt. % CNC loading. This indicates that the viscoelastic properties of the PHB/CNC at low CNC loadings (<2 wt. %) are still dominated by the PHB matrix and with increase of CNC loading, the PHB/CNC may experience a transition from viscous behavior to elastic (solid-like). However, at LAOS conditions the gelation point shifted from lower to higher angular frequency. This is probably due to the formation of some oriented configurations of CNCs in the PHB matrix under non-linear conditions [340,341]. Similar parallel alignment of silicate layers under prolonged application of LAOS conditions has also been reported by Ren and Krishnamoorti et al. [340]. At both 1 and 2 wt. % CNC loading, after the gelation point crossover under LAOS conditions, the G' and G'' formed a plateau like region, which was absent under SAOS conditions. This is probably due to the breakdown of the three dimensional percolation network under prolonged condition of large strain amplitude. Fabrication of polymer nanocomposites through an extrusion process is usually carried out under high shear stress conditions. Therefore, this study could help us predict optimum strain/stress required during extrusion process for fabrication of polymer nanocomposites.

The PHB/CNC nanocomposites were also subjected to heating and cooling cycles of DSC at a scan rate of $1^{\circ}\text{C}/\text{min}$ through a temperature range of $40\text{--}190^{\circ}\text{C}$. The correlation between the thermal and the rheological behavior of PHB/CNC is analyzed near the crystallization temperature. At low temperatures, the polymer displayed solid-like characteristics with the

plateau modulus reaching values greater than 10,000 Pa. Figure 4.10(a), shows the variation of G' at different CNC loadings under cooling scan rates of $1^\circ\text{C}/\text{min}$. It is evident that the decay in modulus is highest for the 5 wt. % PHB/CNC films and least for the 1 wt. % films. This could be due to the formation of proper crystallites in films with 1 wt. % CNC which are absent in films with 5 wt. % CNC due to the agglomeration of CNCs at higher loading. Figure 4.10(b) and (c) shows the comparison of the thermal and rheological behavior of the PHB/CNC films and provides a very good correlation of the crystallization temperature to study the chain dynamics. The DSC endothermic peak, corresponding to crystallization temperature, coincides nicely with the abrupt increase in G' and G'' , which is due to the formation of PHB crystallites in that temperature range.







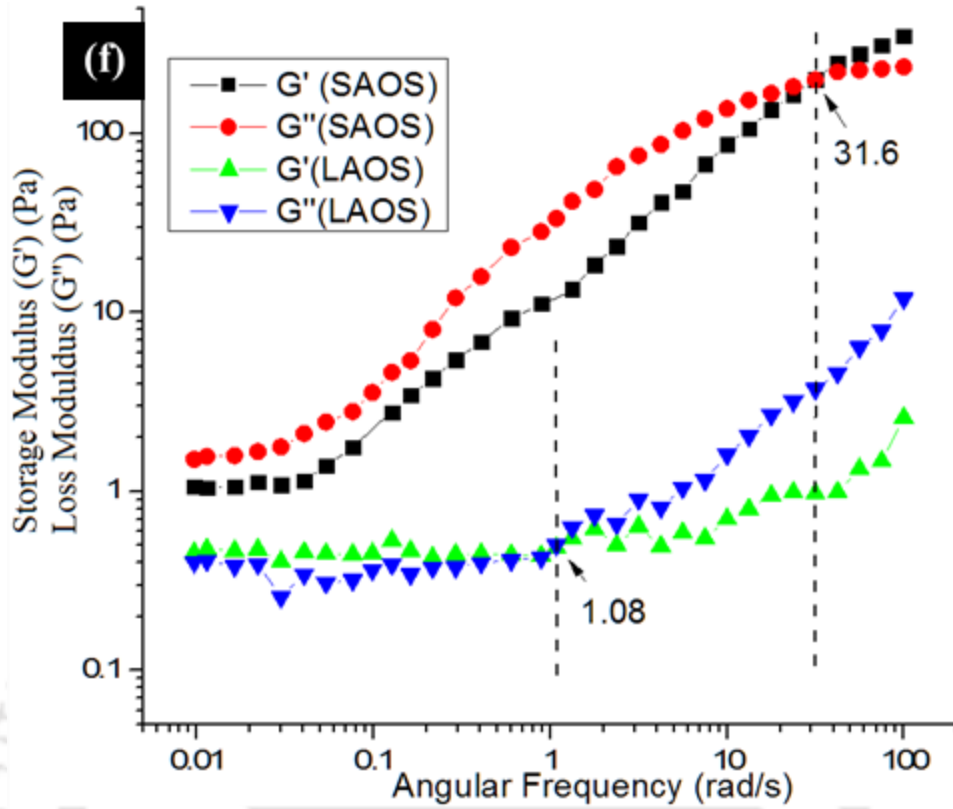
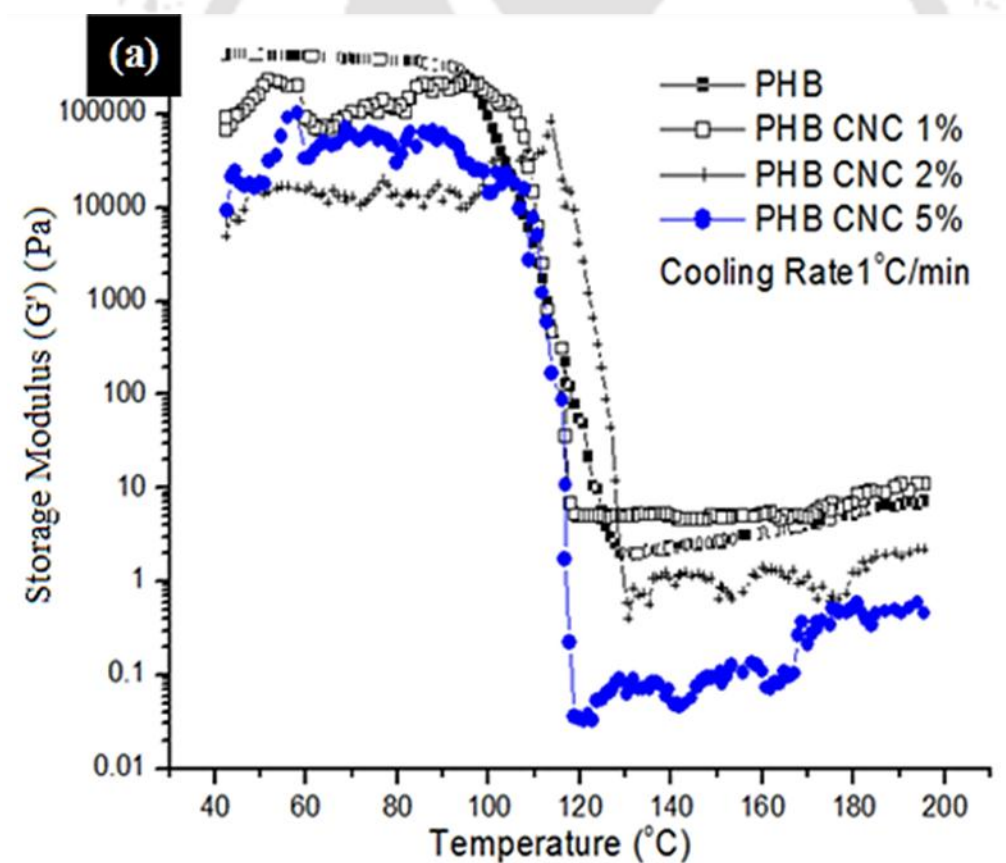


Figure 4.9: (a) Linear viscoelastic region of the PHB/CNC composite films at 180°C. (b) Dynamic storage modulus and (c) complex viscosity for the PHB/CNC samples under LAOS measurements. (d) Comparison of the dynamic storage modulus and complex viscosity under SAOS and LAOS conditions at different loading percentages. (e) Comparison of storage and loss modulus for PHB/CNC 2 wt. % and (f) Comparison of the dynamic storage and loss modulus for PHB/CNC 1 wt. % loadings.

It is evident from Figure 4.10(b) and (c), that the onset temperature of DSC curve matches with the onset of the decay in G' and G'' . As shown in insets of Figure 4.10(b) and (c), during the crystallization phenomenon, the elastic component increases drastically due to transformation of entangled polymer melt to ordered polymer spherulites. However, the viscoelastic component is also present due to the presence of some fraction of amorphous

chains surrounding the crystallites. In 1 wt. % PHB/CNC nanocomposite, the change in G' is comparatively lower compared with neat PHB, due to the presence of CNCs which hinders polymer chain motion necessary for the formation of crystallites. Such observation is also supported by DSC studies which shows decrease in percent crystallinity from approximately 51% to 47% for neat PHB and PHB with 1 wt. % CNC loading, respectively (as discussed in previous section 4.2.5). The rheological and thermal analysis over the temperature range helped to gain insights in the interactions of PHB with CNCs, and understand the effect of CNCs on polymer crystallization phenomenon.



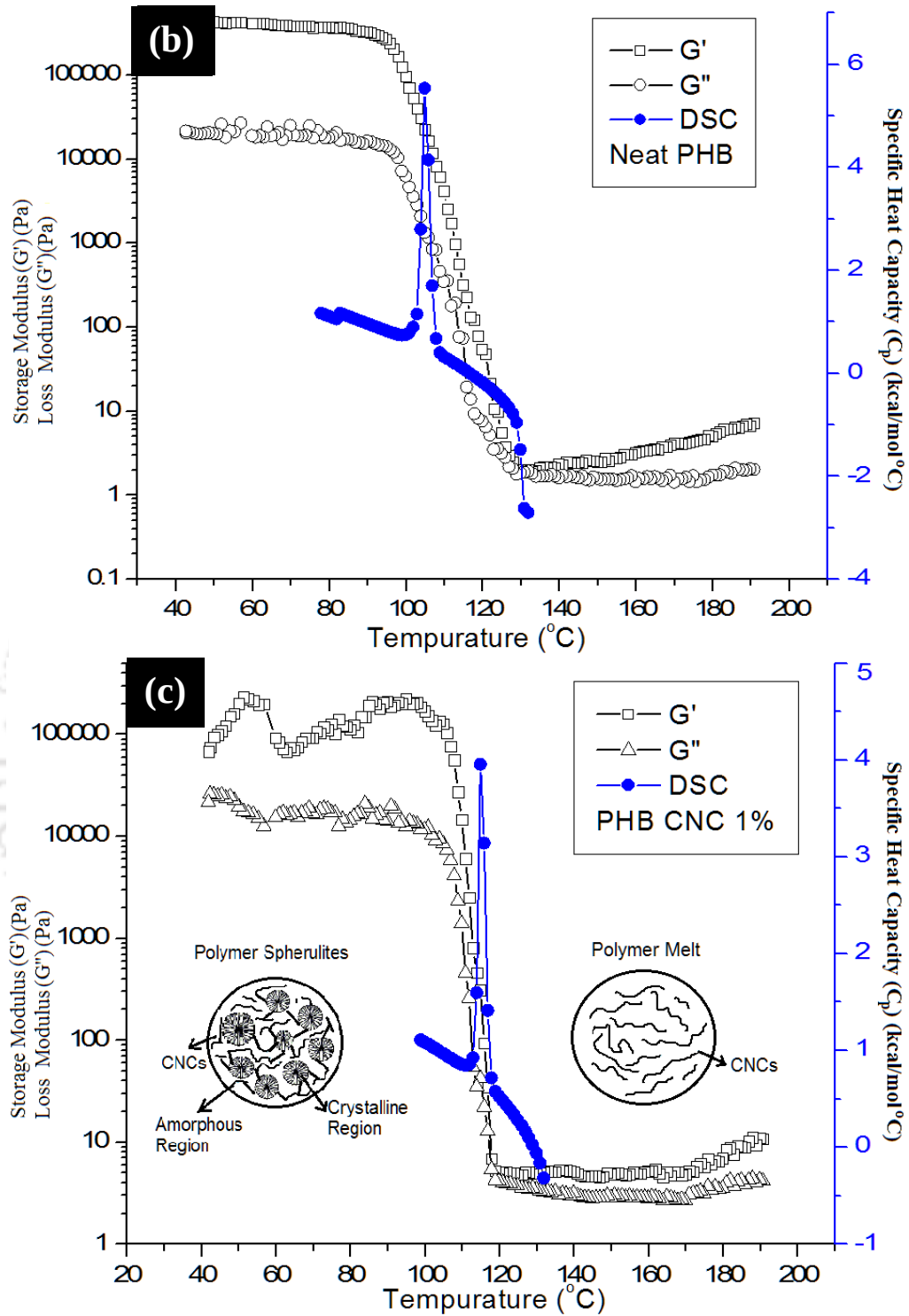


Figure 4.10: (a) Storage modulus for different PHB/CNC loadings at a cooling scan rate of $1^{\circ}\text{C}/\text{min}$. Storage modulus, loss modulus and specific heat capacity as a function of temperature for (b) Neat PHB and (c) PHB/CNC 1 wt. %.

4.2.7 Migration Behaviour of PHB/CNC Nanocomposites: Effect of Food Simulants

Overall migration tests are carried out on PHB/CNC films using ethanol (10% v/v) and isooctane as simulants at incubation temperature 20 and 40°C, respectively, for a period of 2 and 10 days respectively, in order to determine the CNC and PHB fractions that might migrate when these films are brought in contact with food materials (Figure 4.11). In case of ethanol as simulant, maximum level of migration (175 µg/kg) is found with nanocomposites having 5 wt. % CNC loadings, which is much lower than standard migration limits (60 mg/kg) established by the current legislation (Commission Directive, 2002/72/EC). However, a comparison of effect of CNC loading towards migration levels clearly shows that samples with high CNC loadings exhibit significantly higher overall migration. There is a small drop in migration values at 1 and 2 wt. % of CNC due to the restriction of polymer chain movement caused by good adhesion between CNCs and polymeric matrix. But at higher CNC loadings (>3 wt. %), adhesion between hydrophilic CNCs and hydrophobic PHB becomes poor leading to drastic increase in the migration values. Study with isooctane as food simulant has been carried out to determine if the nonpolar solvent gets absorbed into PHB releasing out CNCs or low molecular weight fractions of PHB. Such phenomenon of migration of low molecular weight oligomers has been observed by Schmidt et al. [342] in PLA/clay nanocomposites films when brought in contact with food simulant. The trend in migration levels observed with isooctane as simulant is similar to that observed for ethanol, although the amount of migration is much less.

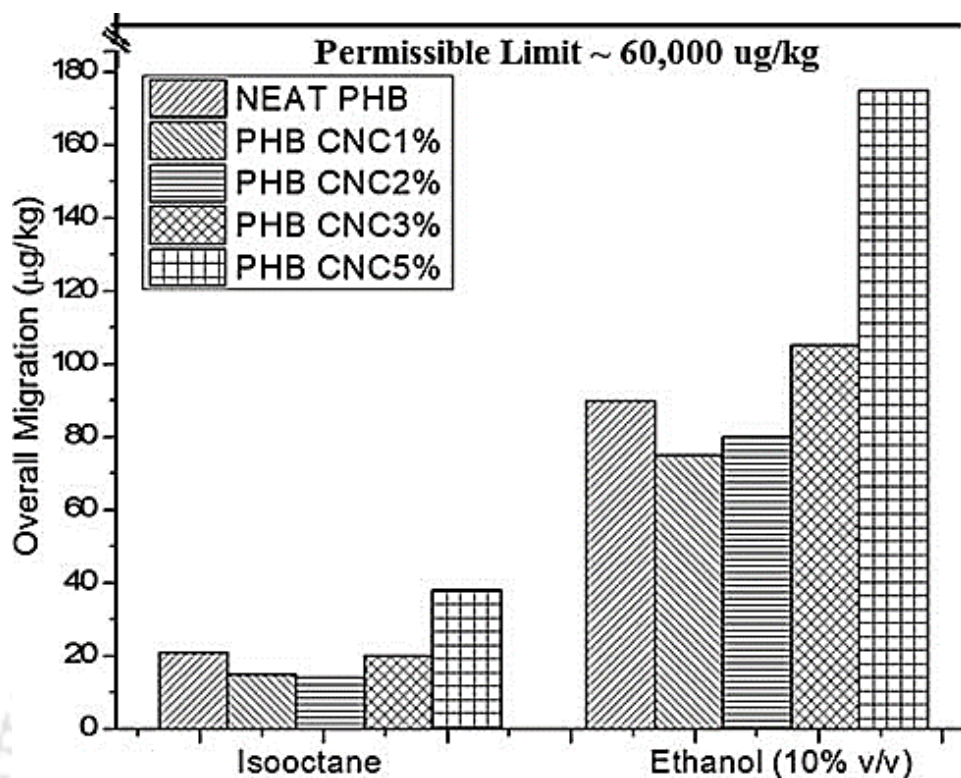


Figure 4.11: Overall migration of PHB and PHB/CNC nanocomposites in two food simulants: isooctane and ethanol (10% v/v) respectively.

The effect of incubation on PHB/CNC films during its interaction with ethanol is analyzed through thermal and physical investigations. After incubation period, visual observation of films shows opacity with whitish appearance and higher brittleness. Such effects could be due to presence of hydroxyvalerate (HV) oligomeric units [343] which may agglomerate with CNCs in presence of simulant (ethanol) leading to the change in the appearance of PHB/CNC films to whitish color. The thermal behavior of PHB/CNC films after incubation in ethanol are characterized (as shown in Table 4.2) to obtain further insight into the effect of simulants on polymer chain dynamics. Significant change in thermal behavior of PHB/CNC nanocomposites is observed after incubation, with formation of new multiple melting peaks and significant change in crystallization temperature. The effect of migration on polymer

chains is clearly observed, as glass transition temperature (T_g) of PHB/CNC nanocomposites increased for all CNC loadings studied (by $\sim 12^\circ\text{C}$ compared to neat PHB). The high T_g , is due to the phenomenon of vitrification which leads to the formation of rigid amorphous fractions, that hinders the polymer chain motion [343].

Table 4.2: Thermal properties of PHB and PHB/CNC composites (a) before and (b) after migration studies in ethanol 10% (v/v).

Samples		T_{cc} ($^\circ\text{C}$)	T_m ($^\circ\text{C}$)	T'_m ($^\circ\text{C}$)	ΔH_{cc} (J/g)	ΔH_m (J/g)	X_{cc} (%)
Neat PHB	(a)	103.8	172.1	-	105.2	86.2	58.7
	(b)	86.3	170.1	162.7	60.3	48.2	32.8
PHB CNC 1%	(a)	97.7	173.0	-	82.5	70.4	48.0
	(b)	84.8	168.5	-	60.7	48.8	33.2
PHB CNC 2%	(a)	96.9	171.2	-	88.9	70.0	47.7
	(b)	88.1	167.6	160.1	68.1	49.8	33.9
PHB CNC 3%	(a)	100.8	171.6	-	106.3	90.5	61.7
	(b)	90.2	169.1	161.5	68.5	55.0	37.5
PHB CNC 5%	(a)	101.3	172.0	-	106.0	84.8	57.8
	(b)	85.0	170.5	162.8	62.7	50.9	34.7

ΔH_m = Enthalpy of melting, T_m = melting temperature, T'_m = second shoulder melting temperature, ΔH_{cc} = Enthalpy of crystallization and T_{cc} = crystallization temperature.

PHB/CNC nanocomposites are found to have multiple melting peaks with the progressive decrease in the melting enthalpy, which may be due to the formation of different lamella or crystallite structure as supported by crystallization studies. The multiple melting peaks (T_m And T'_m) could be attributed to the melting of crystals which have undergone rearrangements or structural reorganization during migration studies to form a wide range of crystallites with different concentrations [344]. Another study reports that PHB lattice usually

contains hydroxyvalerate (HV) units, which could diffuse out of the crystalline region of PHB during migration studies [345]. This exclusion of HV units would lead to the formation of unstable, relatively thinner crystals, which will contribute large surface energy to specific enthalpy, resulting in decreased melting temperature and enthalpy [345]. However, such phenomenon is hindered to some extent by the well dispersed network structure of CNCs in polymeric matrix at lower loading fractions, which even resisted the overall migration of simulants. Neat PHB and PHB/CNC 5 wt. % shows highest decrease in percentage crystallinity (~26% and 23%, respectively) and crystallization enthalpy ($\Delta H_{cc} \sim 44$ J/g) after migration studies. Further, molecular weight (M_w) studies reflects that PHB/CNC films after migration studies shows reduction in molecular weight by ~11–26%, depending on CNC loadings. Gel permeation chromatography (GPC) studies showed the two fractions of polymer segments with $M_w \sim 900$ –1200 kDa and $M_w \sim 1$ –2 kDa after migration studies. The percentage fractions of each segment as shown in Table 4.3 [$M_w \sim 900$ –1200 kDa (R1) and $M_w \sim 1$ –2 kDa (R2)] has been calculated by considering ratio of area of the peaks of PHB/CNC films after migration to the neat PHB film before migration studies. It is observed that R1 decreases in all composition of PHB/CNC films after migration, which is due to the degradation of the large PHB chains into small oligomeric fractions in presence of simulants. However, the progressive decrease in R2, is due to the leaching of low molecular weight fragments into the simulant during migration process (Table 4.3). Therefore, it can be concluded that decrease in M_w leads to the rapid fragmentation of both the crystalline and amorphous segments in PHB/CNC films during migration (in presence of simulants) which predominantly leads to decrease in overall crystallinity. Similar phenomenon of decrease in M_w which subsequently leads to decrease in

crystallinity was also observed during the hydrolytic and alcoholysis based degradation studies of the PHB films[346, 347].

Table 4.3: The change in molecular weight and percentage fractions of polymer segments (M_w ~900-1200 kDa and M_w ~1-2 kDa) in PHB/CNC films of different composition after migration.

Samples	Molecular Weight (M_w)	% M_w Reduction	R ₁	R ₂
Before Migration Studies				
<i>PHB Neat</i>	1164239	-	1.0	1.0
After Migration Studies				
<i>PHB Neat</i>	1036001	11.0	0.35	0.62
<i>PHB CNC 1%</i>	1018951	12.4	0.71	0.78
<i>PHB CNC 2%</i>	996945	14.3	0.12	0.74
<i>PHB CNC 3%</i>	855661	26.5	0.18	0.56
<i>PHB CNC 5%</i>	989501	15.0	0.21	0.48

(R₁ and R₂ is the ratio of area of peaks of PHB/CNC films after migration to the neat PHB films before migration at two peaks corresponding to polymeric segments of M_w ~900- 1200 kDa (R₁) and M_w ~1-2 kDa (R₂))

4.2.8 Gas Barrier Properties of PHB/CNC Nanocomposites: A Mechanistic Study

Table 4.4, lists the oxygen transmission rates (OTR) and permeability parameters such as solubility, diffusivity and permeability for oxygen transport through PHB/CNC nanocomposite films. These results provide insight into the mechanism of oxygen molecule diffusion through the nanocomposites. PHB/CNC nanocomposites show drastic reduction in OTR values upon incorporation of CNCs at very low loading. Reduction in values of both solubility and diffusion coefficients is also observed, which implies increased resistance to permeation of oxygen molecules through CNC network. OTR values show a reduction of ~46% and ~65% on incorporation of 1wt. % and 2 wt. % CNC, respectively. Rheological studies (as discussed

earlier) shows formation of the transient percolation network of CNCs in the polymer matrix through hydrogen bonding. The increased resistance to oxygen permeation offered by CNC percolation network within polymer matrix may be responsible for the reduction in OTR through PHB/CNC films at low CNC loading. Moreover, the possible intercalation of PHB matrix into the CNCs (as suggested by XRD results) may also be responsible for reduction in OTR.

Solubility and diffusivity parameters are predicted from half time values, which also decreased (by ~57 and 17%, respectively) with increase in CNC loading (up to 2 wt. %). The decrease in the solubility coefficient is more pronounced, possibly because of reduction the number of sites in the polymer matrix for physical interaction with oxygen [348]. This is due to the presence of inter-molecular hydrogen bonded network of CNC with hydroxyl groups of PHB which also leads to enhanced resistance to the diffusion of oxygen molecules. However, at higher CNCs loadings (>5 wt. %) such intercalation effect and percolation network could be negligible, which results in higher OTR values. Morphological studies, as discussed in earlier section, showed formation of voids, due to difference in polarity between hydrophilic CNCs and hydrophobic PHB polymer, which could possibly lead to significantly higher solubility coefficient. Even though the diffusivity decreased by ~45% (>5 wt. %), but the effect of solubility coefficient viz., formation of voids due to weak adhesion and absence of hydrogen bonded interaction because of CNC agglomeration, was predominant enough in PHB/CNC 5 wt. %, leading to increased permeability coefficient.

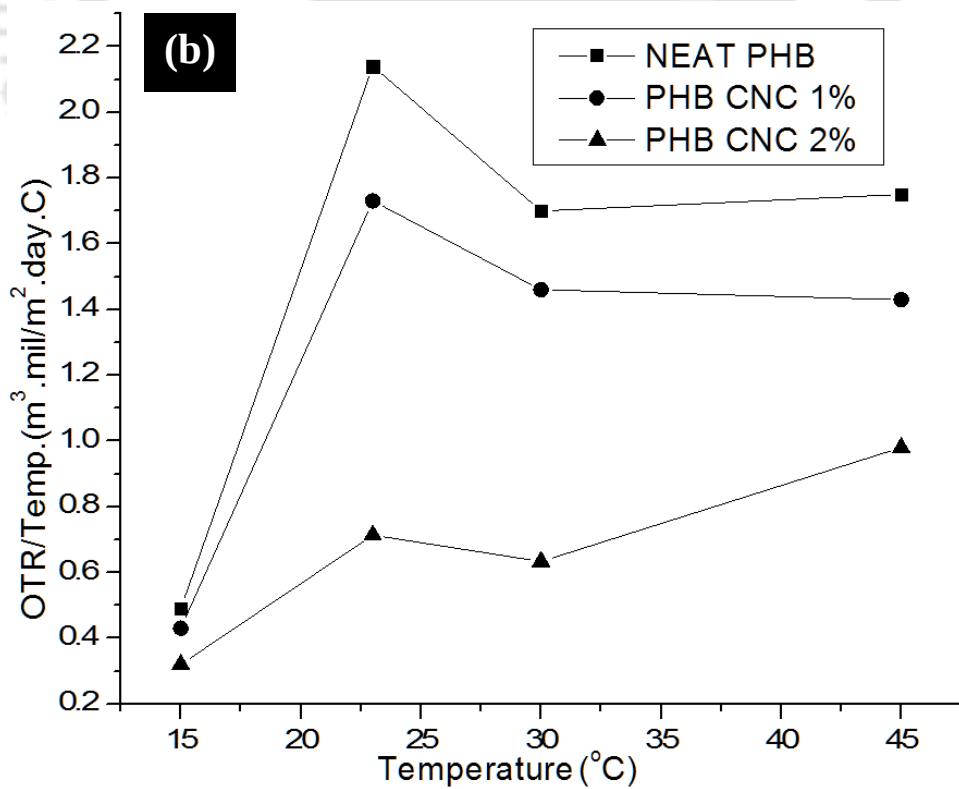
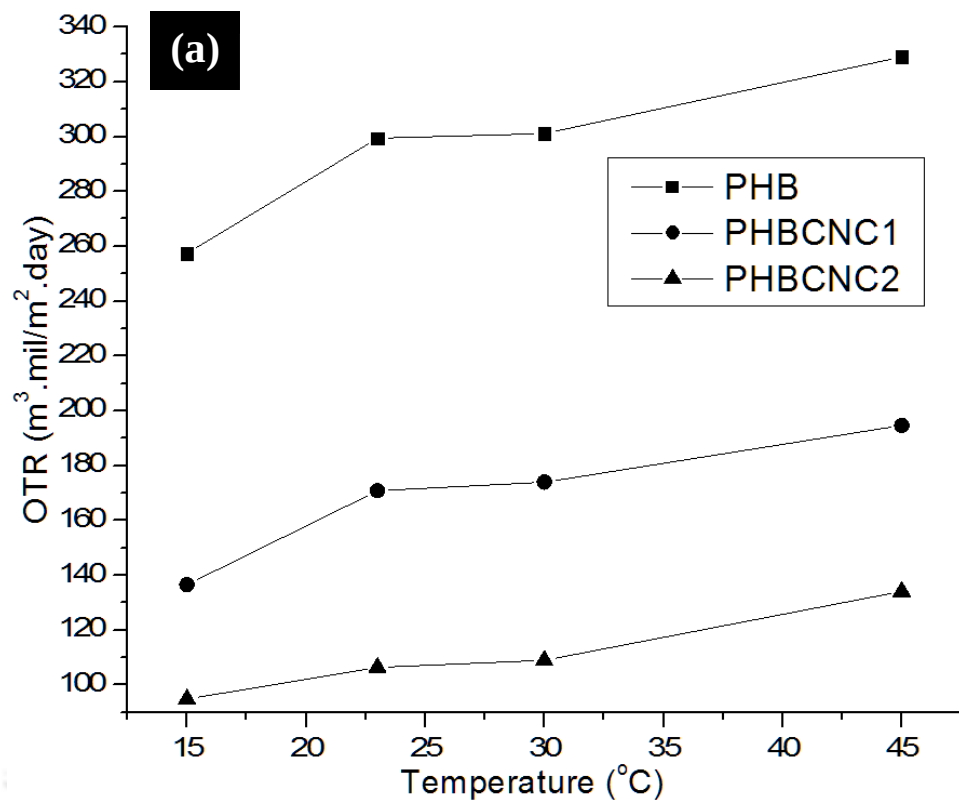
Table 4.4: Oxygen transmission and prediction of permeability parameters at 23 °C and 0% humidity for PHB and PHB/CNC nanocomposites.

Samples	OTR (cm ³ .mil/m ² .day)	Permeability (m ³ .m/m ² .s.Pa) (x 10 ⁻¹⁶)	Solubility (g/g.Pa) (x 10 ⁻⁶)	Diffusivity (m ² /s) (x 10 ⁻¹²)
Neat PHB	299.4	0.3456	3.22	10.7
PHB Literature (31)	350.0	0.3-0.4	-	-
PHB CNC 1%	162.5	0.1976	2.00	9.85
PHB CNC 2%	106.4	0.1231	1.38	8.90
PHB CNC 5%	350.5	0.4056	18.43	2.2

Figure 4.12(a), shows the OTR values for different composition of PHB/CNC films over a range of temperature. Consistent with behavior observed in conventional polymers, increase in temperature caused an increase in oxygen transmission rate. This is because at higher temperature, rate of diffusion of polymer chains/segments increases, which leads to increased OTR values. The rate of change in OTR values with respect to temperature change for neat PHB and PHB/CNC loaded films are shown in Figure 4.12(b). The neat PHB films shows a drastic change in slope whereas change in slope became less pronounced with increase in CNC loadings (at ~2 wt. % loadings). Addition of CNCs to PHB leads to restriction of motion of polymer segments due to chemical interactions between polymer and CNCs through hydrogen bonding. This restricted molecular motion of the polymer chains results in decrease in OTR values with increase in CNC loading (up to 2 wt. %). Therefore, CNCs form stable dispersed network inside the polymer matrix at lower loading fractions (up to 2 wt. %) which increases diffusion path and consequently, reduces the permeability over the entire temperature range studied. To better understand the effect of temperature on gas transport properties of polymer nanocomposites, an attempt was made to fit the Arrhenius expression to the permeability values:

$$P = P' e^{\frac{-E_p}{RT}} \quad (4.1)$$

where P' is the pre-exponential factor (cm.mm/m².day), E_p is the activation energy for permeation (in kJ/mol), R is the universal gas constant (8.314 J/mol.K) and T is the absolute temperature (in K). Figure 4.12(c), shows Arrhenius plot of oxygen permeability versus inverse of temperature for PHB/CNC nanocomposites at different CNC loadings. The slope of graph decreased with increase in CNC loading as diffusivity decreased due to tortuous pathways introduced by the nanofillers. Activation energies for permeation, calculated for different PHB/CNC loading fractions, are 22.6, 35.0 and 46.3 kJ/mol for neat PHB, PHB with 1 wt. % CNC and PHB with 2wt. % CNC respectively. The activation energy values indicate that more energy is required by oxygen molecules to overcome barrier networks formed by CNCs at 1and 2wt. % loading. This provides further evidence of formation of stable networks at low CNC loadings that offer torturous pathways for gas molecule diffusion, thus resulting in lower gas permeation and improved gas barrier properties. As discussed in previous section, the physical interaction between the hydroxyl surface of CNCs and PHB due to hydrogen bonding, is possibly the most predominant factor for decrease in molecular chain motion at higher temperature. Therefore, it can be concluded that, firstly, the most important effect of restriction in molecular mobility of polymer chains due to its interaction with CNCs through hydrogen bonding and secondly due to the creation of tortuous path by CNCs in polymeric matrix leads to significant reduction in activation energy of oxygen permeation [349].



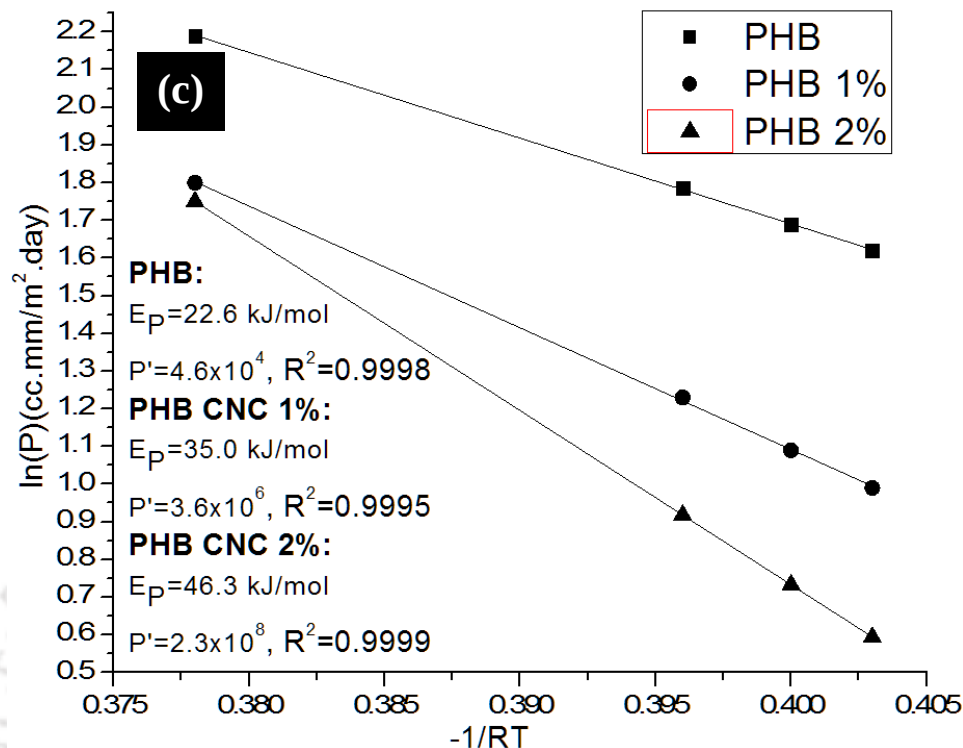


Figure 4.12: (a) Change in oxygen transmission rate (OTR) with temperature at various PHB/CNC loadings. (b) Comparison of rate of change in OTR with respect to temperature for various CNC loadings. (c) Temperature dependence of permeation rate, fitted with Arrhenius equation for various PHB/CNC loading.

4.3 CONCLUSIONS

Nanobiocomposites fabricated using hydrophobic PHB and hydrophilic CNCs showed improved dispersion, and enhancement in mechanical, rheological and barrier properties compared to pristine polymer. Morphological studies showed two different morphological orientation of CNCs in PHB matrix due to improved interfacial interaction between the two biopolymers. Thermal properties studied by DSC showed decrease in the crystallinity with increase in CNC loading which helps in the fabrication of PHB films with better transparency and flexibility. Rheological and mechanical studies showed the formation of the percolation

network of CNCs in PHB matrix at approximately 2 wt. % loading. Rheological studies (linear and non-linear) revealed the crossover behavior of storage and loss modulus for nanocomposites with 1% and 2 wt. % CNC loading, indicating better PHB/CNC interactions. This is due to the intermolecular hydrogen bonding between the CNCs and the PHB matrix which allows CNCs to act as a strong reinforcement agent. Rheological analysis over a wide temperature range and DSC studies showed good correlation between the change in enthalpy and storage modulus during the crystallization phenomenon. A significant change in elastic modulus is observed at the crystallization temperature, highlighting the effect of formation of PHB crystallites on mechanical properties. The percolation concentration measured through rheological and mechanical modelling studies lead us to the conclusion that optimum properties for these polymer nanocomposites are achieved at low CNC loadings of up to approximately 2 wt. %.

Migration studies of films in two food simulants showed that overall migration is within the standards limits as per the legislations (Commission Regulation EU No. 10/2011). Thermal analysis of films subjected to migration studies showed significant decrease in crystallization temperature because of the formation of new crystallites and significant decrease in molecular weight due to leaching of oligomeric units in the presence of food simulants. The oxygen transmission rate drastically decreased by ~65% even at low CNC loading of 2 wt. % compared to neat PHB. Oxygen permeation studies carried out over a range of temperature showed that the activation energy of permeation increases with increase in CNC loading. These attributes (low OTR and migration well within acceptable limits) makes bio-based PHB/CNC nanocomposites a promising material for various applications in the field of food packaging.

Studies on Functionalized Cellulose Nanocrystal-based Polylactic Acid nanocomposites for Packaging Applications

Motivation

Cellulose nanocrystals, are hydrophilic in nature due to which their dispersion in the hydrophobic polymeric matrix becomes a challenging task, hence, requires strategic surface modifications to improve its compatibility. However, alteration of CNC's surface through the utilization of wide varieties of chemicals raises concerns regarding its biocompatibility, biodegradability and toxicity issues. To overcome such problems, we have developed an environmentally sustainable approach to modify the surface of CNCs with various anionic moieties from acids used during hydrolysis step and CNCs with tuned cellulose polymorphism during the processing step (as discussed in chapter 3). Introduction of such surface modified CNCs into polylactic acid (PLA), through solution-casting and industrially viable extrusion- based approaches have shown to improve the physico-chemical, structural and barrier properties of PLA, making it suitable for potential application as biodegradable packagings.

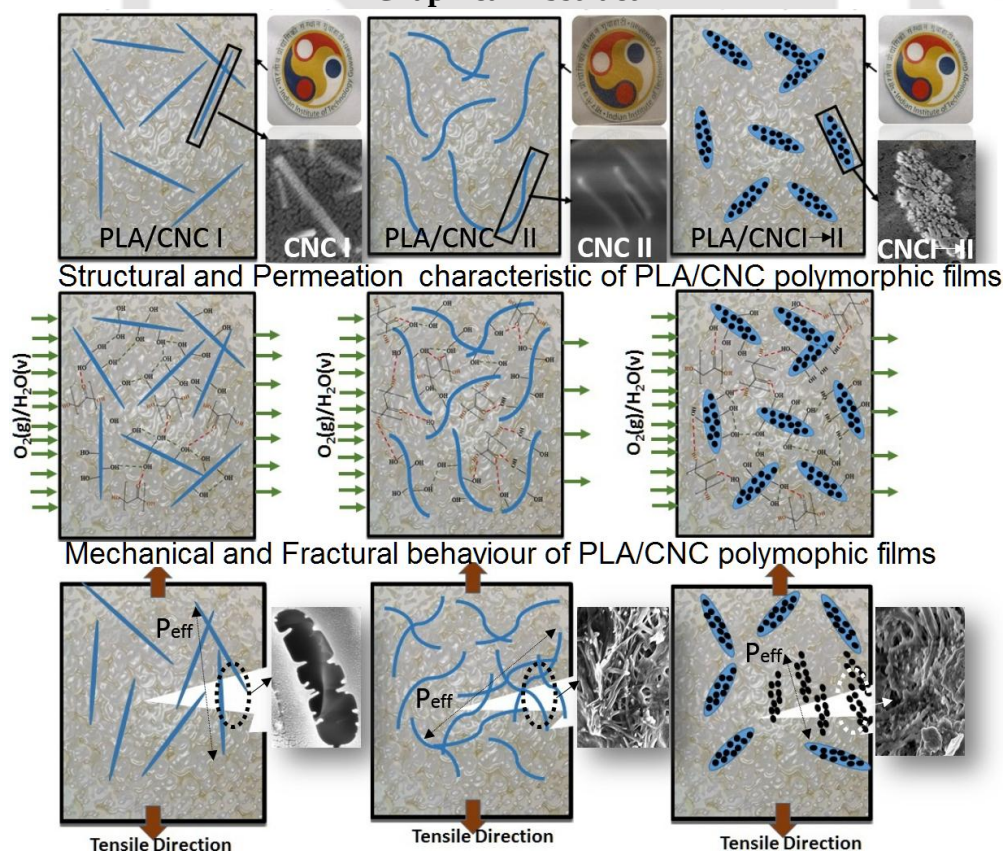
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- Dhar, P., Tarafder, D., Kumar, A., & Katiyar, V. (2015). Effect of cellulose nanocrystal polymorphs on mechanical, barrier and thermal properties of poly (lactic acid) based bionanocomposites. RSC Advances, 5(74), 60426-60440.
- Dhar, P., Bhasney, S. M., Kumar, A., & Katiyar, V. (2016). Acid functionalized cellulose nanocrystals and its effect on mechanical, thermal, crystallization and surfaces properties of poly (lactic acid) bionanocomposites films: A comprehensive study. Polymer, 101, 75-92.

Abstract

Cellulose nanocrystals (CNCs), fabricated using different acids as hydrolysing agents and with different cellulose polymorphs, leads to significant variation in its inherent properties such as morphological properties, crystal structure, surface characteristics, degree of hydrogen bonding and thermal stability. The altered positions of hydroxyl group in CNCs with different cellulose polymorphs, and substitution with anionic moieties in the case of acid- derived CNCs leads to variation in the interfacial interaction of CNC with polylactic acid (PLA) matrix, and thereby its dispersion behaviour. Interestingly, due to tunable aspect ratios and degree of intermolecular hydrogen bonding, both classes of CNCs have substantial effect on mechanical reinforcing efficiency and ability to form percolating network structures. The occurrence and stability of such percolated-network are known to tune the barrier, crystallization and thermal properties of CNC based bionanocomposites. Therefore, the current study provides an insight towards selection of appropriate type of CNCs for manufacturing high performance PLA/CNC nanocomposites with tailored properties.

Graphical Abstract



5.1 INTRODUCTION

Crystalline cellulose exists in four different polymorphs, namely cellulose I, II, III and IV [294] which have significantly altered arrangement of inter- and intramolecular hydrogen bonding. These differences in the inter- and intramolecular hydrogen bonding results in drastic variation in the elastic modulus of the cellulose polymorphs. Elastic moduli of cellulose I, II, III_I, III_{II} and IV are 138, 88, 87, 58 and 75 GPa respectively [281]. When dispersed into a polymeric system, they act as a strong reinforcement agent and lead to significant improvement in mechanical properties. CNCs have been known to intercalate into the polymer matrix forming a dense network like structure, which improves the water vapor and oxygen barrier properties significantly [350]. Due to their attractive physico-chemical and structural properties, CNCs have generated considerable interest in academia as well as industry, that has led to large scale production of CNCs to meet the increased demand [351].

CNCs, with abundant hydroxyl functionality, can be surface modified through different chemical modification approaches such as oxidation, esterification, silylation, etherification and acetylation [352]. Polymer grafted CNCs have been fabricated either through grafting from or onto approach, for improving their dispersion in hydrophilic and hydrophobic biopolymers [1]. However, the above mentioned chemical modification methods lead to good CNC dispersion only through solution casting approaches rather than the commercially viable extrusion process. Studies involving several extrusion-based approaches such as pumping of CNC dispersed in dmac/licl and use of maleated polylactic acid (PLA) coupled with poly (ethylene glycol) (PEG) as compatibilizer have been reported [207]. No significant improvement in the properties of the resulting nanocomposites was observed in such studies. However, the addition of the plasticizer glycerol triacetate (GTA) has been observed to lead to

an improvement in the elongation at break and toughness by ~31 and ~87% respectively [353]. Several studies have been reported in literature which take into consideration the variable aspect ratio, crystallinity and morphology of CNCs or CNFs fabricated from different raw material sources [354], chemical functionalities due to different degree of hydrogen bonding in cellulose polymorphs [17] and variation in the inherent mechanical properties [279] on the reinforcement effects of CNCs in polymeric nanocomposites. Studies by Xu et al. [355] showed that CNFs with larger aspect ratio formed dense fiber entanglements with polyethylene oxide matrix thereby creating a percolated-network structure which significantly improved the modulus of nanocomposites by two times. Saciu et al. [354] reported fabrication of CNCs/CNFs from seven different cellulosic precursors using hydrolysis conditions such as acid based, enzymatic hydrolysis, 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO)-based oxidation and mechanical disintegration followed by detailed investigations of their fundamental properties. CNCs/CNFs fabricated from such precursors were found to have different fractions of crystalline segments with varied polymorphisms, surface functionality, charge and total surface energy. Camarero Espinosa et al. [92] isolated CNCs from different acid sources viz. Sulphuric, phosphoric and hydrochloric acids and investigated their thermal and dispersion stability in polar solvents. Comparison of the thermal behavior showed that phosphoric acid-hydrolyzed CNCs were thermally stable up to ~300°C which was significantly higher compared to sulphuric and hydrochloric acid-hydrolyzed CNCs [92]. Moreover, phosphorylated CNCs when dispersed into polyurethane foams have been found to improve the mechanical strength by ~4.3 times and incorporate flame retardancy characteristics increasing the time of ignition ~2.6 times [356]. Recently, a novel approach of reactive functionalization-cum-blending of polyvinyl acetate (PVAc)/CNCs into PLA showed

improvement in thermal properties and enhancement of mechanical properties by ~19% [278]. Bondeson et al. [167] fabricated PLA nanocomposites by pumping CNCs dispersed in polyvinyl alcohol (PVA) while compounding, which led to agglomeration of CNCs in the PVA phase. Jonoobi et al. [357] used solvent exchanged CNCs and dispersed into PLA through twin screw extrusion process, which led to improvement in storage modulus and increase in tensile modulus by ~21%.

Although extrusion is an industrially scalable and environment friendly (solvent free) approach, some technical challenges still remains related to the proper dispersion of CNCs and drastic reduction in molecular weight of polymer while being processed with CNCs. The processed films shows black/brown coloration and have inferior physico-chemical and structural properties due to reduced molecular weight characteristics. Moreover, modification of CNCs with complex chemical functionalities raises concerns regarding their sustainability, biodegradability and non- toxic behaviour. To overcome the above-mentioned challenges, two different types of surface-modified CNCs, i.e., CNC with different polymorphism and those with substituted anionic moieties are fabricated through a single step processing route (as described in chapter 3, section 3.2.1 and 3.2.2). Variation in cellulose polymorph in CNCs leads to alteration in the packing of cellulosic chains in parallel/anti-parallel directions, rearranging the inter and intra-molecular hydrogen bonding. Similarly, CNCs hydrolyzed with various acids lead to substitution of the hydroxyl groups with different chemical moieties, which might enhance the compatibility with the polymer and subsequently improve the dispersion of CNCs within the matrix. In this chapter, CNCs with different cellulose polymorph and hydrolyzed with different acids are dispersed into PLA through solution casting and melt-compounding routes respectively, followed by the investigation on mechanical,

thermal, barrier and crystallization properties. Therefore, this study is expected to provide proper guidelines in selecting the appropriate aspect ratio, chemical functionality, thermal characteristics and morphology of CNCs to obtain polymer nanocomposites with desired properties for specific end user applications.

5.2 RESULTS AND DISCUSSIONS

5.2.1 *Polymorphic Cellulose Nanocrystal based PLA Nanocomposites Films*

Three different polymorphs of CNCs, namely CNC I (with cellulose I), CNC II (with cellulose II) and CNC:I→II (CNC II from cellulose I crystal structure) are fabricated utilizing raw bamboo pulp as initial cellulosic sources (as discussed in chapter 3). Morphological analysis of the three CNC polymorphs shows distinct variation in its morphology, CNC I with needle-like, CNC II with ribbon-like and CNC:I→II with rice-grain like structures. The significant variation in cellulose I and cellulose II crystal structure was confirmed through FTIR and XRD analysis (present in Section 3.2.1) which shows distinct variation in the d- spacing and hydrogen bonding behavior due to varying molecular arrangement of the cellulose chains. The three polymorphic CNCs are dispersed into PLA matrix through a simple route of solvent exchange-cum-solution casting process which improves the dispersion of CNCs and nullifies the effect of reduction in molecular weight of polymers during processing. The effect of variation in the inherent characteristics of the three CNC polymorphs on their reinforcement efficiencies of biopolymer matrix is discussed in the subsequent sections.

- **Effect of CNC polymorphs on Structural Properties and Dispersion Characteristics**

FTIR analyses was carried out to study the possible interactions and dispersion of CNC polymorphs in the PLA matrix, as presented in Figure 5.1(a). The distinct peaks for the PLA were located at 1228, 1265, 1090, 2997, 2945, 1778 cm^{-1} corresponding to the $-\text{C}-\text{O}-\text{C}-$

asymmetric and symmetric vibrations, $-\text{CH}_3$ asymmetric and symmetric vibrations and $-\text{C}=\text{O}$ vibrations respectively. PLA/CNC I showed distinct peaks at 2857 and 993 cm^{-1} corresponding to $-\text{CH}$ stretching and vibrations of $-\text{C}-\text{OH}$ side groups. PLA/CNC II showed distinct peak at 3440 cm^{-1} representing the hydrogen bonding along the $\text{O}(6)\text{H}-\text{O}(3)$ and $\text{O}(3)\text{H}-\text{O}(5)$ directions of the CNC II. From Figure 5.1(b), it can be observed that intensities of the higher wavenumber peaks at 2830 , 2930 , 3210 and 3300 cm^{-1} (usually representing the hydroxyl functional groups) in PLA/CNC nanocomposites either decreased or shifted compared to neat PLA. The decrease in intensity at 3300 cm^{-1} and presence of new peaks at 3440 and 2930 cm^{-1} alongwith formation of broad hump peak at 3010 cm^{-1} , is more prominent in CNC II compared to other polymorphs, possibly due to formation of strong intermolecular hydrogen bonds with PLA matrix. The broad hump peak at around $\sim 3010\text{ cm}^{-1}$ corresponds to the stretching vibration of the hydrogen bonded $-\text{OH}$ groups in the polymer nanocomposites. These observations indicate the presence of strong hydrogen bonded interaction between the polymer matrix and the CNC polymorphs. Figure 5.1(c) shows the presence of the free $\text{C}=\text{O}$ groups in all the nanocomposites which correspond to the peak situated at $\sim 1778\text{ cm}^{-1}$. The IR band at 1830 cm^{-1} is assigned to hydrogen bonded $\text{C}=\text{O}$ groups originating from the PLA/CNC based interactions. However, the peak shifted by 6 and 4 cm^{-1} on incorporation of the CNC II and CNC: I $\rightarrow$ II, respectively. The degree of hydrogen bonded [358] interactions was predicted using the hydrogen bond fraction parameter ($F_{\text{H-CO}}$). The IR band in the range of $1770\text{--}1850\text{ cm}^{-1}$ was deconvoluted using Gauss/Lorentz functions and the corresponding areas were calculated (shown inset Figure 5.1(c)). $R_{\text{H/a}}$ refers to the specific absorption ratio, which depends upon the strength of hydrogen bonds and should be within the range from $1.2 - 1.75$ [282]. In the present work, $r_{\text{H/a}}$ value was selected to be 1.32 , which was the

absorption ratio of the crystalline to the amorphous peaks of PLA [359] . Figure 5.1(d) shows the change in F_{H-CO} values with change in percentage loading of the three CNC polymorphs in the PLA matrix. Compared to other polymorphs, PLA/CNC II has higher F_{H-CO} values indicating the presence of more extensive improved hydrogen bonding with the PLA matrix. The higher hydroxyl functionality and smaller aspect ratio in CNC II improve the hydrogen bond interaction with carbonyl groups of PLA. The F_{H-CO} value increases with increase in CNC loading (from 1% to 3 wt. %) for all three types CNCs. However, the increase in F_{H-CO} value with loading is not as pronounced for CNC:I→II as it is for the other two CNC polymorphs. This could possibly be due to agglomeration of CNC:I→II at higher loadings. Therefore, it can be concluded that hydrogen bonded interaction between CNC and PLA matrix is most pronounced for CNC II.

The effect of different CNC polymorphs on the crystal structure of the PLA/CNC nanocomposites is shown in Figure 5.2(a). PLA is semicrystalline in nature, due to which it exhibits amorphous hump like peaks at about 16.4° , 19.1° , and 22.6° as per reported literature [357] (marked by square symbols in Figure 5.2(a)). Incorporation of CNC polymorphs at different loadings led to increase in the intensity of the XRD peaks, which suggests an overall increase in the crystallinity of the polymer nanocomposites. However, addition of the CNC polymorphs did not alter the diffraction patterns indicating that although crystallinity increased, there is no change in the crystal structure of PLA. The crystalline peaks for CNCs in the polymer nanocomposites were noticeable only at loading fractions higher than 2%. The degree of crystallinity of PLA/CNC nanocomposites was calculated and compared, as shown in Figure 5.2(b). At lower loading fractions, CNC I improved the crystallinity of PLA, whereas at higher loading fractions, the improvement in crystallinity was small, probably

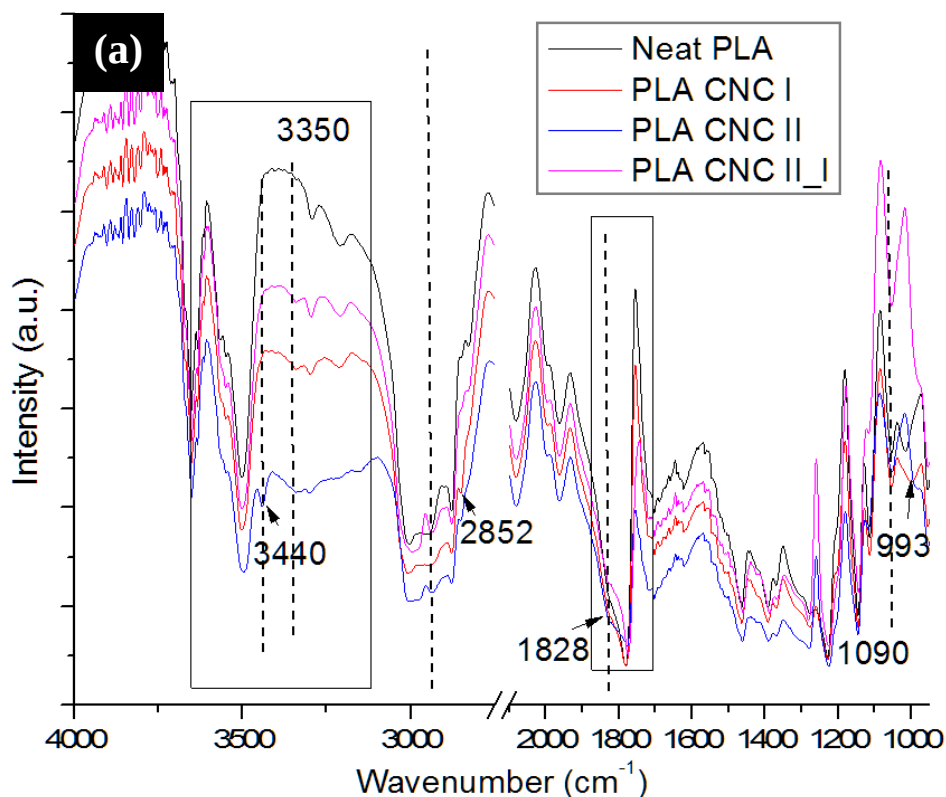
due to agglomeration of CNCs. However, in the case of CNC II and CNC:I→II polymorphs, the crystallinity improved drastically (by ~45%) even at higher loading fractions, which suggests that CNC II exhibits better dispersion in the polymer matrix [360]. Therefore, the crystallinity of biopolymers such as PLA can be tuned by incorporation of different CNC polymorphs depending on the engineering applications.

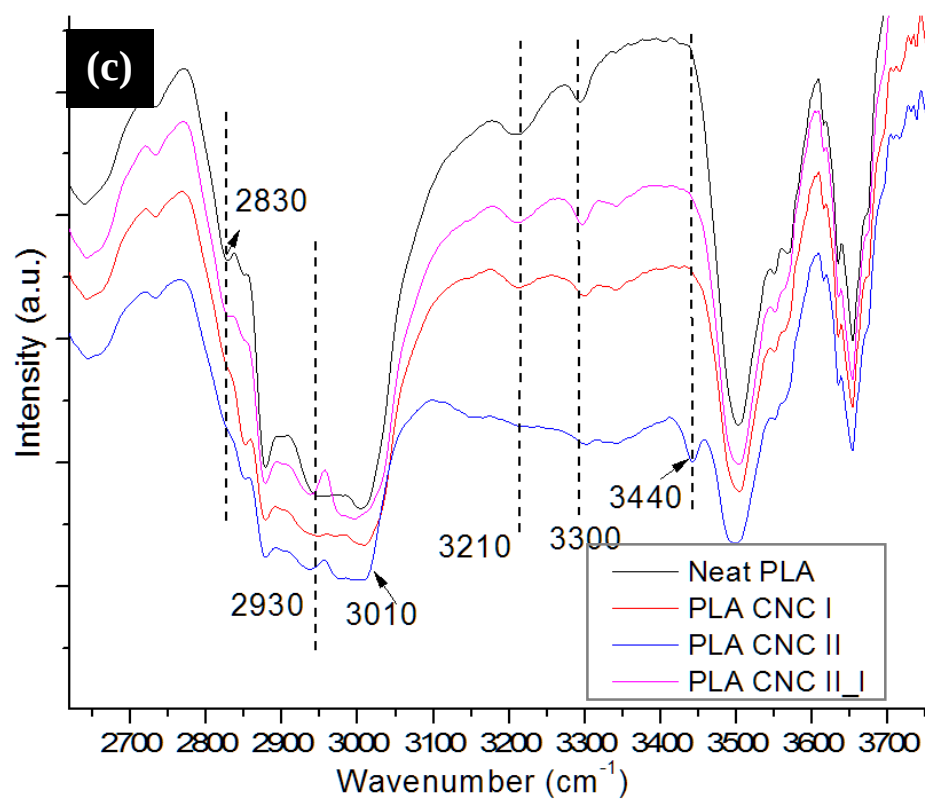
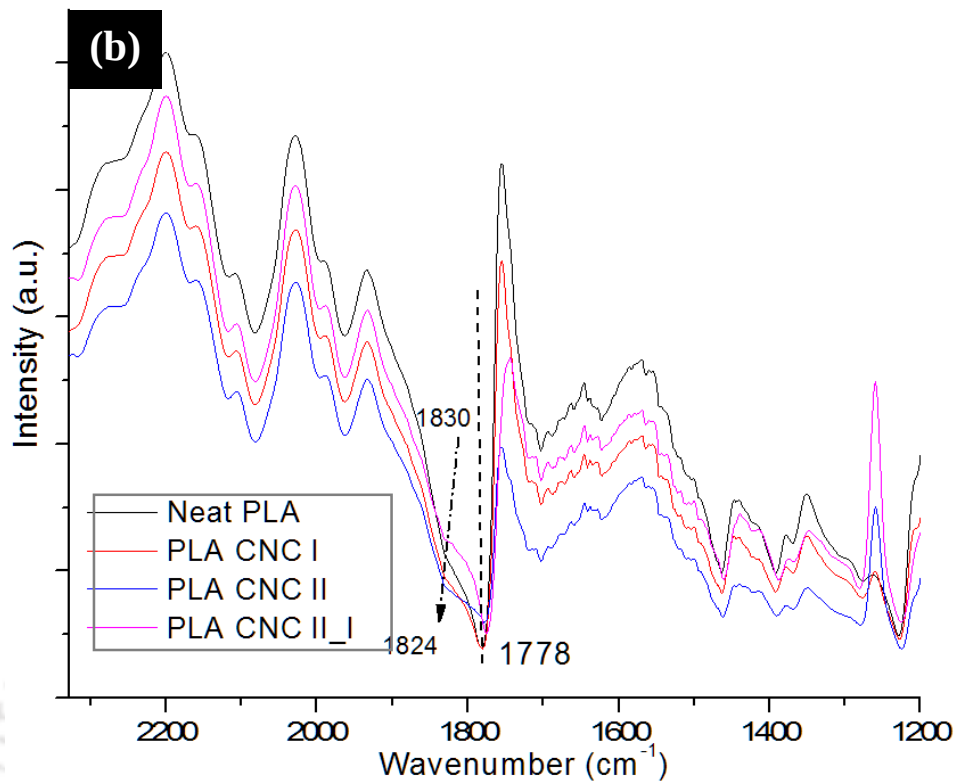
- **Effects of CNC polymorphs on Mechanical Reinforcement**

As discussed in previous section 3.2.1, fabricated CNC polymorphs are found with different aspect ratio, morphology and degree of hydrogen bonding. Therefore, the effect of CNC polymorphs and their loading on the mechanical properties and fractural behavior of resulting PLA/CNC films, have been investigated, as shown in Figure 5.3. Incorporation of 1 wt. % of CNC I, CNC II and CNC:I→II showed an increase in the Young's modulus by ~45%, ~109% and ~71% respectively (Figure 5.3 (a)). Moreover, the ultimate tensile strength improved by ~34%, ~72% and ~74% upon addition of CNC I, CNC II and CNC:I→II respectively (at 1 wt. % loading) compared to pristine PLA film (as shown in Figure 5.3 (b)). The increase in the ultimate tensile strength and Young's moduli of these PLA nanocomposites can be attributed to the reinforcing effect of CNCs. The effective loading of the CNCs appears to be ~2 wt. %.

Interestingly, among all the cellulose polymorphic types, CNC II showed significant improvement in mechanical properties, especially Young's modulus. This is probably due to improved dispersion of CNC II in the PLA matrix compared to the other CNC polymorphs, resulting from enhanced intermolecular hydrogen bonding with the PLA (as evident from the high F_{H-CO} value for PLA/CNC II composites). Although CNC I shows gradual improvement in the Young's modulus with loading, the ultimate tensile strength and percentage elongation

decreased at higher loading, because of the agglomeration due to higher surface energy. Incorporation of all three CNC polymorphs leads to decrease in percentage elongation. The reduction in percentage elongation with loading is higher for CNC II than CNC I, as shown in Figure 5.3 (c). This is probably due to the ribbon-like morphology and tendency of CNC II to form entanglements, which leads to early occurrence of strain hardening, and thus early failure at lower strain percentage. Interestingly, CNC: I→II showed higher ultimate tensile strength at lower loading fractions, due to the presence of strong irreversible hydrogen bonding in the crystalline region among the small spherical aggregates, which is absent in the other cellulose polymorphs.





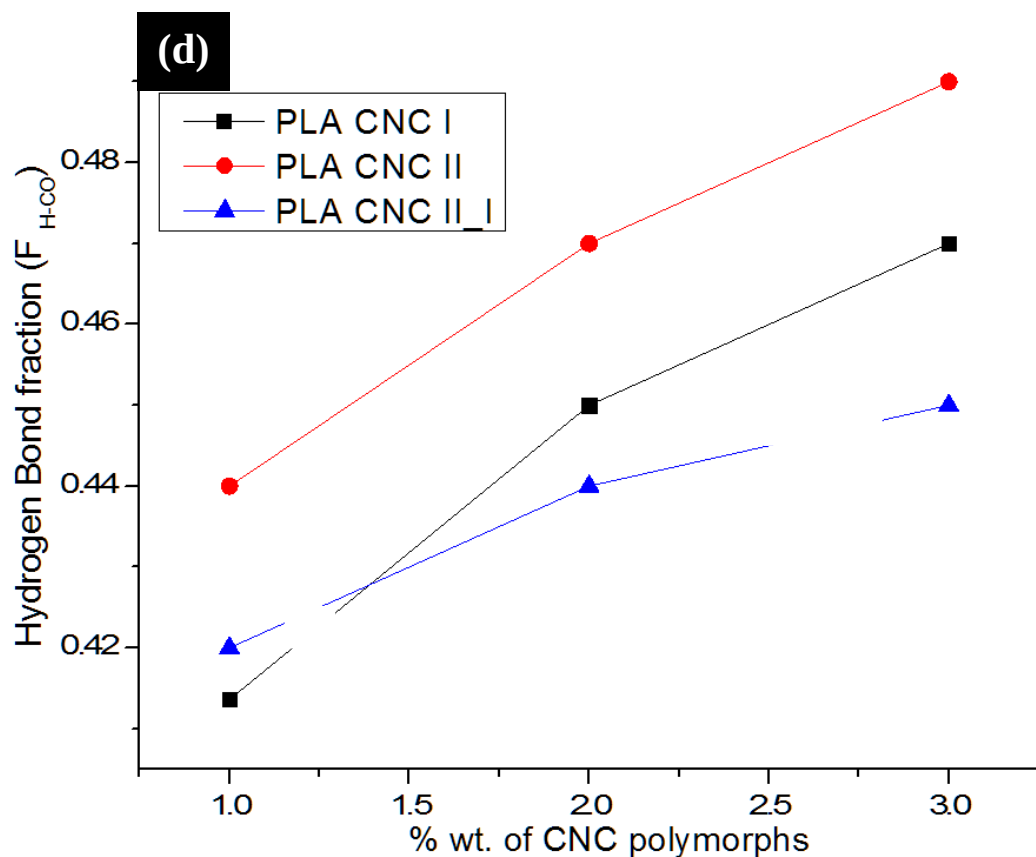
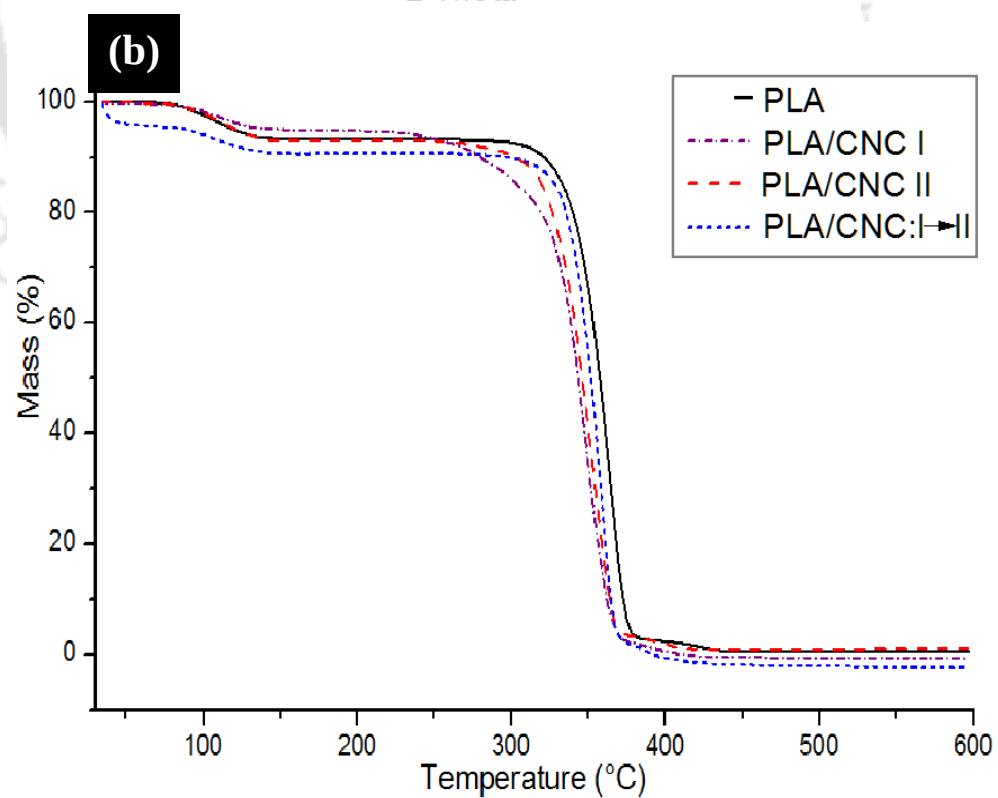
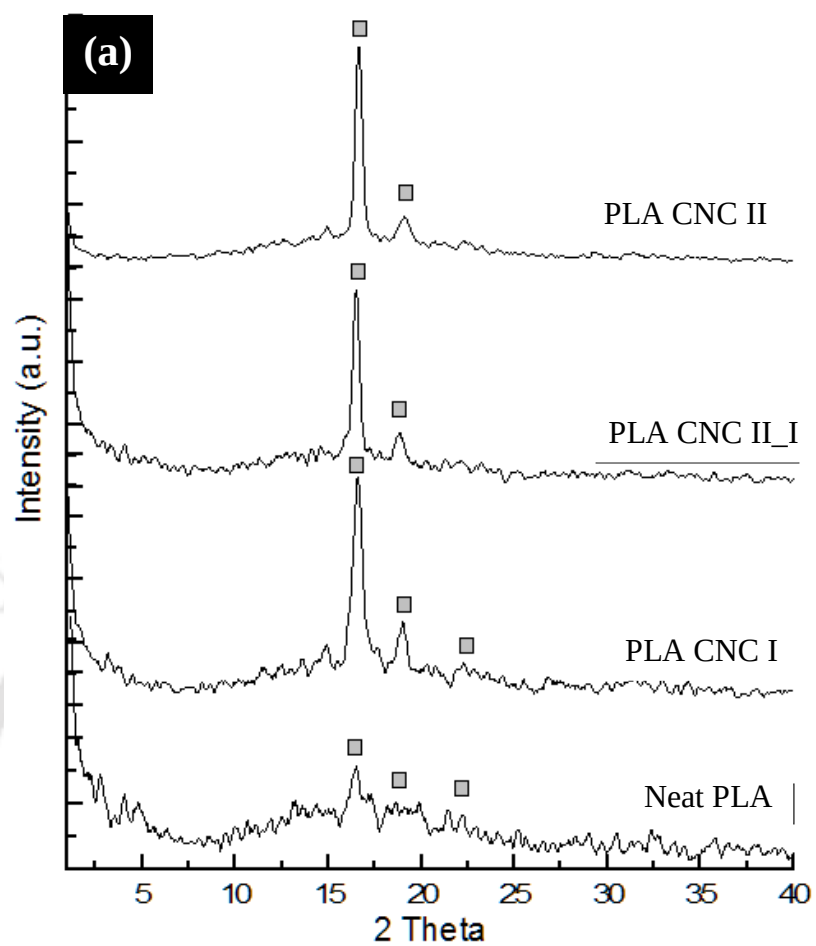


Figure 5.1: FTIR spectra of the Neat PLA and PLA/CNC nanocomposites for different polymorphs at 1wt. % loadings. (b) & (c) Comparison of IR spectra in the range of $2700\text{-}3700\text{ cm}^{-1}$ and $2200\text{-}1200\text{ cm}^{-1}$ respectively. (d) The variation in the hydrogen bonded fraction (F_{H-CO}) at different wt. % of the CNC loadings.



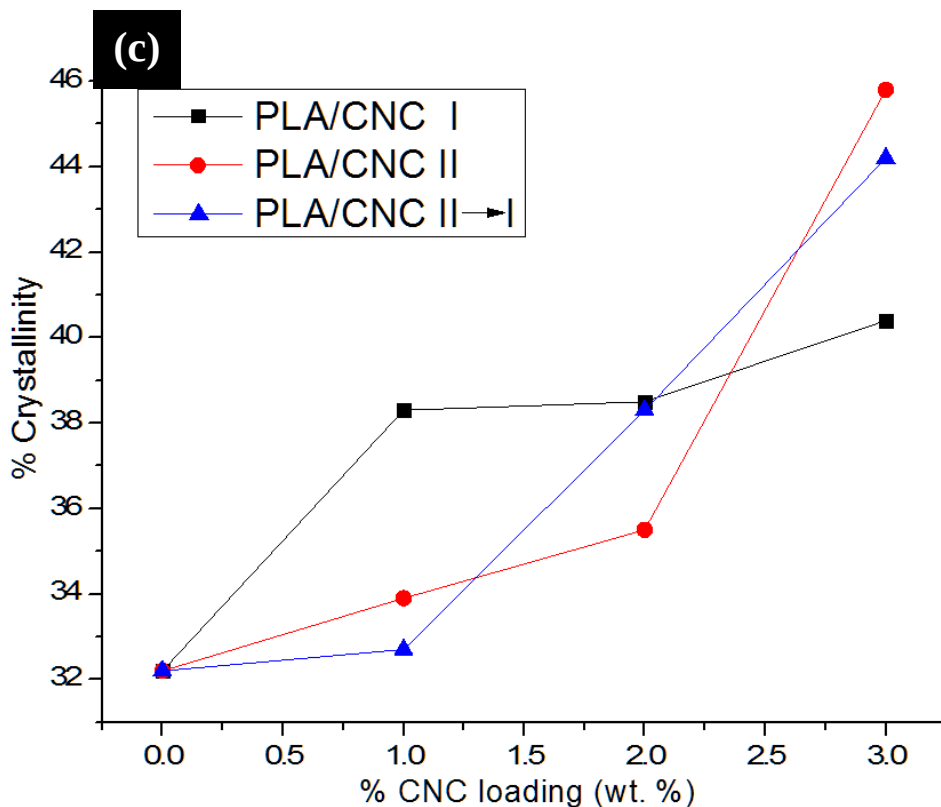
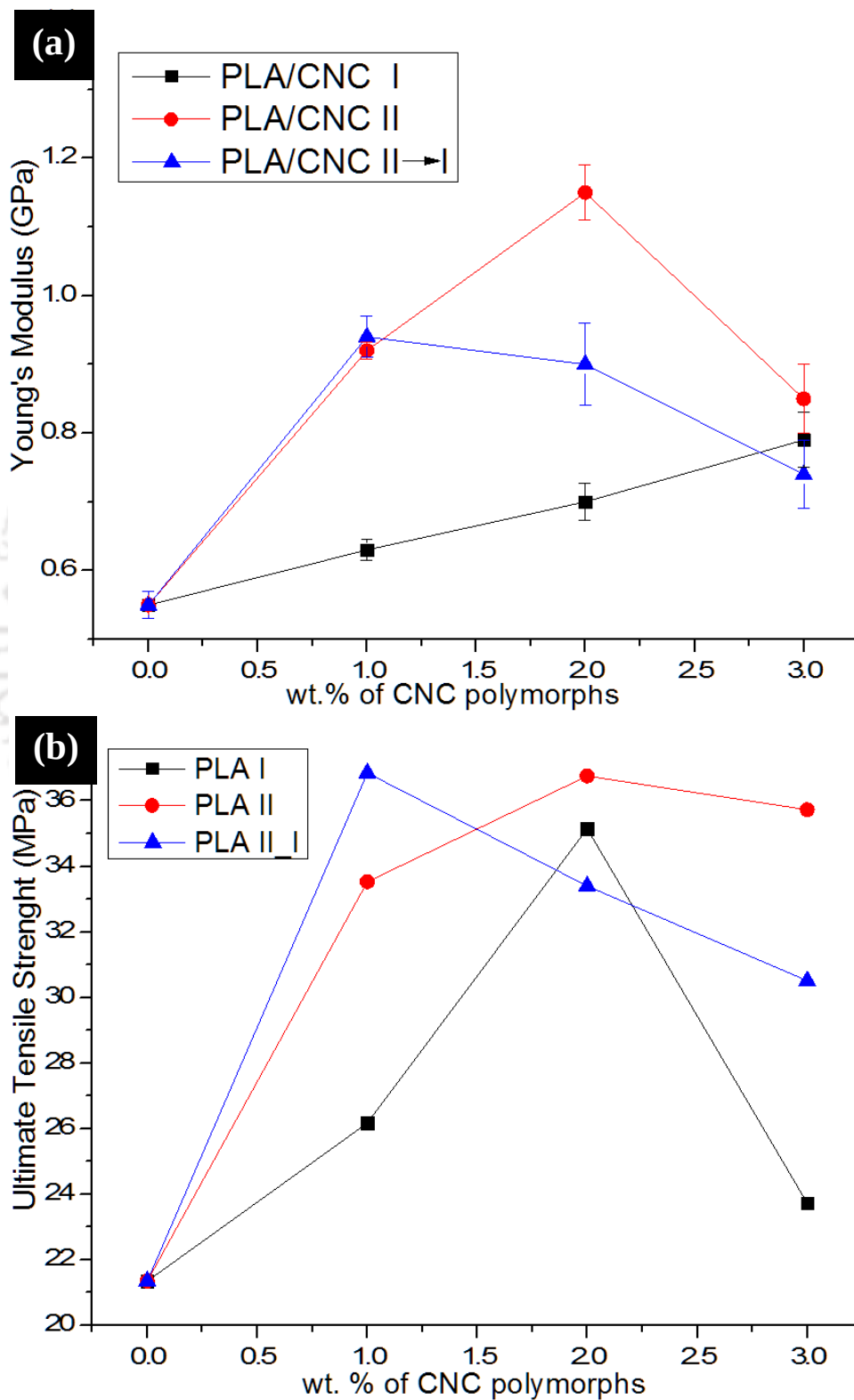


Figure 5.2: (a) Comparison of XRD spectra and TGA plots for different PLA/CNC polymorphs films at 1wt. % CNC loading. (b) The percentage change in crystallinity of PLA/CNC composites at different wt. % of CNC loadings.

However, elongation decreased drastically with increase in loading of CNC:I→II, which could be due to two possible reasons. Firstly, CNC:I→II behaves more like a real fiber structure which usually has lower elongation percentage as reported in literature [305]. Secondly, the weaker intermolecular hydrogen bonding of CNC: I→II with the polymer chains, as evident from the low F_{H-CO} values, causes the PLA/CNC: I→II debonding to occur easily. In summary incorporation of either CNC: I→II or CNC II polymorphs leads to improvement in the Young's modulus and tensile strength of PLA. Therefore, mechanical

properties such as tensile modulus and percentage elongation at break of PLA can be readily tuned by incorporation of different mixtures of CNC polymorphs.



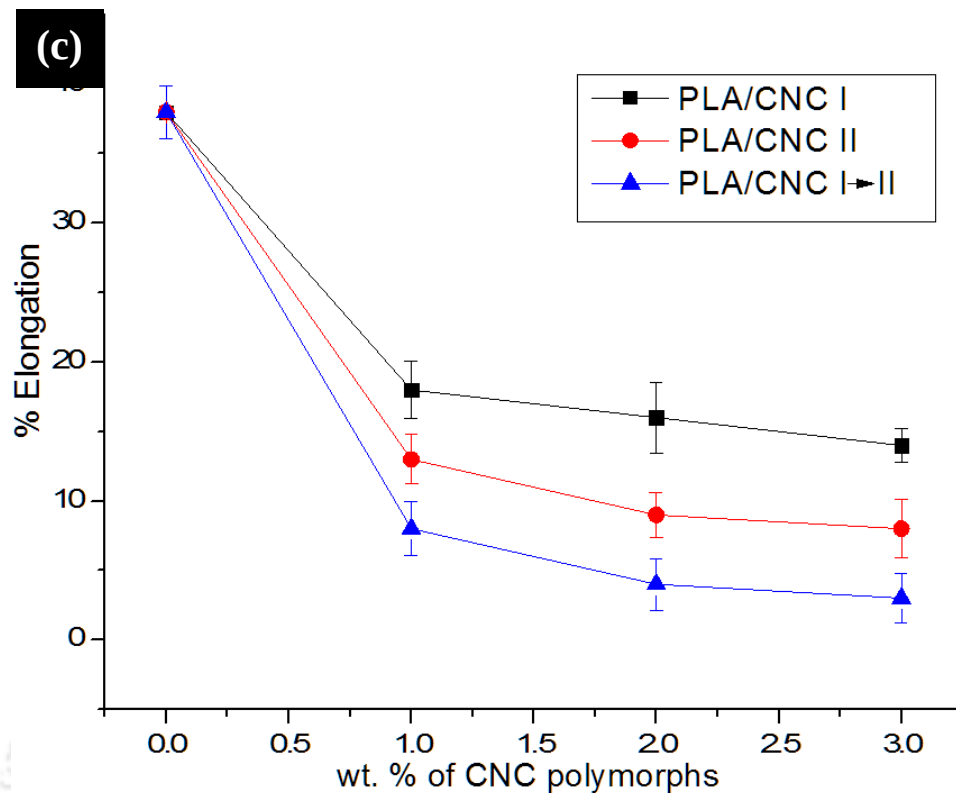
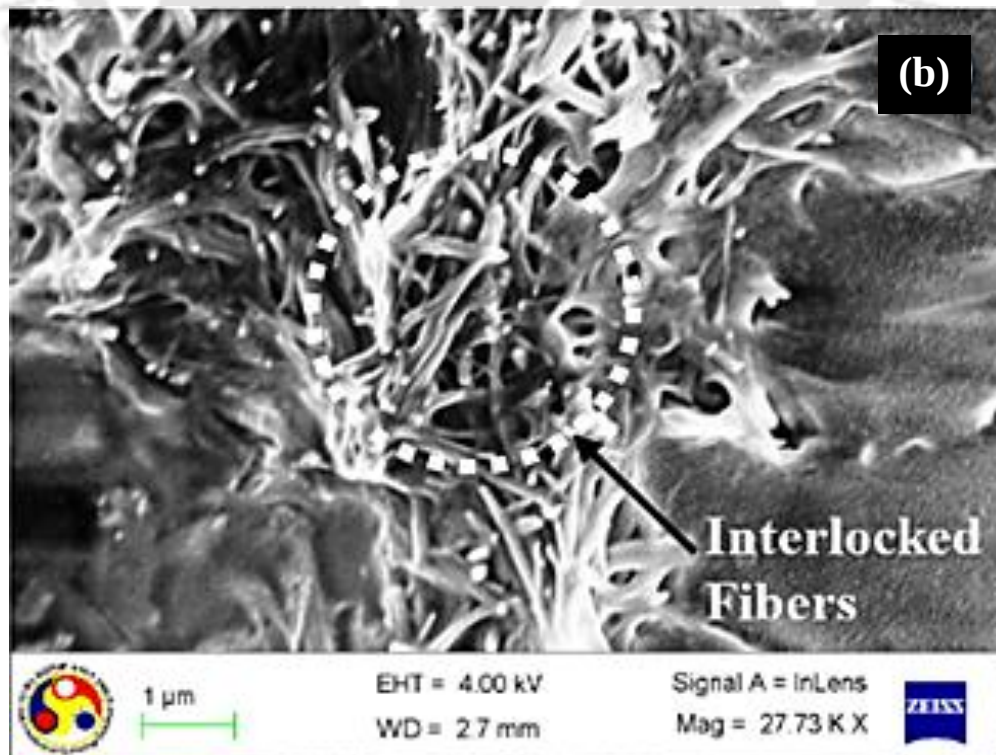
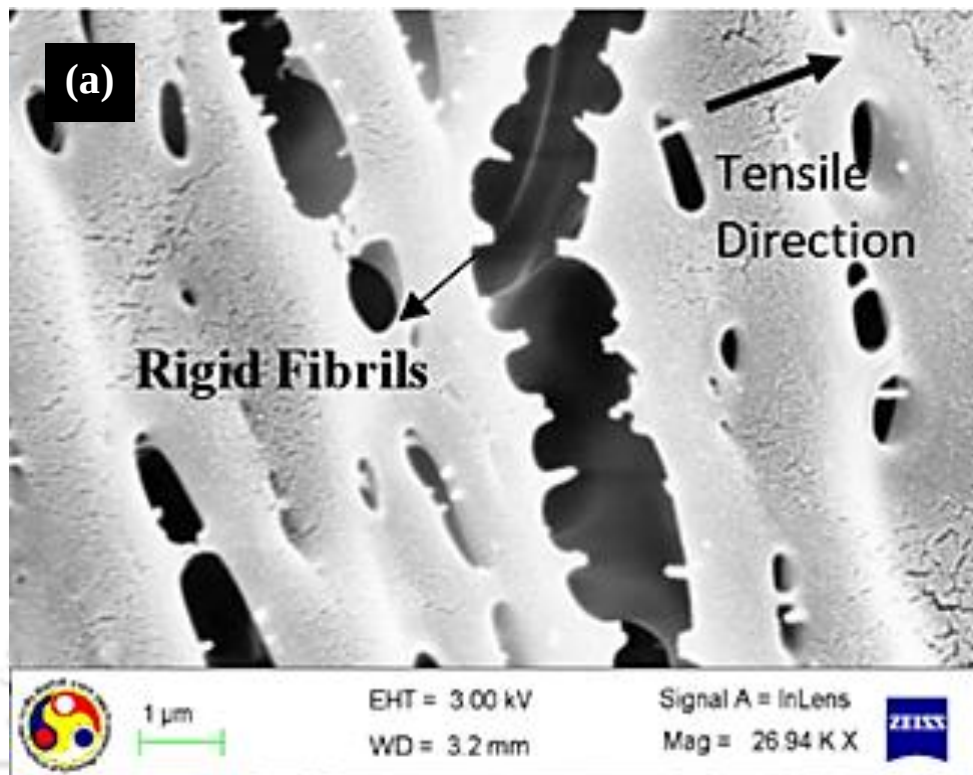


Figure 5.3: Comparison of the (a) Young's Modulus (b) Ultimate Tensile Strength and (c) percentage elongation at break for PLA/ CNC I, PLA/CNC II and PLA/CNC:I→II nanocomposites at 1wt. % loadings.

- **Effect of polymorphs on the Fractural Behavior of PLA/CNC Nanocomposites.**

Morphological investigations of the tensile samples for the three different PLA-based CNC nanocomposites are shown in Figure 5.4. During tensile tests, the samples undergo transition to white color as strain is increased, which is possibly due to the stress induced crystallization during interfacial debonding. The fractured surfaces of the three CNC polymorphs in PLA matrix leads to fibrillation when cracks propagate. PLA films with CNC I polymorph show rigid fibrils which bridges along the tensile direction (in Figure 5.4(a)). Such rigid nanofibrils may represent PLA embedded with CNC I, and are able to withstand higher elongation/strain at break, as the sample undergoes swift matrix failure. The tensile samples of PLA films with

CNC II polymorphs show higher fibril formation. Due to presence of such interfibrillar entanglement, the PLA/CNC II nanocomposites are able to withstand higher stress compared to other CNC polymorphs. However, such entanglements led to the formation of fiber interlocking (as shown in Figure 5.4(b)) and agglomeration of CNC II (at higher concentrations) which subsequently caused weakening of the polymer-CNC II adhesive interaction. Presence of such interactions in the PLA/CNC II nanocomposites is due to the enhanced hydrogen bonding, as discussed before, which leads to decrease in percentage elongation at failure. Investigation of the tensile samples of PLA/CNC: I→II nanocomposites shows the presence of intense agglomeration of spherical nanoparticles (Figure 5.4(c)). The initial rice grain-like self-assembled spherical particles were absent, which may be due to debonding of the irreversible hydrogen bonded crystalline segments under high tensile loading conditions. The presence of such agglomerated spherical nanoparticles caused the strain at failure to decrease drastically at higher concentrations. It is due to presence of such agglomerated spherical nanoparticles the strain at failure decreased drastically at higher concentrations. Therefore, the three PLA/CNC nanocomposites followed different mechanisms of crack propagation, which are influenced by the morphology and degree of inter/intramolecular hydrogen bonding between CNC-polymer matrix.



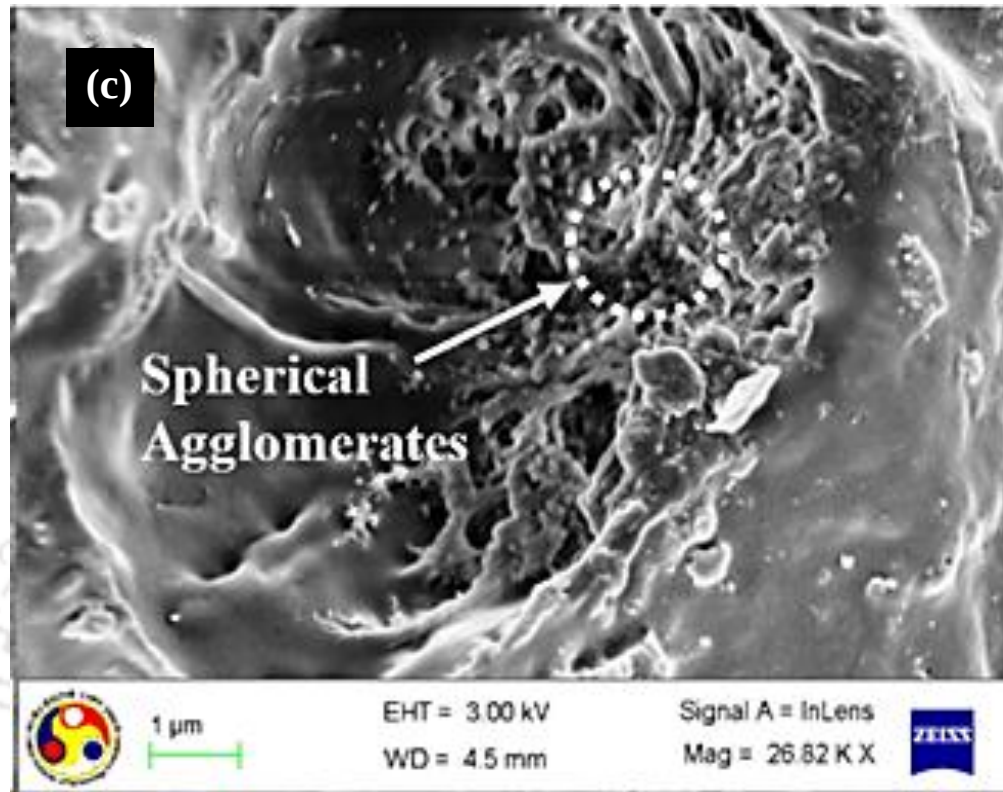


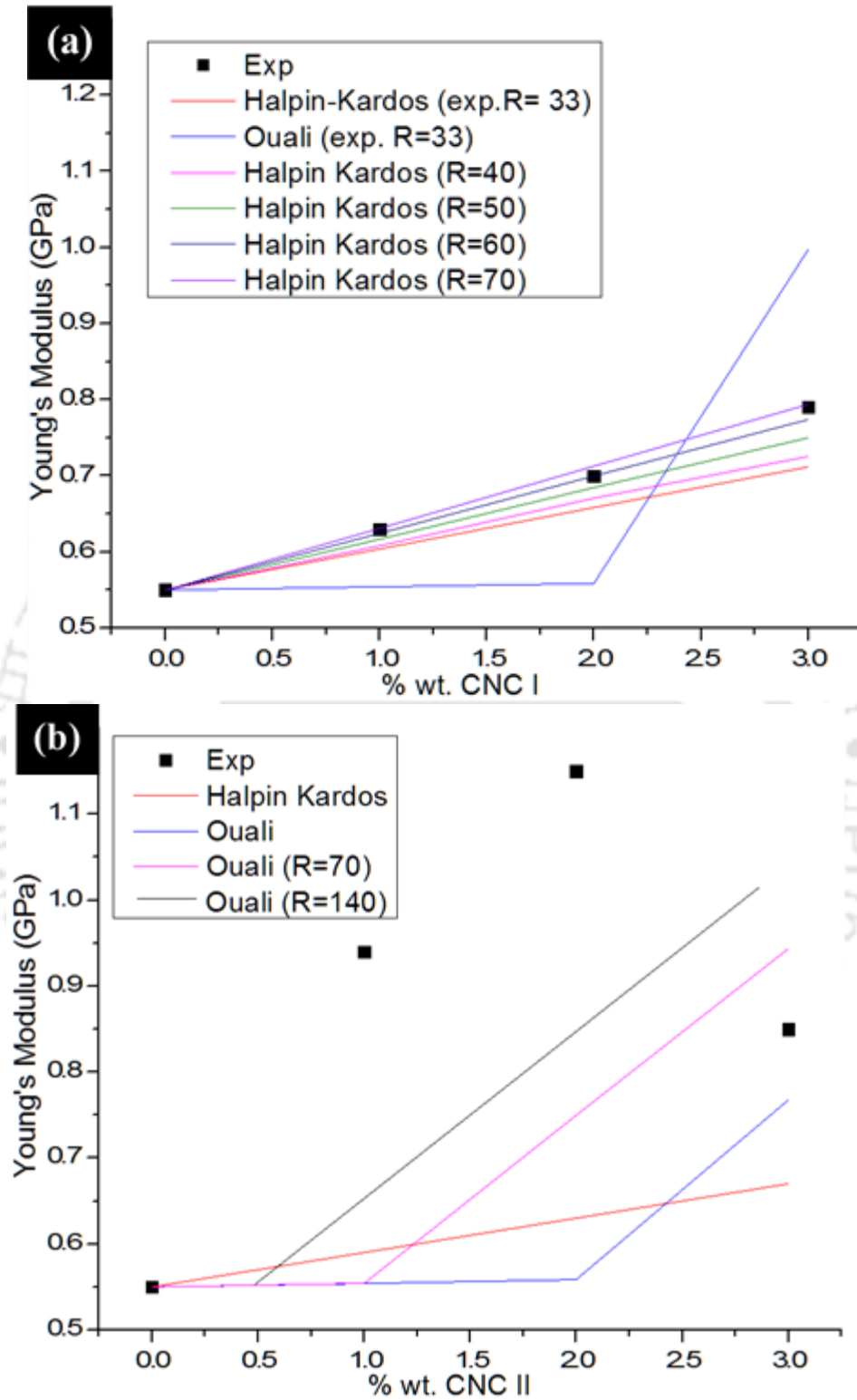
Figure 5.4: FE-SEM images of the fractures surface of (a) PLA/CNC I, (b) PLA/CNC II and (c) PLA/ CNC: I→II after tensile tests.

- **Mechanical Property Modelling of Polymorphic CNC based PLA nanocomposite**

Figure 5.5, shows the comparison of the experimental ($E_{c \text{ exp}}$) and theoretical elastic modulus of PLA composites based on the Halpin-Kardos, Ouali and Cox–Krenchel models for the three different CNC polymorphs. Aspect ratio for the CNC polymorphs using FESEM and AFM micrographs are found to be ~ 33 , ~ 25 and ~ 6 for CNC I, CNC II and CNC:I→II respectively. CNC loading for the formation of percolation network in the PLA matrix, calculated using Ouali model, are found to be 3.3, 4.3 and 17.9 wt. % for CNC I, CNC II and CNC: I→II respectively. For CNC I, the Halpin-Kardos model showed good agreement with the experimental modulus; e.g., $E_{c \text{ exp}} = 0.63$ and $E_{c \text{ HK}} = 0.60$ for 1% CNC loading at $P = 33$ (where P is the aspect ratio). Further, the theoretical prediction of elastic modulus was

examined for different aspect ratios, $P = 40, 50, 60$ and 70 . Halpin-Kardos model gave best fit with the experimental elastic modulus at aspect ratios of 60 and 70 respectively, as shown in Figure 5.5(a). This is probably due to the partial alignment of agglomerated CNCs in the hydrophobic PLA matrix, which subsequently leads to the increase in the effective aspect ratio. Due to the presence of such agglomerations, the effective aspect ratio (P_{eff}) of the CNCs in the PLA matrix was higher than that predicted from the FESEM and AFM micrographs for CNCs dispersed in solution. Similar phenomenon of increase in aspect ratio due to aggregations was observed by Khoshkhava et al. [361] for freeze dried CNCs in the polypropylene matrix. However, Halpin-Kardos model could not predict the $E_{c \text{ exp}}$ of the PLA/CNC II and PLA/CNC: I→II nanocomposites (Figures 5.5(b) and (c)). As discussed earlier, CNC II and CNC: I→II have higher intra- and intermolecular hydrogen bonded interactions; however, such filler-filler interaction is not taken into consideration in the Halpin-Kardos model. For the case of CNC II, $E_{c \text{ exp}}$ shows good agreement with the modulus obtained from Cox–Krenchel model. It is probably because Cox–Krenchel model considers the perfect elastic-like adhesion between the fiber and polymer, which possibly is a better representation of the more extensive interaction between the CNC II and the PLA matrix through intermolecular hydrogen bonding. Therefore, such composites show improved dispersion of CNC II resulting in better transfer of the tensile modulus of CNC II to the PLA matrix, thus leading to higher composite modulus [362]. Although the elastic modulus of CNC II considered for theoretical calculation was 88 GPa which is lower than that of CNC I, the experimentally calculated elastic modulus for PLA/CNC II composites was higher (~ 0.6 GPa for PLA/CNC I and ~ 1.1 GPa for PLA/CNC II at 2 wt. % CNC loading) [363]. However, Ouali model under predicts the $E_{c \text{ exp}}$ presumably because it does not take into account the intermolecular hydrogen bonding prevalent in CNC II

which leads to formation of stable percolating network. Presence of such network enhances the modulus of nanocomposites significantly, which could be predicted from theoretical modelling. In case of PLA/CNC: I→II nanocomposites, Ouali model closely predicted the trend of the $E_{c_{exp}}$ Values, probably due to lesser degree of hydrogen bonding prevalent in its networks. For lower loadings of CNC: I→II, Ouali model predicts well at aspect ratio of $P = 140$, whereas at higher loadings, aspect ratio of $P = 70$ better matches the experimental results (Figure 5.5(c)). This is due to the combined effect of two phenomena: (i) aggregation of CNC: I→II in polymer matrix, and (ii) strength of percolating network formed at lower loadings, which depends on the degree of hydrogen bonding. In summary, the morphology and aspect ratio of the CNC polymorphs and their degree of hydrogen bonding interaction with PLA matrix significantly affected resulting nanocomposite modulus. Moreover, Halpin Kardos, Cox–Krenchel and Ouali model closely predicted the $E_{c_{exp}}$ values of PLA/CNC I, PLA/CNC II and PLA/CNC: I→II nanocomposites respectively.



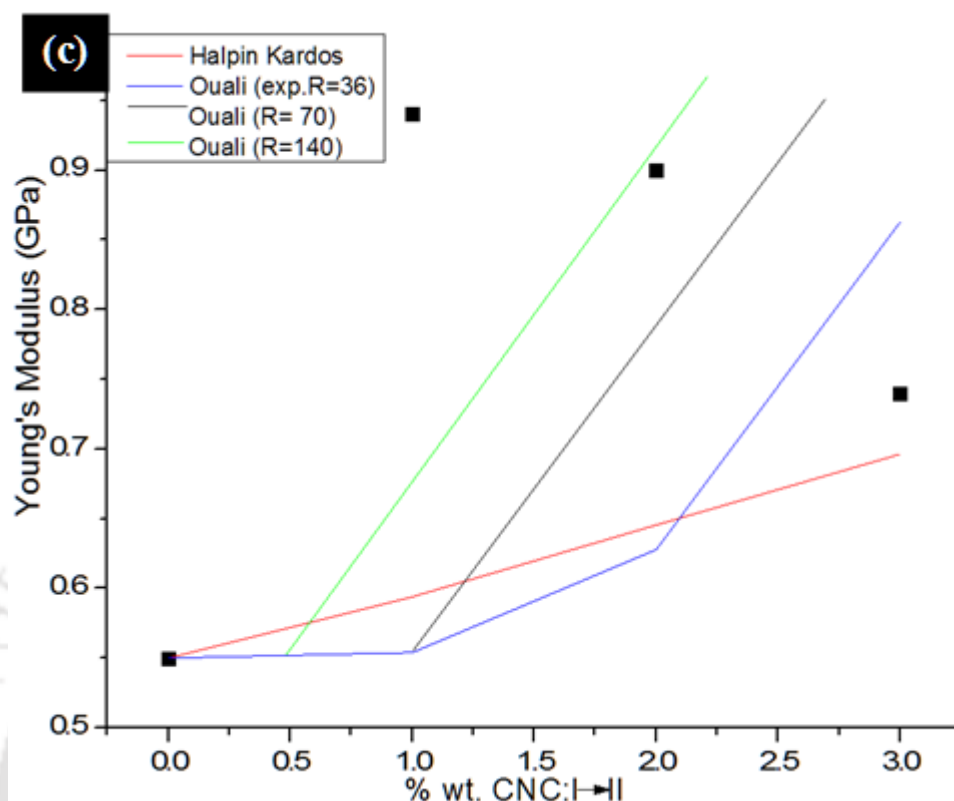


Figure 5.5: Comparison of the experimentally predicted and theoretically calculated modulus from Ouali and Halpin Kardos model for the (a) PLA/CNC I, (b) PLA/CNC II and (c) PLA/CNC: I→II nanocomposites.

- **Effect of CNC polymorphs on Thermal Properties of PLA nanocomposite**

The three CNCs with different cellulose polymorphs were used for the fabrication of PLA-based nanocomposites and their thermal properties were evaluated as shown in Figure 5.2(c). Up to the processing temperature of PLA (~200 °C), all the three PLA/CNC nanocomposites show high thermal stability with maximum reduction in weight by ~5%. This loss of weight is possibly due to removal of trapped solvent and moisture present in the PLA composite films. However, the onset degradation temperatures varied depending on the CNC polymorph, with values of 310, 330 and 280 °C for CNC I, CNC II and CNC: I→II-based PLA nanocomposites (Figure 5.2(c)). The DTG peak temperatures at which maximum degradation occurred for

PLA-CNC I, CNC II and CNC: I→II composites are 352, 358 and 349 °C respectively. Though CNC: I→II has higher thermal stability compared to CNC I, it enhances the degradation rate of PLA. Therefore, the thermal stability, degradation rate and processing temperature of biopolymers can be tuned by appropriately selecting from among different CNC polymorphs.

- **Effect of CNC polymorphs on Barrier Properties of PLA nanocomposite**

Water vapor permeability (WVP) and oxygen permeability (OP) studies were carried out to determine the effect of the three CNC polymorphs on the barrier properties of the PLA/CNC nanocomposite films. The WVP value of the neat PLA film matches well with the reported values in the literature [364]. PLA/CNC polymorphic films showed significant drop in the WVP values in all the nanocomposites, with highest reduction observed in PLA/CNC II at 3 wt. % loading (Table 5.1). On comparing the three different CNC polymorphs, CNC II and CNC:I→II show significantly higher reduction in WVP compared to CNC I. At 1 wt. % loading of CNC I, the WVP decreases by ~38%, probably due to the improvement in polymer crystallinity and dispersion of CNCs at lower loadings in PHB matrix. However, at higher loadings the reduction in WVP was not significant, probably due to the agglomeration of the CNC I at higher loadings. Incorporation of CNC II reduces the WVP by ~31% at lower loadings (~1 wt. %) and the percentage reduction interestingly improves to ~51% at higher loading (~3 wt. %). This is because of entangled morphology of the ribbon-like CNC II coupled with its enhanced dispersion within the polymeric matrix due to higher degree of hydrogen bonding. CNC:I→II significantly reduces the WVP by ~40% even at low loading fraction (~1 wt. %) and by ~48% at higher loadings (~3 wt. %) due to the hornification process. It was also observed that rewetting of such structures did not cause the CNCs to revert to the original spherical aggregate-like morphology, with CNC: I→II behaving more like real fibers [305].

Because of such fiber-like behavior, CNC: I→II probably forms strong hydrogen bonded network with the polymer matrix, thereby hindering the permeation of water molecules. Formation of hydrogen bonded network in CNC II and CNC:I→II with such irreversible structures (in the presence of water) probably decreases the number of active hydrophilic sites for polymer-water interactions. Therefore, CNC II and CNC:I→II show greater reduction in WVP even at higher loadings, compared to CNC I in which presence of water molecules disrupts the formation of such network.

Table 5.1: Water vapor permeability coefficient and % reduction in WVTR for the different PLA/CNC nanocomposites.

Sample	Water Vapor Permeability (WVP) (g.mm/m ² .day.kpa)	% reduction in WVP
Neat PLA	1.23	-
PLA/CNC I (1%)	0.76	38.0
PLA/CNC I (2%)	0.86	30.4
PLA/CNC I (3%)	0.86	30.4
PLA/CNC II (1%)	0.85	31.2
PLA/CNC II (2%)	0.71	42.5
PLA/CNC II (3%)	0.60	51.4
PLA/CNC: I→II (1%)	0.75	39.3
PLA/CNC: I→II (2%)	0.69	44.1
PLA/CNC: I→II (3%)	0.64	48.2

Table 5.2, summarizes the oxygen permeability (P) and percentage reduction of OP for neat PLA and PLA/CNCs under various loadings. The permeability coefficient and oxygen permeability of neat PLA are found to be in agreement with values reported in literature [171]. PLA-based CNC nanocomposites showed reduction in the OP for all the films; however, the optimum concentrations varied significantly with the type of CNC polymorph. For CNC I, the optimized condition was 1 wt% loading at which the OP reduced by ~69%. Similarly as in WVP studies, at higher loadings the reduction in OP did not improve because of the

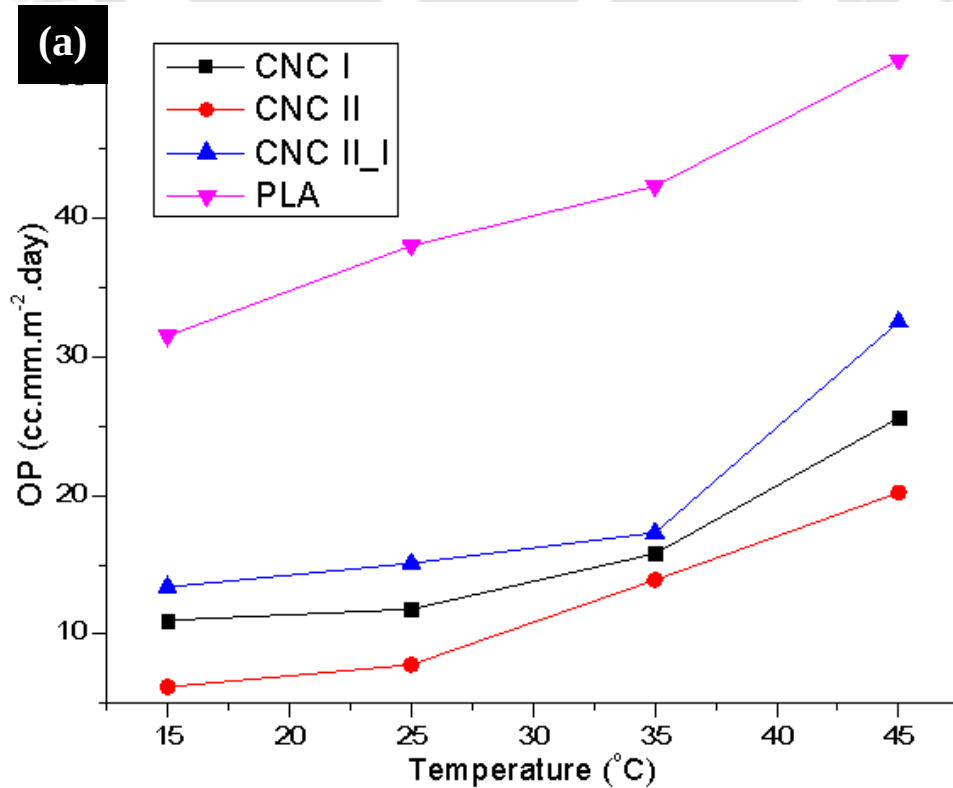
agglomeration of CNC I. Presence of such agglomerates creates interphases due to the difference in polarity of hydrophilic CNCs and hydrophobic PLA, thereby creating preferential pathway for oxygen molecule diffusion. However, presence of such interfacial regions is hindered when nanofillers such as CNCs form a network like structure in the polymer matrix due to hydrogen bonding, as is the case with CNC II and CNC:I→II. CNC II showed a 64% reduction in OP at 1 wt% loading and ~80% reduction at 3 wt% loading. As discussed earlier, because of favorable interaction and increase in number of sites for hydrogen bonding in CNC II, the polymer probably is partially immobilized on the CNC surface, thereby creating a smooth interface even at higher loadings. CNC:I→II showed less reduction in permeability compared to CNC I and CNC II, with maximum % reduction of ~63% at 2 wt. % loading. This is probably due to the presence of inter-spacing between the self-assembled spherical aggregates which have formed into fibrillar structures. Presence of such interparticle spacings of even few nanometers may lead to the permeation of the small oxygen molecules, thereby increasing the permeability.

The change in OP of PLA based CNC nanocomposites has been studied over a range of temperatures to better understand the effect of CNC polymorphs on polymer chain dynamics. Figure 5.6(a) shows the comparison of OP for neat PLA and PLA/CNC polymorph films at 1 wt. % loadings over the temperature range of 15-45°C. Among the CNC polymorphs, CNC II has better capability to restrict polymer chain motion. This is probably due to the presence of stable hydrogen bonded percolated network in nanocomposites with CNC II in the temperature range of 15-45°C. However, such behavior was absent in CNC: I→II, probably due to the presence of interparticle spacing among the spherical aggregates, which didn't form stable percolated network in the higher temperature range. Further, to understand the effect of

CNC polymorphs on the gas transport properties of biopolymer such as PLA, an attempt was made to fit the Arrhenius equation to the predicted OP values. Figure 5.6(b), shows the Arrhenius plot of PLA/CNC nanocomposites filled with CNC I, CNC II and CNC:I→II polymorphs at 1wt. % loadings. The activation energies calculated are 15.1, 21.4, 29.9 and 19.4 kJ/mol for neat PLA and PLA/CNC nanocomposites of CNC I, CNC II, CNC: I→II respectively. As discussed in previous section, probably due to the presence of hydrogen-bonded networks in PLA/CNC II nanocomposites, oxygen molecules have to overcome higher barrier potential (29.9 kJ/mol) compared to that in the neat PLA and the other two nanocomposites. The activation energy values could be used as a measure of predicting the strength or stability of the percolation network as discussed in mechanical modelling [349]. Thus, the hydrogen-bonded percolation network was most stable in CNC II followed by CNC I and CNC : I→II over the temperature range studied. The variable morphology, degree of intra- and intermolecular hydrogen bonding, aspect ratio and extend of dispersion in the polymer matrix of the CNCs strongly affect the barrier properties of polymer. From the above discussion, it can be concluded that the barrier properties of PLA could be tuned by incorporating different CNC polymorphs. Recent studies have shown that incorporation of inorganic fillers such as clays reduces the OP and WVP values of PLA by 62% and 41% respectively [365]. The PLA/CNC nanocomposites considered in the present work show similar barrier properties, and can be used for food packaging applications because of their non-toxicity and bio-based origin. Therefore, this study on CNC polymorphs will help in designing new CNC-based polymer nanocomposites with tunable barrier properties for food packaging applications.

Table 5.2: Oxygen permeability coefficient (OP), oxygen permeability (P) and percentage reduction of OP for neat PLA and PLA/CNC nanocomposites.

Sample	Permeability Coefficient (OP) (cc.mm.m ⁻² .day)	Permeability (cc.mm.m ⁻² .day.kpa) × 10 ⁻²	% reduction in OP
Neat PLA	38.0	19	-
PLA/CNC I (1%)	11.8	5.9	68.9
PLA/CNC I (2%)	13.7	6.8	63.9
PLA/CNC I (3%)	14.0	7.0	63.1
PLA/CNC II (1%)	13.8	6.9	63.6
PLA/CNC II (2%)	9.8	4.9	74.2
PLA/CNC II (3%)	7.8	3.9	79.4
PLA/CNC: I→II (1%)	15.1	7.5	60.2
PLA/CNC: I→II (2%)	14.2	7.1	62.6
PLA/CNC: I→II (3%)	14.5	7.2	61.8



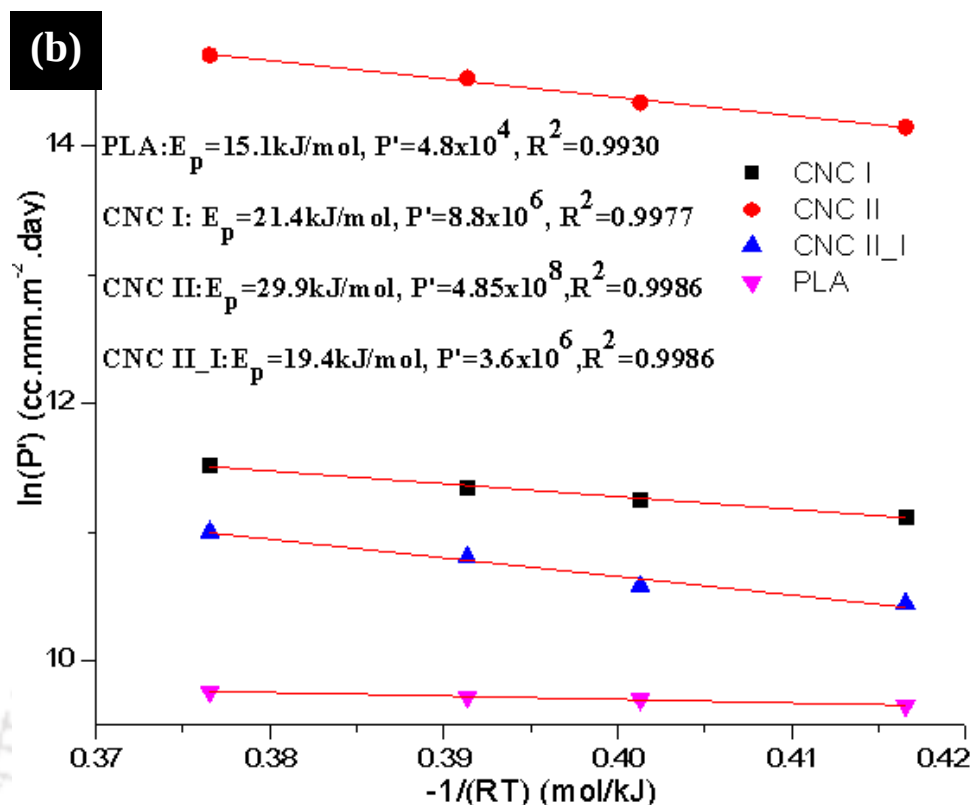


Figure 5.6: (a) Comparison of the oxygen permeability (OP) for PLA/CNC I, PLA/CNC II and PLA/CNC:I→II at 1 wt. % loadings. (b) Arrhenius plot of the temperature dependence of oxygen permeation (OP) in PLA/CNC I, PLA/CNC II and PLA/CNC:I→II polymorphic composites.

5.2.2 Acid-hydrolyzed Cellulose Nanocrystals based PLA Nanocomposites Films

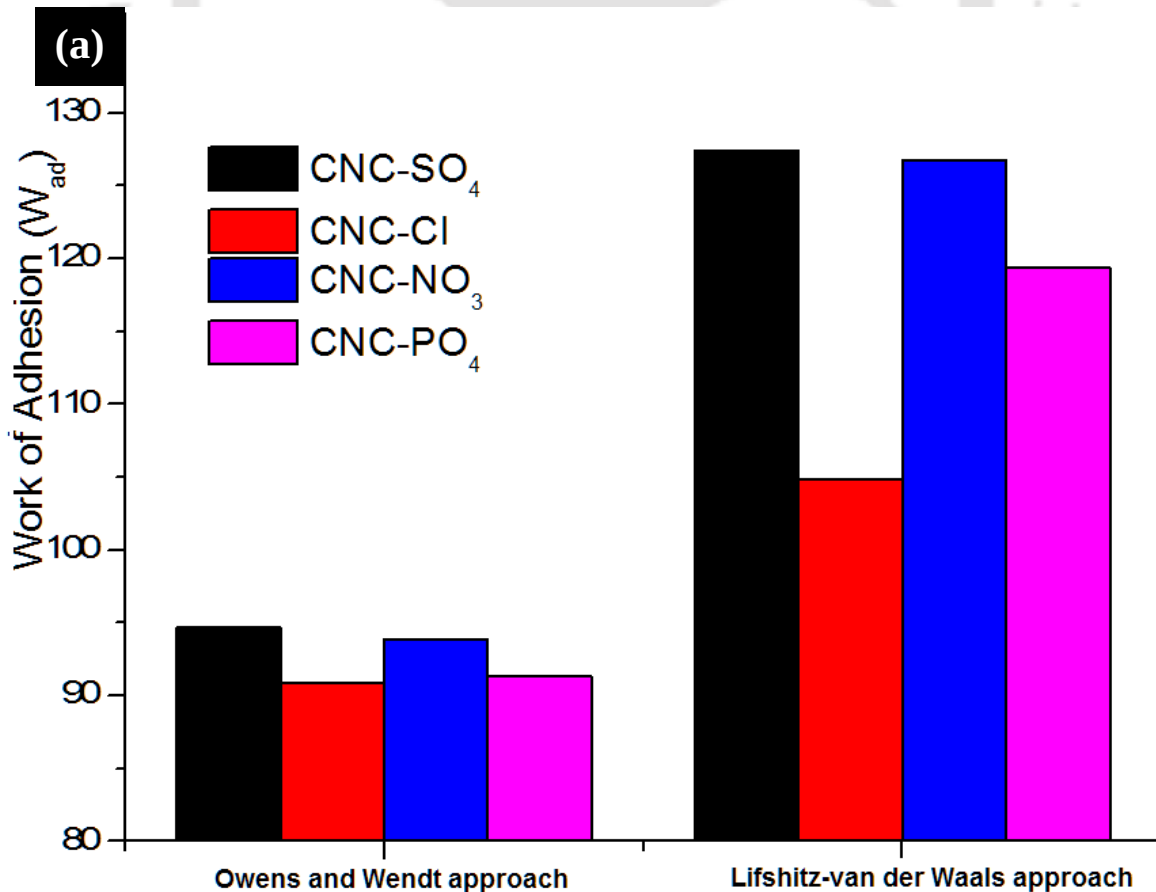
The four different acid-derived CNCs utilizing sulphuric, hydrochloric, phosphoric and nitric acids as hydrolysing agents are fabricated from Whatmann filter paper as cellulosic source (as discussed in chapter 3, section 3.2.2). The morphological analysis shows distinct variation with needle-like, small rod-like, rice grain-like and thin fibrillar structures for CNCs fabricated using sulphuric, hydrochloric, phosphoric and nitric acids as hydrolysing agents respectively. The hydroxyl groups of the CNCs undergo various degrees of substitution as confirmed from elemental analysis. The presence of 1.2 wt% SO_4 , 0.3 wt% Cl , 2.8 wt% PO_4

and 0.1 wt% NO_3 functional groups on the CNCs derived from sulphuric, hydrochloric, phosphoric and nitric acids respectively, significantly alters the surface behavior of the acid- derived CNCs. Even though all the CNCs maintain cellulose I crystal structure, significant variations occur in percentage crystallinity and crystallite dimensions, which is expected to affect the crystallization behaviour of prepared nanocomposites. The substituted anionic moieties in CNCs change its interfacial interaction with the PLA matrix, thereby altering the dispersion characteristics, thermal and structural properties, as discussed in subsequent sections.

- **Effect of Acid-derived CNCs on Dispersion Characteristics and Structural Properties**

Incorporation of different chemical moieties on the CNC surface during acid hydrolysis is expected to significantly alter its dispersion behavior in the polymer matrix. The extent of dispersion would depend on the interfacial tension between the acid-derived CNCs and the PLA chains, which has been evaluated using the thermodynamic parameter, work of adhesion (W_{ad}) [312]. The W_{ad} values decreased with decrease in the polarity of the acid-derived CNCs, which is probably due to reduction in the interfacial surface tension with the polymer. The decrease in free energy between the CNCs and PLA matrix would lead to better interfacial adhesion, and with the application of the shear forces during extrusion process, would lead to improved dispersion. Both CNC- PO_4 and CNC-Cl show comparatively lower W_{ad} values (by Owens and Wendt approach) suggesting that fabrication of nanocomposites with CNCs derived from these acids would lead to better interfacial adhesion with PLA matrix (Figure 5.7(a)). However, CNC- SO_4 and CNC- NO_3 with higher W_{ad} values would probably lead to agglomeration due to poor interfacial tension with the PLA matrix. Therefore, the thermodynamic parameter W_{ad} can be used as an effective tool to predict the interfacial surface

energy and the dispersion of CNCs in polymeric matrix. Similar trend are observed in the W_{ad} values evaluated using the Lifshitz-van der Waals/acid-base approach, which takes into consideration the hydrogen-bonded interactions between the CNCs and PLA matrix. In this case, CNC-PO₄ shows slightly higher W_{ad} values, probably due to the enhanced hydrogen bonding with PLA compared to CNC-Cl, which may lead to improved mechanical reinforcements. Even though CNC-Cl has lower W_{ad} values, due to the absence of the surface charges on the chlorine functional moieties, CNC-Cl tends to agglomerate in PLA nanocomposites thereby leading to deterioration in its properties. Although W_{ad} values can act as an effective tool to predict the degree of dispersion of CNCs into the polymer matrix, the dispersion of CNCs in polymeric matrix would depend upon several factors such as the processing technique followed, microstructural properties of the polymer, thermal stability and chemical functionalities of CNCs, which are discussed in the subsequent sections.



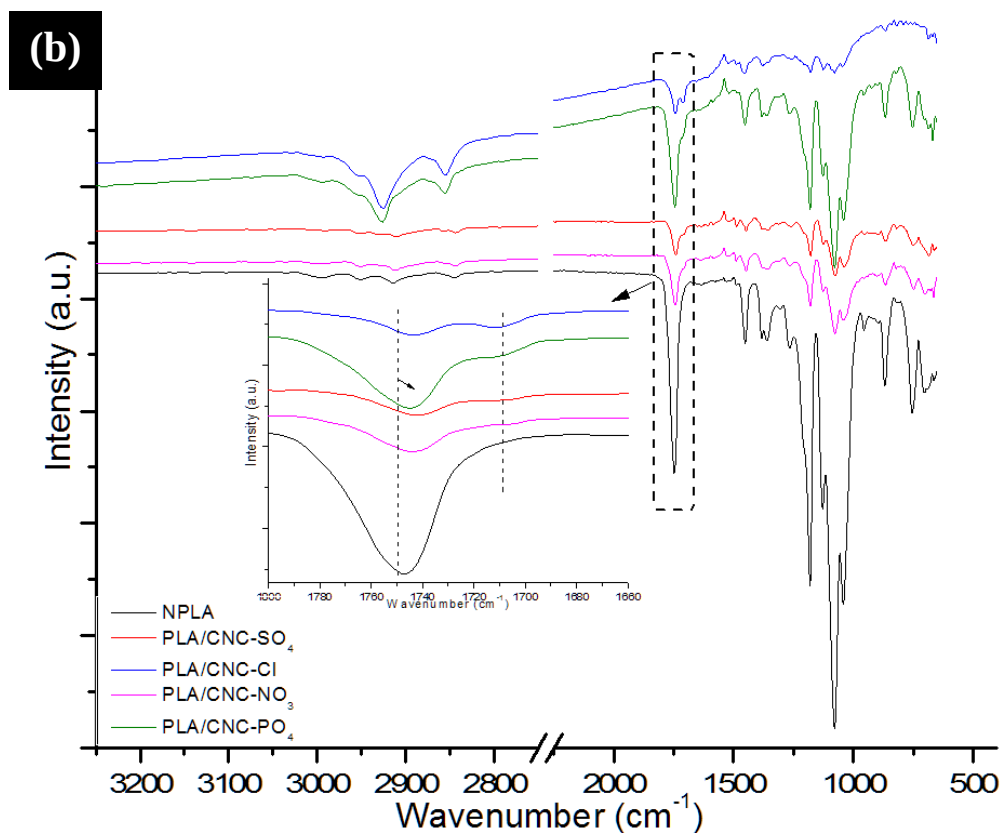


Figure 5.7: (a) Work of adhesion for the various acid-derived CNCs measured using the Owens and Wendt approach and Lifshitz-van der Waals/acid-base approach for their interfacial interaction with the PLA system, and (b) FTIR spectra of the PLA/CNC nanocomposites containing various acid-derived CNCs (inset: FTIR spectra for the carbonyl peaks of PLA in the range of 1800–1700 cm^{-1}).

The chemical structure analysis of PLA/CNC nanocomposite films are carried out to understand the possible interaction of different acid-derived CNCs with the PLA matrix (Figure 5.7(b)). FTIR spectra for the neat PLA films show characteristic peaks at 1746, 1452, 1358, 1180, 1128, 1076, 1042 and 954 cm^{-1} which correspond to the C=O stretching, CH_3 asymmetric and symmetric vibrations, C–O–C asymmetric stretching, C–OH side group vibrations, C–O vibrations, –CH stretching and C–C vibrations respectively [17]. The peaks at

2854 and 2894 cm^{-1} represent the asymmetric and symmetric- CH_2 vibrations of PLA whose intensities increased in the nanocomposites due to the presence of-CH groups in CNCs. Incorporation of the different acid-derived CNCs led to shift in carbonyl peak, C-O-C stretching and the C-OH side group vibrations of PLA which suggests the presence of possible hydrogen bond-based interactions. However, the degree of hydrogen-bonded interactions, evaluated using the hydrogen bonded fraction ($F_{\text{H-CO}}$) parameter, was found to vary significantly with the different acid-derived CNCs. The carbonyl peak for the PLA at 1746 cm^{-1} shifted to 1744 cm^{-1} for the PLA/CNC- NO_3 and PLA/CNC-Cl respectively and to 1742 cm^{-1} for PLA/CNC- SO_4 and PLA/CNC- PO_4 respectively. Further, the C-O-C stretching vibrations and free hydroxyl (C-OH side groups) of PLA at 1180 and 1128 cm^{-1} shifted to 1176 and 1126 cm^{-1} in case of PLA/CNC- SO_4 and PLA/CNC- PO_4 respectively. The $F_{\text{H-CO}}$ parameter is found to be ~ 0.40 for PLA, which increased to 0.45 for PLA/CNC- NO_3 , 0.47 for PLA/CNC-Cl, 0.48 for PLA/CNC- SO_4 and 0.53 for PLA/CNC- PO_4 . The significant shift in the peaks and high $F_{\text{H-CO}}$ values for the PLA/CNC- PO_4 (followed by PLA/CNC- SO_4) suggests the presence of enhanced intermolecular hydrogen bonding with the PLA matrix compared to the rest of the acid-derived CNCs. Similar observations for the enhanced compatibility between the phosphate groups present in hydroxyapatite and PLA matrix was also observed by Kasuga et al. [366] leading to its improved dispersion. Moreover, assessment of the surface properties of CNC- PO_4 from the γ_s^{P} and W_{ad} parameters, as discussed in previous section 3.2.2, suggests their improved interaction with the hydrophobic polymeric systems. Thus, it can be concluded that the CNC- PO_4 undergoes enhanced dispersion compared to the rest of acid derived CNCs, possibly due to the increased compatibility and presence of interfacial hydrogen bonding with PLA matrix. On the other hand, surface properties of CNC-Cl and CNC- NO_3 are

found to be more hydrophilic in nature and the absence of substantial hydrogen bonding with PLA leads to their agglomeration. Although PLA/CNC-Cl systems have higher W_{ad} values, the agglomeration of CNC-Cl has been reported [102] which is due to the absence of negative surface charges that are instrumental in introducing repulsive behavior in CNC rods and increasing their stability. Therefore, it could be inferred that the different functional moieties introduced during acid hydrolysis on the CNCs play a significant role in the degree of inter/intramolecular hydrogen bonding, thereby affecting their dispersion within the PLA matrix.

Figure 5.8 (inset) shows the physical appearance of the extruded strips of PLA/CNC nanocomposites using different acid-derived CNCs (under higher CNC loading of ~3 wt. %) at similar processing conditions. PLA/CNC-SO₄ films shows the presence of light brownish coloration with black dots which was however found to be absent in rest of the nanocomposites. The black coloration is probably due to the lower thermal stability of CNC-SO₄ which undergoes degradation during extrusion process. Compared to other acid-derived CNCs, CNC-SO₄ has lower T_{onset} ~220°C, as the PLA nanocomposite processing was carried out at ~180°C inside a co-rotating twin-screw extruder, it underwent thermal-cum-shear induced degradation. The molecular weight analysis further revealed that the sulphate groups on the CNCs catalyzed the degradation of the PLA chains which led to significant decrease in the weight average (M_w) and number average (M_n) molecular weights (by 28% and 23% respectively) compared to neat PLA. The PLA/CNC nanocomposites prepared from the other acid-derived CNCs did not show any such coloration and led to the formation of transparent films at lower CNC loadings and whitish coloration at higher CNC loadings. The high thermal stability of the CNC-Cl, CNC-PO₄ and CNC-NO₃ with T_{onset} in the range of

~270–350°C prevented the degradation of the CNCs and the PLA significantly. However, gel permeation chromatography (GPC) studies showed that PLA/CNC-Cl undergoes higher reduction in M_w by ~20% compared to only ~15% and 17% in the case of PLA/CNC-PO₄ and PLA/CNC-NO₃ respectively. This suggests that along with the higher thermal stability of the nitrate and phosphate chemical moieties, they do not have any catalyzing effect on the depolymerization/degradation of PLA chains during the extrusion process. Therefore, the chemical functionalities introduced during hydrolysis with different acid types have significant effect on the molecular chain characteristics, which in turn affects the properties of the fabricated nanocomposites significantly.

Morphological investigations of the PLA/CNC films fabricated using different acid- derived CNCs are carried out to understand their dispersion behavior and interfacial interaction with the PLA matrix as shown in Figure 5.8. The degree of dispersion of the freeze- dried CNCs in the nanocomposite was found to vary depending on the type of CNCs; interestingly, the CNCs were found to retain their intrinsic morphology in the PLA matrix. The needle-like morphology of CNC-SO₄ was found to be present when it was dispersed in the polymer (as shown with black arrows in Figure 5.8(a)), but due to their hydrophilic nature the CNC-SO₄ particles accumulated together in the hydrophobic PLA matrix. In case of PLA/CNC-Cl, small rod-like CNC-Cl is present (shown with black arrows in Figure 5.8(b)) but with higher degree of agglomeration (shown in black boxes) probably due to poor intermolecular hydrogen bonding with PLA as discussed in previous section 3.2.2. Interestingly, CNC-PO₄ was found to be uniformly dispersed in the hydrophobic PLA matrix with least agglomeration compared to other CNCs, which is evident from the presence of rice grain-like particle morphology in the nanocomposite (Figure 5.8(c)). Such improved dispersion in PLA/CNC-PO₄ is due to its

hydrophobic surface properties and enhanced intermolecular hydrogen bonding which also leads to smooth interface with PLA matrix. CNC-NO₃ with hydrophilic surface behavior is not compatible with the PLA matrix, and the particles are bundled into large bunches due to intermolecular hydrogen bonding. Therefore, we conclude that the surface properties of CNCs can be tuned during hydrolysis step itself, thereby avoiding the surface modification steps required to improve compatibility with the polymer matrix.

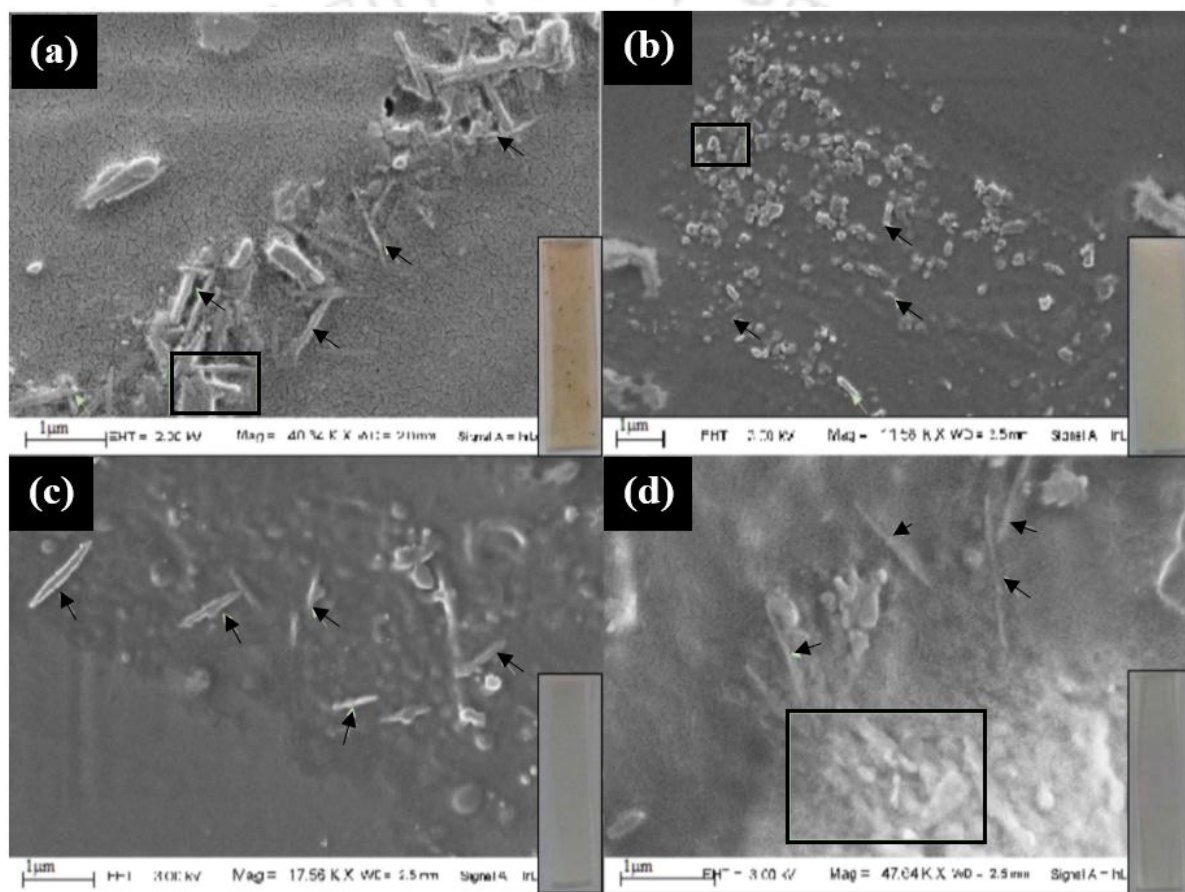


Figure 5.8: FESEM micrographs and pictorial representation of the extruded strips (inset) of the (a) PLA/CNC-SO₄, (b) PLA/CNC-Cl (c) PLA/CNC-PO₄ and (d) PLA/CNC-NO₃ nanocomposites (at 1 wt. % loadings). (With individually dispersed CNCs being marked in black arrows and agglomerated CNCs marked in black boxes).

- **Effect of Acid-derived CNCs on Mechanical Reinforcement of PLA/CNC Nanocomposites**

Thermo-mechanical properties of PLA/CNC nanocomposites fabricated using different acid-derived CNCs were evaluated to understand their influence on the storage (E') and loss modulus (E'') over a wide temperature range of 20–90 °C (Figure 5.9 (a)-(d)). The acid-derived CNCs are found to have variable aspect ratios, morphological dimensions, surface properties and degrees of hydrogen bonding which tend to affect the dispersion and mechanical behavior of the nanocomposites significantly. Figure 5.9(a) shows the storage moduli of the PLA/CNC nanocomposites filled with 1 wt% of the different acid-derived CNCs. Introduction of CNC-PO₄ to PLA shows a significant increase in the E' values by 1.75 times, compared to 1.5 and 1.4 times in the case of CNC-Cl and CNC-SO₄ respectively, with the least being ~1.01 times for CNC-NO₃. As discussed in previous section 3.2.2, the W_{ad} values predicted for the PLA/CNC-PO₄ suggested possibly better interfacial interaction and improved compatibility with the PLA matrix. Presence of such improved interactions is expected to transfer the elastic modulus of the CNCs to the polymeric matrix effectively, thereby improving the storage modulus of the nanocomposites significantly. The high aspect ratio of CNC-PO₄ compared to the other acid-derived CNCs could also be responsible for higher reinforcement efficiency at lower volume fractions, resulting in improved stress transfer from CNCs to the PLA matrix. Moreover, at higher loadings (from 1 to 3 wt%) drastic increase in the complex modulus (E_c) of nanocomposites is observed probably due to enhancement in F_{H-CO} values which suggests improved hydrogen bonding and more uniform distribution of CNC-PO₄ in the PLA matrix. Although the W_{ad} values for the PLA/CNC-Cl system are lowest, effective stress transfer to the matrix was not possible due to the absence of intermolecular hydrogen bonding with the

PLA matrix (as is evident from the FTIR studies) and the lower aspect ratio of CNC-Cl. This was also evident from the FESEM micrographs which showed higher degree of agglomerations resulting in the decrement of the E_c values of PLA/CNC- Cl nanocomposites. Both CNC-SO₄ and CNC-NO₃ have comparatively similar W_{ad} values but the difference in the E' is probably due to variation in the γ_s^p parameter (Table 3.2). CNC-NO₃ has higher γ_s^p values that suggests enhanced hydrophilicity compared to CNC-SO₄, thus leading to agglomeration in the hydrophobic PLA matrix. Therefore, the fabrication of CNCs with different acids can effectively tune the morphological dimensions, surface properties and hydrogen bonding characteristics of CNCs, thereby altering the mechanical properties of the prepared polymer nanocomposites.

To further measure the reinforcing efficiency of the different acid-derived CNCs in the PLA matrix, the filler effectiveness coefficient (C_{FE}) was calculated as,

$$C_{FE} = \left(\frac{E'_g}{E'_r} \right)_{comp} / \left(\frac{E'_g}{E'_r} \right)_{matrix} \quad (5.1)$$

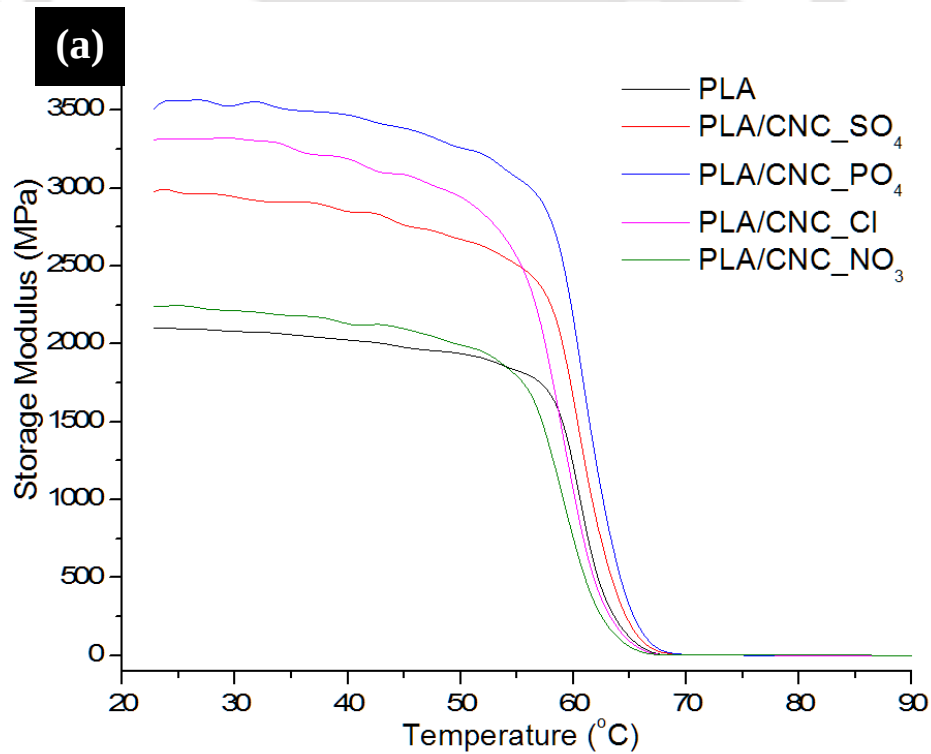
Where E'_g And E'_r Represent the storage modulus of the PLA/CNC based nanocomposites in the glassy (46°C) and rubbery (85°C) region. The nanocomposites with lower C_{FE} values are expected to have highest reinforcement efficiency from the dispersed nanofillers [367]. Comparison of the C_{FE} values (at constant ~1 wt. % CNC loading) suggests that PLA/CNC- PO₄ has highest reinforcing efficiency followed by PLA/CNC-SO₄, PLA/CNC-Cl and PLA/CNC-NO₃, consistent with the storage modulus studies (Table 5.3). At higher loadings for the respective acid-derived CNCs, drastic rise in the C_{FE} values are observed which suggests poor reinforcing behavior probably due to agglomeration of the hydrophilic

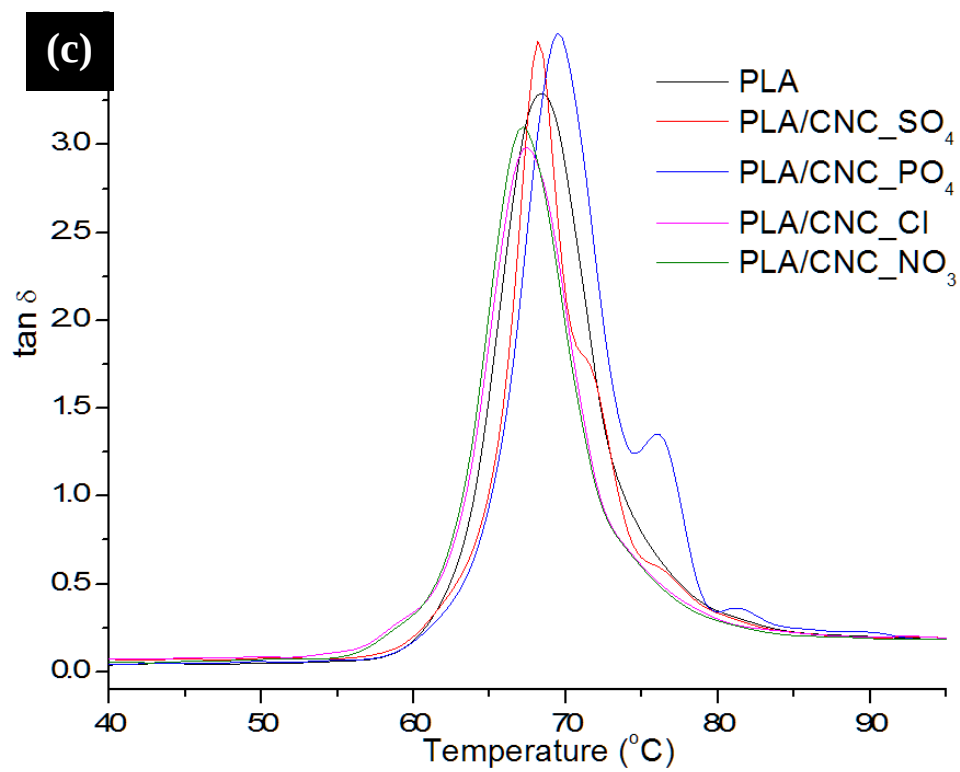
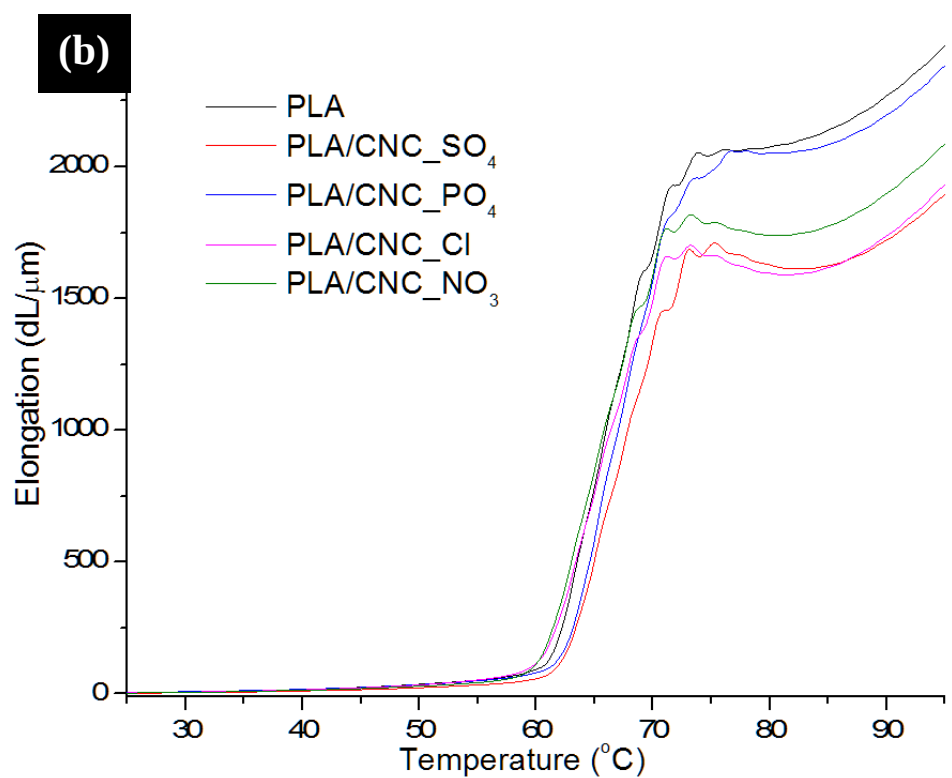
CNCs in hydrophobic PLA matrix. Compared to the other acid derived CNCs, CNC-PO₄ shows exceptionally low C_{FE} values even at higher loadings. This is probably due to several reasons such as enhanced intermolecular hydrogen bonding, higher aspect ratio and improved surface characteristics, which act synergistically in enhancing the composite modulus of the PLA/CNC-PO₄ nanocomposites.

Table 5.3: Comparison of the thermo-mechanical properties of the PLA/CNC nanocomposites fabricated with various acid derived CNCs at different loading.

Sample	Storage Modulus E'(MPa) @46°C	Glass Transition Temp. (T _g) (°C)	Storage Modulus E'(MPa) @85°C	C _{FE}
Neat PLA	2204.0	68.2	1.4	-
PLA/CNC-SO ₄ -1p	2746.2	68.7	2.2	0.88
PLA/CNC-SO ₄ -2p	3180.3	69.2	2.2	1.02
PLA/CNC-SO ₄ -3p	3355.6	69.5	2.0	1.15
PLA/CNC-Cl-1p	3052.6	67.2	2.7	0.80
PLA/CNC-Cl-2p	2235.0	67.3	1.5	1.00
PLA/CNC-Cl-3p	3045.5	69.5	2.5	0.84
PLA/CNC-NO ₃ -1p	2071.1	67.1	1.4	0.99
PLA/CNC-NO ₃ -2p	3026.6	69.1	2.1	1.01
PLA/CNC-NO ₃ -3p	3297.6	69.2	2.1	1.17
PLA/CNC-PO ₄ -1p	3363.4	69.5	5.0	0.59
PLA/CNC-PO ₄ -2p	3452.7	69.4	3.1	0.79
PLA/CNC-PO ₄ -3p	4156.2	70.2	3.3	0.89

The micro-mechanical Halpin–Kardos model and the Ouali model based on percolation theory are used to compare the experimentally and theoretically predicted modulus of the nanocomposites over a wide range of aspect ratio. The optimum CNC loading for the formation of the percolating network are found to be ~3%, 9%, 2.7% and 7% by weight for CNC-SO₄, CNC-Cl, CNC-PO₄ and CNC-NO₃ respectively. Above the threshold percolated concentrations of CNCs, it is known that the modulus of nanocomposites is governed by strong filler-polymer interactions which is due to the presence of intermolecular hydrogen bonding between the CNCs and PLA chains in this case. Up to ~3 wt. % loadings, complex modulus of the PLA/CNC-Cl and PLA/CNC-NO₃ are found to increase linearly with CNC loadings because of the absence of percolated CNC network. The calculated ϕ_c For PLA/CNC-Cl and PLA/CNC-NO₃ are found to be significantly higher (~9 and 7 wt. % CNC loadings) than the experimental limit (~3 wt. % CNC loadings) above which agglomerations of CNC occurs.





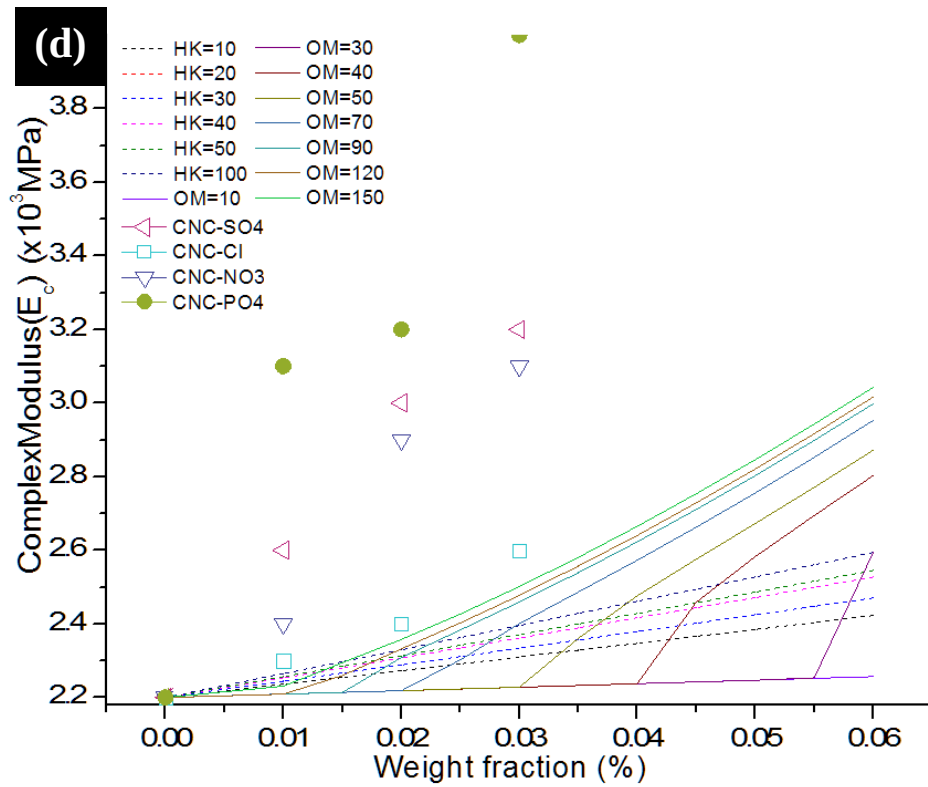


Figure 5.9: The effect of the four different acid hydrolyzed CNCs on the (a) storage modulus, (b) elongation behavior and (c) $\tan \delta$ properties of the PLA/CNC nanocomposites (with CNC loadings kept constant at 1wt. %) measured over a temperature range of 20-90°C. (d) Comparison of the experimentally predicted and theoretically calculated modulus of the PLA/CNC nanocomposites using the Halpin-Kardos (HK) and Ouali models (OM) at different aspect ratio of CNCs.

As discussed earlier, the drastic jump in the E' values at ~3 wt. % loadings is probably due to the presence of the percolating network-like structure in PLA/CNC-SO₄ and PLA/CNC-PO₄. Presence of such stable networks leads to increase in the glass transition temperature (measured from the $\tan \delta = E''/E'$ values) by ~1.3 and 2.0°C for PLA/CNC-SO₄ and PLA/CNC-PO₄ respectively. Both the models (Halpin-Kardos and Ouali) could predict the trend of experimentally calculated modulus of the PLA/CNC-Cl, PLA/CNC-NO₃ and

PLA/CNC-SO₄, (to some extent) albeit at higher aspect ratio of CNCs. This is probably because of CNC aggregations which form an interconnected structure leading to higher aspect ratio. It is also evident from the FESEM micrographs of PLA/CNC-SO₄ in which the individual CNCs get agglomerated in certain aligned direction thereby increasing the effective aspect ratio of CNCs inside PLA matrix [368]. Compared to neat PLA, the elongation behavior for all the acid derived CNC based PLA nanocomposites decreased (Figure 5.9 (b)). The decrease is more pronounced in the case of PLA/CNC-NO₃, PLA/CNC-SO₄ and PLA-CNC-Cl compared to PLA/CNC-PO₄. The improved elongation behavior of PLA/CNC-PO₄ is due to improved interfacial adhesion between the CNC-PO₄ and PLA matrix, which is absent in the other acid-derived CNC nanocomposites where higher degree of agglomeration is present. Therefore, the mechanical behavior of PLA/CNC nanocomposites can be finely tuned by selecting the hydrolyzing acids used for CNC fabrication appropriately.

- **Effect of Acid-derived CNCs on Thermal degradation Behavior of PLA/CNC Films**

The effect of different chemical functional moieties introduced onto the surface of CNCs during acid hydrolysis on the thermal properties of the fabricated PLA/CNC nanocomposites are shown in Figure 5.10. The different acid-derived CNC-based PLA nanocomposites are processed into strips through extrusion at ~180°C under identical processing conditions. Up to a processing temperature of ~300°C (applicable for most of the thermoplastics), PLA/CNC- SO₄ shows the maximum weight loss (of ~4%) compared to the rest of the nanocomposites.

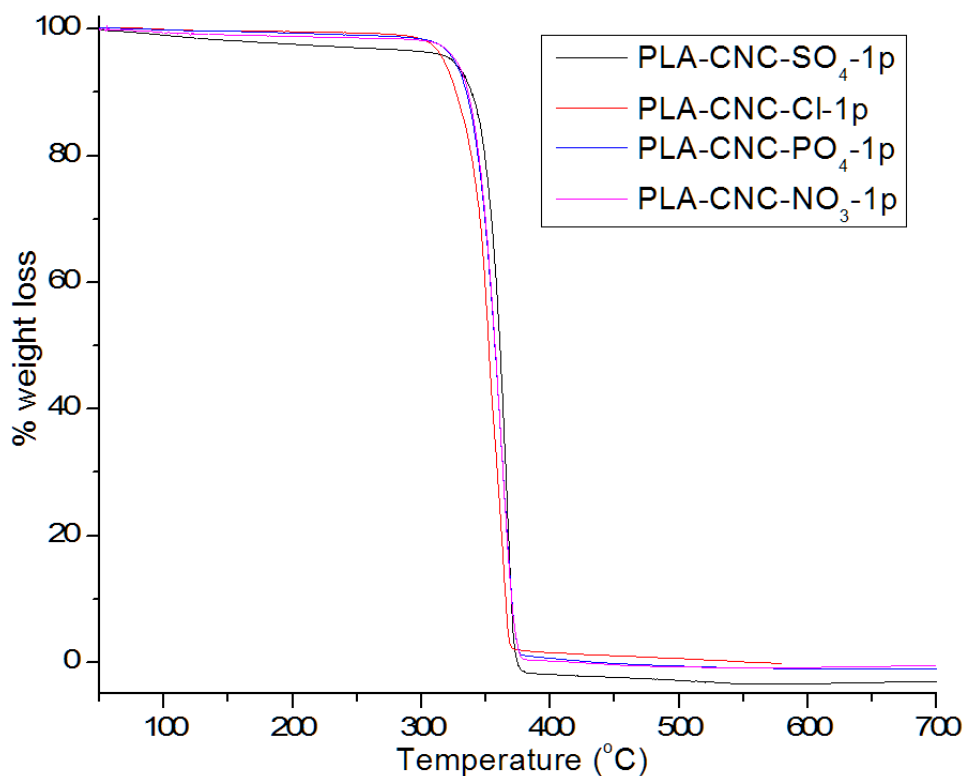


Figure 5.10: TGA profiles of the PLA/CNC nanocomposites fabricated with different acid derived CNCs at a constant loadings of 1 wt. % CNCs.

The intrinsic thermal properties of the CNCs fabricated from different acids (as discussed in previous section) were found to be integrated in the nanocomposites which following the same trend. The onset degradation temperature of the nanocomposites are ~ 320 , ~ 330 , ~ 335 and ~ 337 °C for PLA/CNC-SO₄, PLA/CNC-Cl, PLA/CNC-PO₄ and PLA/CNC-NO₃ respectively. The maximum degradation temperature (T_d) evaluated from the DTG plots also shows a similar trend of $T_d \sim 355$, ~ 362 , ~ 360 and ~ 362 °C for PLA/CNC-SO₄, PLA/CNC-Cl, PLA/CNC-PO₄ and PLA/CNC-NO₃ respectively. The residue content (at ~ 500 °C) is found to be ~ 0.4 , ~ 1.9 , ~ 1.75 and ~ 0.65 wt. % for PLA/CNC-SO₄, PLA/CNC-Cl, PLA/CNC-PO₄ and PLA/CNC-NO₃ respectively. The thermal stability of PLA/CNC nanocomposites followed the order: PLA/CNC-NO₃ > PLA/CNC-PO₄ > PLA/CNC-Cl > PLA/CNC-SO₄. Therefore, it can be

inferred that the thermal properties for the fabricated polymer-CNC nanocomposites are dependent on the inherent thermal properties of different acid-derivatized CNCs.

- **Effect of Acid-derived CNCs on Crystallization Kinetics of PLA/CNC Nanocomposites**

The variable intrinsic properties of different acid-derived CNCs (such as aspect ratio, morphology and surface characteristics) are expected to change the crystallization dynamics of fabricated nanocomposites significantly. Figure 5.11(a) shows the comparison of DSC thermograms (second heating cycles) for the PLA/CNC nanocomposites with 1 wt. % loading of different acid-derived CNCs. The melting properties of the prepared nanocomposites almost remain unaltered in comparison to the pristine PLA, with the maximum improvement in T_m by $\sim 1.7^\circ\text{C}$ only for the PLA/CNC- PO_4 nanocomposites under 3 wt. % loading. However, at higher CNC loadings (~ 3 wt. %), the formation of multiple melting peaks with the presence of peak broadening suggests the formation of imperfect crystals (during crystallization process) and possible degradation of PLA chains during processing. Incorporation of the various acid-derived CNCs shows slight improvement in the T_g values (by $\sim 2^\circ\text{C}$ only) especially at higher CNC loadings (see Table 5.4). The cold crystallization behavior, especially the enthalpy of crystallization and crystallization temperature (T_{cc}), are found to vary significantly with the type of acid-derived CNC and its loading fractions. Decrease in T_{cc} values is observed for all the PLA-based nanocomposites fabricated with different acid-hydrolyzed CNCs in comparison to neat PLA, suggesting the possible nucleating effect of CNCs [265]. The PLA/CNC-Cl nanocomposites show a significant drop in the T_{cc} values by ~ 7 and $\sim 11^\circ\text{C}$ at 1 and 3 wt. % loadings respectively. For other nanocomposites, T_{cc} did not change significantly with a decreases of only ~ 1.9 , 4 and 2°C for PLA/CNC- SO_4 , PLA/CNC- NO_3 and PLA/CNC- PO_4

nanocomposites respectively (at 1 wt. % loading). This indicates that the intrinsic characteristics of CNC- Cl such as low aspect ratio and improved compatibility within PLA matrix (as evident from the W_{ad} values) have significant effect on the crystallization behavior of the PLA, which have been investigated through isothermal crystallization kinetics studies. The percentage crystallinity (X_c) increases on incorporation of different acid-derived CNCs especially at higher loadings, with the maximum improvement by ~10% for PLA/CNC-Cl and PLA/CNC-PO₄ nanocomposites (Table 5.4). This is probably due to improved dispersion of the CNC-Cl and CNC-PO₄ with the PLA matrix as well as lower degree of thermally induced degradation during extrusion process of the nanocomposites.

Table 5.4: Crystallization properties of the PLA/CNC nanocomposites fabricated with various acid-derived CNCs at 1–3 wt% CNC loadings.

Sample	T _g (°C)	T _{cc} (°C)	ΔH _{cc} (J/g)	T _m (°C)	ΔH _a _m (J/g)	X _c (%)
PLA	60.9	128.8	53.6	168.1	-42.0	45.1
PLA/CNC_SO ₄ -1p	61.0	126.9	54.4	168.3	-43.9	47.2
PLA/CNC_SO ₄ -2p	61.5	123.7	50.4	167.7	-44.4	47.7
PLA/CNC_SO ₄ -3p	61.6	123.0	51.0	167.9	-45.7	49.1
PLA/CNC_Cl-1p	60.8	121.7	51.8	167.3	-41.0	44.1
PLA/CNC_Cl-2p	61.2	121.5	51.4	167.3	-43.7	46.9
PLA/CNC_Cl-3p	61.4	116.2	50.4	169.8	-50.4	54.2
PLA/CNC_NO ₃ -1p	60.9	124.8	52.5	167.3	-35.2	37.8
PLA/CNC_NO ₃ -2p	60.9	123.6	53.8	166.8	-37.3	40.1
PLA/CNC_NO ₃ -3p	61.2	122.3	52.5	166.9	-42.2	45.3
PLA/CNC_PO ₄ -1p	61.0	126.8	63.4	168.2	-45.1	48.4
PLA/CNC_PO ₄ -2p	61.4	126.1	54.8	167.8	-47.3	50.8
PLA/CNC_PO ₄ -3p	62.1	121.8	50.0	167.9	-51.1	54.9

Isothermal crystallization kinetics are carried out to further understand the influence of various acid-derived CNCs on their degree of nucleation behavior when dispersed into PLA matrix. Figure 5.11(b), shows the DSC thermograms for the isothermally crystallized

PLA- CNC nanocomposites at 120 °C (at a constant 1 wt% CNC loading). The introduction of CNCs in the PLA matrix decreases the peak time for crystallization with the formation of narrow sharp crystallization peaks compared to broad hump-like peaks in the case of pristine PLA. Incorporation of CNC-Cl and CNC-PO₄ significantly reduced the peak crystallization time thereby enhancing the rate of PLA crystallization. As evident from the surface properties of CNCs (i.e., the W_{ad} values), improved interaction of CNC-Cl and CNC-PO₄ with the PLA matrix led to their effective dispersion. Presence of such interactions increases the molecular mobility of PLA chains to crystallize into an ordered arrangement of crystals as well as increases the number of sites for nucleation [369]. The relative crystallinity (X_t) of the nanocomposites, which takes into account the heat released during the isothermal crystallization process during different time intervals, is calculated using eq. (5.2),

$$X_t = \frac{\int_0^t \left(\frac{dH}{dt} \right) dt}{\int_0^\infty \left(\frac{dH}{dt} \right) dt} \quad (5.2)$$

where t and H refer to the crystallization time and the amount of heat released during the crystallization process. Figure 5.11(c) shows the effect of different acid-derived CNCs on the percentage relative crystallinity of the nanocomposites at various time intervals. The sigmoidal curves represent the primary stage of crystallization viz. The nucleation and radial growth of the crystallites followed by plateau region corresponding to the secondary crystallization. The pristine PLA shows a significantly slower crystallization process compared to the PLA/CNC nanocomposites which follow the order of PLA/CNC-Cl > PLA/CNC-PO₄ > PLA/CNC-SO₄ and PLA/CNC-NO₃. This could be attributed to the improved compatibility of the PLA chains with the CNCs which reduces the time required for chain folding during the crystallization process

(under isothermal conditions) thereby leading to fast crystallization process (especially for PLA/CNC-Cl and PLA/CNC-PO₄). Due to the hydrophilic surface characteristics of CNC-SO₄ and CNC-NO₃ (higher γ_s^p values), they tend to agglomerate in the PLA matrix thereby hindering the crystallization phenomenon.

The isothermal crystallization kinetics of the PLA based CNC nanocomposites were studied through classical approach using the Avrami model, which takes into consideration the effect of relative crystallinity (X_t) with respect to time, as follows eq.(5.3),

$$(1 - X_t) = \exp(kt^n) \quad (5.3)$$

where n is the Avrami index which represents the type of nucleation and k is the growth constant which depends on the mechanism of crystallization process. These parameters are predicted from the slope and intercept of the double log plots of the Avrami eq. (5.5), $\ln(-\ln(1 - X_t))$ vs $\ln t$ (Figure 5.11(d)). The half crystallization time ($t_{1/2}$) represents the rate of crystallization process which is calculated from eq. (5.4),

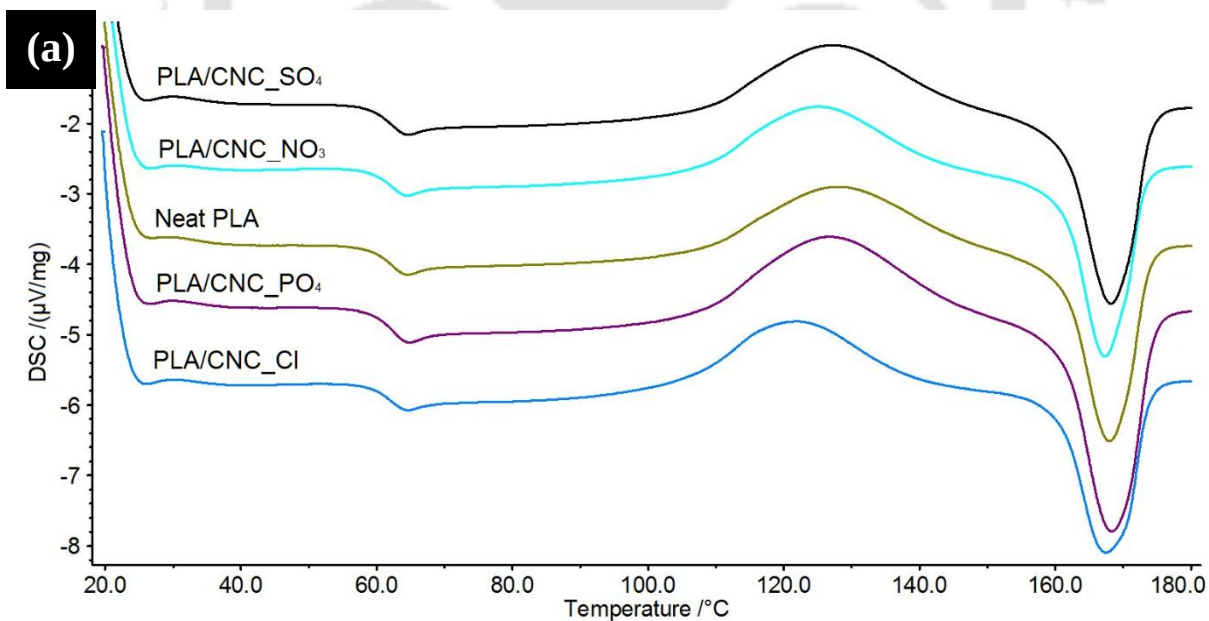
$$t_{1/2} = \left(\frac{\ln 2}{k} \right)^{1/n} \quad (5.4)$$

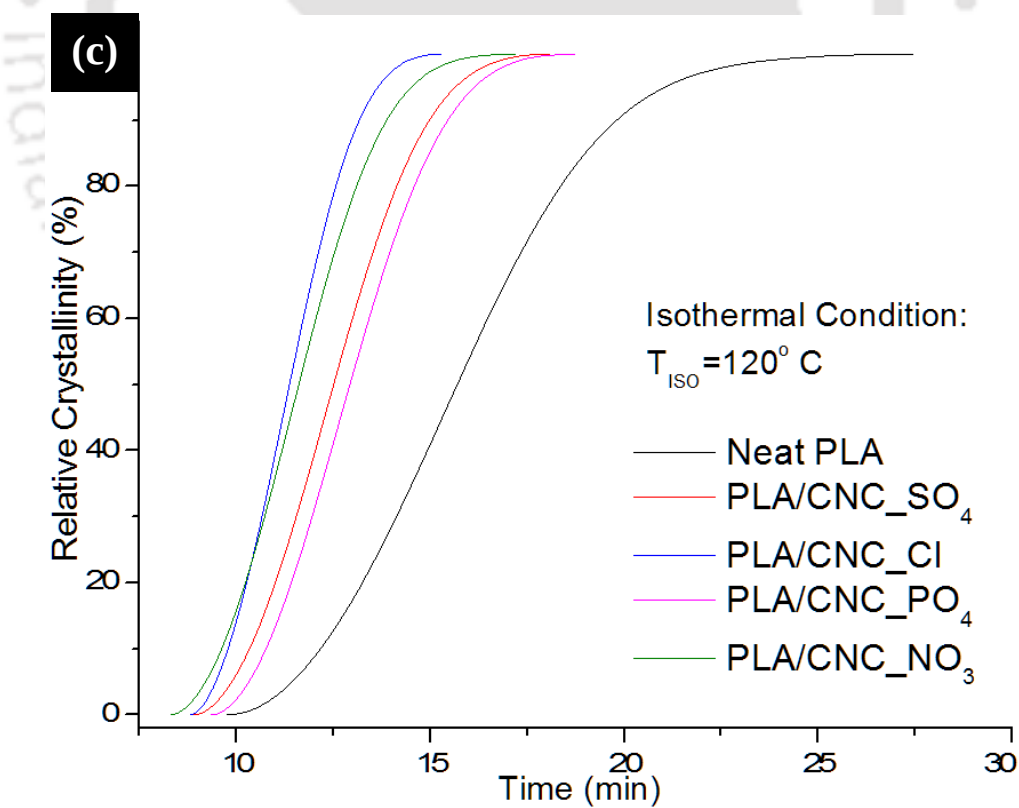
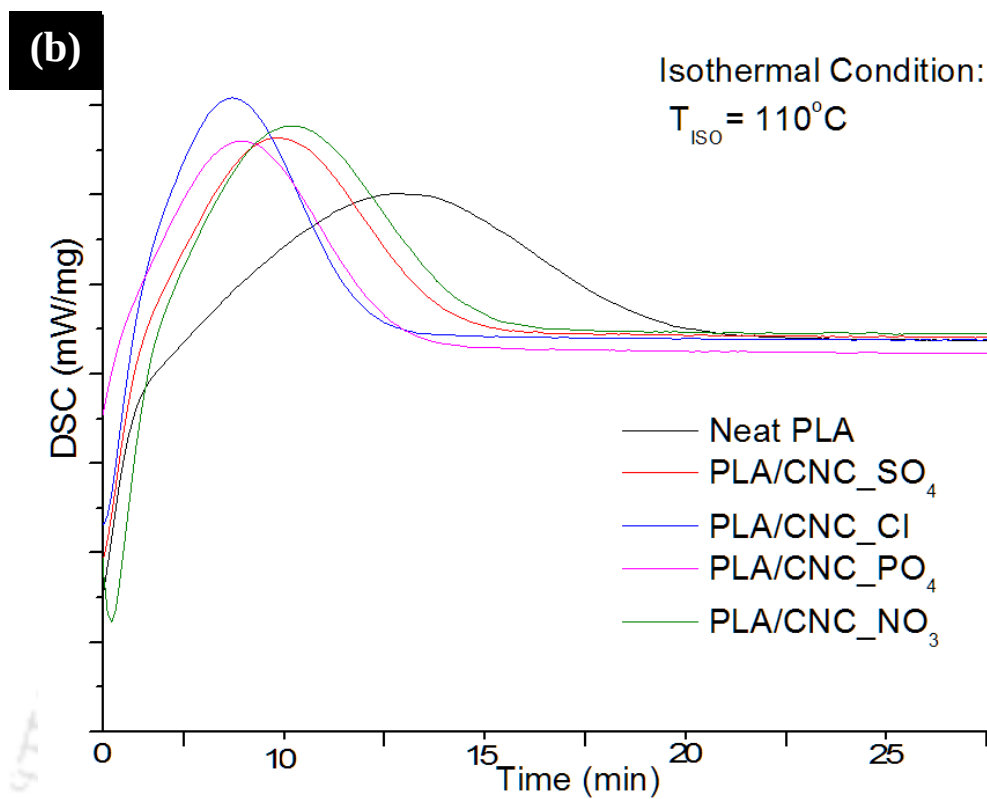
Table 5.5, summarizes the Avrami parameters calculated for the PLA/CNC nanocomposites derived from different acids at two different temperatures, 120 and 130°C respectively. Figure 5.11(d) shows the Avrami plots for the PLA nanocomposites fabricated with various acid- derived CNCs at 1 wt. % loadings, from which the parameters n and k are calculated using linear regression. The Avrami index was found to vary in range of $n= 2-3$ for all the acid-derived CNCs, which suggests the occurrence of heterogeneous nucleation phenomenon followed by growth of spherulites in three dimensions. Therefore, similar mechanism of nucleation and growth of PLA crystallites is observed in nanocomposites containing the different acid-derived CNCs. However, significant difference in the crystallite growth rate and

size are observed. The Avrami crystallization rate constant ' k ' is an important parameter which is used to predict the nucleation ability of the nanofillers. The higher ' k ' values for the PLA/CNC-Cl nanocomposites suggest that CNC-Cl are the effective nucleating agents compared to the other CNCs. In terms of the nucleation efficiency, the different acid-derived CNCs follow the order $\text{CNC-Cl} > \text{CNC-PO}_4 > \text{CNC-SO}_4 \sim \text{CNC-NO}_3$. With increase in crystallization temperature, the time for peak crystallization process increased, and the crystallization rate constant ' k ' decreased significantly for all the PLA-based CNC nanocomposites. This possibly suggests that with increase in temperature, homogenous nucleation of PLA is predominant which results in decrease in the rate of crystallization. The half time $t_{1/2}$ for PLA crystallization was found to be ~ 5.96 min, which decreased significantly to 2.56 min for PLA/CNC-Cl, 3.3 min for PLA/CNC-PO₄, 3.51 min for PLA/CNC-NO₃ and 3.55 min for PLA/CNC-SO₄. As discussed in the previous sections, the lower W_{ad} values for the PLA/CNC-Cl and PLA/CNC-PO₄ led to their effective dispersion which consequently enhanced the crystallization kinetics [369]. Further, the improvement in the crystallization behavior of PLA/CNC-Cl could be attributed to the structural characteristics such as morphology, aspect ratio and dimensions of the CNC-Cl. The small rod-like CNC-Cl particles with lower aspect ratio have greater interfacial area of interaction with the PLA matrix, and thereby induce a faster nucleation rate. Similar improvement in the PLA crystallization kinetics was also observed by Xu et al. [370] on introduction of carbon nanotubes with lower aspect ratio, probably due to improved binding with the polymer backbone.

Table 5.5: The predicted Avrami parameters for the PLA/CNC nanocomposites (at 1wt. % loadings) fabricated with different acid hydrolyzed CNCs at isothermal conditions $T_{iso} = 120$ and 130°C .

Samples	120°C			130°C			$E_a(\text{Avrami})$ (kJ/mol)	U (J/mol)
	K (min^{-1})	N	$T_{1/2}$ (min)	K (min^{-1})	N	$T_{1/2}$ (min)		
PLA	0.012	2.24	5.96	0.009	2.25	6.89	-37.8	1213.2
PLA/CNC-SO ₄ -1p	0.038	2.29	3.55	0.017	2.18	5.47	-105.9	3575.4
PLA/CNC-Cl-1p	0.080	2.29	2.56	0.051	2.22	3.24	-31.9	1137.9
PLA/CNC-NO ₃ -1p	0.040	2.27	3.51	0.023	2.20	4.70	-72.8	2951.4
PLA/CNC-PO ₄ -1p	0.045	2.29	3.30	0.031	2.20	4.10	-49.1	2874.7





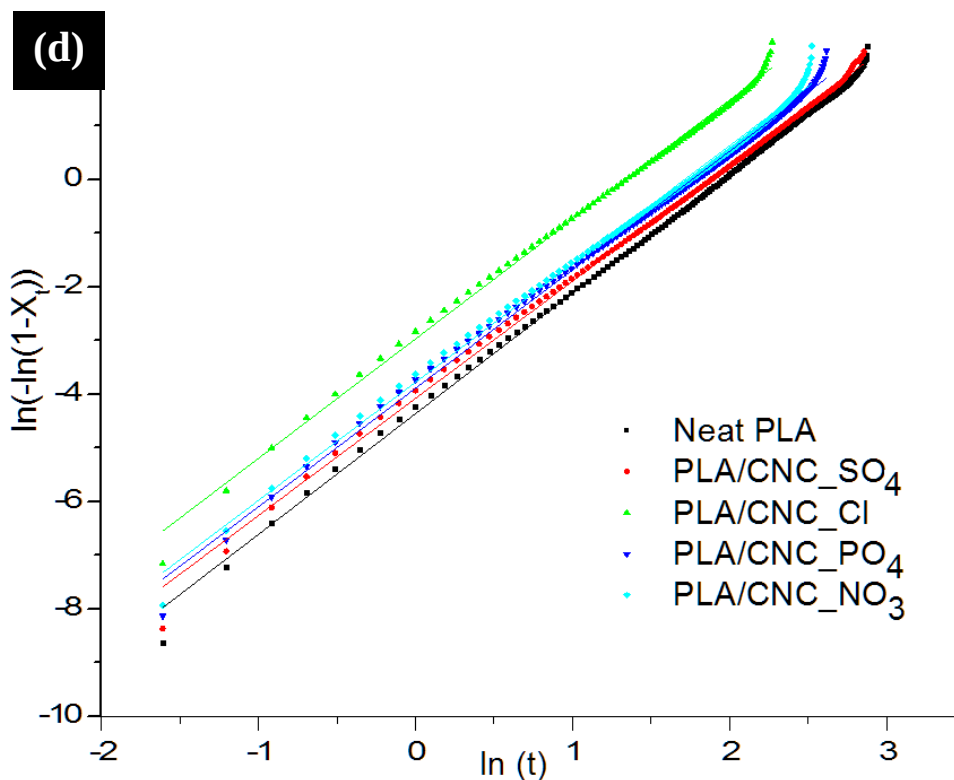


Figure 5.11: (a) DSC thermograms showing the second heating cycles of the PLA/CNC nanocomposites fabricated with 1wt. % loading of the various acid derived CNCs (at a scan rate of 10°C/min). (b) Comparison of DSC curves for isothermally crystallized PLA/CNC nanocomposites at temperature of 120°C (the loading of various acid derived CNCs are fixed at 1 wt. %), (c) The evaluation of the relative crystallinity with time and (d) Avrami log-log plots for the pristine PLA and PLA/CNC nanocomposites (at ~1wt. % CNC loadings) when isothermally crystallized at 120°C.

The effective activation energy (ΔE_x) represents the minimum energy required by the polymeric chains to overcome the barrier of crystallization process and is obtained from the model free iso- conversional method, as per eq. (5.5)

$$\frac{d(-\ln t_{x,t})}{d(1/T_{c,t})} = -\frac{\Delta E_X}{R} \quad (5.5)$$

The ΔE_X Energy barrier takes into consideration two effects: firstly, the minimum diffusion energy required by the polymer chains to reach the site of crystallization and secondly, the energy required to form the crystallites of a critical size from which the radial growth starts. As shown in Table 5.5, ΔE_X Calculated from the Avrami parameters, interestingly shows higher $E_{a(\text{Avrami})}$ values for the PLA/CNC-SO₄, PLA/CNC-NO₃ and PLA/CNC-PO₄ compared to PLA/CNC-Cl. This is probably due to the presence of higher aspect ratio CNC-PO₄ (~60), CNC-SO₄ (~57) and CNC- NO₃ (~24), which act as chain diffusion barrier thereby preventing the formation of ordered packing state. The lower $E_{a(\text{Avrami})}$ values for PLA/CNC-Cl are due to the low aspect ratio of CNC- Cl (~17) along with its improved interaction with PLA, due to the which the energy barrier required to initiate the crystallization process is significantly reduced [370]. Therefore, the effective activation energy of crystallization of the PLA/CNC nanocomposites, which is a temperature dependent term, was found to depend on the morphology and dimensions of the respective acid- derived CNCs.

The effect of different acid-derived CNCs on the mechanism of crystallization process, which involves formation of nuclei followed by the growth of spherulites, is determined using polarized optical microscopic (POM) studies at three different isothermal conditions. Figure 5.12 shows the POM micrographs at different time intervals for the PLA-based CNC nanocomposites (with 1 wt. % CNC loading) isothermally crystallized at 120°C. Addition of CNCs accelerates the rate of nucleation process compared to neat PLA. The rate of nucleation and density of spherulites however depends upon the type of acid-derived CNCs. The small rod-like CNC-Cl nanoparticles, because of their improved dispersion in PLA matrix along with their lower aspect ratio, increased the number of nucleating sites and enhanced the crystal

nuclei density (at $t = 5\text{min}$). The compatibility of CNCs with the PLA matrix and their aspect ratio are found to be key factors which control the nucleation process. CNC-SO₄ and CNC- PO₄ have almost similar aspect ratio, but PLA/CNC-PO₄ showed comparatively increased nucleation rate probably due to improved dispersion in PLA matrix as evident from the W_{ad} values. PLA/CNC-NO₃ shows the presence of least number of nucleation sites (at $t = 5\text{ min}$) with the presence of homogenously dispersed large size spherulites (at $t = 20\text{ min}$). Figure 5.14(a) shows the effect of the different acid-derived CNCs on the morphological dimensions of the PLA spherulites. Agglomeration in the PLA/CNC-NO₃ system decreases the interfacial area of interaction with the PLA molecules leading to lesser effect on the polymer chain mobility. This subsequently decreases the rate of crystal nucleation process and enhances the growth of spherulites due to lesser hindrance from the neighboring spherulites. For PLA/CNC- PO₄ and PLA/CNC-Cl, increase in the crystallization time (at $t = 20\text{ min}$) led to significant increase in the crystal nuclei density, with the formation of spherulites comparatively smaller in size. This indicates the presence of heterogeneous nucleation phenomenon probably due to the presence of large number of nucleating sites. Presence of such high density of crystals hinders the spherulite growth rate (at $t = 20\text{ min}$) due to the formation of molecular chain entanglements leading to overlapping of crystals and distortion in their shape and structures. Figure 5.13 shows the effect of the isothermal crystallization temperature (at $T_{cc}=110, 120\text{ and }130\text{ }^{\circ}\text{C}$) on the spherulite morphology and dimensions after crystallization time of 10 min. At $T_{cc} = 110^{\circ}\text{C}$, a rapid eruption of spherulite growth was observed in case of all the PLA/CNC nanocomposites due to which the distinct crystal morphology was found to be absent. With increase in the crystallization temperature, the process of folding of polymer chains into ordered crystalline state is delayed which

subsequently decreases the spherulite growth rate. At higher $T_{cc} = 120^{\circ}\text{C}$, the density of spherulites decreases, especially in the case of the PLA/CNC- NO_3 , probably due to its poor dispersion, which further delays the crystallization process.

The Lauritzen-Hoffman (LH) nucleation theory [371] was used to understand the effect of the crystallization temperature (T_{cc}) on the nucleation and growth rate (G) of the spherulites,

$$G = G_o \text{Exp} \left[-\frac{U^*}{R(T_c - T_{\infty})} \right] \text{Exp} \left[\frac{K_g}{f T_c \Delta T} \right] \quad (5.6)$$

where G_o Represents the pre-exponential factor and U^* Is the activation energy required to transport the polymeric chains to the site of crystallization. $\Delta T = (T_m^o - T_c)$ is the undercooling, where T_m^o Is the equilibrium melting temperature and T_c Is the crystallization temperature respectively. T_{∞} Is the hypothetical temperature at which the motion of polymeric chains is considered to be stagnant. f is the correction factor represented as $f = \frac{2T_c}{(T_c + T_m^o)}$, which takes into account the change in enthalpy when the temperature is reduced below T_m^o . K_g , the secondary nucleation parameter, represents the minimum energy required for the formation of critical nucleus size which is required to initiate the crystallization process. K_g is a function of lateral surface energy of crystals (σ) and calculated using eq. (5.7) for the PLA/CNC nanocomposites:

$$K_g = \frac{i\sigma\sigma_e b_o T_m^o}{\Delta H_f k_B} \quad (5.7)$$

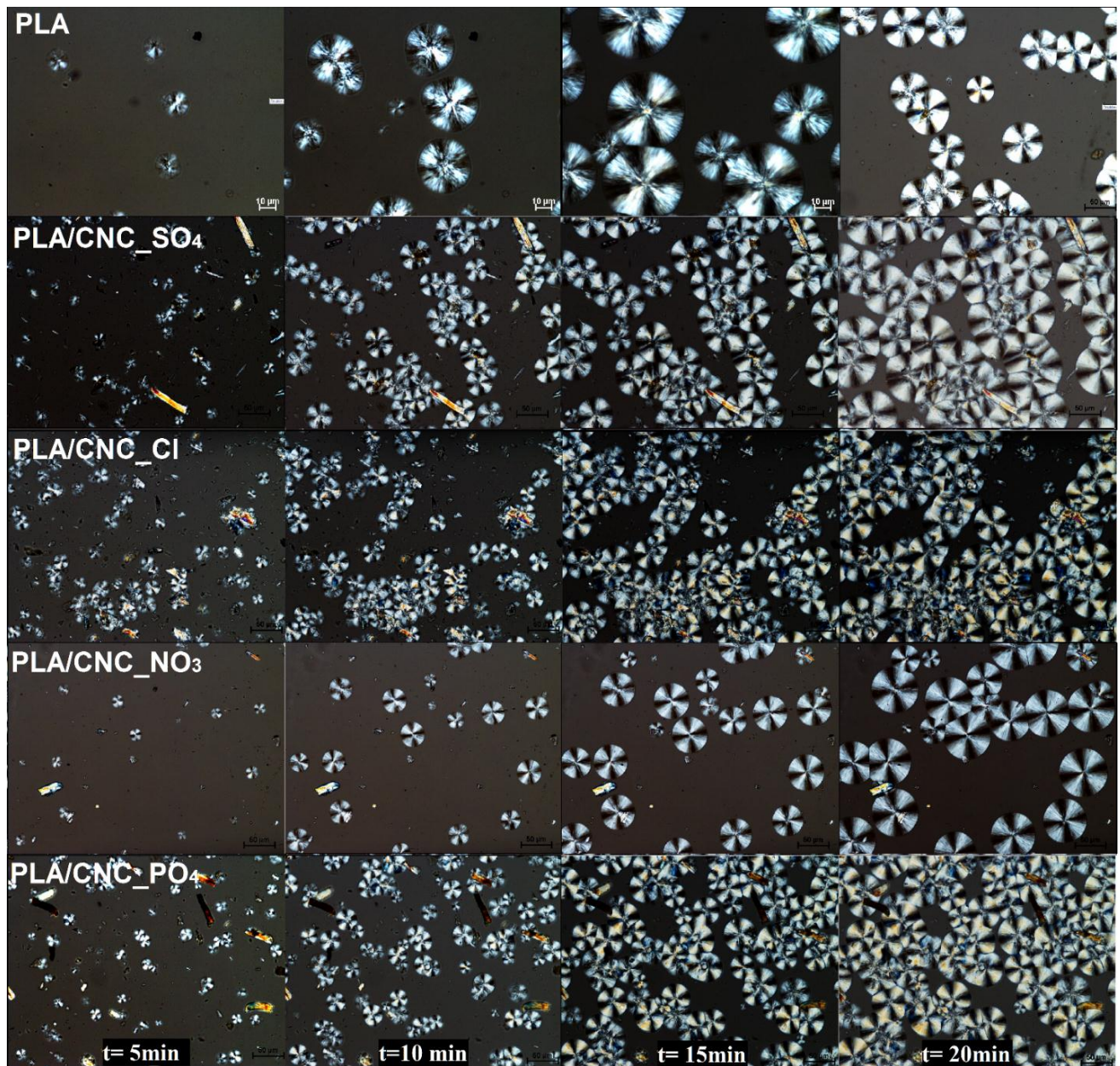


Figure 5.12: POM micrographs of the PLA and PLA/CNC nanocomposites fabricated with different acid hydrolyzed CNCs isothermally crystallized at $T_{ISO}=120^{\circ}\text{C}$ taken at different time intervals ($t= 5, 10, 15$ and 20 min).

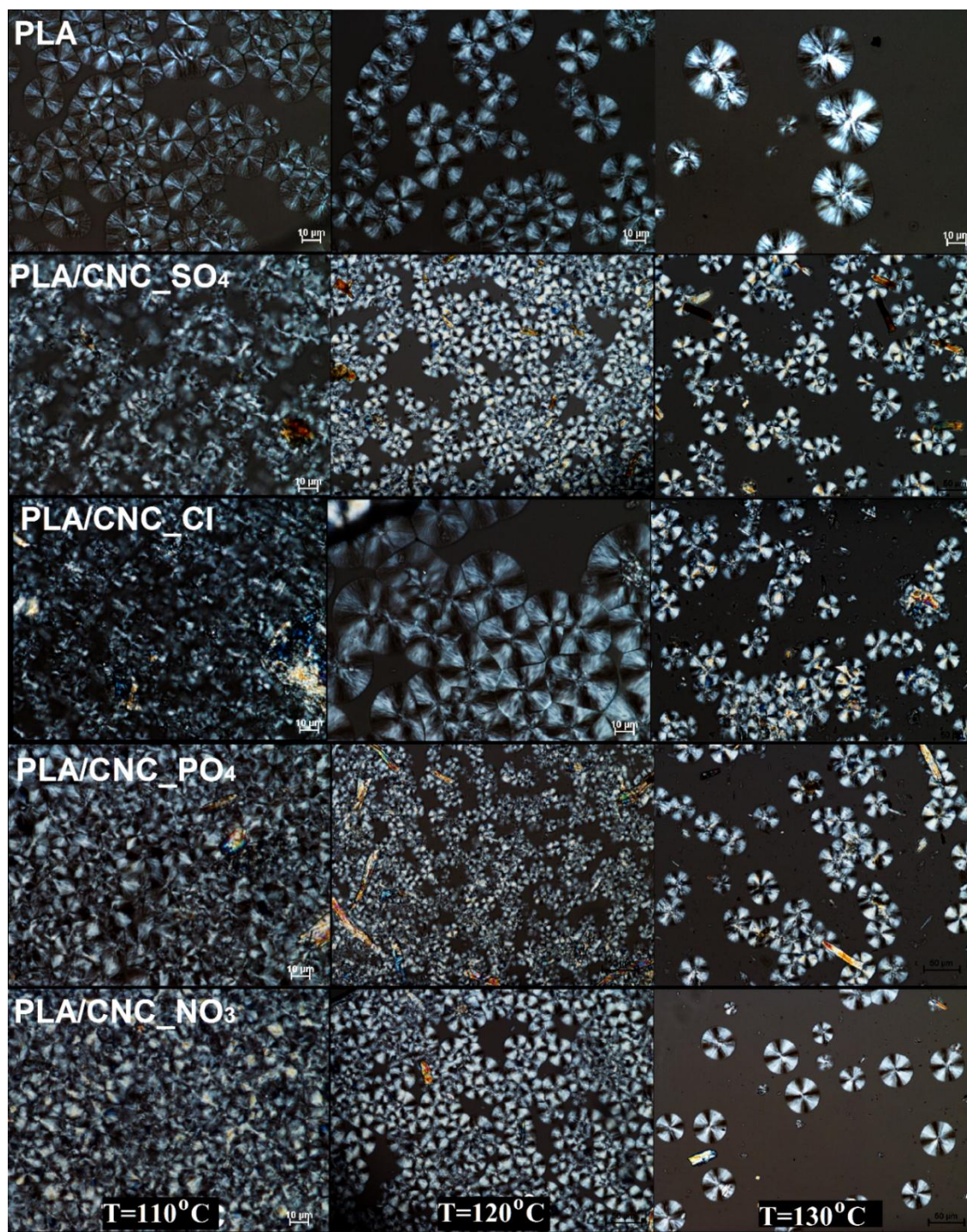


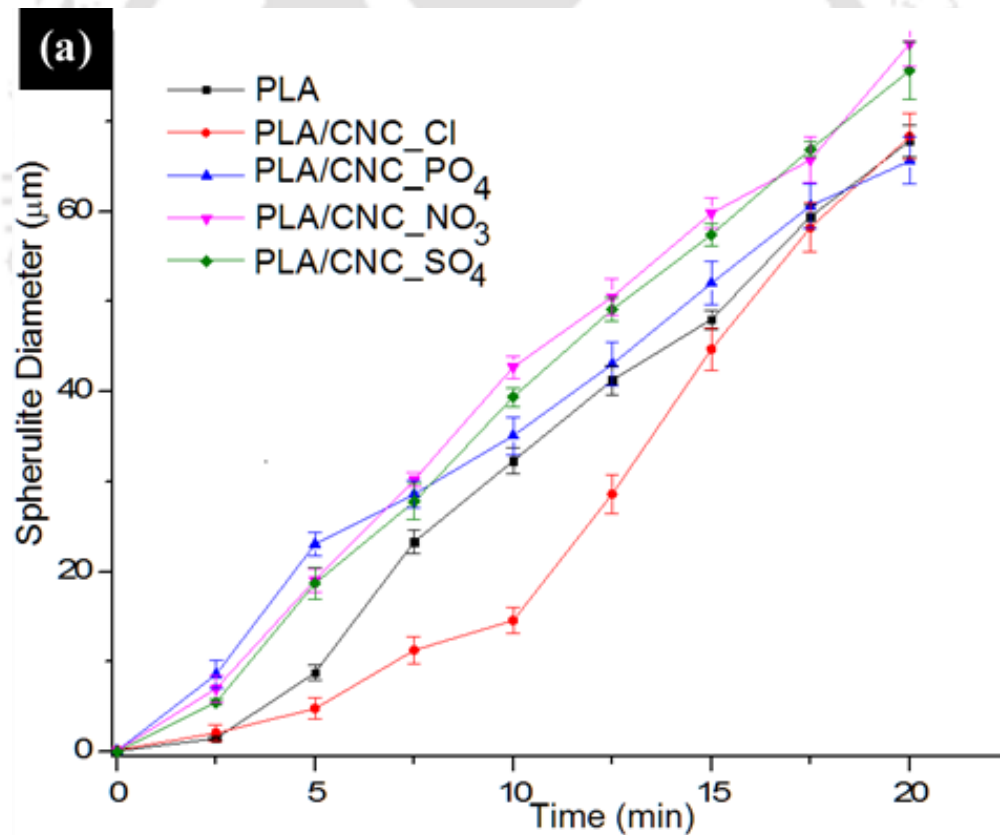
Figure 5.13: POM micrographs of the PLA and PLA/CNC nanocomposites fabricated with various acid-derived CNCs isothermally crystallized at three temperatures $T_{ISO}=110, 120$ and 130°C , captured at crystallization time of $t=10$ min.

where for the PLA systems b_o Represents the surface nucleus thickness (taken to be 5.17×10^{-10} m), σ lateral surface free energy $\sim 12.03 \times 10^{-3}$ J/m², σ_e Represents the fold surface energy ($\sim 60.89 \times 10^{-3}$ J/m²), i is the crystallization regime ($i = 2$) for PLA system as crystallization generally takes place in regime II [372], ΔH_f Is considered as 93 J/g and k_B Represents the Boltzmann constant (1.38×10^{-23} J/K) [373].

Figure 5.14(b) shows the logarithmic plots of eq. (8), semi-log plots of $\ln G + \frac{K_g}{fT_c\Delta T} \nu S \frac{1}{R(T_c - T_\infty)}$ from which the activation energy values U is calculated from the slope of the linear fit to the data by employing eq. (5.8), for the various acid-derived CNC-based PLA nanocomposites. Incorporation of CNC-Cl into PLA nanocomposites results in the reduction of U values for the PLA/CNC-Cl system (~ 1137.9 J/mol) in comparison to the pristine PLA (~ 1213.2 J/mol). Lowering of the U values suggests that the transportation activation energy required by PLA chains to interact with the CNC-Cl interface for the initiation of crystallization process is reduced significantly.

As explained in the previous section, this could be due to improved dispersion of CNC-Cl in the PLA matrix which acts as an efficient nucleating agents. However, the higher values of U for the PLA/CNC-SO₄ (3575.4 J/mol), PLA/CNC-NO₃ (2951.4 J/mol) and PLA/CNC-PO₄ (2874.7 J/mol) suggests that the CNCs restricted the molecular mobility of the PLA chains into ordered crystallite state. Therefore, it can be concluded that although CNC-SO₄, CNC-NO₃ and CNC- PO₄ are efficient nucleating agents, they hindered the radial growth of the PLA spherulites effectively which limited the crystal perfection [374]. Such phenomenon is possibly due to two important factors: firstly, the presence of high aspect ratio CNCs (especially in case of CNC-PO₄) and secondly, the poor interfacial interaction with PLA matrix (in case of

CNC- SO_4 and CNC- NO_3), restricted the lamella growth thus requiring more activation energy to initiate the chain folding process [375,376]. Therefore, the crystallization mechanism of PLA- based CNC nanocomposites strongly depends on the intrinsic characteristics of CNCs which can be finely tuned during hydrolysis with different acids.



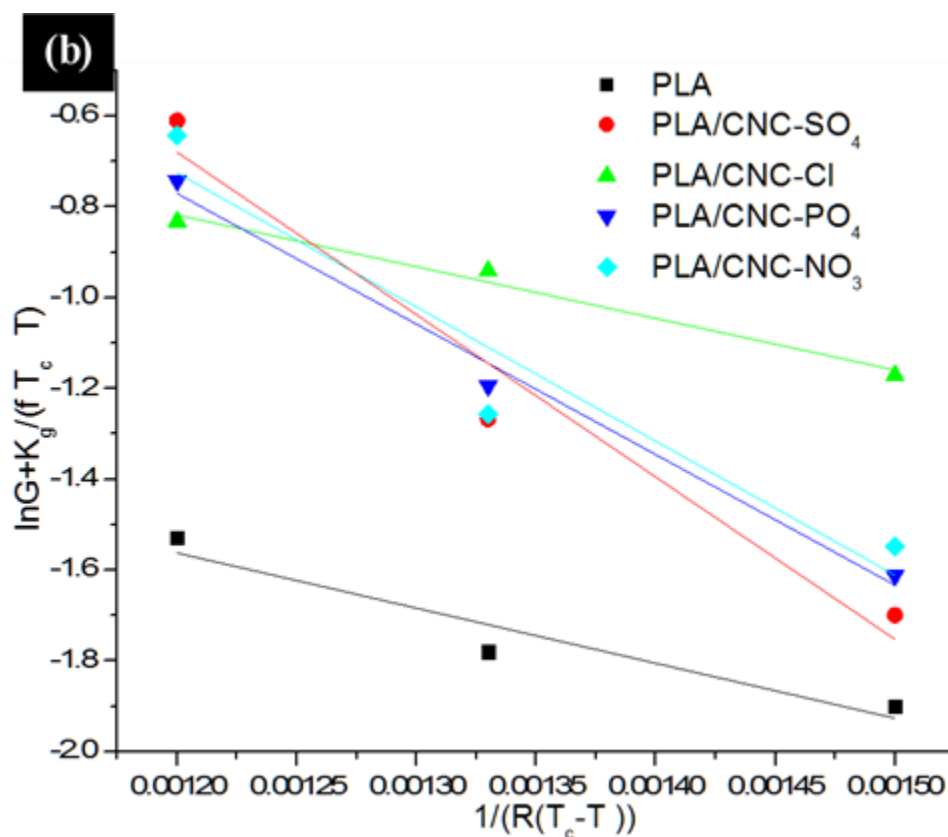


Figure 5.14: (a) Comparison of the growth of PLA spherulites in the presence of various acid-derived CNCs when isothermally crystallized at $T_{ISO}=120$ °C at different time intervals and (b) Lauritzen-Hoffman plots to determine the effect of crystallization temperature (T_c) on the growth kinetics of the PLA crystallites, in the presence of the different acid-derived CNCs.

5.3 CONCLUSIONS

The main aim of this study is to investigate the effect of CNCs with different cellulose polymorph and modified with various anionic moieties incorporated during acid hydrolysis step on the physico-chemical, thermal, barrier, structural and crystallization behaviour of PLA based nanocomposites. Investigation of the mechanical properties shows that at similar CNC concentrations, CNC II shows comparatively higher Young's modulus (~ 0.6 GPa for

PLA/CNC I, ~ 1.1 GPa for PLA/CNC II and 0.9 GPa for PLA/CNC: I $\rightarrow$ II at 2 wt. % CNC loading), due to the formation of hydrogen bonded entangled network and better adhesion with the polymer matrix. PLA/CNC II has lower elongation at break due to fiber entanglements compared to PLA/CNC I, which undergoes swift matrix failure, due to orientation of CNCs along the tensile direction. Further, predicted values of the modulus of the composites using Halpin-Kardos and Ouali models showed good agreement for PLA/CNC I and PLA/CNC: I $\rightarrow$ II at higher aspect ratio. Cox–Krenchel model showed good agreement with the predicted modulus for PLA/CNC II films. For the case of acid derived CNCs, higher aspect ratio CNC- PO₄ (~ 60) with long needle-like morphology improved the elastic modulus and elongation behavior of PLA/CNC nanocomposites even at higher loading fractions. On the basis of the micro-mechanical models, the optimum loadings for the formation of percolating network structure in PLA matrix are found to be ~ 3.3 , 4.3 and 17.9 wt% for PLA/CNC I, PLA/CNC II and PLA/CNC: I $\rightarrow$ II respectively. For the different acid-derived CNCs, the corresponding values for PLA/CNC-SO₄, PLA/CNC-Cl, PLA/CNC-PO₄ and PLA/CNC-NO₃ are ~ 3 , 9, 2.7 and 7 wt. % respectively. The high aspect ratio fiber entangled network in CNC II also helped in improving the oxygen and water vapor barrier properties by $\sim 80\%$ and $\sim 51\%$ respectively. As confirmed from the FTIR studies, all CNC polymorphs formed hydrogen- bonded percolating network at different loadings. The stability of such networks was qualitatively measured using the activation energy (E_a) values calculated from the Arrhenius equation, which showed the order CNC II > CNC: I $\rightarrow$ II > CNC I over a wide temperature range (15–45°C). Comparison of the thermal stability of the PLA-based CNC nanocomposites shows the trend PLA/CNC: I $\rightarrow$ II > PLA/CNC II > PLA/CNC I for the

different CNC polymorphs, and the order PLA/CNC-NO₃ > PLA/CNC-PO₄ > PLA/CNC-Cl > PLA/CNC-SO₄ for the different acid- derived CNCs.

The different acid-derived CNCs are observed to modify the microstructural characteristics and molecular properties of PLA chains drastically during the extrusion-based processing of nanocomposites under high temperature and shear conditions. CNCs with low aspect ratio, such as CNC-Cl (~17) with small rod-like morphology, enhance the nucleation phenomenon during the crystallization process. Isothermal crystallization kinetics studies were carried out using the classical Avrami models which showed that the crystallization process in all the PLA/CNC nanocomposites are nucleation-controlled. Evaluation of the Avrami parameters and the crystallization half time ($t_{1/2}$) values shows that the nucleation efficiency of the various acid-derived CNCs follows the order CNC-Cl > CNC-PO₄ > CNC-SO₄ ~ CNC-NO₃. Lauritzen- Hoffman nucleation theory shows that incorporation of CNCs into PLA matrix hindered the growth of spherulites due to which it required higher activation energy to initiate the nucleation process. Presence of such restrictions led to the formation of imperfect crystals, and with increase in crystallization time, overlapping and distortion of the spherulite structures took place. Interestingly, the calculated activation energy values ' U ' for different PLA/CNC systems are found to be dependent on the aspect ratio of the CNCs as well as on their compatibility with the PLA matrix. Such detailed comparative study will aid in rationally selecting the appropriate cellulose polymorph and hydrolyzing acid for the fabrication of CNCs with desired characteristics, which can be used for manufacturing high performance PLA/CNC nanocomposites with properties tailored for targeted applications.

Processing of Polylactic Acid based Cellulose Nanocrystal Films through Reactive Extrusion Approach

Motivation

Increased environmental concern, rising price of petrochemicals along with strict rules and regulations have resulted in increased focus on the development of sustainable and biodegradable products from abundantly available and naturally derived inexhaustible renewable resources. However, commercialization of such biodegradable polymers as products has been limited due to their high production cost, low yield as well as non-recyclability. To overcome such techno-economic challenges, a novel reactive extrusion-based processing of polylactic acid (PLA)-based cellulose nanocrystal films has been developed which is successfully tested on a pilot-plant scale extruder. The developed process shows promising results using a wide variety of organic or inorganic nanofillers, with the formation of high molecular weight cross-linked gel-like structures which prevented thermal and shear induced degradation of PLA. Moreover, the processed films are recyclable, show dimensional stability along with improved physicochemical, structural, thermal and barrier properties, making them adequate for the fabrication of biodegradable food packaging, with the potential to replace the petroleum-derived non-degradable plastics.

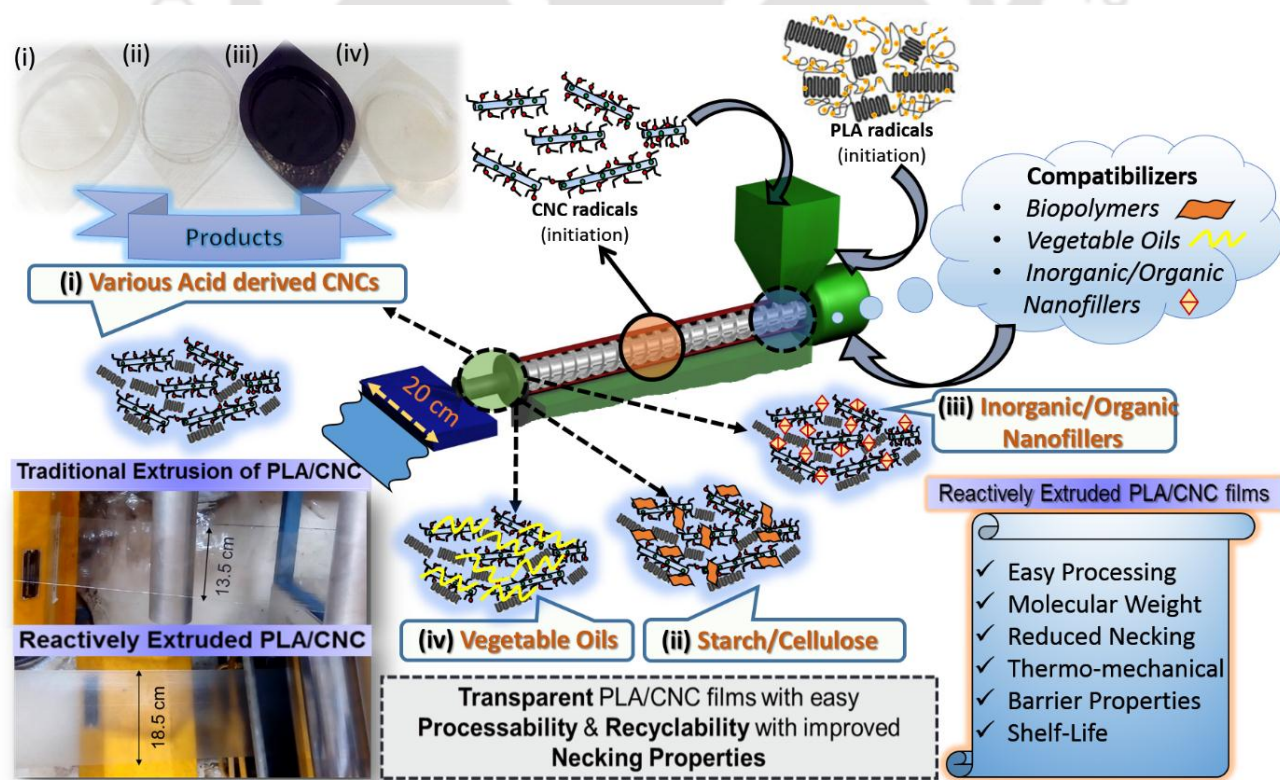
The work in this chapter are published as:

- Dhar, P., Tarafder, D., Kumar, A., & Katiyar, V. (2016). Thermally recyclable polylactic acid/cellulose nanocrystal films through reactive extrusion process. *Polymer*, 87, 268-282.
- Dhar, P., Gaur, S., Soundararajan, N., Gupta, A., Bhasney, S. M., Kumar, A., & Katiyar, V. (2017). Reactive Extrusion of Polylactic Acid /Cellulose Nanocrystal Films for Food Packaging Applications: Influence of Filler type on Thermo-mechanical, Rheological and Barrier Properties. (Accepted at Industrial & Engineering Chemistry Research).
- Katiyar, V., Kumar, A., Dhar, P., “One Step Reactive Processing of Cellulose based Polymeric Nanocomposites Sheets”. (Patent Application No: 201631022079).

Abstract

This chapter demonstrates a single step industrially scalable reactive extrusion strategy of fabricating recyclable PLA/CNC based cast films with improved melt strength, structural, thermo-mechanical, and barrier properties. The developed films are transparent with reduced necking behavior and enhanced dimensional stability, as evident from the improved necking or thickness swell ratio, film inhomogeneity index values as well as from the rheological response. Recycling of PLA-graft-CNC films doesn't alter the molecular weight, thermal, crystallization and mechanical properties of the nanocomposites significantly. The reactively extruded PLA/CNC films shows reduction in both oxygen (20-65%) and water vapor barrier properties (27-50%) as well as improvement in thermo-mechanical properties, which strongly depends upon the type of compatibilizers used. Utilization of these films as packages for storage of oil and dairy based products shows a shelf-life of ~5 months and ~2 weeks respectively and are within the standard migration limits as per the set legislations. Therefore, the current study provides a novel approach for fabricating CNC-reinforced-biodegradable PLA films which can be recycled and have promising applications in development of food packagings as a potential replacement to petroleum-derived polymers.

Graphical Abstract



6.1 INTRODUCTION

Poly(lactic acid) (PLA) is a bio-based thermoplastic polymer derived from renewable resources (e.g. corn, starch, sugarcane, etc.) which has the potential to replace the petroleum-derived polymers and solve the problems related to their sustainability. Among all the bio-based polymers, PLA has attracted considerable interest because of its easy processability and comparable mechanical and thermal properties in comparison to conventional synthetic polymers. However, the brittle nature, inferior oxygen or water vapor barrier properties and heat distortion temperature ($\sim 55^{\circ}\text{C}$) [161] limits its application for manufacturing commodity products. Therefore, modifications of PLA through physical approaches like incorporation of nanofillers [162,163], plasticizers [377,353] and blending [378] as well as through chemical approaches like polymerization through different routes such as grafting onto and from approaches [164,165] have been extensively researched. Introduction of different class of inorganic or organic nanofillers such as nanoclays (montmorillonite (MMT), cloisite, hydrotalcite etc.) [364], carbon-based nanomaterials (graphene and its derivatives, carbon nanotubes or nanofibers [369] etc.), metallic nanoparticles (silver, iron etc.), inorganic nanoparticles (alumina, nanosilica, polyhedral oligomeric silsesquioxane etc.) [379] or biopolymeric nanoparticles (nanocellulose and its derivative, chitosan, starch etc.) have been carried out to improve the properties of PLA. Although the above mentioned approaches have addressed the problems to certain extent, there are still important engineering problems which occurs specifically during processing of PLA at industrial scale and needs to be solved to make the processing of PLA more economically viable [380]. These includes heat shrinkage of films at the die end which leads to necking behavior of cast films at die head causing dimensional instabilities in the processed films. Moreover, melt extrusion (under high temperature and

shear forces) in the presence of such nanofillers leads to significant reduction in molecular weight due to several factors such as hydrolysis in presence of trace amount of moisture/water (in case of hydrophilic nanofillers), depolymerization or random chain scissions and intermolecular/intramolecular transesterification forming monomeric or oligomeric units (in presence of nanoclays or inorganic nanoparticles) [162]. Incorporation of carbon-based nanomaterials (such as graphene nanoplatelets) or nanoclays leads to heat shrinkages in films alongwith significant decrease in melt extrudate swell ratio. The biopolymer-based nanofillers (such as cellulose, chitosan or starch), which are commonly hydrophilic in nature, tends to agglomerate in the PLA matrix and due to abundant functionalities, induce degradation during extrusion forming black/brown coloration in the films [381,124]. The large number of hydroxyl functional groups in these biopolymeric nanofillers act as active sites for the initiation of degradation of PLA backbone [381]. In addition to the reduction in molecular weight, the processed films undergo drastic heat shrinkages leading to dimensional instabilities in the processed films [382, 341]. These are responsible for generation of wastages of PLA during processing which needs to be addressed for successful processing of dimensionally stable PLA cast films at industrial scale with economical and commercial viability. Although the incorporation of various nanofillers improves the structural, thermal and barrier properties of nanocomposites significantly, the deterioration in their processability restricts their applications in fabrication of value-added products through industrially viable extrusion process.

Recently, several studies have been reported on reactive extrusion of PLA in the presence of various chain extenders and radical initiators which lead to improvement in melt strengthening thereby widening the processing window of PLA. Marsilla & Verbeek [25] grafted itaconic anhydride on PLA through radical grafting process, which led to improvement

in the tensile strength and elongation at break (~60 %). Spinella et al. [26] developed a green approach using titanium tetrabutoxide for reactive blending of PLA with poly (ω -hydroxytetradecanoic acid) compatibilizing through trans-esterification reaction. This led to significant improvement in the elongation at break (by ~140%) and impact strength (by ~2.4 times). Interestingly, it was found that reactive extrusion based PLA composites retained the inherent biodegradable nature and were readily compostable and degradable under hydrolysis conditions [383]. Similarly, *in situ* compatibilization of PLA with poly (butylene adipate-co-terephthalate) lead to improvement in the impact toughness and elongation at break (by ~300%) [383]. Yang et al. [28] achieved significant increase in thermal stability of PLA by incorporating trace amount of crosslinking agent. Blending of PLA with poly (3-hydroxybutyrate-co-4- hydroxybutyrate) in the presence of dicumyl peroxide (DCP) initiator resulted in high crosslinking which showed typical toughening with improvement in tensile strength [384]. Grafting of maleic anhydride in the presence of an initiator showed slight increase in molecular weight of PLA, possibly due to crosslinking or increase in chain entanglements [385]. Recent studies reporting the effect of radical initiators on the rheological properties of PLA confirms that the increase in branching leads to improvement in the melt strength by approximately three times with higher shear sensitivity [386,387,388]. However, studies on reactive extrusion of polymers in presence of fillers or nanofillers have been rarely reported. Wang et al. [34] grafted bamboo flour onto PLA using benzoyl peroxide and *tert*- butyl peroxy benzoate (as initiators) and glycidyl methacrylate (as chain extender). Improved compatibility between the two phases was observed which led to enhancement in the mechanical strength and modulus by 44 and 135% respectively, alongwith increase in the thermal properties. Recent studies [273] on *in situ* reactive extrusion of PHB with cellulose in

presence of DCP led to improvement in the glass transition temperature and onset degradation temperature (by $\sim 22^{\circ}\text{C}$), alongwith significant decrease in the crystallinity.

Processing of PLA/CNC nanocomposites through melt extrusion process is a challenging task as it leads to the agglomeration of hydrophilic CNCs in PLA matrix due to the presence of strong intermolecular hydrogen bonding [124]. CNCs self-assemble to form non-dispersible agglomerates (under freeze or air drying conditions) in dry state [368] and the sulfate groups of CNC surface (acquired during sulphuric acid hydrolysis) act as catalyzing agent for the degradation of both CNCs and PLA during extrusion [389]. Polymeric nanocomposites (fabricated from either fossil fuel or bio-derived resources) undergo drastic reduction in the film dimensions (especially width) which is termed as ‘necking’ phenomenon, when they are stretched above their glass transition temperature [390]. During twin-screw extrusion-cum film casting (EFC) process, the polymer melt swells after leaving the die-head and then passes through a chilled roll under extensional forces followed by rolling up onto a take up roll in presence of air [382]. Throughout this process the polymer films are above their glass transition temperature, henceforth, the polymeric chains reorients themselves into parallel planes along the stretch direction. Presence of such parallel alignment reduces the inter-chain frictional forces between the polymeric chains leading to severe contraction, the degree of necking, however, depends upon the drawing ratio applied [391]. Alongwith it, another phenomenon known as ‘edge-beading’ also occurs, which leads to thickening of films on their edges and thinning at the center portion, leading to inhomogeneity in the films. Presence of both the ‘necking’ and ‘edge-beading’ phenomenons limits the processing window (for both uni or bi-axial drawings) creating defects in fabricated films and limits their applications, even though they have significantly improved structural or barrier properties [382]. Till date seldom studies, have

been reported in literature, which addresses the technical issues and engineering aspects of processing biobased polymers such as PLA and improving their extrudability or physico- chemical properties (such as molecular weight, necking/width swell ratio, heat shrinkages etc.), especially in presence of nanofillers which is an essential requirement for successful commercialization of the PLA based products at industrially viable scale. Also, to the best of our knowledge, studies reporting recycling of polymer-CNC nanocomposites (without altering the polymeric properties significantly) haven't been reported in the literature till date.

In this chapter, we discuss in detail the reactive extrusion-based processing of PLA graft CNC (PLA-*g*-CNC) nanocomposites is carried out in the presence of DCP (as radical initiator) and the effect of recycling on their physico-chemical, mechanical, thermal and crystallization properties are extensively studied. This reactive extrusion based processing of PLA has been tried and tested in a pilot-plant scale extruder with the wide range of the fillers (such as various acid derived CNCs, biopolymers, vegetable oils, inorganic nanoparticles and carbon based nanomaterials) which have been never reported elsewhere. Processing of the biodegradable polymeric films with such improved dimensional stability, economic viability, easy scalability alongwith the other structural properties are of keen interests for the researchers as well as industrialists working in development of biodegradable packages for potential commercial applications. The grafting is confirmed through FTIR and the possible mechanism is explained from NMR analysis. XRD and DSC studies are carried out to understand the effect of grafting parameters on the change in crystallinity of PLA. The effect of grafted CNCs on the mechanical properties of the nanocomposites is investigated with DMA and tensile studies. To better understand the potential applications of the fabricated films as food packaging products, we

carried out oxygen/water vapor barrier property investigations, migration studies in the presence of several food simulants, and estimated the shelf-life for stored oil- and dairy-based food products. Therefore, this study provides a novel one-step process of fabricating PLA/CNC films through reactive extrusion approach with both improved properties and biodegradable characteristics, making them a suitable alternative to conventional polymers for possible food packing applications.

6.2 RESULTS AND DISCUSSIONS

6.2.1 *Reactive Extrusion of PLA-based CNC Nanocomposites through in situ Grafting Approach*

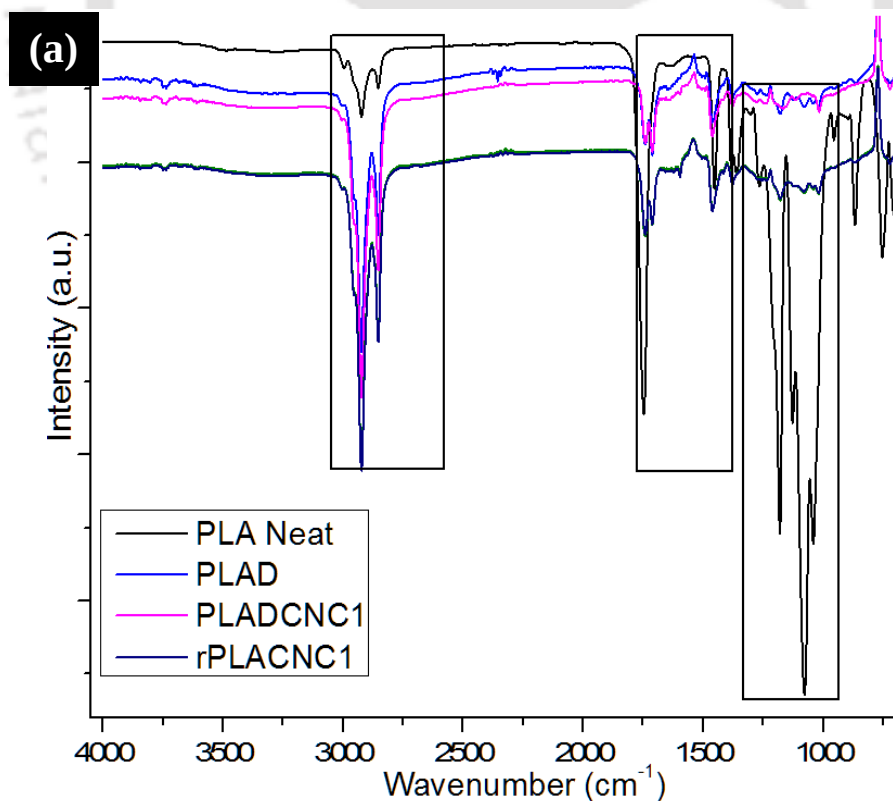
- **Reaction Mechanism, Grafting Parameters and Molecular Weight studies**

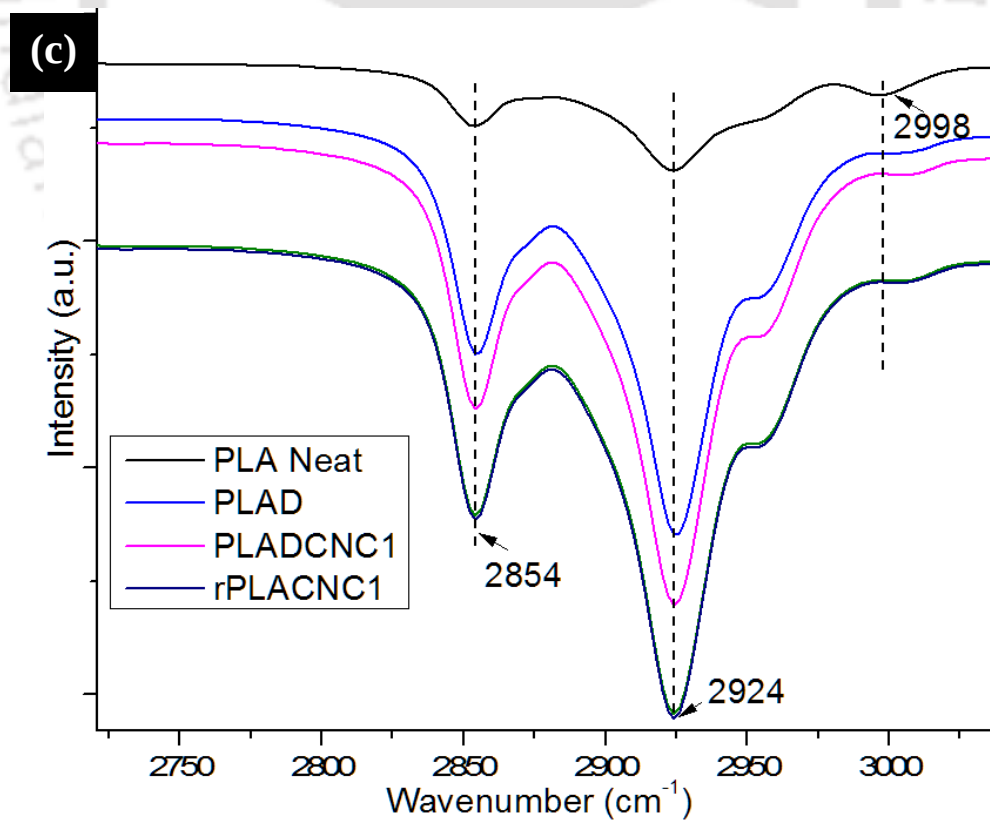
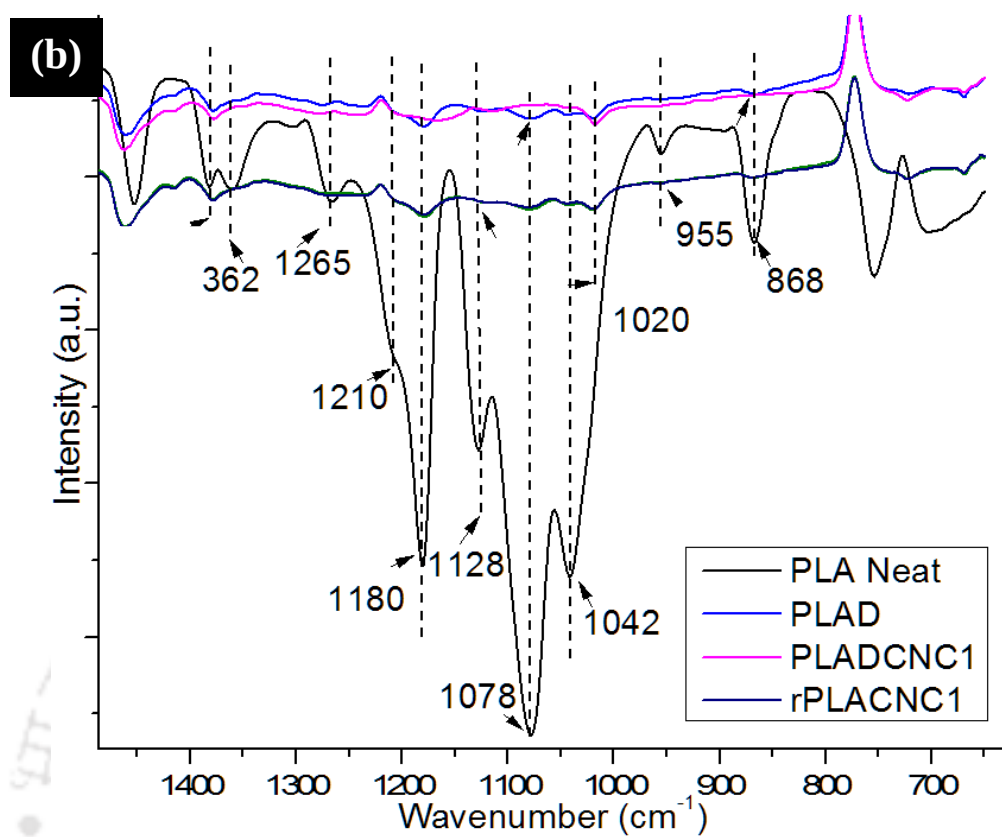
Reactive grafting of PLA on CNC (PLA-g-CNC) surface was successfully carried out through extrusion process in presence of DCP which decomposes into free radicals at high temperature. The activity of the DCP initiated peroxide radicals (as shown in Scheme 6.1 (a)) is short (~180s) [392, 393], due to which grafting reaction took place during the initial 5 minutes of the extrusion process under high shear conditions. To achieve effective grafting, optimization of the reactive extrusion process in terms of parameters such as screw speed, temperature and DCP content was carried out. It was found that a screw speed of 50 rpm, extrusion temperature of 180°C, and DCP content of 1.2% was effective in achieving high molecular weight. A residence time of about 5 min was selected because of the short life of DCP radicals as well as previous studies also report maximum gel yield under similar conditions [273]. At such optimized conditions, the effect of different CNC loadings (1– 3 wt. %) on grafting parameters and properties of PLA fabricated through extrusion

process were investigated in detail. The PLA-*g*-CNC nanocomposites extruded in the presence of DCP and with various loadings of CNCs (1–3wt. %) are represented as PLAD, PLADCNC1, PLADCNC2 and PLADCNC3, respectively. During the reactive extrusion process, it was found that the complex viscosities of these samples (~50–70 Pa.s at various CNC loadings) were higher in comparison to neat PLA (~40 Pa.s), probably due to the formation of branched and cross-linked structures [394]. The PLA-*g*-CNC formed micro gel-like structure which was filtered out and extracted in chloroform, to remove the unreacted PLA. It was found that the incorporation of CNCs into PLA during reactive extrusion process enhances the formation of gel and grafting percentage. Table 6.1 summarizes the effect of CNC loading on the percentage gel yield, graft percentage, graft efficiency, weight conversion, specific rotation and optical rotation respectively during the reactive extrusion process. The maximum grafting efficiency and gel yield % was found to be 40.7 and 76.8 % respectively for PLADCNC1 (Table 6.1). Gel yield decreases at higher CNC loadings, probably due to thermally induced chain scission during extrusion, catalyzed by the high content of sulfate groups present on CNC surface which suppress the grafting reaction. Moreover, the agglomeration of CNCs at higher loadings decreases the number of available –CH₂OH groups on CNC surface for the grafting reaction to take place. The specific and optical rotation of PLA-*g*-CNC changes compared to neat PLA, by ~21° and 0.1° respectively. This is possibly due to the grafting of CNCs on the chiral center carbon of PLA which hindered the specific rotation [395].

To confirm the grafting of PLA on the CNC surface, chemical structure analyses were carried out through FTIR and NMR spectroscopy. PLA showed characteristic FTIR peaks at 1748, 1180, 1128, 1078, 1042, 868 and 954 cm⁻¹ corresponding to C=O stretching, C–O–C asymmetric stretching, C–OH side group vibrations, C–C vibrations and –CH stretching

[396](Figure 6.1). CNC showed characteristic FTIR peaks at 3402, 2908, 1647, 1427, 1319, 1203, 1163, 1031 and 896 cm^{-1} corresponding to $-\text{OH}$ stretching, $-\text{CH}$ stretching, $-\text{OH}$ bending, $-\text{CH}_2$ bending, $-\text{CH}_2$ wagging, C-OH bending in plane at C-6, $-\text{C-O-C}$ asymmetric stretching of β -glucosidic linkage, C-OH stretching in plane at C-6 and $-\text{C-O-C}$ asymmetric bending of β -glucosidic linkage respectively [17]. The FTIR peaks at 2854, 2924 and 1380 cm^{-1} , corresponding to the symmetric, asymmetric stretching and deformation of $-\text{CH}_3$ groups, were present in all the samples. (Figure 6.1(b)). The peak at 1180 cm^{-1} corresponding to C-O-C stretching, were present in PLA Neat, PLAD and rPLACNC1(1.35) (with decreased in intensity compared to pristine PLA due to grafting), however, it shifted to 1164 cm^{-1} in case of PLADCNC1. The peak at $\sim 1128 \text{ cm}^{-1}$ corresponding to CH_3 rocking, is visible for the PLA Neat, however, slight shift in peaks were found for PLAD to $\sim 1124 \text{ cm}^{-1}$, for rPLACNC1(1.35) to $\sim 1122 \text{ cm}^{-1}$ and PLADCNC1 to $\sim 1122 \text{ cm}^{-1}$.





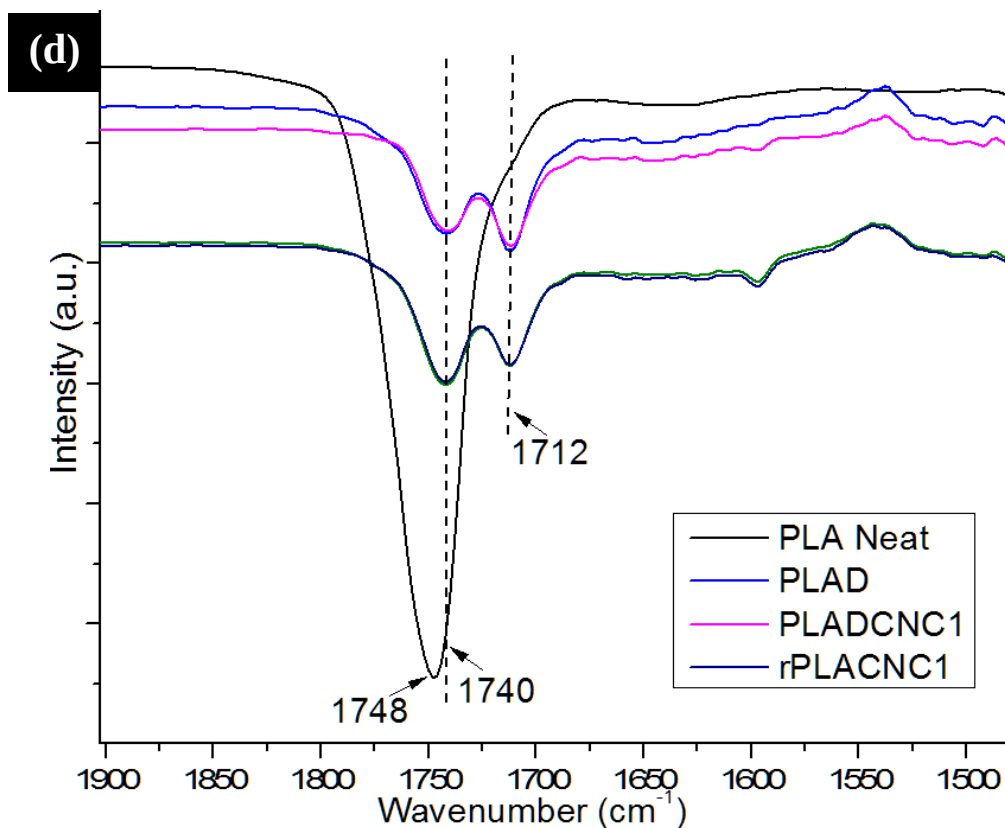
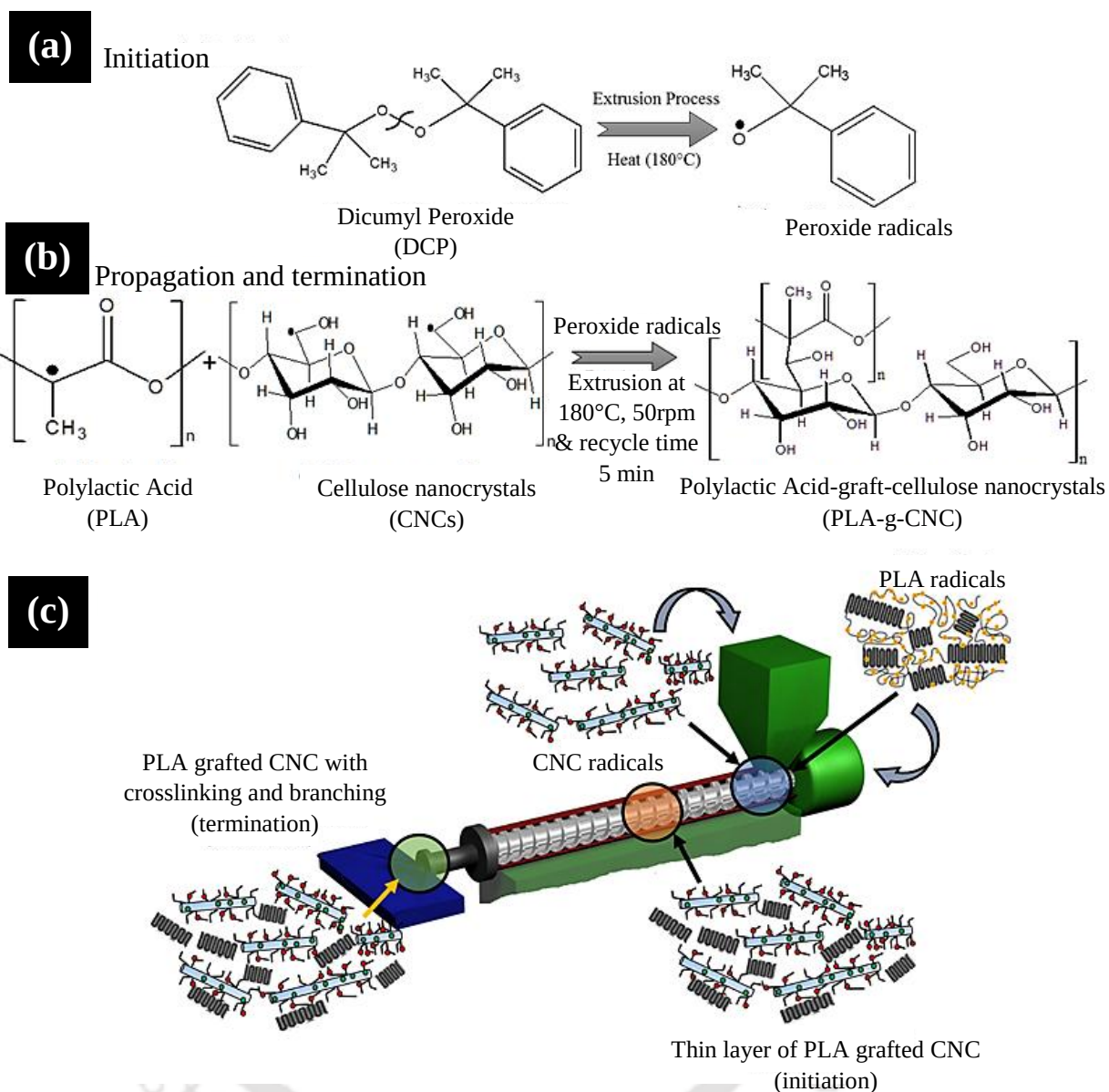


Figure 6.1: (a) Comparison of FTIR spectrograms of neat PLA, PLAD, PLADCNC1 and rPLA CNC1(1.35) with the selected regions (marked in black boxes) analyzed at (b) 3600–2700 cm⁻¹, (c) 1900–1400 cm⁻¹ and (d) 1500–600 cm⁻¹ wavenumber range and the representative peaks marked with black arrows.



Scheme 6.1: (a) Mechanism of thermal decomposition of the DCP into peroxide radicals during extrusion at $T=180^\circ\text{C}$ (initiation step), (b) Generation of CNC and PLA radicals followed by reactive extrusion at screw speed = 50 rpm and recycle time = 2 min (propagation step) leading to the formation of PLA-g-CNC structures (termination step). (c) Pictorial representation of the grafting mechanism of initiation, propagation and termination of the reactive extrusion process for PLA- g-CNC along the different zones of the extruder.

Similarly, the peak at $\sim 1454\text{ cm}^{-1}$ corresponding to CH_3 asymmetric bending for neat PLA and PLACNC1, shifted to $\sim 1464\text{ cm}^{-1}$ for the PLA-*g*-CNC gels and reprocessed rPLACNC samples. The shift in peaks of C–O–C stretching, $-\text{CH}_3$ rocking and bending is probably due to the grafting of PLA with CNCs alongwith the formation of high molecular weight cross-linked PLA-*g*-CNC branched nanocomposites [273]. The peaks at 2998 and 1362 cm^{-1} present in PLA and PLACNC1, corresponding to the $-\text{CH}$ vibration and stretching respectively, were significantly diminished in all the extruded samples (in the presence of DCP) (Figure 6.1(b) and (d)). In the case of the PLADCNC1 and rPLACNC1, the $-\text{CH}$ stretching peak of PLA at 1042 cm^{-1} was absent and a new peak at 1020 cm^{-1} corresponding to formation of new C–C linkage due to grafting between the PLA and CNCs was found. CNCs show their characteristic peaks at 1028 and 1203 cm^{-1} for the C–OH stretching and bending in plane at C-6 which were found to be present in the PLA-*g*-CNC gels and reprocessed samples, however, slightly shifted to 1020 and 1208 cm^{-1} respectively. Presence of such peaks confirms the formation of the grafting between the C(6)–OH of the CNCs with the methine groups of PLA (as shown in Scheme 6.1). Moreover, the carbonyl peak for pristine PLA shifted from 1748 cm^{-1} to 1740 cm^{-1} during extrusion (in presence of DCP only) (Figure 6.1(c)), due to the grafting of $-\text{CH}_2\text{OH}$ groups in CNCs onto the methine groups of PLA. ^1H NMR spectroscopy analysis of PLA-*g*-CNC samples (PLADCNC1) also showed new characteristic peaks at 3.68 ppm and in the $4.1\text{--}4.5\text{ ppm}$ range [397] (see Figure 6.2(a)) which confirms the grafting. The new peak at 3.68 ppm corresponds to the methine protons formed in the PLA-*g*-CNC structure (marked as ‘a’ in the inset of Figure 6.1(a)). Presence of such methine protons was also reported in case of PLA grafted maleic anhydride based wood flour composites and PLA grafted with polyethylene glycol systems in presence of DCP ($\sim 3.6\text{ ppm}$) [398, 399]. The peaks in the range of $4.1\text{--}4.5\text{ ppm}$

correspond to the methylene protons of the cellulose backbone, which confirms the presence of CNCs in the grafted structure [42]. In the ^1H NMR spectra of pristine PLA (Figure 6.2(b)), the peak at 3.68 ppm was found to be absent and the peaks at 5.19 and 1.46 ppm which represent the CH and CH_3 protons, were found to have no significant shift/changes in comparison to ^1H NMR spectra of PLA-g-CNC. The grafting efficiency was calculated for PLA-g-CNC under various CNC loadings (1–3 wt. %) by integrating the peak area at 3.68 ppm. It was found that the grafting efficiency was highest in the case of 1 wt. % CNC loading and decreased with increase in CNC loading, consistent with the calculations for gel yield as shown in Table 6.1. The peroxide radicals generated at high temperature react with both PLA and CNC under high shear conditions, abstracting hydrogen from both leading to the formation of stable micro radical structures (Scheme 6.1(b)). These radicals are highly reactive and diffuses effectively in molten PLA (during extrusion) thus generating higher concentrations of these radicals in the PLA phase. Due to the impermeable crystalline nature of CNC, the generated PLA radicals (containing DCP) could only react with surface $-\text{CH}_2\text{OH}$ groups of CNCs forming a thin overlapping layer of PLA surrounding the CNCs (Scheme 6.1(c)). Formation of such thin layer of PLA chains, grafted onto the CNC surface, through this radical approach is an instantaneous process and provides better interfacial compatibility between PLA and CNC. The FTIR peaks of PLA-g-CNC samples (see Figure 6.1(d)) showed that peaks corresponding to the amorphous segments of PLA at 1265 and 955 cm^{-1} disappeared while the low intensity peak at 1210 cm^{-1} , corresponding to crystalline segments of PLA [400], remained. The amorphous chains of PLA which are randomly entangled (in the molten state) on grafting with the CNCs organized into ordered positions along the surface of nanocrystals (which are highly ordered stacks of cellulose

(a) PLA-g-CNC

Chemical structure of PLA-g-CNC is shown, featuring a cellulose backbone with PLA chains grafted onto it. The chemical structure is labeled with 'a' and 'n'.

The ^1H NMR spectra are displayed below the structure. The top spectrum shows the cellulose backbone region (3.3-4.4 ppm) with peaks labeled 'a' and 'n'. The bottom spectrum shows the full range (0-10 ppm) with peaks labeled 'a' and 'n'. The peaks are assigned to the following chemical shifts (ppm): 7.28, 7.29, 5.21, 5.20, 5.19, 5.15, 5.16, 3.68, 1.70, 1.69, 1.60, 1.26, 1.24, 0.90, 0.89, 0.85, 0.84, 0.08, and 0.01.

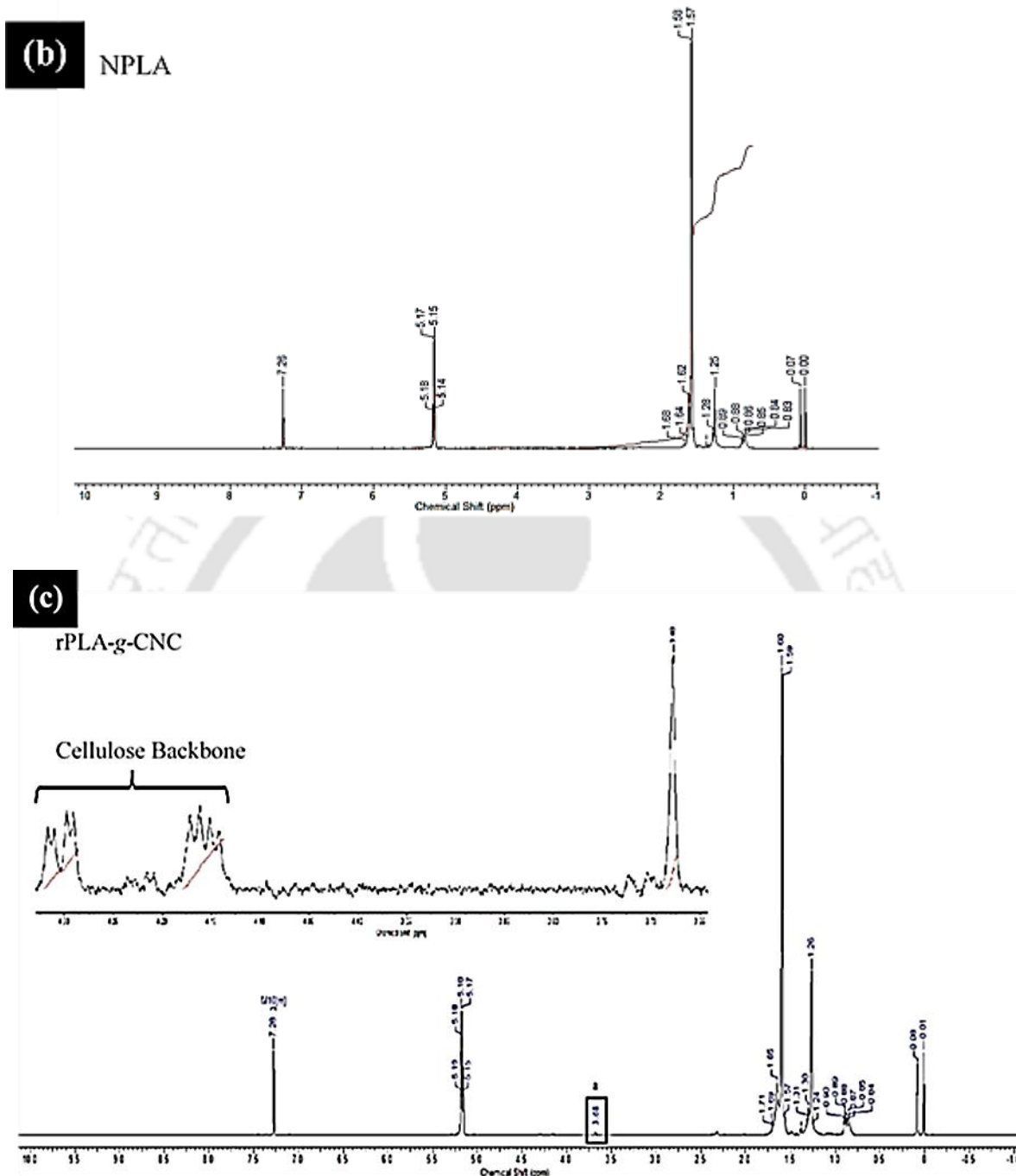


Figure 6.2: ^1H NMR spectra of (a) PLA-g-CNC (b) neat PLA and (c) reprocessed PLA-g-CNC films (rPLA-g-CNC) with 1wt. % CNC loadings.

Table 6.1: Calculated grafting parameters, namely gel yield percentage (Gel Yield), graft percentage (%GP), grafting efficiency (%GE) and weight conversion (%WC), for the reactively extruded PLA/CNC nanocomposite strips in the presence of DCP.

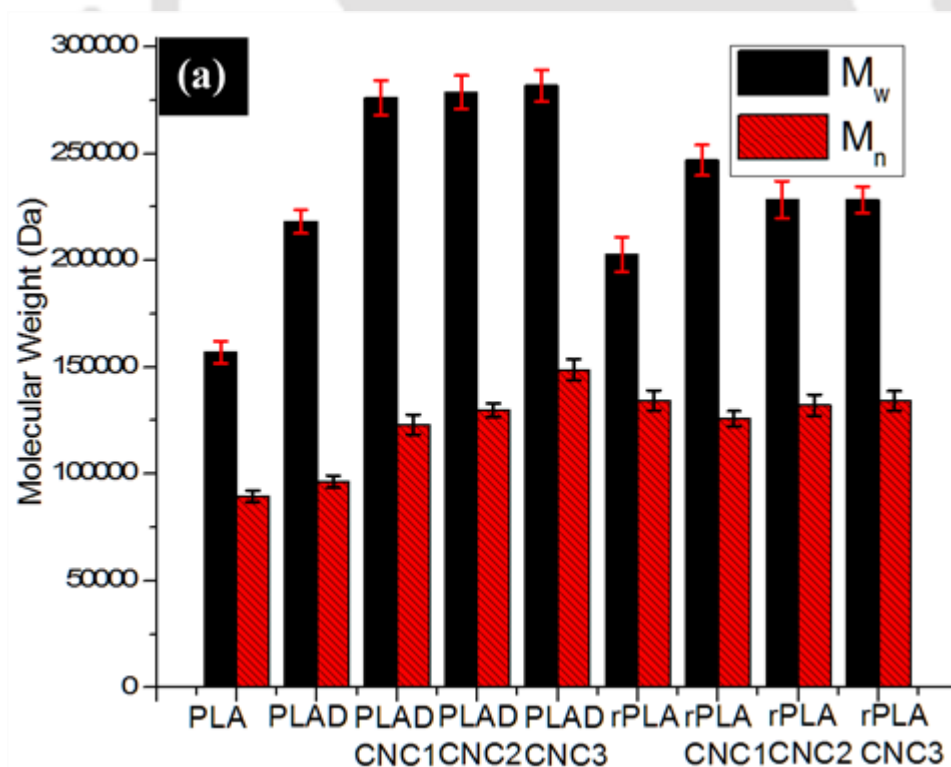
Samples	Gel Yield (%)	Graft (%)	Graft Efficiency (%)	Weight Conversion (%)	Specific Rotation (°)	Optical Rotation (°)
PLA	-	-	-	-	-158.37	-1.582
PLAD	52.2(±0.6)	-	-	50.8(±1.8)	-145.07	-1.509
PLADCNC1	74.2(±0.9)	7706.2(±10.3)	76.8(±2.9)	73.6(±2.1)	-137.24	-1.329
PLADCNC2	61.0(±1.2)	2900.1(±25.5)	57.6(±5.1)	59.6(±1.9)	-140.73	-1.419
PLADCNC3	58.1(±1.8)	1840.0(±21.3)	54.7(±2.8)	57.7(±1.7)	-145.22	-1.475

Figures 6.3(a) & (b) show the weight average (M_w) and number average (M_n) molecular weight and polydispersity index (PDI) of reactively extruded PLA/CNC films at different CNC loadings. Reactive extrusion of PLA with DCP in the presence of CNC (1–3 wt. % range) shows an increase in both M_w and M_n by ~40 %. Such drastic increase in MWD is however absent during reactive extrusion of PLA in the presence of DCP alone. The PDI of the PLA-g-CNC did not changed significantly and was found to be ~ 2, which suggests that the chain length of branched PLA were formed with uniform distribution even at different CNC loadings. The GPC chromatograms (Figure 6.4) for PLA-g-CNC were broader compared to those for PLA and show the presence of two small distinct shoulders both at high and low molecular weight regions. This can be attributed to two possible phenomenon occurring during the reactive extrusion process: chain extension in the presence of DCP (leading to chromatogram shift to high MWD region) and chain scission in the presence of CNCs (leading to shift to low MWD region). In the former case, as shown in Figure 6.4, the molecular weight corresponding to shoulder peak in PLA-g-CNC was found at least two times higher than neat

PLA. This indicates that the PLA chains cross-linked to form a tree-like or more complex branched structure [401]. The area of the high molecular weight peak (retention time ~ 11 min) increased and formed small shoulder for PLADCNC1 (~88%) and PLADCNC2 (~90%) in comparison to the neat PLA (~78%). The increase in MWD in the presence of CNCs is due to the presence of $-\text{CH}_2\text{OH}$ groups in CNC which act as active sites for the generation of radicals, which leads to the propagation and growth of the PLA chains on CNC surface (as discussed in Scheme 6.1(b) & (c)). However, on increasing the CNC loading (~3 wt. %), the area of the high molecular weight peak decreased (~70%) and that of the low molecular weight peaks (at retention time ~ 16.4 and 17.2 min) increased (~12 and 16% respectively). This is probably due to the presence of high concentration of sulfate groups or agglomeration of CNCs (at higher loadings), which interfere during reactive extrusion process thereby degrading the PLA at higher processing temperatures. It is noteworthy to mention that the area of low molecular weight fractions (at retention time ~16.7 and 17.3 min) for PLADCNC1 (~1.7 and ~10.3% respectively) and PLADCNC2 (~4 and 5% respectively) decreased compared to neat PLA (~8.4 and ~13% respectively) (Figure 6.5). This is probably because grafting of PLA chains on the $-\text{CH}_2\text{OH}$ groups of CNC moieties shields the sulfate and hydroxyl groups which are responsible for chain scission and alongwith it DCP acts as cross-linking agent which led to the increase in the molecular weight of the PLA chains due to propagation reaction (Scheme 6.1). Also, on comparing the GPC chromatograms of the NPLA and PLAD, it is found that reactive processing in presence of DCP helps in preventing the thermal induced degradation of PLA during extrusion process significantly.

- **Effect of Reprocessing of the PLA-grafted-CNC Gels on Structure and Molecular Weight.**

Extracted PLA-g-CNC gel was vacuum dried and subsequently extruded into strands using process conditions detailed in the experimental section. the reprocessed PLA-g-CNC samples for all weight percent of CNCs (1– 3wt. %) are represented as rPLA, rPLACNC1(1.35), rPLACNC2(3.33) and rPLACNC3(5.15) with the numbers in bracket representing the probable CNC wt. % present in the samples after the ungrafted PLA have been removed (calculated using eq. (2.14)). The FTIR and NMR spectra of the reactively extruded and reprocessed PLA-g-CNC composites did not show any significant difference in chemical structure (see Figure 6.1 and Figure 6.2 (c)), which is due to the presence of C–C bonds that are stable enough during reprocessing.



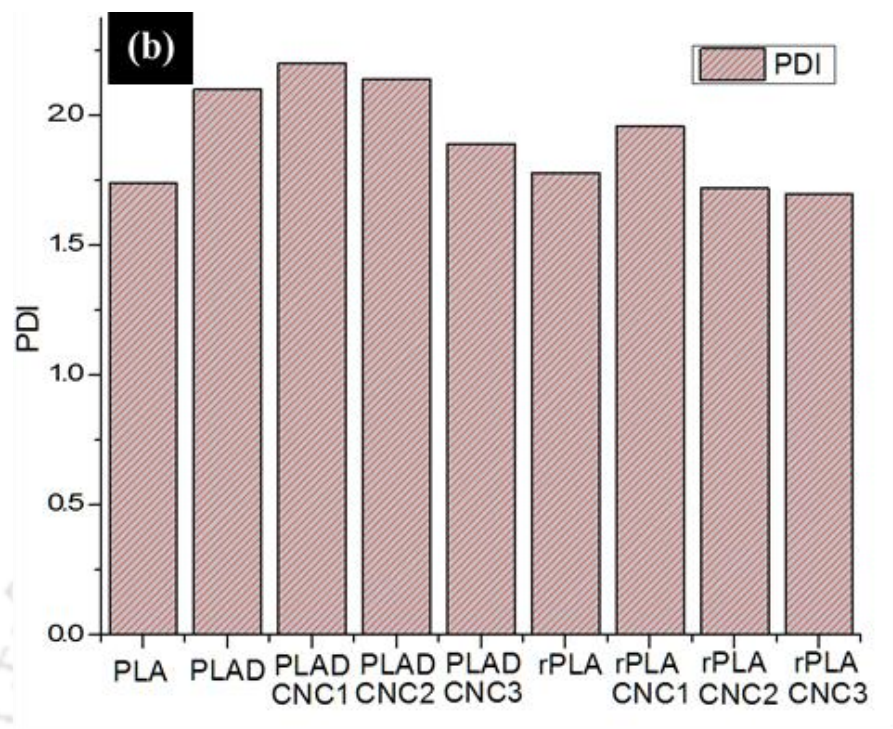


Figure 6.3: (a) Weight average (M_w) and number average (M_n) molecular weight and (b) polydispersity index (PDI), reactively extruded PLA/CNC nanocomposites.

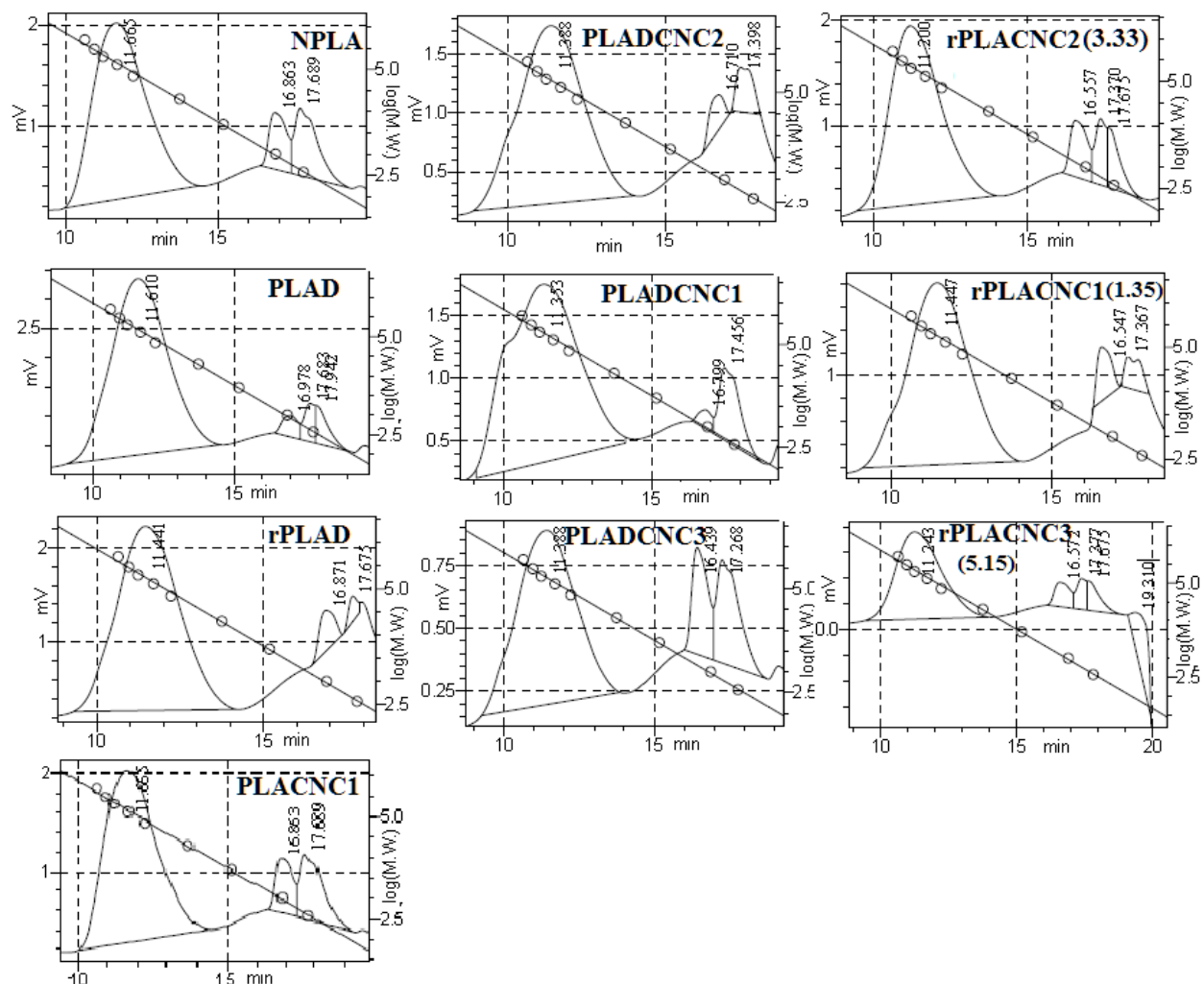


Figure 6.4: GPC chromatograms showing the molecular weight distribution of the high and low molecular weight peak fractions (at retention time of ~11 min and ~16-17 min respectively) for reactively extruded and reprocessed PLA-g-CNC samples.

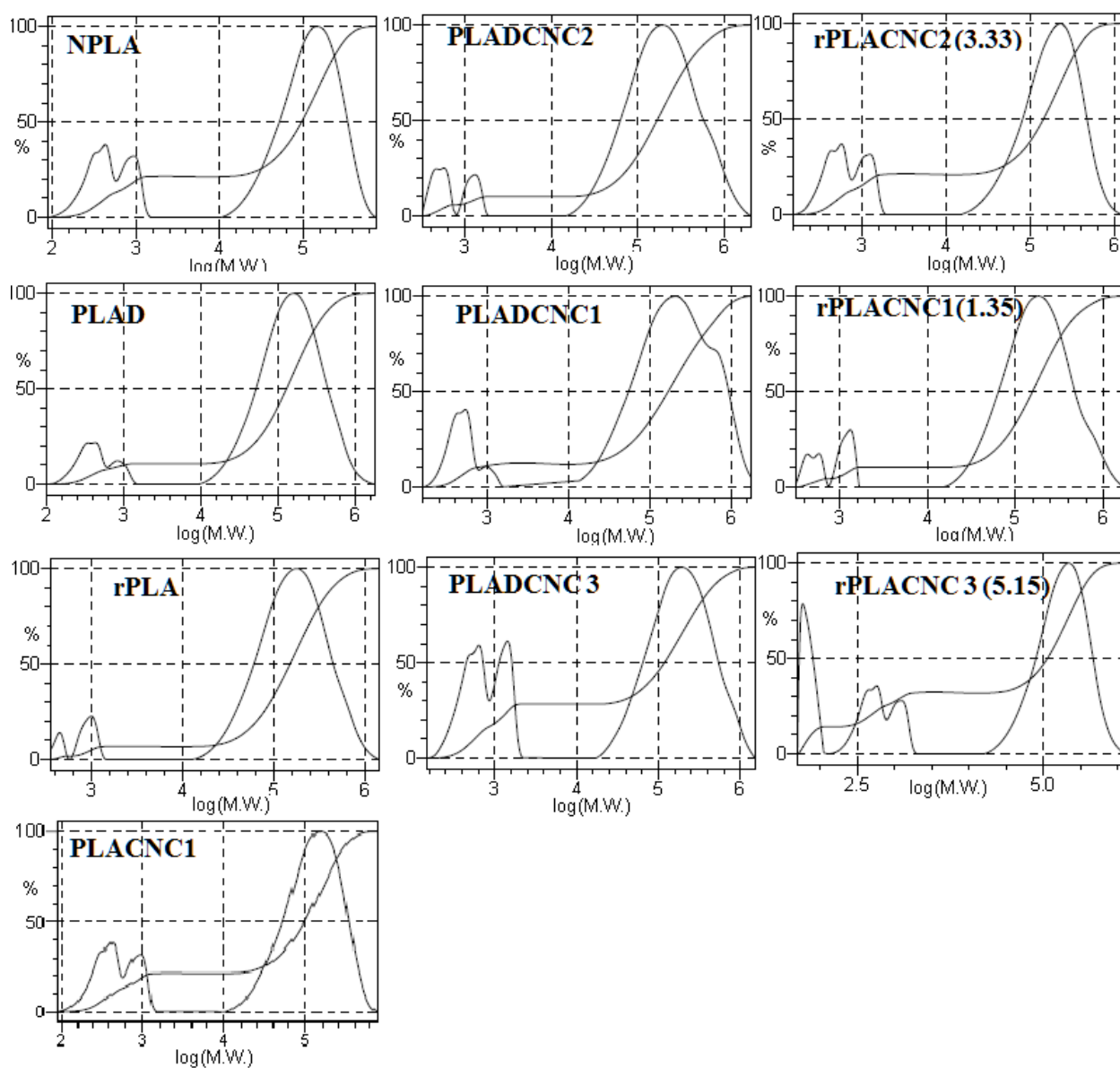


Figure 6.5: GPC chromatograms showing the weight fractions of high and low molecular weight segments for reactively extruded and reprocessed PLA-g-CNC samples.

GPC studies showed that recycling of the PLA-g-CNC gel led to decrease in M_w and M_n by ~18 and 15% only (at ~3wt. % loading) (Figure 6.3(a)), which is less compared to traditional PLA/CNC extrusion (~22 and 18% at 1wt. % CNC loadings during first time extrusion). The reactive extrusion based grafting approach effectively masks the interaction of sulfate and hydroxyl groups in CNCs with PLA, thereby preventing the drastic reduction in molecular weight. The PDI was found to be ~1.8 for recycled samples, which was lower than that of PLA-g-CNC probably due to chain scission of the long branched PLA chains during extrusion. However, after reprocessing the GPC chromatogram was found to be sharper, with a decrease in the shoulder area for high molecular weight peak and increase in number of peaks in the low molecular weight region. The area of the high molecular weight shoulder peak decreased from ~89 to 68% for 1 and 3 wt. % CNC loadings respectively. Moreover, three low molecular weight peaks were found (at retention time ~16.5, 17.3 and 17.6 min), whose area increased with the higher CNC loadings (~8.46, 6.4 and 6.1% respectively at 3 wt. % CNC loading) (Figure 6.4). This is possibly due to lower grafting efficiencies at ~3 wt. % CNC loadings, which enhances the thermal degradation of PLA due to presence of sulfate and hydroxyl groups. However, the decrease in MWD is not significant enough compared to the traditional approach of PLA/CNC processing and recycled polymers with $M_w \sim 250$ kDa finds potential engineering applications.

- **Morphological Studies of Reactively Extruded and Recycled PLA-g-CNC Films.**

Figure 6.6(a) compares the physical appearance of the reactively extruded PLA, PLADCNC1 and rPLACNC1(1.35) with the PLA/CNC 1 wt. % extruded by simple melt blending at similar processing conditions. Extrusion of the freeze dried CNCs with pristine PLA showed the formation of black agglomerated particles, which is probably due to the

degradation of the sulfate groups in CNCs at high shear and temperature. Moreover, the FESEM micrograph for the PLA/CNC1 nanocomposite processed through traditional approach showed the presence of large agglomerations of the freeze dried CNCs (marked in square box Figure 6.6(b)). However, the reactive extruded PLA-*g*-CNC strips were transparent and didn't produced such black agglomerated particles during extrusion, at all CNC loadings. Even the recycled strips were found to be stable and transparent. As explained earlier, the reactive extrusion being an instantaneous process, the radicals generated on CNCs surface reacts with PLA chains thereby shielding the sulphate groups which are responsible for degradation during extrusion process. Moreover, the presence of such PLA encapsulated CNC showed better interfacial compatibility with the PLA matrix which led to improved dispersion (as shown in Figure 6.6(c) and (d)). Figure 6.6(c), shows the presence of micro-gel like domains of branched PLA-*g*-CNC (in square box) alongwith the rod-like CNCs which are uniformly distributed in the matrix. Magnified micrograph of the circular region (inset Figure 6.6(c) at ~20KX) shows the presence of smooth interphase between the micro-gel domains of PLA matrix with CNCs. The CNCs fabricated through sulphuric acid hydrolysis were 700 ± 50 nm in length and 30 ± 8 nm in width. However, the dimensions of the PLA-*g*-CNCs after reactive extrusion changed significantly with increased length of $1.5 \pm .3$ μ m and diameter of 120 ± 8 nm. The increase in the dimensions is probably due to the presence of grafting between the hydrophilic CNCs with the hydrophobic PLA through reactive extrusion in presence of DCP. Re-extrusion of the extracted PLA-*g*-CNC gel, shows smooth PLA matrix with absence of the micro-gel domains in the interphase (Figure 6.6(d)). The freeze dried CNCs (marked in black arrows) are randomly distributed without any phase separation. It suggests that the

grafted CNCs even after reprocessing were stable and unaltered, which suggests its enhanced compatibility and dispersion in PLA matrix.

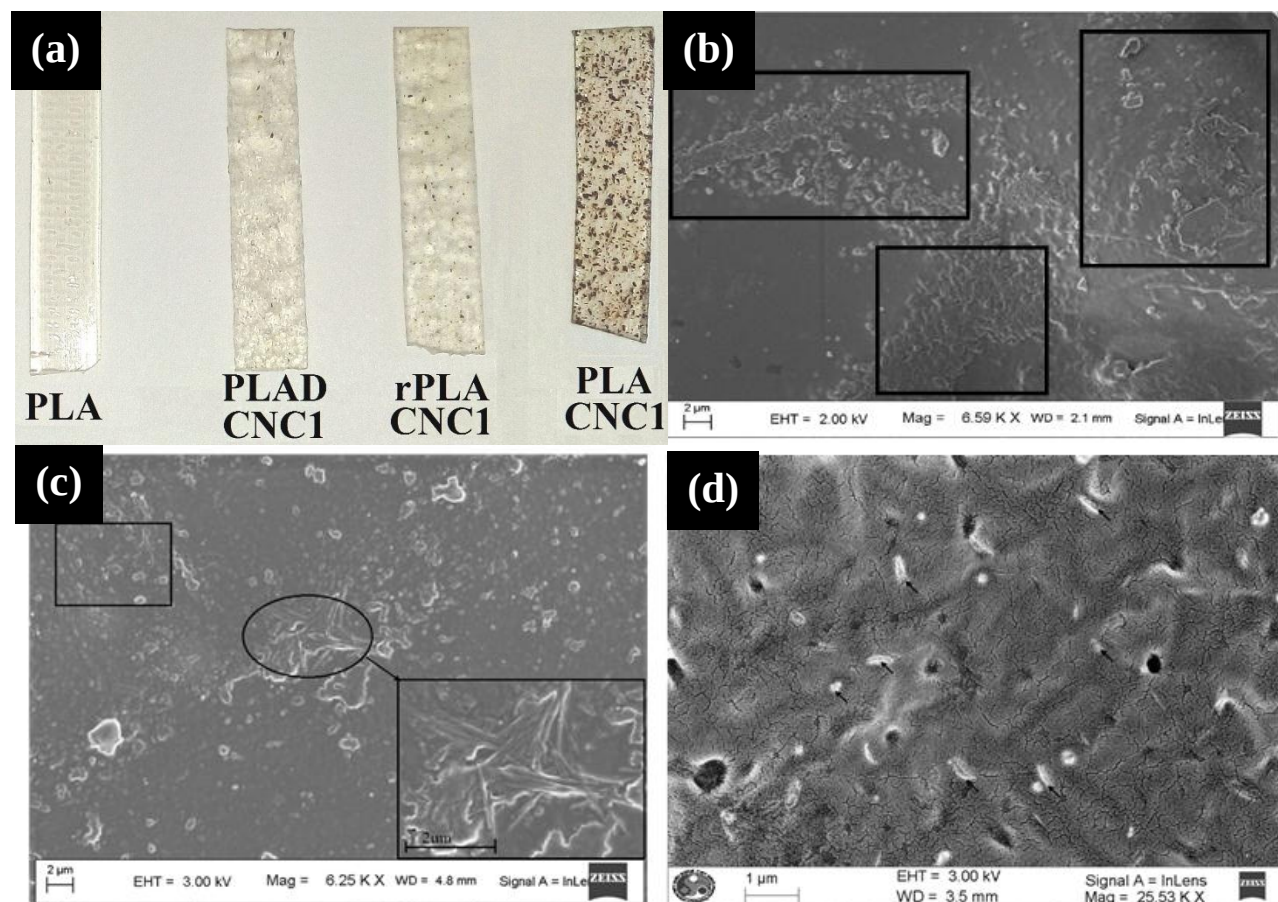


Figure 6.6: (a) Pictorial comparisons of the neat PLA, PLADCNC1 and rPLACNC1 strips with PLA/CNC 1wt. % extruded by simple melt blending (traditional approach), FESEM micrographs (b) PLADCNC1 and (c) PLADCNC1 and (d) rPLACNC1(1.35).

- Thermal Properties of the Reactively Extruded and Recycled PLA-g-CNC Films.**

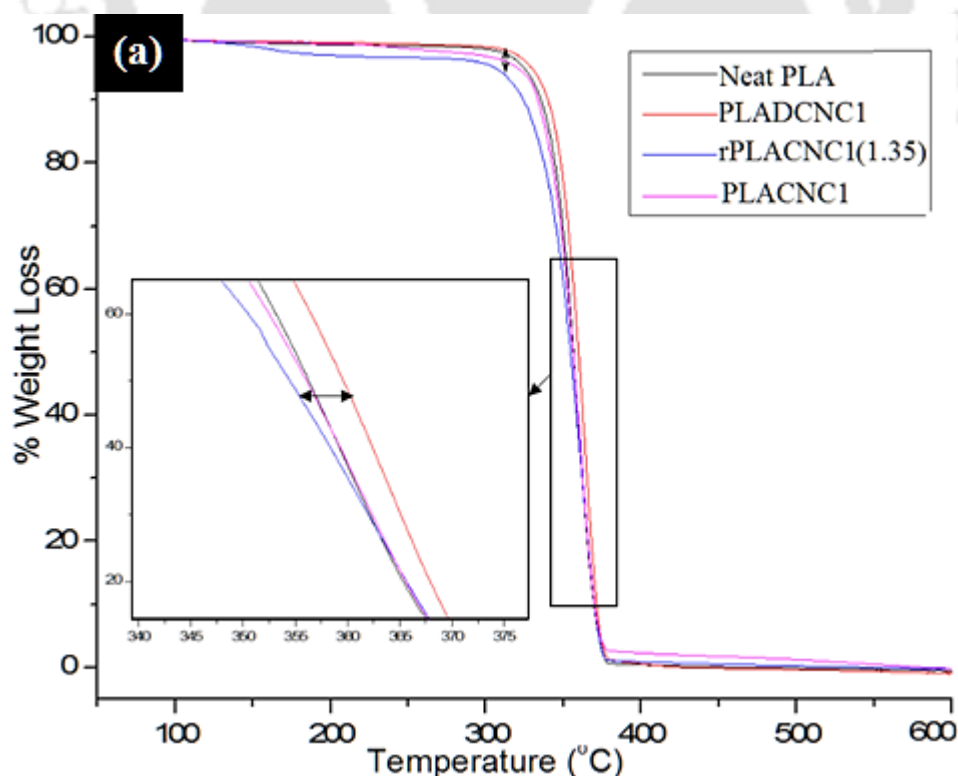
Thermal degradation behavior of PLA-g-CNCs before and after recycling are investigated to predict the onset degradation temperature (T_{onset}), temperatures at which 10% and 50% reduction occur ($T_{10\%}$ and $T_{1/2}$) and % reduction weight at 300°C. PLA showed a single step degradation profile, with a T_{onset} of ~306 °C and $T_{1/2}$ of 356°C which is due to hydrolysis and

oxidative chain scission of PLA chains (Figure 6.7) [278]. In our previous studies [17], simple dispersion of CNCs into PLA matrix didn't showed any improvement in the thermal properties. The PLACNC1 nanocomposite prepared through traditional approach showed a slight decrement in $T_{1/2}$ $\sim$ 1-2°C compared to neat PLA. However, grafting CNCs onto PLA in presence of small amount of DCP through reactive extrusion approach led to improvement in thermal stability of PLA. The T_{onset} and $T_{1/2}$ of PLADCNC1 was found to be improved by $\sim$ 12°C and $\sim$ 5°C respectively, compared to neat PLA (Figure 6.7(a)). The percentage reduction in weight during the initial processing (upto $\sim$ 300°C) was also reduced significantly (only $\sim$ 1.9 wt. %) compared to traditional process. This is due to the formation of PLA encapsulated CNCs initially during reactive extrusion, which masks the sulfate and hydroxyl groups of CNCs, thereby delaying the degradation process. Also, the formation of C–C bond between the PLA and CNCs leads to enhanced thermal stability as higher activation energy is required to break such bridged linkages. Reprocessing the PLA-*g*-CNC nanocomposites led to the formation of low molecular weight fractions due to chain scission under thermal stress conditions. This led to a decrease in the T_{onset} and $T_{1/2}$ of rPLACNC1(1.35), by 24°C and 8°C respectively, alongwith a reduction of $\sim$ 5 % weight at 300°C in comparison to PLADCNC1. Moreover, the reprocessed (rPLACNC1 (1.35)) sample shows a weight loss $\sim$ 4% at a temperature $\sim$ 150°C, which is probably due to the presence of the of low molecular weight PLA chains as evident from the GPC chromatograms in Figure 6.4 with peaks at retention time 16.7 and 17.4 min ($\sim$ 13.4 and $\sim$ 17.5% area of GPC chromatograms respectively) (Figure 6.4) . In comparison to the other PLA-*g*-CNC nanocomposites (Figure 6.7(a)), rPLACNC(1.35) has the highest fractions of the low molecular weight PLA chains($\sim$ 30% area of the GPC chromatogram), hence it showed a significant weight loss at $\sim$ 150°C. Presence of such low molecular weight fractions is probably

due to the chain scissions during thermal based recycling of PLA-*g*-CNCs in the extruder, which subsequently leads to decrement in the thermal properties. As shown in the inset of Figure 6.7(a), the reprocessed rPLACNC1(1.35) has almost similar degradation profile in the temperature range of 345–375°C. Therefore, it can be concluded that the thermal properties of the PLA- *g*- CNC films do not alter significantly on reprocessing which is desirable for product fabrication.

- **Crystallization Studies of the Reactively Extruded and Recycled PLA-*g*-CNC films.**

XRD and DSC studies are carried out to understand the effect of both grafting and branching in PLA-*g*-CNC nanocomposites on their crystal structures. XRD diffractogram for the neat PLA showed representative peaks at 16.4° and 18.8° (see Figure 6.7(b)), which correspond to the α -form of the PLA crystal with (010) and (110/200) planes [352]. Presence of such interaction probably leads to ordered arrangement of the amorphous PLA chains on the CNC surface, which subsequently enhances the crystallinity of the nanocomposites.



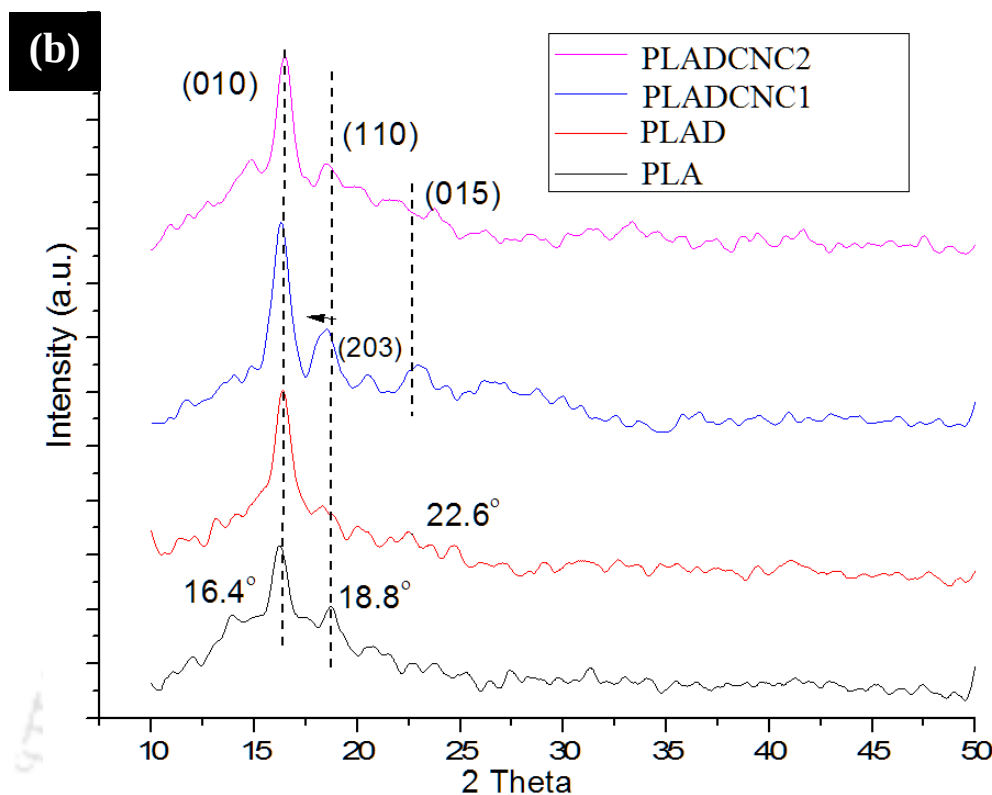


Figure 6.7: (a) Comparison of the thermogravimetric (TGA) plots for the neat PLA, PLADCNC1 and rPLACNC1(1.35) respectively with the inset showing the TGA profile in the temperature range of 345–375°C and (b) XRD diffractograms for the extruded neat PLA and reactively extruded PLA-g-CNC films, represented as PLAD, PLADCNC1 and PLADCNC2.

Similar observation of increase in crystallinity of the PLA based cellulose composites, due to uniformly dispersed cellulose microcrystal which in turn led to ordered arrangement of the PLA chains was reported by Lin et al. [402] and Mukherjee et al. [352]. The grafting was further confirmed by increase in d-spacing corresponding to the shift of peak $2\theta = 18.8^\circ$ for neat PLA to a lower 2θ value of 18.3° for PLA-g-CNC (2 wt. %) nanocomposites. Similar phenomenon of shift in 2θ values to lower angles was also observed by Choi et al. [398],

which suggests possible intercalation of CNCs into the PLA crystal structure during reactive grafting. The presence of CNCs in PLA-g-CNC films is confirmed from the low intensity peak at $2\theta=22.6^\circ$, corresponding to cellulose I crystal structure [17]. It might be due to the presence of high molecular weight PLA chains grafted onto the CNC surface, that the intensity of peak at $\sim 22.6^\circ$, is not very sharp in case of the PLA-g-CNC nanocomposites. The XRD diffractograms do not change significantly for the reprocessed rPLACNC films, which suggests that the crystal structure does not change significantly after recycling.

DSC studies were carried out to understand the effect of heating and cooling cycles on the crystallization dynamics of reactively grafted PLA/CNC nanocomposites, as shown in Figure 6.8; the corresponding thermal parameters are listed in Table 6.2. The glass transition temperature (T_g) of PLA was found to be $\sim 61.6^\circ\text{C}$. On grafting CNCs reactively with PLA, the change in T_g was not significant (from second heating cycle) in comparison to neat PLA, probably because grafting restricted the motion of PLA chains. PLA grafted CNCs have shown lower T_g (first heating cycle) in comparison to neat PLA; which was suppressed with increase of CNC loading. This is probably due to the presence of ungrafted PLA chains or grafting of small PLA chains onto CNCs which have enhanced mobility thereby decreasing the T_g . Moreover, presence of branching leads to formation of inter-spacing between the PLA chains, which undergoes shrinkage during first heating cycle thereby increasing chain mobility and decreasing T_g [403]. The specific heat (C_p) values corresponding to the change in the slope of T_g thermograms is determined through peak integration for all the PLA-g-CNC samples. Subsequently, the percentage of PLA chains that are grafted onto CNCs (% g-PLA) are calculated from the ratio of the C_p values for corresponding PLA-g-CNC samples to the C_p value of the neat PLA (Table 2). PLADCNC1 has the highest g-PLA value of 66%, which

is in-line with its graft efficiency and gel yield of 40.7 and 74.2% respectively. It was found that on increasing the CNC loading, both the grafting efficiency (calculated from gel fraction) and g-PLA (calculated from DSC) decreases. It is noteworthy to mention that such approaches for determining the weight percentage of polymers grafted on CNCs have been reported elsewhere [273,404]. The decrease in g-PLA is probably due to the agglomeration of CNCs at higher loadings, which reduces the effective $-\text{CH}_2\text{OH}$ groups available for grafting of PLA chains. We can vary grafting length of PLA chains on CNC surface by tuning the reactive extrusion process conditions, which in turn can result in significant change in polymer properties.

Figure 6.8(a) and (b) shows the DSC thermograms for the first cooling and second heating cycles of reactively extruded PLA-g-CNC films. In the cooling cycle, neat PLA does not show any exothermic peak corresponding to the crystallization process. However, on reactive grafting of CNCs, sharp intense peaks corresponding to crystallization can be seen during the cooling cycle. This is due to the grafting of amorphous PLA segments on the CNCs through C–C crosslinks, which act as nucleating sites for crystal growth. The crystallization peak for PLAD occurs at 127.3°C which decreases to 124.3°C with increase in the CNC concentration (~ 3 wt. %). At higher CNC loadings, the % gel yield decreases and only 50% of PLA chains are grafted onto CNC surface; therefore, the ungrafted chains undergo rearrangement to overcome the energy barrier to form crystals at lower temperature. Formation of sharper crystallization peaks alongwith higher ΔH_c values (~ 29 J/g) with increased CNC loadings (~ 3 wt. %) also suggest that higher fractions of ungrafted PLA chains have rearranged to form crystals [403]. The crystallinity (X_c) of extruded PLA films is found to be $\sim 28\%$. With incorporation of 2 wt. % CNCs, the % crystallinity (X_c) values increased slightly, because of

the formation of C–C grafted sites with the amorphous sections of PLA which also led to improved dispersion of CNCs. Figure 6.8(b), shows slight change in the slope near T_g with complete absence of the cold crystallization peak for PLA-g-CNC in comparison to the neat PLA. Occurrence of such phenomenon is probably due to formation of enhanced cross-linked structures of the amorphous PLA with the CNC domains which restricts the amorphous polymer chain mobility to form crystalline lamella. The high density of cross-linked PLA chains with crystalline CNC through C-C bond formation, leads to better interfacial adhesion, subsequently reduces the interspaces causing reduction in the free volume. During the 2nd heating cycle, relatively more amorphous PLA chains became crystallized at $T_{cc} \sim 125^\circ\text{C}$; however, cold crystallization peaks were absent in case of grafted PLAs for all CNC loadings due to already developed crystallites during the first cooling as shown in Figure 6.8(a). These thermographs clearly depict that the activation energy of chain folding is lower in PLA-g-CNC compared to pristine PLA due to which grafted PLA chains could overcome the energy barrier and crystallize upon cooling whereas neat PLA chains could not [384]. The melting peaks for PLA-g-CNCs showed a narrow and sharper peak compared to PLA which suggests formation of stable and uniform size crystals during reactive extrusion process. These findings are in line with GPC studies where relatively uniform MWD of the PLA-g-CNC chains was observed due to which these samples show the single melting phenomenon compared to pristine PLA.

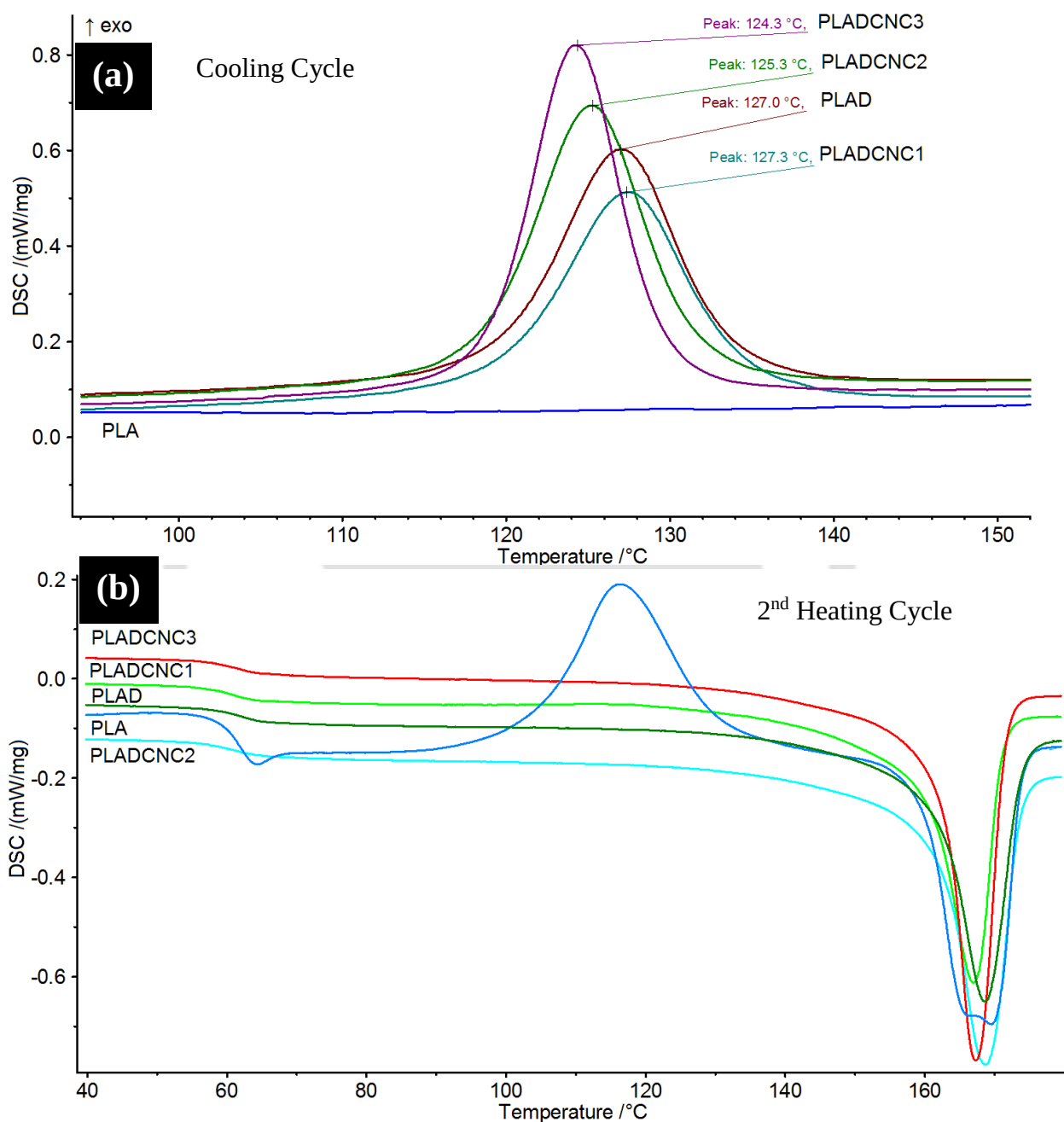


Figure 6.8: DSC thermograms for the (a) first cooling cycle and (b) second heating cycle for the reactively grafted PLA-g-CNC nanocomposites.

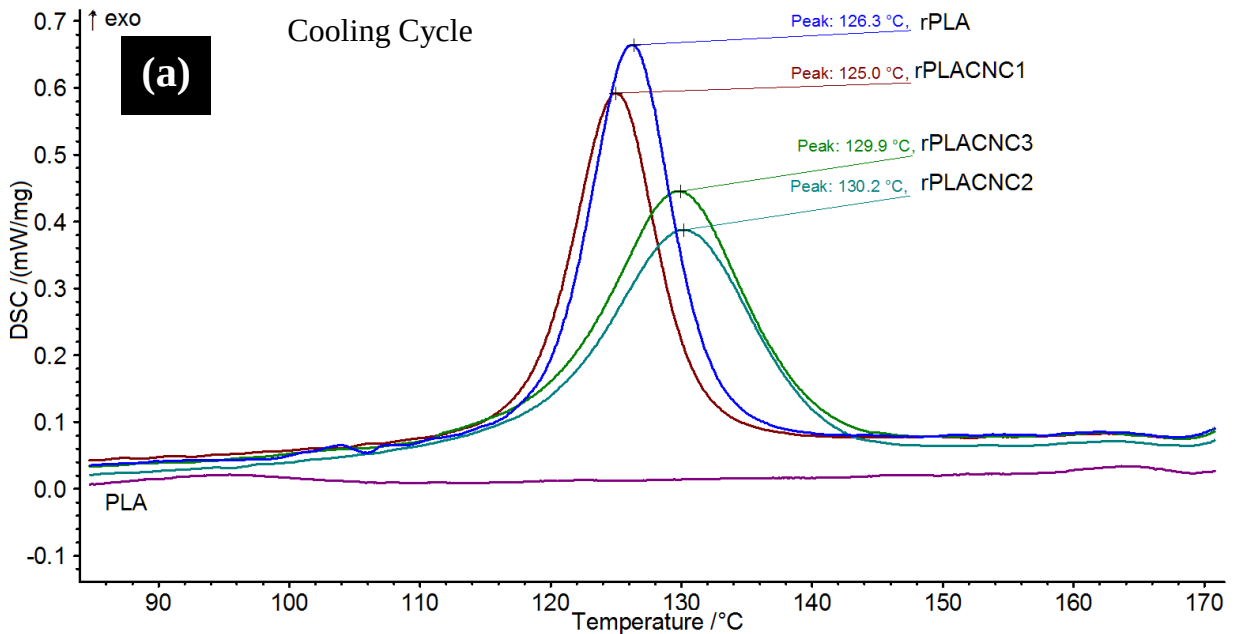
Table 6.2: Thermal and crystallization properties of the reactively extruded PLA-g-CNC nanocomposites and reprocessed PLA-g-CNC gels.

Sample	T _g (°C)	ΔC _{p,Tg} (J/g.°C)	T _{cc} (°C)	ΔH _{cc} (J/g)	T _c (°C)	ΔH _c (J/g)	T _m (°C)	ΔH _m ^a (J/g)	X _c (%)	g-PLA (g-rPLA) (%)
PLA	61.6	1.638	112.5	26.2	-	-	170.2	-26.1	28.1	100
PLAD	54.5	0.780	91.6	13.8	127.0	27.4	168.6	-29.2	31.3	47.6
PLADCNC1	52.7	1.080	88.0	12.1	127.3	24.0	165.5	-31.6	34.0	66.0
PLADCNC2	55.0	0.823	93.3	16.6	125.3	28.8	169.1	-32.5	35.0	50.2
PLADCNC3	55.8	0.875	93.5	15.5	124.3	28.9	169.8	-31.2	33.5	53.4
rPLA	53.0	0.944	91.5	14.7	126.3	29.3	169.0	-29.2	31.3	-
rPLACNC1 (1.35)	57.0	0.885	94.2	14.1	125.0	26.0	169.7	-26.7	28.7	54.0 (93.7)
rPLACNC2 (3.33)	56.0	0.850	90.6	11.7	130.2	24.7	167.7	-30.6	32.9	51.9 (90.0)
rPLACNC3 (5.15)	55.8	0.781	90.6	12.9	129.9	29.2	166.4	-31.0	33.3	47.6 (82.7)

(where T_g= glass transition temperature, ΔC_{p,Tg}=specific heat corresponding to glass transition temperature calculated from first heating cycle, g-PLA=represents the % of PLA chains grafted onto CNC surface (measured as the ratio of ΔC_{p,Tg} (for PLA-g-CNC gels)/ ΔC_{p,Tg} (for PLA)), g-rPLA= represents the % of PLA chains grafted onto CNC surface after reprocessing of the PLA-g-CNC gels (measured as the ratio of ΔC_{p,Tg} (for reprocessed rPLACNC nanocomposites)/ ΔC_{p,Tg} (for rPLA)), T_{cc}= cold crystallization temperature, ΔH_{cc}= enthalpy for cold crystallization, T_c= crystallization temperature (cooling cycle), T_m= melting temperature, ΔH_m^a= melting enthalpy corresponding to 2nd heating cycle, ΔH_m^p= melting enthalpy for 100% crystalline PLA was taken as 93 J/g [26] and X_c=degree of final crystallinity calculated from ratio of ΔH_m^a and ΔH_m^p.)

Further, DSC analysis was done for extracted PLA-g-CNC gel domains which are reprocessed under similar extrusion conditions (Figure 6.9(a) & (b)). The re-extruded PLA- g- CNC showed drastic reduction in the % g-PLA and X_c values (Table 6.2), which is due to chain scissions under high shear thermal stressed conditions. The % of PLA chains grafted onto the CNC surface after reprocessing of the PLA-g-CNC gels (g-rPLA) was calculated from the ratio of C_p value of the reprocessed rPLACNC nanocomposite to the C_p value of reprocessed PLA corresponding to their T_g values. The reduction in g-rPLA values suggests possible debonding/ degradation of the high molecular weight amorphous PLA chains

grafted on the CNC surface, which subsequently leads to decrease in X_c . The crystallization peak temperature increases with increase in CNC loading for the reprocessed samples. It is probably because on re- extrusion of PLA/CNC nanocomposites the fraction of gel domains containing agglomerated CNCs increased and it underwent molecular weight reduction due to thermal degradation. Presence of such agglomerations and gel fractions hindered the crystallization phenomena which led to shift in $T_c \sim 126.3^\circ\text{C}$ for rPLA to 130.2°C for rPLACNC2(3.33). However, the reprocessed PLA/CNC nanocomposites do not show any significant alteration in melting behavior compared to PLA-g-CNC. Therefore, it can be concluded that while reactive grafting of CNCs leads to significant change in the crystallization properties, both the integrity and stability of the crystal structures are maintained after reprocessing.



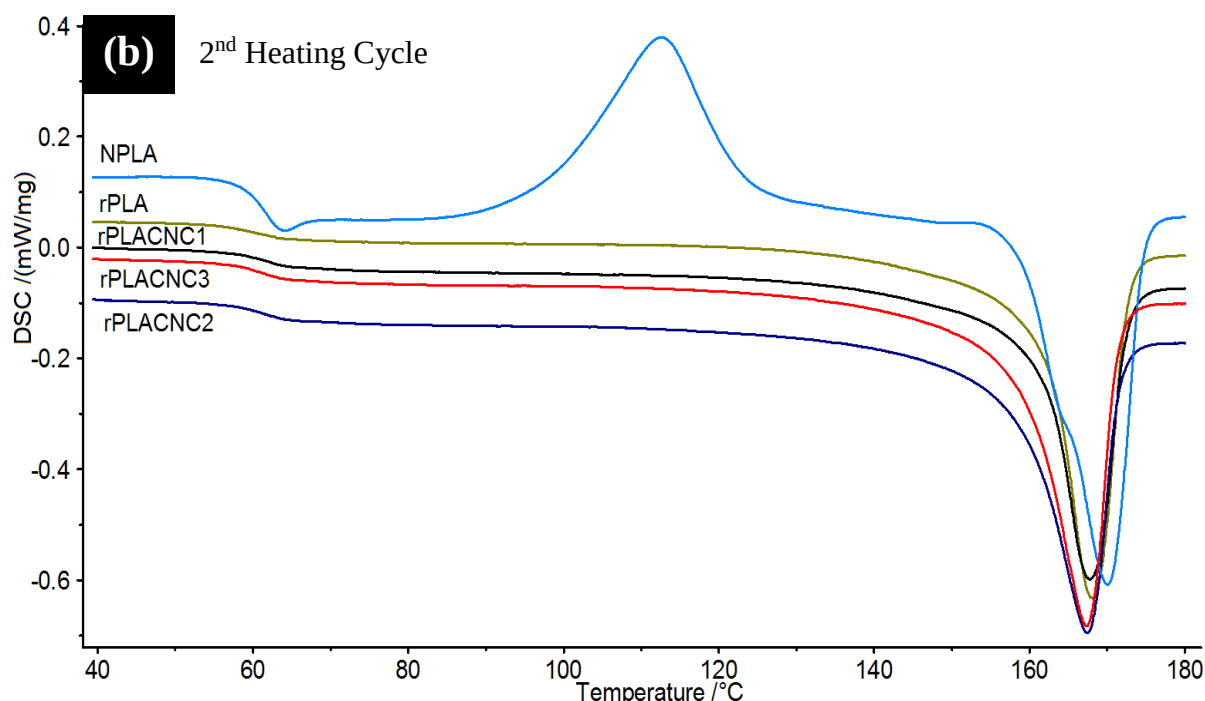


Figure 6.9: DSC thermograms for the (a) first cooling cycle and (b) second heating cycle for the re-extruded PLA-g-CNC gels.

- **Mechanical Property Studies of Reactively Extruded and recycled PLA-g-CNC Films.**

The mechanical properties of *in situ* reactive compatibilized PLA-g-CNC were investigated to study the effect of grafting nanofillers like CNC in the presence of DCP. The neat PLA extruded strips have an ultimate tensile strength (UTS) of 36.2 MPa and elongation at break of 9%, comparable with the reported literature [405]. The PLA extruded strips with 1wt. % of CNC (PLACNC1) in the absence of the DCP as cross-linking agent, showed improvement in the UTS Young's modulus (YM) by ~14 and ~100% respectively. Reactive grafting of CNCs into PLA matrix led to significant increase in the tensile strength and YM of nanocomposite by ~41% and ~490% respectively (Figure 6.10(a) & (c)). However, the elongation at break is reduced significantly (by ~ 70%) for all the loading fractions of CNCs

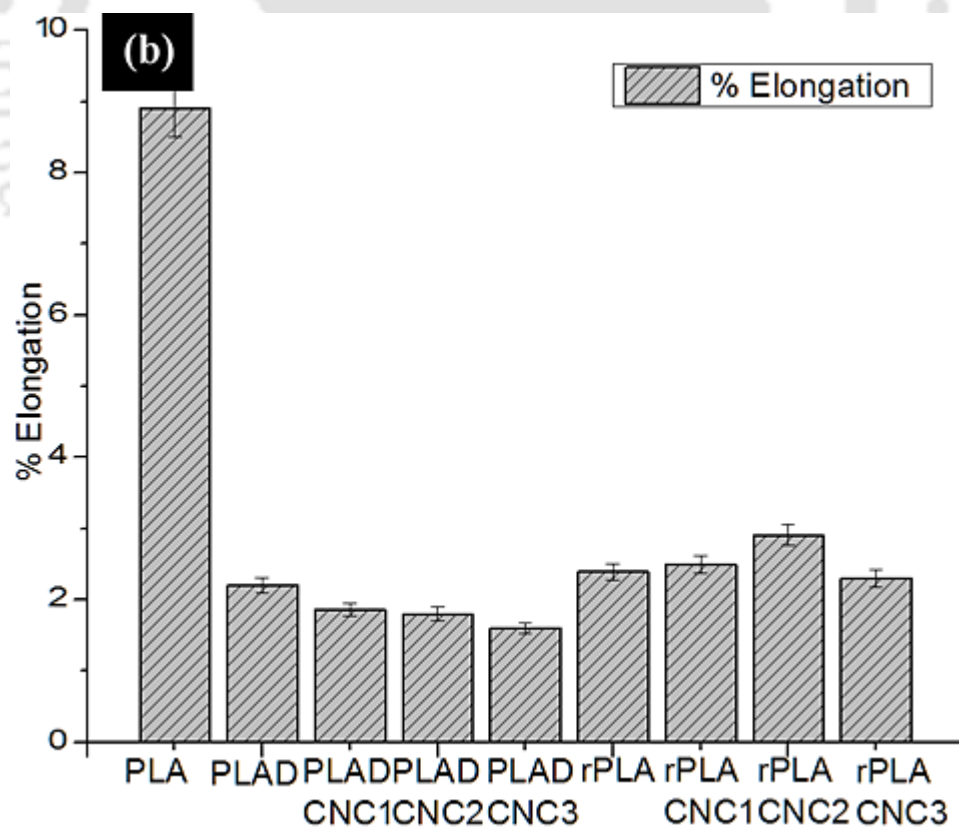
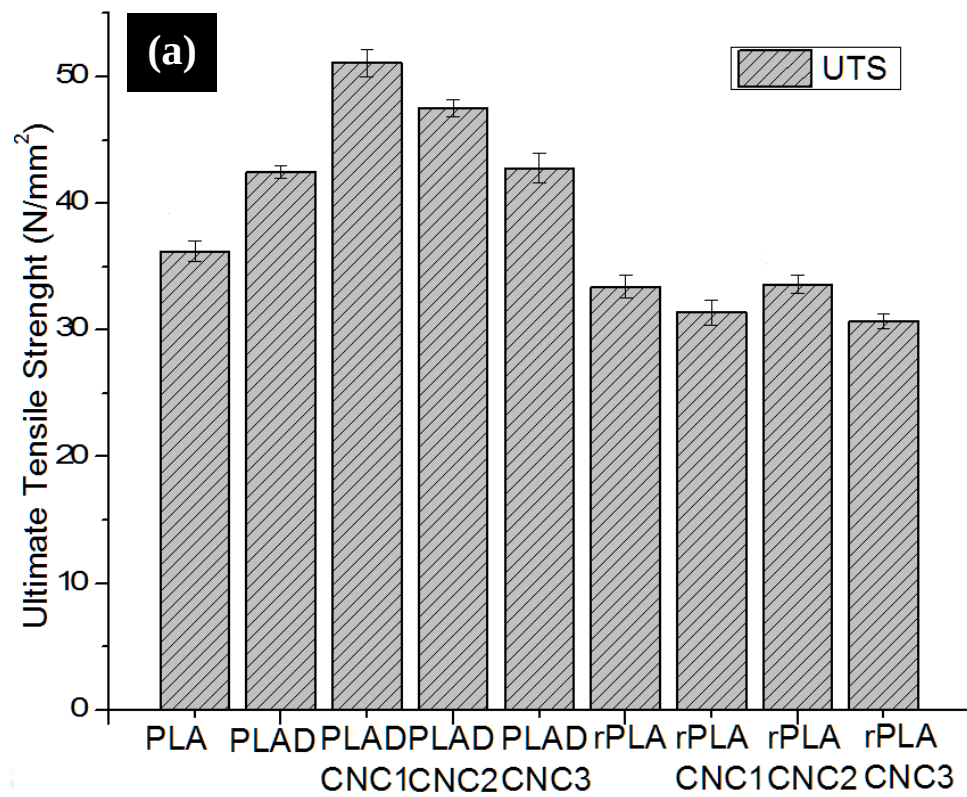
studied. Restriction in the elongation may be due to the crosslinking between CNCs and the polymer chains through the formation of C–C bond linkages (Figure 6.10(b)). However, the improvement in modulus can be explained by the following two phenomena: firstly, reactive grafting of PLA chains on CNC surface leads to uniform dispersion and enhances interfacial adhesion of PLA-modified-CNCs with the bulk PLA matrix (as explained in previous section), and secondly, the formation of C–C bonds between the PLA and CNC could act as an effective linkage for transferring the developed stresses (transverse elastic modulus of 18–50 GPa and anisotropic elastic modulus of 140–220 GPa [297] of individual CNCs) between them. Also, increase in the MWD during reactive extrusion and formation of branched network like structures in PLA-*g*-CNC probably contributes to the increase in the modulus. Moreover, as explained from the FTIR studies, grafting of crystalline segments of CNCs probably took place on the amorphous domains of PLA, which subsequently led to increase in the crystalline fractions in the PLA-*g*-CNC nanocomposites (further confirmed by XRD studies). Presence of such crosslinking with the amorphous segments restricts the free mobility of PLA chains to dissipate energy under tensile force, resulting in the increased brittleness and reduction in the % elongation at break. However, on increasing the % CNC loading (up to ~3wt. %) in PLA- *g*- CNC nanocomposites, grafting efficiency decreases due to agglomeration of CNC which subsequently leads to deterioration of mechanical properties. It is noteworthy to mention that similar phenomenon of decrease in elongation for such cross-linked structures was also observed in case of reactive extruded polyhydroxybutyrate and polypropylene samples [406].

When the extracted PLA-*g*-CNC gels were reprocessed into strips under identical processing conditions (at 180°C, 50 rpm and retention time of 5 mins), the UTS and modulus of the rPLA-*g*-CNC films decreased by ~28 and 56% respectively (Figure 6.10 (a) & (c)). This

is probably because degradation of bulk PLA chains (both branched and linear) takes place either during thermal reprocessing or due to the presence of sulfate groups on the agglomerated CNCs (at higher loadings), as confirmed by the MWD studies. It is noteworthy to mention that the UTS for the reprocessed samples at different wt. % CNC loadings are comparable to neat PLA or PLACNC1, probably due to shielding of sulphate groups. Presence of grafted PLA chains on the CNC surface helped overcome the problems related to thermal induced PLA degradation during extrusion in presence of CNCs especially at higher loadings. MWD analysis of the reprocessed PLA-*g*-CNC films shows decrease in M_w and M_n by ~18 and 15% respectively and decrease in the area of the high molecular weight peaks from ~89 to 68% (Figure 6.4), which could be responsible for the decrease in mechanical properties. However, the increase in overall content of the CNCs are represented in brackets for the reprocessed PLA-*g*-CNC samples (due to removal of ungrafted PLA chains while washing). It could help in improving the UTS of the nanocomposites but its contribution towards the nanocomposites have been found to be negligible. The reprocessed films showed slight improvement in elongation at break (by ~75%), probably because of the presence of low molecular weight fractions (increase in the area of low molecular weight fractions after reprocessing) which act as plasticizers under tensile load. The UTS and modulus values of the reprocessed/recycled PLA-*g*-CNC films are comparable or even better than PLA (especially Young's modulus). Therefore, the approach of grafting PLA chains onto CNCs through reactive extrusion process allows the recycling of PLA-*g*-CNC nanocomposite films even after service life.

Fracture behavior of the stretched PLA-*g*-CNCs films prepared using original and reprocessed PLA/CNC samples was studied. FESEM micrographs of PLADCNC1 films showed presence of large polymer fibrillation which is caused by the formation of enhanced

cross-linking (Figure 6.11(a)). Small micro-gel beads of PLA-*g*-CNC were also found alongwith fibrils, consistent with reported studies for reactively grafted polymers [384]. Branching of PLA chains in the presence of DCP leads to formation of entangled fibrils which hinders early matrix failure, thereby increasing its toughness effectively. Therefore, it could be concluded that both enhancement in interfacial adhesion and formation of such entangled branched structures are responsible for the improvement in mechanical properties of PLA- *g*- CNC. However, on reprocessing of the extracted gels, rPLACNC1(1.35) films showed smooth prominent rigid lines parallel to the direction of the tensile load (Figure 6.11(b)). Presence of such aligned fibrils in reprocessed films is due to the mobility of branched polymer chains which causes certain improvement in the elongation properties. Therefore, it can be concluded that reactive extrusion of PLA/CNC nanocomposites by varying the DCP and CNC concentrations will lead to different degree of chain grafting and branching which can be used to tune the mechanical properties.



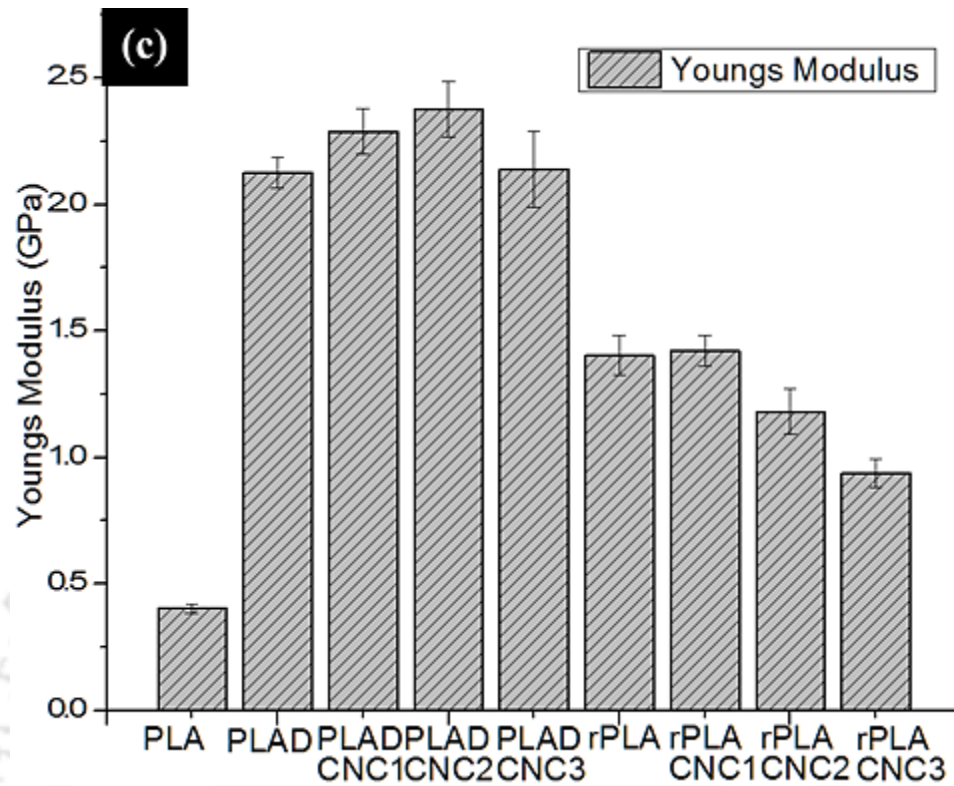


Figure 6.10: Mechanical properties: (a) ultimate tensile strength, (b) % elongation and (c) Young's modulus of the reactively extruded PLA-g-CNC nanocomposites and reprocessed PLA-g-CNC gels.

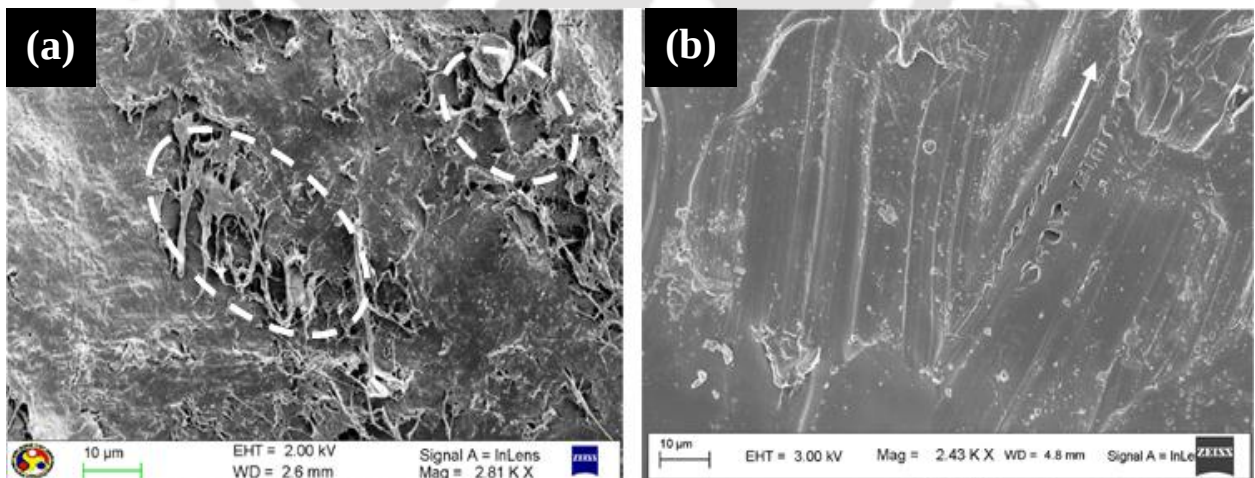


Figure 6.11: FESEM micrographs of the fractured samples of (a) PLAD/CNC1 and (b) rPLA/CNC1(1.35).

- **Thermo-mechanical studies of reactively extruded and recycled PLA-g-CNC films**

Dynamic mechanical analysis (DMA) was carried out to measure the change in the storage modulus (E') and $\tan(\delta)$ before and after reprocessing of the PLA-*g*-CNC nanocomposites (at ~2 wt. % CNC loading) over a temperature range of 25–90°C (Figure 6.12(a) & (b)). The E' increased on incorporation of CNC (at 2 wt. %) in presence of DCP due to the formation of cross-linked structure and better interfacial adhesion with the polymer matrix. E' values for reactively grafted CNCs is found to be significantly higher than the ungrafted PLA/CNC films. This suggests that grafting of CNCs with polymer through the formation of the C–C bond leads to effective transfer of CNC modulus to the PLA matrix. The effectiveness of the grafted CNCs as reinforcement agent is further measured by the filler effectiveness coefficient C_{FE} :

$$C_{FE} = \frac{\left(\frac{E'_g}{E'_r}\right)_{comp}}{\left(\frac{E'_g}{E'_r}\right)_{matrix}} \quad (6.1)$$

where E'_g and E'_r represent the storage moduli for the glassy and rubbery state of PLA and the PLA-*g*-CNC nanocomposite, measured at constant frequency of 1Hz and at temperature 46°C and 85°C respectively [278]. The nanofillers which have lower C_{FE} values are considered to be the most effective reinforcing agent for the given polymer matrix. The C_{FE} values for the PLA/CNC films with ~2 wt. % CNC (PLACNC2) fabricated through traditional approach was 0.836, compared to 0.721 for the reactively grafted PLA-*g*-CNC (at ~2 wt. %). Therefore, it can be concluded from the C_{FE} data that effective dispersion of CNCs could be achieved using the proposed reactive grafting approach.

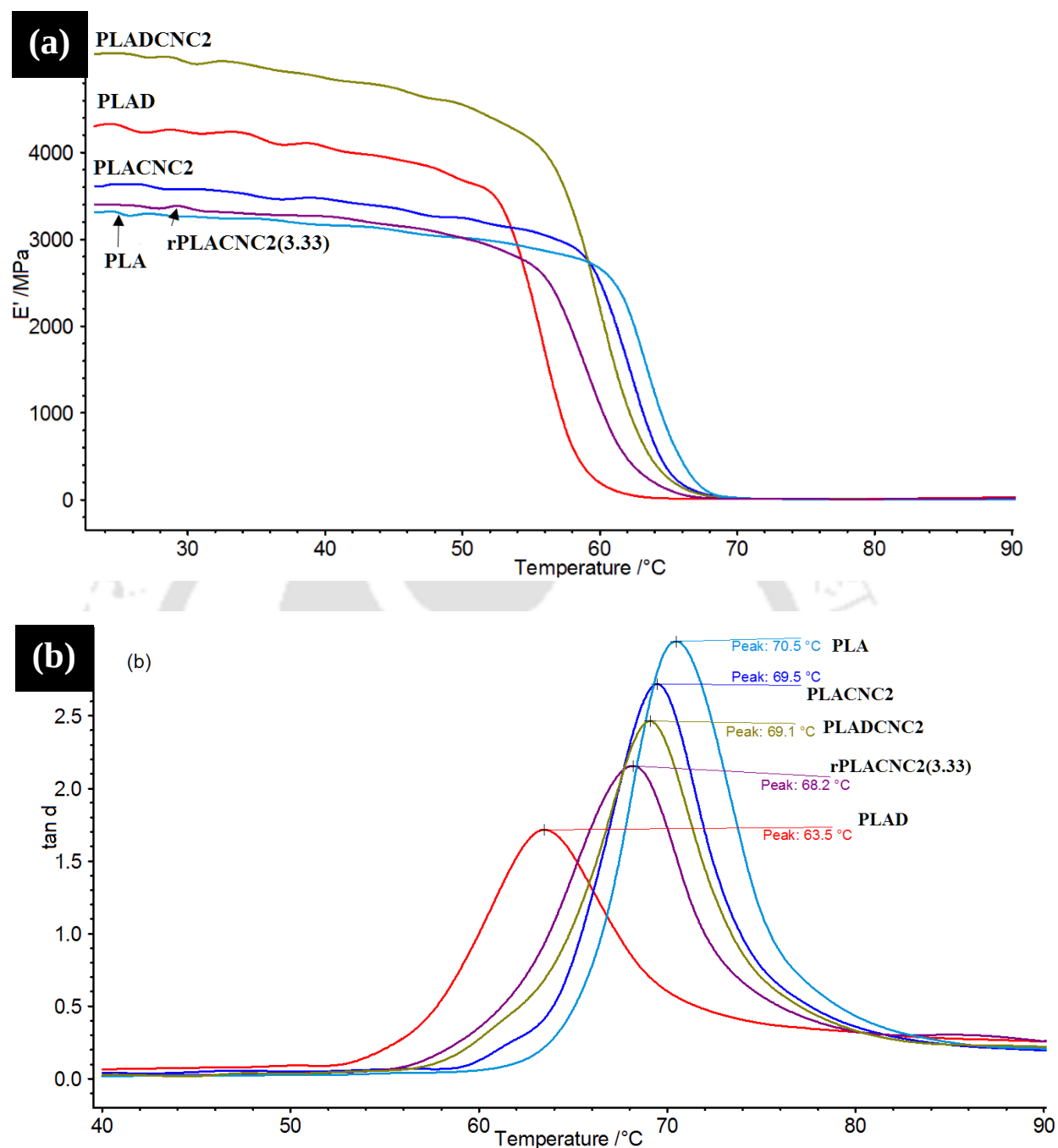


Figure 6.12: (a) DMA and (b) $\tan \delta$ plots for the reactively grafted and reprocessed PLA- g- CNC films (~ 2 wt. % CNC loading).

Figure 6.12(b) shows the comparison of the $\tan (\delta)$ plots for which the peak temperature was selected to be the glass transition temperature for the nanocomposite. It is found that the

glass transition temperature decreases for the reactively extruded PLA-*g*-CNC nanocomposites in comparison to the neat PLA. This is probably because the cross-linked PLA-*g*-CNC gels usually undergoes heat shrinkage at T_g (as explained in previous section), which depends on the degree of branching as per the reported literature [403]. The decrease in T_g was further confirmed by DSC analysis and similar phenomenon has been previously observed for the reactive grafting of maleic anhydride on PLA in the presence of DCP [385].

On reprocessing E' decreases significantly, probably due to the thermal degradation which is accompanied by a decrease in the molecular weight and formation of large fractions of low molecular weight chains which are less effective at storing energy under tensile load. However, C_{FE} values do not change significantly during reprocessing of the PLA-*g*-CNC films suggesting that there is no significant change in the grafted CNC structure, as discussed earlier. Therefore, it can be concluded from the thermo-mechanical studies that reactive grafting of CNCs results in the formation of thermally stable and reprocessable PLA films suited for high performance engineering applications.

6.2.2 Reactive Grafting of PLA-based CNC Nanocomposites in the Presence of Various Compatibilizers:

- **Reaction Mechanism, Grafting Parameters and Molecular Weight Studies**

PLA/CNC nanocomposites are chemically grafted through reactive extrusion approach inside a pilot plant scale extruder–cum–sheet casting unit, the degree of grafting was, however, tuned by the presence of different types of compatibilizers. The different compatibilizers used in this study are classified into four categories depending upon their chemical functionalities: (i) different acid derived CNCs (fabricated using sulphuric (CNC-SO₄) and hydrochloric acids (CNC-Cl)), (ii) biopolymers (such as cellulose and starch) which are chemically active with

abundant hydroxymethyl groups, (iii) inorganic/organic nanofillers (clays such as montmorillonite (MMT), alumina, nanosilica and carbon fiber) which are not chemically interacting during reactive extrusion process and (iv) vegetable oils (such as coconut oil (coco) and olive oil) with both saturated and unsaturated fatty acid chains are chemically reactive during extrusion process (abbreviation mentioned in Materials and Methods, section 2.2.7). DCP used as grafting agents decomposes into peroxide radicals (with half-life of ~ 180 s) under high temperature and shear conditions inside a twin-screw extruder. As reported in our previous study [367], the generated peroxide radicals abstracts nascent hydrogen from both PLA and CNC surface which leads to formation of chemically reactive radical moieties. The different compatibilizers used in this study have various chemically interacting or non-interacting active sites which significantly altered the radical activity and its propagation behavior. The abundantly generated radicals on PLA surface (in melt phase) causes PLA chains to undergo branching alongwith the formation of cross-linked structures with both CNCs and compatibilizers, which alters the grafting parameters and mechanism significantly. Therefore, reactive grafting of PLA/CNCs with compatibilizers led to the formation of micro gel-like domains that were separated-out from the unreacted PLA chains to predict the grafting parameters such as percentage gel yield, graft efficiency and graft percentage (Table 6.3 and 6.4). Among two acid derived CNCs, CNC-Cl shows comparatively higher gel yield and graft efficiency (GE) of $\sim 39\%$ and 38% respectively. The decrease in the grafting percentage for PLA/DCNC-S is probably due to presence of the sulfate groups which are known to degrade PLA during extrusion as well as subdue the chemical grafting process [407]. Incorporation of biopolymers such as cellulose and starch (as compatibilizers) increases the gel yield and grafting efficiency to $\sim 28\%$ and 35% respectively, probably due to the presence of abundant

hydroxymethyl groups which are known to act as active sites for grafting of PLA chains [367] (Scheme 6.2(ii)). This suggests that DCP radicals can generate radical moieties from both the CNCs and as well as from the compatibilizers, if they have any chemically interactive sites. The inorganic/organic nanofillers such as MMT, alumina, nanosilica and carbon fiber lacks any such sites, from where protons could be abstracted by the generated DCP radicals. Nanosilica and clays, such as MMT are composed of aluminum or magnesium silicate hydroxides [408] which are found to inhibit the grafting process with reduced gel yield and GE percentage of ~16 and 14% respectively. This is probably because the Al and Mg metal oxides and hydroxides are known to act as catalyst for initiating the depolymerization of PLA backbone during melt extrusion process [409], due to which the grafting process is inhibited. Vegetable oils used in this study have significant variation in their chemical composition with ~70% unsaturated fatty acids in case of olive oil and ~90% of saturated fatty acids in coconut oil. The unsaturated oils are expected to be more reactive in generation of radicals which can subsequently graft with either the PLA or CNC radicals. However, the gel yield and graft efficiency were comparatively lower for PLA/DCNC-S/Olive (~19% and 17% respectively) than PLA/DCNC-S/Coco (~58% and 56% respectively). Such decrease in the grafting parameters is possibly due to the rancidification process which leads to hydrolysis of oils into shorter chains [410]. Further, olive oil may chemically react with CNC or PLA radicals and interfere with the grafting between PLA and CNCs, which are responsible for the formation of the gel fractions. Interestingly, both CNF and coconut oil are found to have increased gel fraction yield of ~68% and 58% respectively. Both CNF and coconut oil are known to undergo enhanced dispersion in PLA matrix and shows improved physical interaction with the hydrophilic CNCs. Both of these compatibilizers synergistically improves the dispersion of

CNCs in polymeric matrix, enhancing the number of grafting sites between PLA and CNCs which subsequently increases the gel yield. Therefore, the extent of chemical grafting during reactive extrusion could be tuned through incorporation of various compatibilizers which are expected to alter the physio- chemical and structural properties of fabricated nanocomposites.

Processing of PLA based CNC nanocomposites through industrially viable melt extrusion approach (under high temperature and shear conditions) leads to significant degradation in molecular weight which subsequently limits their application in fabricating value-added products. In this study, melt extrusion of PLA in the presence of CNC-SO₄ and CNC-Cl through traditional process with pilot plant scale extruder shows a drastic drop in M_w by~ 55% and 72% respectively and M_n by~66% and 82% respectively (compared to that of pristine PLA). Such deterioration in molecular properties can be effectively controlled through the reactive extrusion process in presence of radical initiator DCP which simultaneously improves the dispersion characteristics of CNCs in PLA matrix [367]. Furthermore, to understand the proposed reactive extrusion process in detail, we used different compatibilizers (such as clays, alumina, nanosilica, starch etc.) that are known to have improved interfacial interaction with PLA but causes its degradation during melt processing. Reactive extrusion of PLA/CNC nanocomposites with CNC-SO₄ and CNC-Cl in presence of the DCP radical initiator shows improvement in molecular weight values alongwith the percentage area of the gel permeation chromatograms (at lower retention times corresponding to high M_w fractions). PLA/DCNC-Cl shows enhancement in M_w by~71% and 79% and M_n by~74% and 83% at 1 and 2 wt. % loadings respectively, compared to the traditional approach of extrusion (for PLA/CNC-Cl-1p). However, for PLA/DCNC-S the M_w increased by~48% and 46% and M_n by~35 and 8% at 1 and 2 wt. % loadings respectively, which was lower than enhancement observed for the

PLA/DCNC-Cl system. CNCs hydrolyzed with sulphuric acid have higher substitution of the hydroxymethyl groups with the sulphate moieties forming $\text{CH}_2\text{-SO}_3\text{H}$ end groups, which are absent in case of CNCs hydrolyzed with hydrochloric acids where the $\text{CH}_2\text{-OH}$ end groups are in excess [102]. Presence of such sulphate moieties are known to acts as catalyst for depolymerization of PLA and simultaneously inhibits the grafting reaction, thereby, decreasing the M_w and M_n values (in comparison to PLA/DCNC-Cl). From GPC chromatograms (Figure 6.13) it can be observed that the percentage area of high molecular weight peak (at retention time $\sim 11\text{min}$) increases for both PLA/DCNC- $\text{SO}_4\text{-1p}$ ($\sim 78\%$) and PLA/DCNC-Cl-1p ($\sim 82\%$) in comparison to neat PLA ($\sim 72\%$), suggesting lower degradation of PLA chains into oligomeric units during reactive based melt processing. Introduction of biopolymers such as cellulose and starch increases the number of grafting sites (due to increases in hydroxymethyl groups) which significantly improves the M_w by $\sim 49\%$ and 53% and M_n by $\sim 54\%$ and 29% respectively. This suggests that the reactive extrusion based approach could be used to effectively disperse hydrophilic bio-fillers into hydrophobic polymers with significant improvement in the molecular properties. The inorganic /organic nanofillers which are known to degrade the PLA during extrusion through traditional approach [409,411,365] are used to understand their effect on PLA/DCP/CNC nanocomposites during reactive extrusion. Incorporation of alumina or clays such as MMT did not show any inhibitory effects or decrement in M_w and M_n values of the fabricated nanocomposites. As discussed previously, vegetable oils with higher degrees of unsaturation such as olive oil show lower grafting efficiency and comparatively less improvement in the molecular properties (but comparable to PLA). These observations suggest that the inhibitory effects of nanofillers i.e. the degradation of PLA chains could be overcome to some extent through the proposed novel reactive extrusion

process. However, certain nanofillers such as nanosilica or CNF and coconut oil shows remarkable improvements in the M_w values by ~58%, 60%, 59 % and M_n by ~51%, 38%, 56 % respectively. This is probably due to the synergistic effects of both CNCs and nanofillers that improves the dispersion of CNCs in PLA and thereby causes increase in both gel fraction as well as molecular weight. Therefore, incorporation of different compatibilizers can effectively tune the grafting mechanism with improved gel fraction yield and molecular weight characteristics of the prepared nanocomposites, required for fabrication of commercial products.

Table 6.3: Prediction of the molecular weight distribution, weight average (M_w) and number average (M_n) and polydispersity index (PDI) of extruded PLA, reactively extruded PLA/CNC nanocomposites in presence of different compatibilizers.

Samples	M_w	M_n	High M_w (% Area)	Low M_w (%Area)	PDI
Neat PLA extruded	182000	124000	72.9	27.1	1.46
<i>Acid derived CNCs</i>					
PLA/CNC-S-1p	81408	41261	65.9	34.1	2.36
PLA/CNC-Cl-1p	85578	45673	75.5	24.5	2.25
PLA/DCNC-S-1p	158000	63600	78.2	21.8	2.48
PLA/DCNC-S-2p	152400	45000	85.6	14.4	3.38
PLA/DCNC-Cl-1p	180600	84400	82.1	17.9	2.14
PLA/DCNC-Cl-2p	245000	127800	83.1	16.9	1.91
<i>Biopolymers</i>					
PLA/DCNC-S/Cellulose	160500	91000	88.5	11.4	1.76
PLA/DCNC-S/Starch	180700	104900	81.1	18.8	1.72
<i>Inorganic/Organic Nanofillers</i>					
PLA/DCNC-S/MMT	169000	97500	87.5	12.5	1.78
PLA/DCNC-S/Alumina	153600	75700	94.3	5.7	2.02
PLA/DCNC-S/Nanosilica	195600	85500	85.1	14.9	2.28
PLA/DCNC-S/CarbFiber	207900	67500	84.0	15.9	3.07
<i>Vegetable Oils</i>					
PLA/DCNC-S/Coco	200400	68700	89.3	10.7	2.91
PLA/DCNC-S/Olive	160600	95900	87.1	12.8	1.67

(The High and Low M_w represents the fractional area covered by GPC chromatograms at retention time of ~10-11 and ~16-17 minutes, respectively shown in Figure 6.13).

Table 6.4: Predicted grafting parameters, namely gel yield percentage (Gel Yield), graft percentage (%GP), grafting efficiency (%GE) and weight conversion (%WC), for the reactively extruded PLA/CNC nanocomposite in the presence of different compatibilizers.

Samples	Gel Yield (%)	Graft (%)	Graft Efficiency (%)	Weight Conversion (%)	Specific Rotation (°)	Optical Purity (%)
Neat PLA extruded	-	-	-	-	- 156.2	100
<i>Different Acid derived CNCs</i>						
PLA/DCNC-S-1p	26.2	2536.6	25.3	26.3	-130.6	83.6
PLA/DCNC-S-2p	25.1	1155.1	23.1	25.1	-131.5	84.1
PLA/DCNC-Cl-1p	39.9	3800.3	38.0	39.0	-140.8	90.1
PLA/DCNC-Cl-2p	30.4	1420.3	28.4	30.4	-141.2	90.3
<i>Biopolymers</i>						
PLA/DCNC-S/Cellulose	29.8	1390.4	27.8	29.1	-124.7	79.8
PLA/DCNC-S/Starch	36.6	1730.5	34.6	36.6	-148.4	95.0
<i>Inorganic/Organic Nanofillers</i>						
PLA/DCNC-S/MMT	16.2	710.2	14.2	16.2	-141.2	90.3
PLA/DCNC-S/Alumina	26.3	1214.5	24.3	26.3	-124.7	79.8
PLA/DCNC-S/Nanosilica	16.4	720.4	14.3	16.3	-125.7	80.4
PLA/DCNC-S/CarbFiber	68.9	3347.6	67.0	68.9	-148.4	95.0
<i>Vegetable Oils</i>						
PLA/DCNC-S/Coco	58.0	2758.1	55.9	58.0	-141.7	90.7
PLA/DCNC-S/Olive	19.3	865.7	17.3	19.3	-121.6	77.8

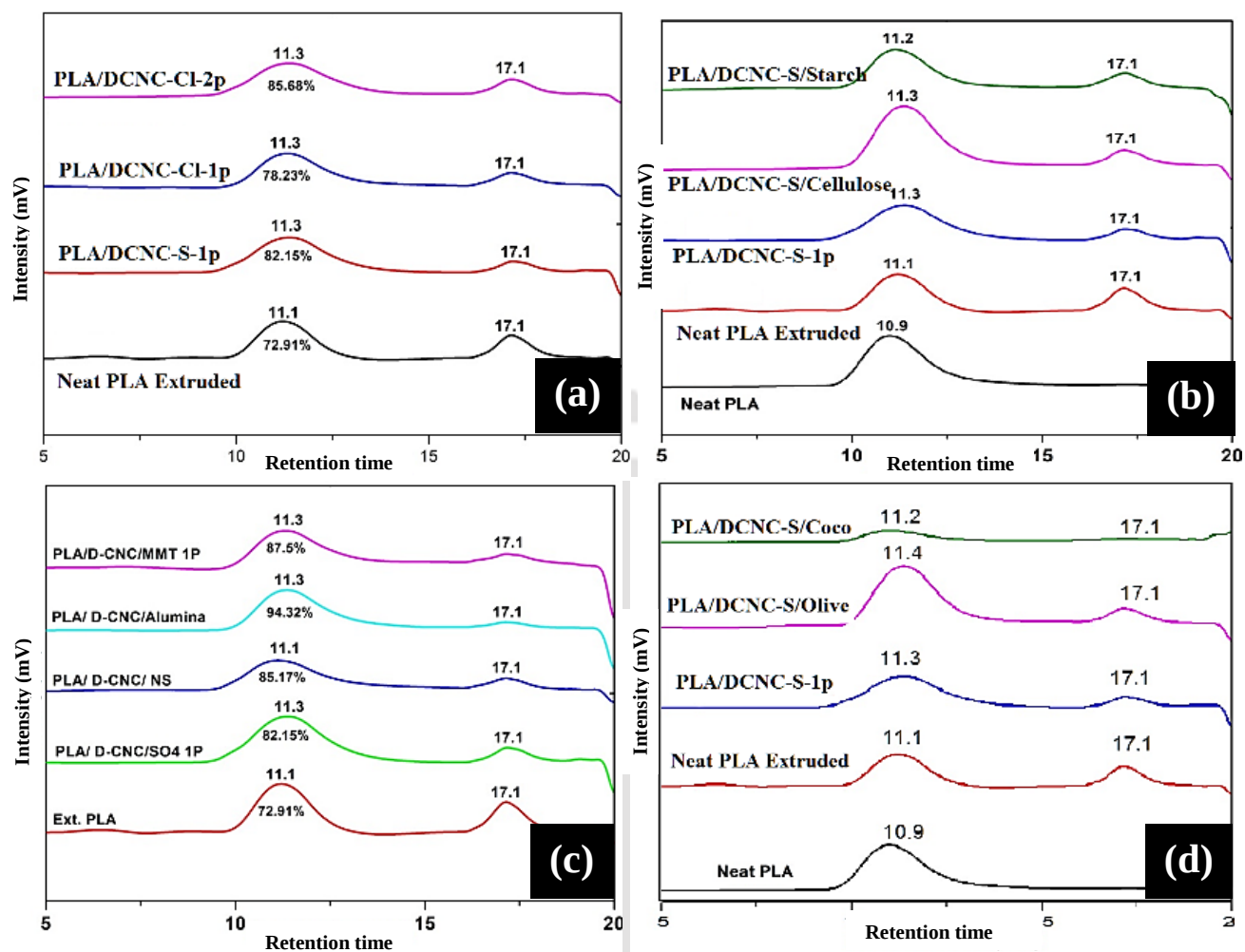
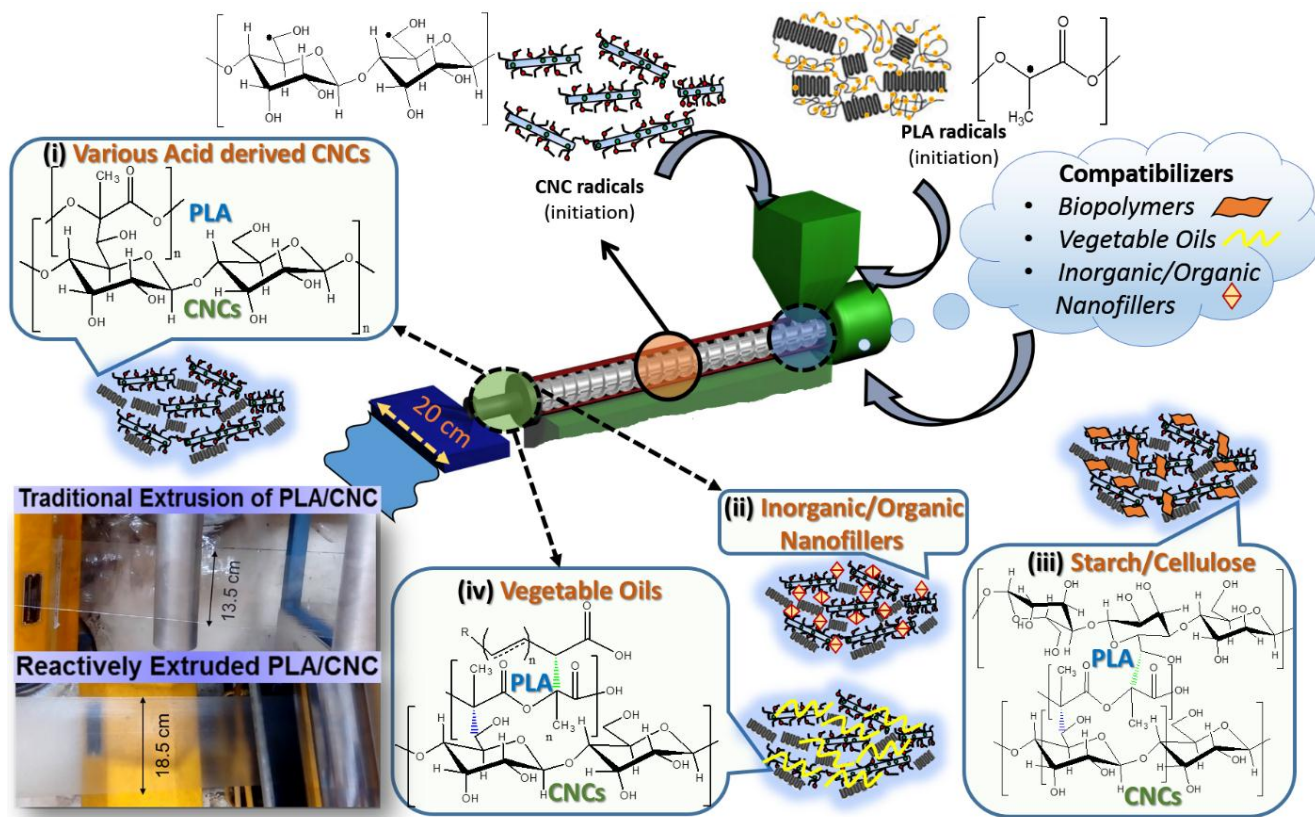


Figure 6.13: Gel permeation chromatograms (GPC) showing the molecular weight distribution of the high and low molecular weight peak fractions (at retention time of ~10-11 min and ~16-17 min respectively) with their weight fractions for reactively extruded PLA/CNC nanocomposites in presence of different compatibilizers.



Scheme 6.2: Mechanistic investigations on the generation of the radicals onto the PLA and CNC surface during the reactive extrusion and their possible chemically grafted structures with the different compatibilizers (i) sulphuric and hydrochloric acid derived CNCs, (ii) biopolymers, (iii) inorganic/organic nanofillers and (iv) vegetable oils (n =represent the mono or poly-saturated or unsaturated fatty acids), respectively.

To better understand the mechanism of chemical grafting of CNCs with PLA in the presence of different compatibilizers FTIR spectroscopy studies were carried out as shown in Figure 6.14(a)-(d). During reactive extrusion the $-\text{CH}_2\text{OH}$ groups in CNCs chemically grafts with the methine groups of PLA forming a $\text{C}-\text{C}$ bond (Scheme 6.2(i)) [367]. Introduction of different compatibilizers especially the non-reacting species such as the inorganic/organic

nanofillers are not expected to change the chemical grafting mechanism of PLA and CNCs. Although, the different acid derived CNCs, biopolymers and vegetable oils are expected to react during reactive extrusion process but the grafting sites for both PLA and CNC remains the same. FTIR spectrograms for pristine PLA in Figure 6.14 shows the characteristics peaks at ~ 1746 , 1180 , 1128 , 1078 , 1042 , 868 and 954 cm^{-1} which represents the C=O stretching vibrations, asymmetric C-O-C stretching, vibrations of C-OH side groups, C-C vibrations and -CH stretching respectively [396]. The -CH₃ symmetric or asymmetric stretching and deformations corresponding to PLA remain unaltered (in all reactively extruded samples) which suggests that the -CH₃ end groups do not participate in chemical grafting process. For all the PLA based acid derived CNC nanocomposites, the peak corresponding to -CH stretching and bending vibrations at ~ 2998 , 1360 , 1040 and 954 cm^{-1} are found to be almost absent (or present with reduced intensity). The presence of new peak at $\sim 1002\text{ cm}^{-1}$ for PLA/DCNC-S samples and at $\sim 1022\text{ cm}^{-1}$ for PLA/DCNC-Cl samples possibly denotes the formation of C-C linkages between PLA and CNCs during reactive extrusion. The carbonyl peak of PLA at $\sim 1746\text{ cm}^{-1}$ is found to be shifted towards 1740 cm^{-1} and characteristic peaks of CNCs representing C-OH stretching ($\sim 1028\text{ cm}^{-1}$) and bending (1208 cm^{-1}) in plane at C-6 are found to be absent. Further, the intensity of peaks signifying amorphous PLA chains at ~ 1264 and 955 cm^{-1} diminished and with presence of low intensity peak at $\sim 1214\text{ cm}^{-1}$ (representing crystalline segments of PLA) [400], suggests that more fractions of amorphous PLA chains chemically grafts onto CNCs. This is probably due to the enhanced mobility of the amorphous segments, causing the generated DCP radicals to easily abstract more fractions of nascent hydrogen from the PLA melt compared to crystalline domains of CNCs. Therefore, both hydrochloric and sulphuric acid derived CNCs are able to chemically graft onto the PLA

surface through the formation of C–C linkages, albeit with different degrees of grafting efficiency and gel fraction yields. Incorporation of the reactively compatible biopolymers such as cellulose and starch shows enhanced gel yield with improved relative molecular weight suggesting possible chemical grafting with PLA during reactive extrusion. For the case of PLA/DCNC-S/Cellulose, significant changes in the FTIR spectrum was not observed compared to the PLA/DCNC-S which suggests that the grafting mechanism remains unaltered with the introduction of micron size cellulosic fibers (Scheme 6.2(iii)). For the case of starch which is almost structurally similar to cellulose except with α orientation of C-O-C linkages [412], intense PLA peaks (intense carbonyl peak at $\sim 1748\text{cm}^{-1}$, -CH stretching and bending at 1452 or 1360cm^{-1}) are observed in the FTIR spectrograms possibly due to the formation of higher molecular weight polymer and increased gel yield of grafted PLA/CNC (as discussed in earlier section). This suggest that $-\text{CH}_2\text{OH}$ groups present in the hydrophilic starch could efficiently act as grafting sites, thereby, improving the dispersion characteristics and physico-chemical properties of reactively extruded PLA/CNC nanocomposites (Scheme 6.2(iii)). Introduction of vegetable oil such as olive and coconut oils which usually contains different amount of unsaturated and saturated fatty acids improves the dispersion and molecular weight properties. FTIR spectrograms of PLA/DCNC-S/Coco (Figure 6.14(d)) shows the presence of low intensity peaks at $\sim 1708\text{ cm}^{-1}$ representing the carbonyl stretch and peaks at ~ 1126 or 965cm^{-1} representing the characteristic absorption band for triglycerides present in different compositions for both the oils [413] (Scheme 6.2(iv)). Figure 1(c), shows the FTIR spectra of the reactively extruded PLA/CNC nanocomposites in presence of different inorganic nanofillers (evident from distinct FTIR peaks) which are found to inhibit the gel fraction yield

but with molecular properties comparable to PLA. Presence of nanosilica and montmorillonite are evident from the low intense peaks at ~ 1126 and 800 cm^{-1} region representing the Si-OH

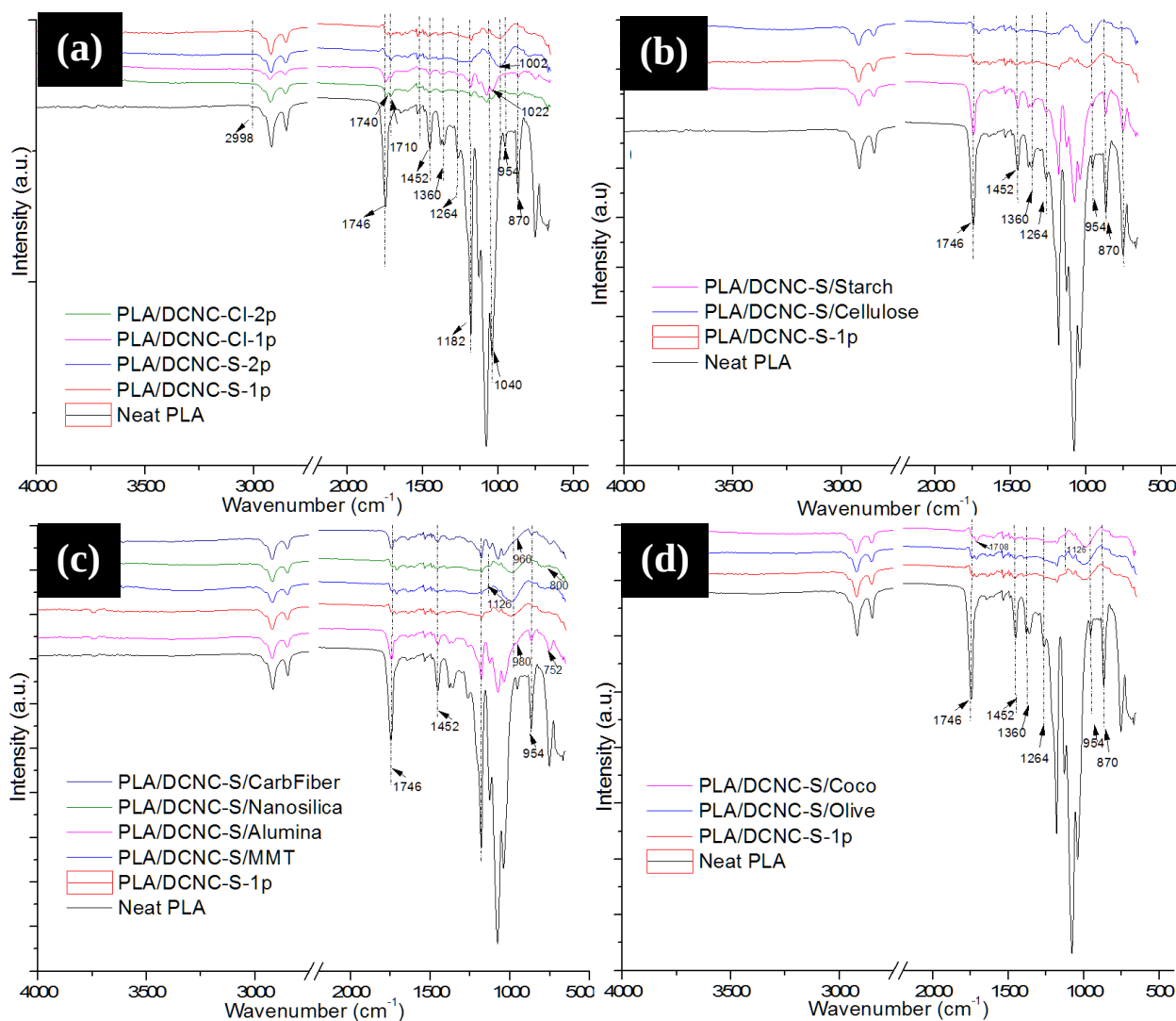


Figure 6.14: FTIR spectrographs of the reactively extruded PLA/CNC films with differently added compatibilizers (a) sulphuric and hydrochloric acid derived CNCs, (b) biopolymers, (c) inorganic/organic nanofillers and (d) vegetable oils, respectively.

bond stretching and Si-O symmetric stretching respectively [414,415]. Both alumina and CNFs shows the representative peaks of PLA with greater intensity probably due to higher gel yield

as well as molecular weight properties (as explained earlier). PLA/DCNC-S/Alumina shows the representative peak of Al-O-Al at 752 cm^{-1} and the C-C peak formed due to reactive grafting between PLA/CNCs shifted to 980 cm^{-1} . This is probably due to formation of DCP and Al_2O_3 bi-dispersed particles in PLA matrix which was similarly observed by Tangboriboon et al.[416] CNFs shows extraordinarily high gel yield and molecular weight properties which possibly led to shifting of the FTIR peak corresponding to C-C bond formation from 1002 to 960 cm^{-1} (Scheme 6.2(ii)). As already reported, CNFs are known to have improved intermolecular interactions with the polymeric matrix [11] as well as with CNCs which possibly leads to synergistic effect in improving the molecular behavior as well as structural properties of the nanocomposites significantly (discussed in subsequent section). Therefore, it can be concluded from physio-chemical studies that reactive grafting of PLA/CNC nanocomposites in presence of different compatibilizers significantly alters the grafting parameters as well as grafting mechanism, which could be used as a tool to effectively tune the polymeric properties.

- **Morphological Studies and Film Properties after Reactive Extrusion of PLA/CNC films in Presence of Compatibilizers**

The effect on physical and micro-structural properties of PLA/CNC films, when reactively extruded inside a pilot-plant scale extruder in the presence of different compatibilizing agents was studied in details. Significant variation in the micro-structural features such as transparency, necking or thickness swell ratio and normal stress difference values are observed in reactively extruded PLA/CNC nanocomposite films. Figure 6.15, shows the transparency values of films measured in the UV-Vis region which helps us understand the dispersion effect of different compatibilizers physically. The neat PLA films shows the transparency of ~80%,

which is a thickness dependent parameter, hence, the transparency values for the rest of the films are normalized with respect to PLA. Incorporation of the hydrochloric acid derived CNCs, improved the transparency to ~90% (for PLA/DCNC-Cl-1p) which is probably due to higher gel yield as well as improved dispersion of the CNC-Cl. However, PLA/DCNC-S-1p, shows a drastic decrease in the transparency to ~55%, which possibly suggests the presence of agglomeration and may also be responsible for the decrease in gel yield and molecular weight characteristics. For both the acid-derived CNCs at higher loadings, the transparency of the films decreased, suggesting the plausible agglomerations of CNCs due to strong inter- molecular hydrogen bonding. Introduction of biopolymers as well as vegetable oils into PLA through reactive extrusion process shows noteworthy improvement in the transparency of the films (~95%), compared to the traditional processing approach [417,418]. This suggests that effective dispersion of the hydrophilic biopolymers and vegetable oils could be carried out through reactive grafting based approach (as also evident from FTIR spectroscopic studies). The improvement in percentage transparency values compared to native PLA is possibly due to the formation of high molecular weight gel domains of PLA with the presence of branching and cross-linking with the fillers during reactive extrusion. Moreover, due to the similar refractive indices of PLA and CNCs (~1.54 and 1.46 respectively), the formed branched or cross-linked gels have reduced scattering of light through interfacial sites, thereby improving the transparency of films significantly. The different inorganic/organic nanofillers, as discussed, in previous sections acts as an inhibitory effect on gel fraction yield and molecular weight characteristics, also altered the percentage transmittance values of films significantly. The PLA/DCNC-S/Carbfiber shows the least percentage transmittance values of ~40% due to the black coloration effect from the carbon nanofibers followed by PLADCNC-S/Alumina. The

improvement in transparency of PLA/DCNC-S/MMT is due to improved compatibility of the clays such as MMT with PLA. However, for the films containing nanosilica, the transparency remains unchanged in comparison to PLA. Similar, observation of exceptionally improved optical transparency was also observed by Haraguchi et al. [419] for the cross-linked hydrophobic poly (2-methoxyethyl acrylate) (PMEA) and hydrophilic clays (hectorite) due to the presence of polymer-clay network morphology formed during radical polymerization. Therefore, transparency of PLA/CNC films (at ~1wt.% loadings) can be finely tuned through reactive extrusion process by incorporating different compatibilizers that alters the gel fraction and molecular behavior of the fabricated nanocomposite.

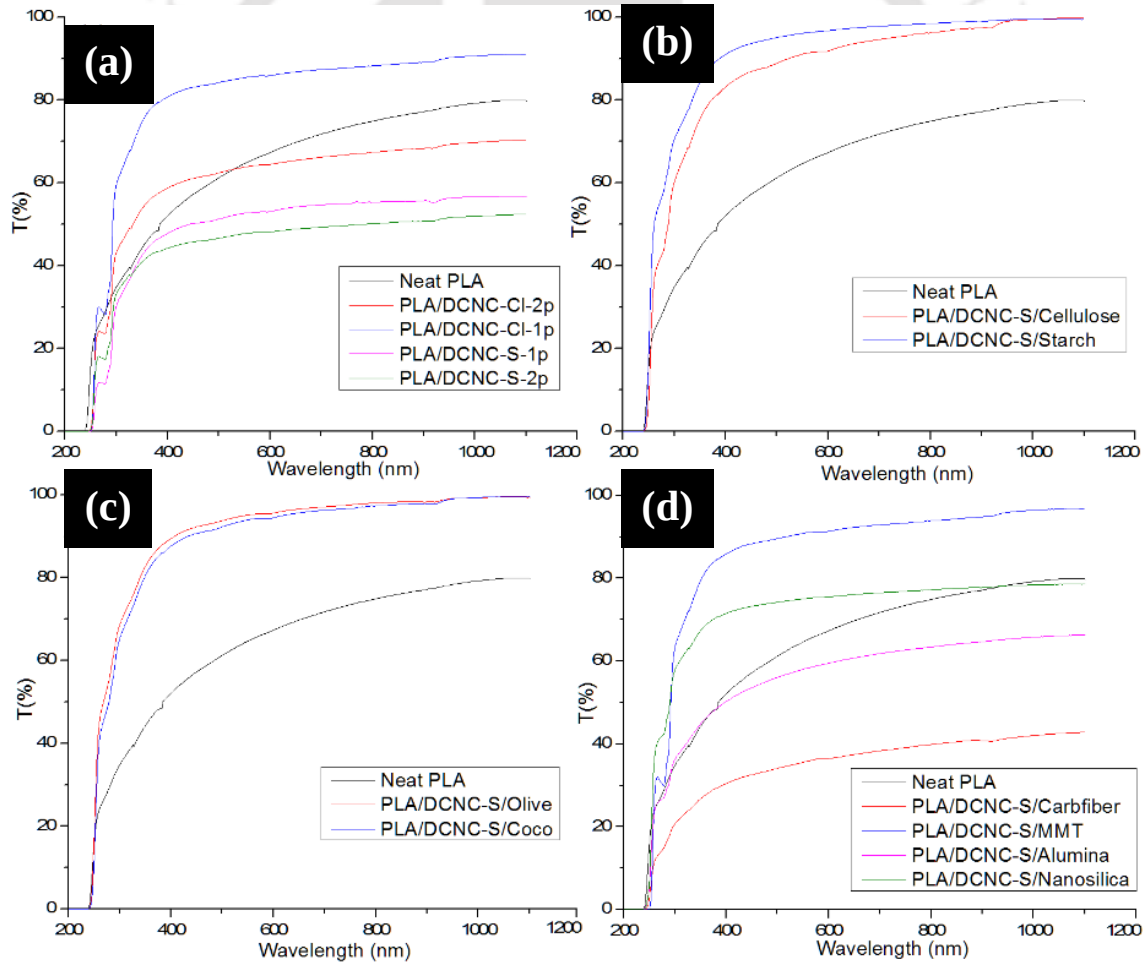
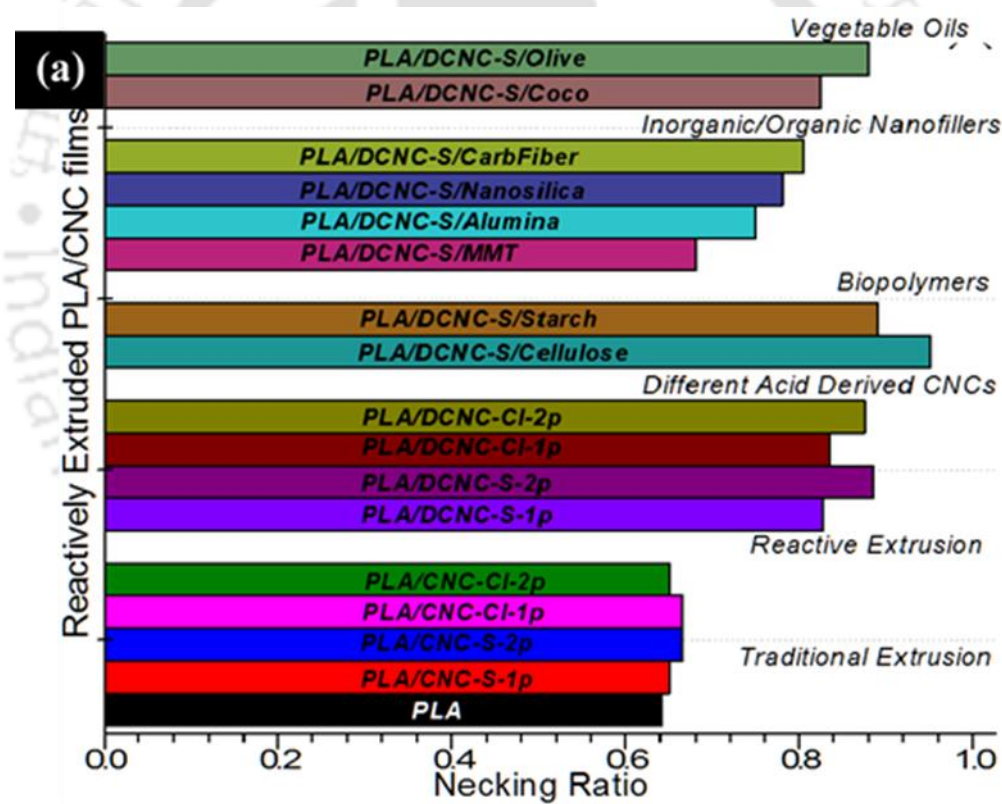


Figure 6.15: Transparency of PLA/CNC films with compatibilizers (i) different acid hydrolyzed CNCs (ii) bio-based fillers (iii) inorganic nanofillers and (iv) vegetable oil.

The variations in molecular architecture of PLA/CNC films arising due to the formation of branched or cross-linked structures are predicted from macroscopic properties such as necking ratio, thickness swell ratio and film inhomogeneity index (as shown in Figure 6.16(a) and (b)) when reactively extruded under constant draw ratio. PLA or PLA/CNC nanocomposites when extruded through traditional approach in a pilot scale twin-screw extruder shows a drastic necking behavior with a necking ratio of ~ 0.6 , which was also prevalent in presence of the both acid derived CNCs at different loadings. This is possibly due to improved adhesion between the PLA and CNCs which forms localized junctions that leads to alignment of polymeric chains in a particular stretched direction, initiating neck in extruded films [420] (Scheme 6.3(i)). Moreover, the extruded PLA/CNC films (through traditional approach) shows drastic drop in the molecular weight properties, with increased fractions of low M_w polymeric chains, which could possibly enhance necking phenomenon. Interestingly, reactive extrusion of the PLA/CNC nanocomposites shows improved processability with significantly reduced necking behaviour which, however, depends upon the type of compatibilizers used. With presence of different acid derived CNCs, both CNC-SO₄ and CNC-Cl results in improvement of necking ratio to ~ 0.82 (~ 1 wt. %) and ~ 0.88 at higher (~ 2 wt. %) CNC loadings. For the different biopolymers used as compatibilizers, PLA/DCNC-S/Cellulose shows the highest necking ratio of ~ 0.95 followed by PLA/DCNC-S/Starch ~ 0.90 . Similar, behavior is observed when vegetable oils such as olive or coconut oil are used as compatibilizers, resulting in improved necking ratio of ~ 0.88 and 0.82 respectively. The enhanced processability of PLA/CNC films through reactive extrusion can be attributed to numerous key factors that may act synergistically or independently for improving the necking or film inhomogeneity index values. Among several parameters, the crucial ones include: (i) degree of branching or cross-

linking and their relative fractions present in the PLA/CNC films, (ii) extent of improvement in molecular weight characteristics, (iii) gel fraction yield during reactive extrusion and (iv) relative fractions of degraded low M_w fragments (produced during extrusion due to shear and thermal induced degradations). Formation of high molecular weight PLA chains in relatively high proportions (~80-94%) during reactive extrusion, as evident from the GPC chromatograms, leads to enhancement in relaxation time (τ) for the PLA/CNC films [382]. According to the reptation theory [421], the high M_w PLA forms entangled chains which reduces its mobility and increases the relaxation time which is proportional to the cubic exponent of M_w ($\tau \sim M_w^3$). Moreover, reactive grafting of PLA with CNCs led to formation of gels with different fractional yields depending upon fractions of CNC loadings [367]. Presence of such cross-linked gels or high M_w entangled PLA chains increases frictional forces between the PLA interlayers that restricts orientation of polymeric chains or CNCs [390] (as shown in Scheme 6.3 (iii-vi)). Due to presence of aforementioned effects, necking of PLA/CNC films are highly restricted when they are passed from the die head and stretched through the chiller roll during the reactive extrusion process. The thickness swell ratio and film inhomogeneity index which represents the degree of uniformity in terms of film thickness are also found to be improved following the reactive extrusion-based process (as shown in Figure 6.16). The film inhomogeneity index values for PLA is found to be ~5.1% which increased to 9-12% for the PLA/CNC-SO₄ and CNC-Cl (fabricated through traditional approach), possibly due to the edge beading effect [382]. Presence of such effect leads to the thinning of the films at the center and thickening at their edges which could effectively be controlled through reactive extrusion [422] that reduced the inhomogeneity index values to 5-9% and improved thickness swell ratio to 1.4-2.1 in presence of the different compatibilizers. Furthermore, PLA or PLA/CNC films

processed through traditional approach shows formation of the low molecular weight oligomeric PLA chains which are present in significant higher weight fractions (~24-34%). Such low molecular weight oligomeric chains have relatively shorter relaxation time[423] (and with presence of such high weight fractions in films), easily undergoes orientation in the stretched directions (when passed through chiller and take up roll) which causes dimensional instability in films. Therefore, proposed reactive extrusion approach may potentially overcome technical challenges associated with biopolymer processing through an industrial scale extruders with easy recyclability to fabricate PLA based products.



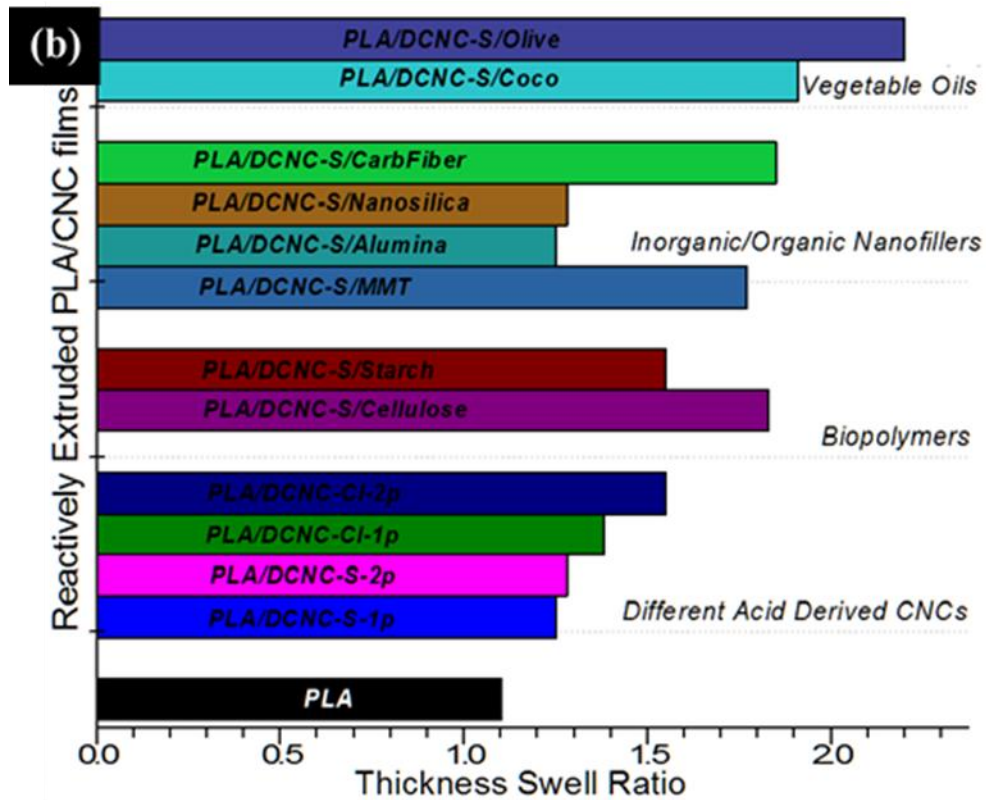


Figure 6.16: (a) Necking ratio, and (b) thickness swell ratio properties of the reactively extruded PLA/CNC nanocomposites in the presence of different compatibilizers

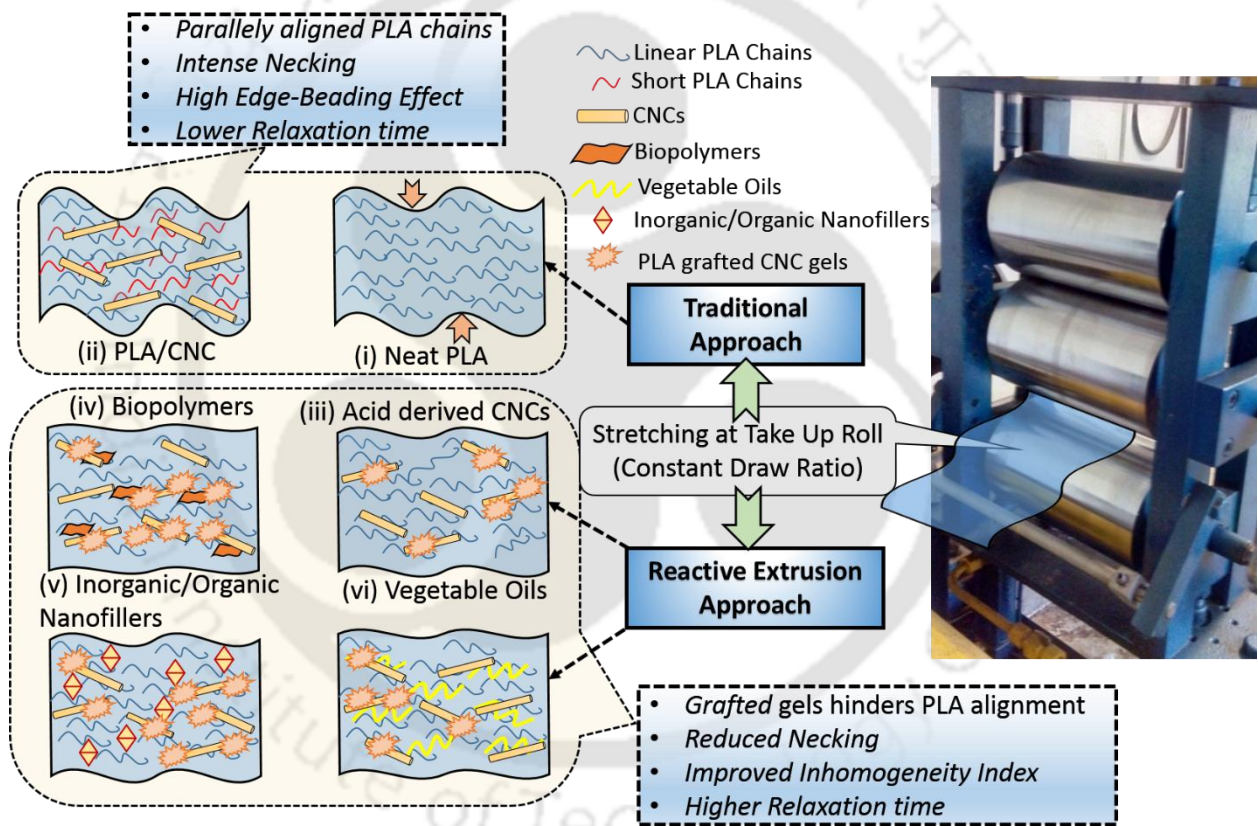
To better understand the necking or thickness swelling phenomenons, in terms of the practical aspects of extrusion based-processing for manufacturing of value-added products the influence of normal forces under steady shear flow was studied. The normal forces measured through parallel plate geometry are usually normal stress differences $\Delta N = N_1 - N_2$ (where N_1 represents $(\tau_{11} - \tau_{22})$ and N_2 represents $(\tau_{22} - \tau_{33})$), where τ_{ii} corresponds to normal stresses acting in (1) flow, (2) flow gradient and (3) vorticity directions [424]. These components provides a tool to determine the elastic energy stored in polymer melts when they are flowed, stretched or aligned during extrusion along the directions of flow streamlines, which also results in generation of normal thrust forces on die-heads [425]. CNCs have high elastic modulus [426] and with extensive hydroxyl functionalities have the ability to form percolated network-like

structures in PLA matrix [17] forming a jammed solids or dispersed gels during processing. In this study, the gel-like structures are high molecular weight fractions containing branched or cross-linked PLA grafted with CNCs, which modify the molecular architecture of PLA/CNC films fabricated through reactive extrusion. Figure 6.17(a), shows the comparison of the normal stresses induced in the polymeric melts at different shear rates for the PLA/CNC films extruded through traditional and reactive extrusion based approaches, containing different acid derived CNCs. For PLA or PLA/CNC films fabricated through traditional extrusion process, the ΔN values are found to be independent of the shear rate over a wide range. However, the reactively extruded PLA/CNC films for both acid derived CNCs shows significant variation in the ΔN values especially at higher shear rates. The positive ΔN values with increased magnitude at higher shear rates for PLA/DCNC-SO₄-1p is possibly due to the high M_w of entangled or branched PLA grafted CNCs as well as increased gel fraction yields (Figure 6.17(a) & Scheme 6.3(iii)). Moreover, as discussed previously, CNCs or PLA graft CNC gels could form percolated network-like structure that generates the normal thrust (between the parallel-plates) and leads to increase in the relaxation time of polymeric melts. The positive ΔN values are also responsible for improved necking or thickness swell ratio and inhomogeneity index values, thereby, suggesting that entangled or cross-linked polymers arising due to reactive grafting helps in reducing polymer melt instabilities (Scheme 6.3(iii)). Similar, behavior have been reported for the high M_w polypropylene or high density polyethylene [427] or carbon-nanotube/polypropylene based nanocomposites [428] in which large ΔN values are observed due to presence of the gel-like assemblies. The morphological differences between the CNC-SO₄ and CNC-Cl in terms of the aspect ratio are also responsible for the variation of ΔN values. CNC-SO₄, with higher aspect ratio~50 could form higher degree

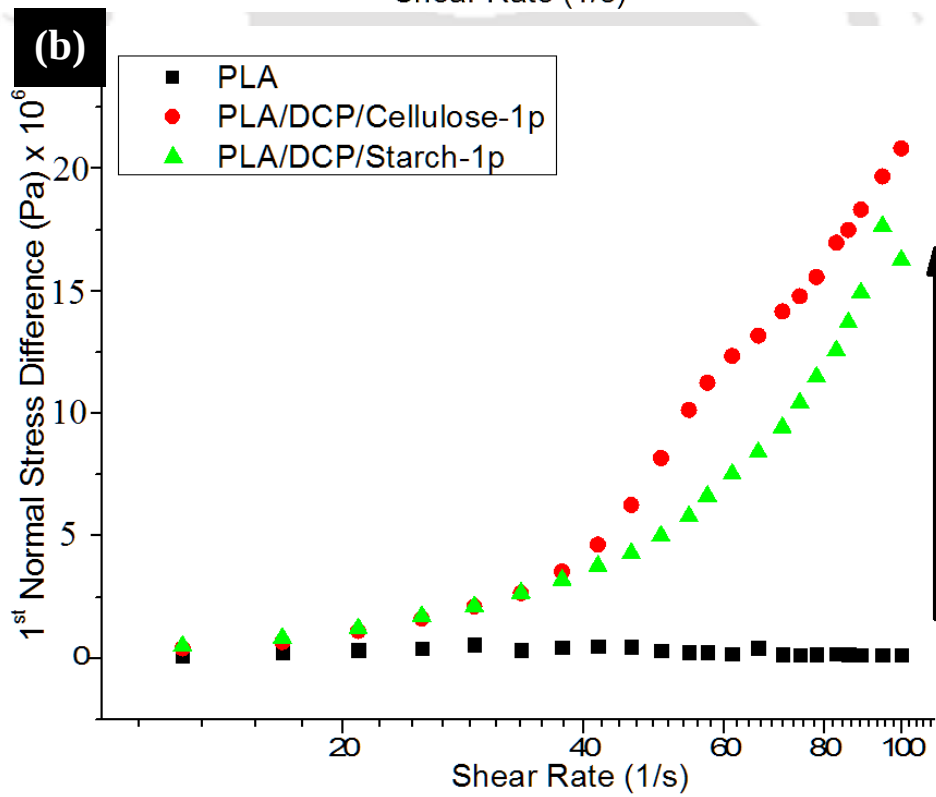
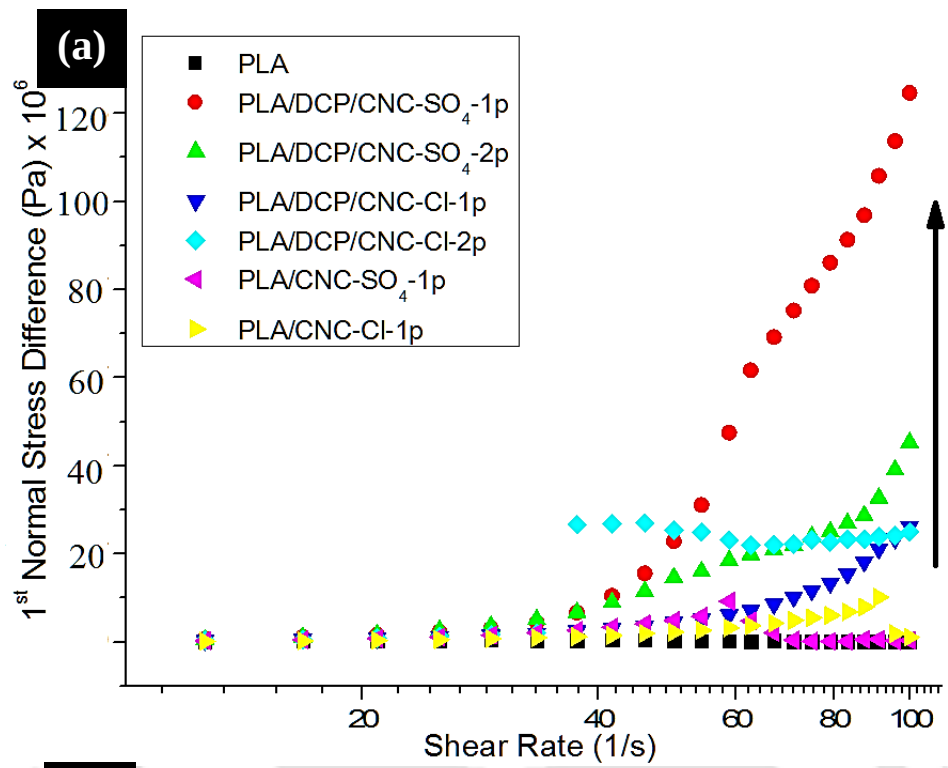
of entanglements with the PLA chains during reactive extrusion which are either difficult to break or could form new entanglements when extruded at high shear rates. However, CNC-Cl with low aspect ratio~17, has lower degree of interactions, hence, it could orient in the direction of shear flow thereby disrupting the percolated network easily. Formation of such oriented CNC-Cl nanostructures leads to shrinkages of films i.e. lowering of necking ratio values which was similarly observed by Xu et al. [429] with the low aspect ratio carbon nanotube based isotactic polypropylene nanocomposites. Therefore, it can be inferred that increasing the aspect ratio of CNCs alters stability of percolation network, which significantly alters the induced normal stress as well as film dimensions.

For different compatibilizers used during reactive extrusion, the normal stress differences are significantly altered which is expected to depend on several parameters such as molecular weight, degree of grafted gel fractions as well as on chemical interaction between PLA/CNC and fillers. Incorporation of vegetable oils and biopolymers (Scheme 6.3(iv), (vi)) shows improved ΔN values at higher shear rates (see Figures 6.18(c), (d)) due to the presence of high M_w fragments and grafted gels fractions but with relatively lower ΔN magnitudes in comparison to CNC-SO₄ (Scheme 6.3(iii) & (iv)). This is possibly due to improved interactions of vegetable oils and cellulose with PLA or CNCs, which causes the percolated network formed to be mobile and are easily deformed at higher shear rates. However, the improved necking ratio for these compatibilizers is due to the increased gels fractions alongwith high molecular weight fractions which increases the relaxation time of the PLA grafted CNCs, thereby preventing the rapid heat induced shrinkages. For inorganic/organic nanofillers, PLA/DCNC/MMT-1p and PLA/DCNC/Alumina-1p shows lowered ΔN values which led to shrinkages in films dimensions and lowering of the necking ratio values (comparatively

lowered than PLA/CNC films fabricated through traditional approach) (Scheme 6.3). The improved ΔN values for PLA/DCNC/Nanosilica-1p and PLA/DCNC/CarbFiber-1p is possibly due to significantly higher M_w fractions (as discussed previously) which increases the relaxation time. In summary, the proposed reactive extrusion approach provides a unique opportunity to fabricate transparent PLA/CNC films (with different compatibilizers added to it) which improves dimensional stability and physio-chemical properties, even during processing with industrial scale extruders.



Scheme 6.3: Plausible mechanism governing the improved processability for the (i) PLA, (ii) PLA/CNC films processed through traditional approach, and in presence of the different compatibilizers (iii) sulphuric and hydrochloric acid derived CNCs, (iv) biopolymers, (v) inorganic/organic nanofillers, and (vi) vegetable oils, respectively.



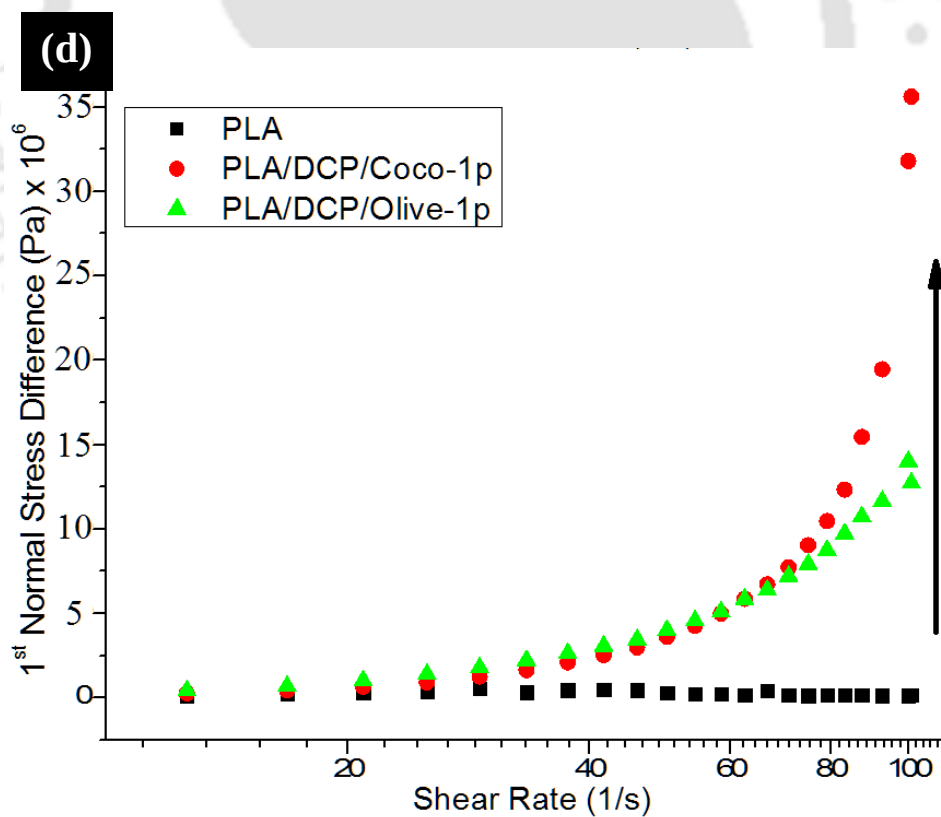
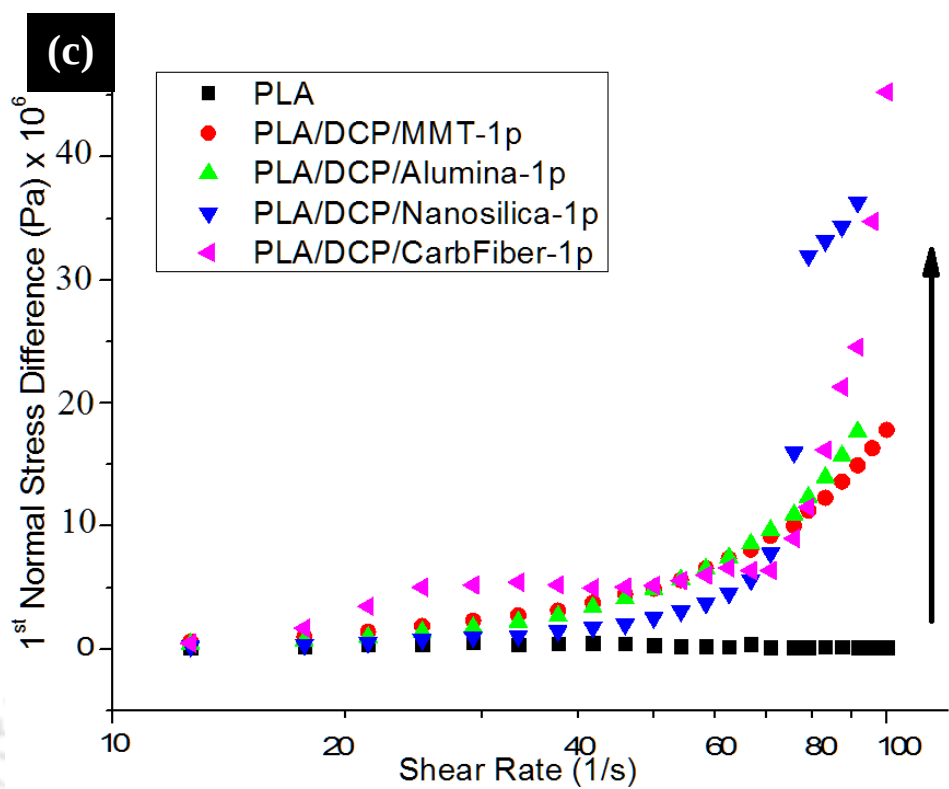


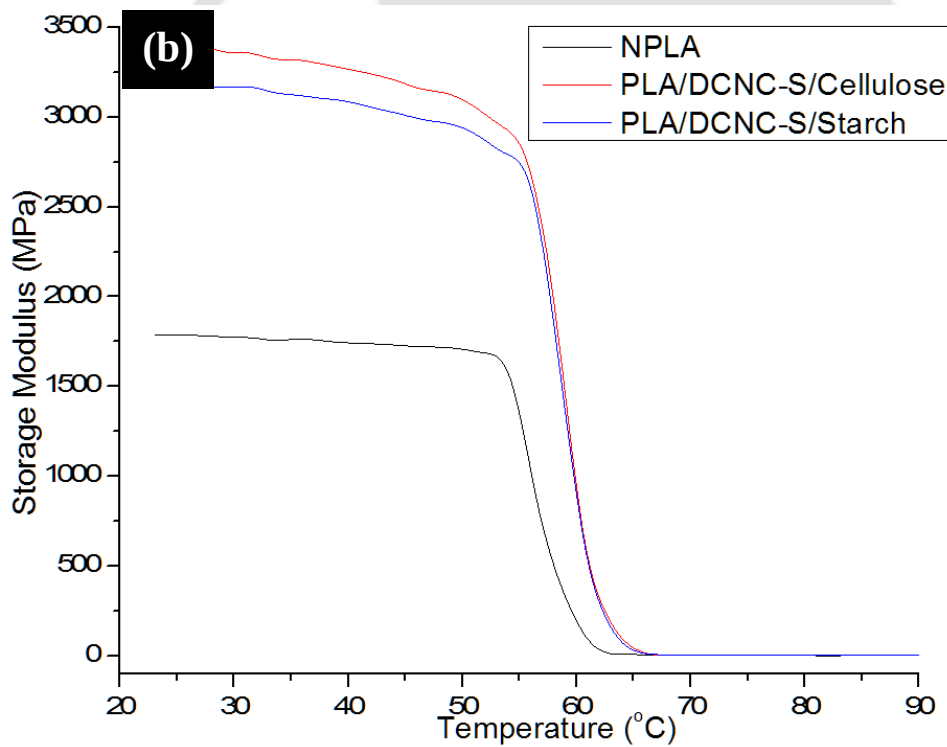
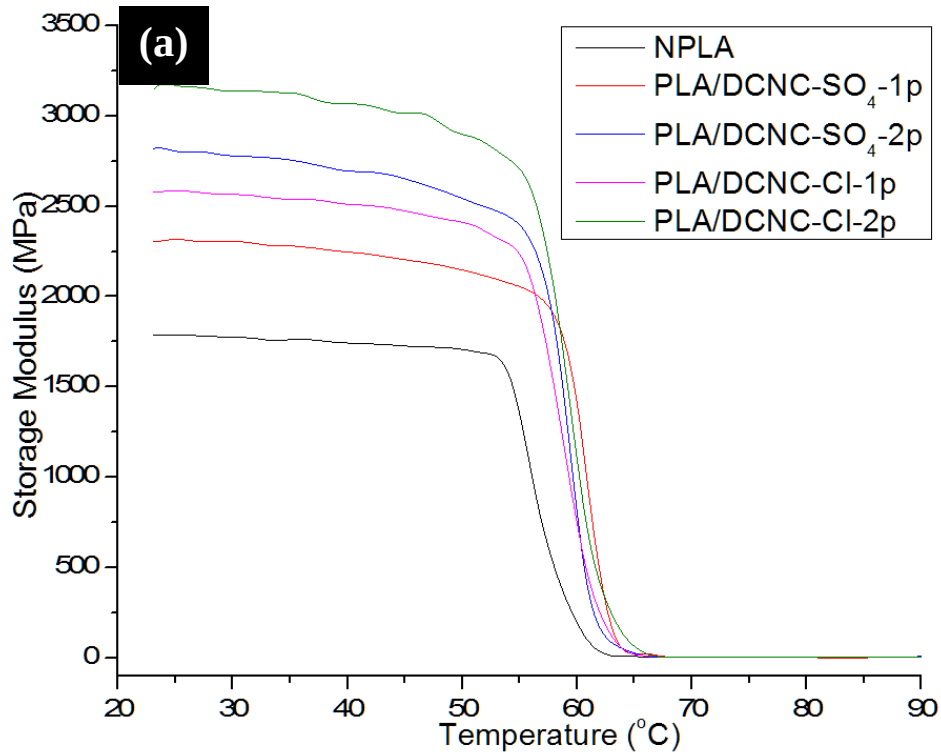
Figure 6.17: Normal Stress differences measured for the reactively extruded PLA/CNC films with differently added compatibilizers (a) sulphuric and hydrochloric acid derived

CNCs, (b) inorganic/organic nanofillers (c) biopolymers, and (d) vegetable oils, respectively.

- **Mechanical Properties of Reactively Extruded PLA/CNC Films in the Presence of Compatibilizers.**

The thermo-mechanical behavior of reactively graft PLA/CNC films were studied to understand the effect of various added compatibilizers and degree of grafting between PLA and CNCs on the storage (E') and loss modulus (E'') measured over a wide temperature range of 20-90°C (Figure 6.18(a-d)). CNCs are well-known for their strong reinforcement effect in the polymeric nanocomposites and interestingly high anisotropic mechanical properties with Young's modulus of ~140-220GPa along the axis and ~ 2-50GPa in transverse direction [1]. The reactive grafting leads to the formation of C–C linkages which enhances the interfacial adhesion of CNCs with bulk PLA and also acts as an efficient network for transfer of CNC's modulus to fabricated nanocomposites. However, mechanical properties of reactively extruded PLA/CNC films will strongly depend on the degree of branching or cross-linking which leads to improved molecular weight, grafting efficiency, gel fraction yield, physical properties (such as aspect ratio) and dispersion characteristics or interfacial interaction of the added compatibilizers with PLA. To better understand the reinforcing effect of CNCs in presence of different compatibilizers the filler effectiveness coefficient (C_{FE}) is calculated using equation (6.1). It is known that PLA/CNC films (with different compatibilizers added) which have lower C_{FE} values are most effective reinforcing agent [278]. Introduction of various acid derived CNCs, improves E' by several order of magnitudes even at higher loadings with significant improvement observed for the case of CNC-Cl compared to CNC-SO₄. The higher grafting efficiency of PLA chains onto CNCs alongwith the higher M_w and gel fraction yields (due to

enhanced cross-linking or branching) for PLA/DCNC-Cl possibly leads to improvement in the modulus. Further, low C_{FE} values for PLA/DCNC-Cl (~1.96 and 1.85 at 1 and 2 wt. % loadings) suggest that CNCs or PLA graft CNC gels undergoes uniform dispersion in the PLA matrix and thus acts as an efficient reinforcing agents (Table 6.5). Addition of different biopolymers such as cellulose and starch, also showed lower C_{FE} values and higher E' which suggests that the hydrophilic biopolymers can be efficiently dispersed in PLA systems. This is possible because during reactive extrusion PLA graft CNC gels of high M_w are formed which improves their compatibility with PLA matrix thereby preventing the agglomeration of hydrophilic fillers. The vegetable oils such as olive and coconut oil showed slight decrease in E' and improvement in elongation properties due to their plasticizing effect. Therefore, formation of C–C linkages between PLA graft CNC gels and chemically interacting fillers such as biopolymers and vegetable oils (as explained earlier in Scheme 6.2 (iii) and (iv)) creates strong stress bearing zones and results in effective reinforcement. Although, the different organic/inorganic nanofillers are chemically non-reactive but they undergo enhanced dispersion alongwith the PLA graft CNC gels due to their improved compatibility with the PLA matrix. As discussed in previous section, PLA/DCNCS/CarbFiber and PLA/DCNCS/Nanosilica shows high M_w PLA graft CNC gel fractions due to their synergistic interaction with the CNCs. Due to the presence of such enhanced interfacial interactions and high M_w gel fraction units, the fabricated nanocomposites shows lowered C_{FE} values and high E' (compared to neat PLA). Therefore, the generation of PLA graft CNC gels during reactive extrusion ensures significantly enhanced interfacial interaction with various added compatibilizers which in combination induces strong reinforcement effect in processed PLA/CNC films.



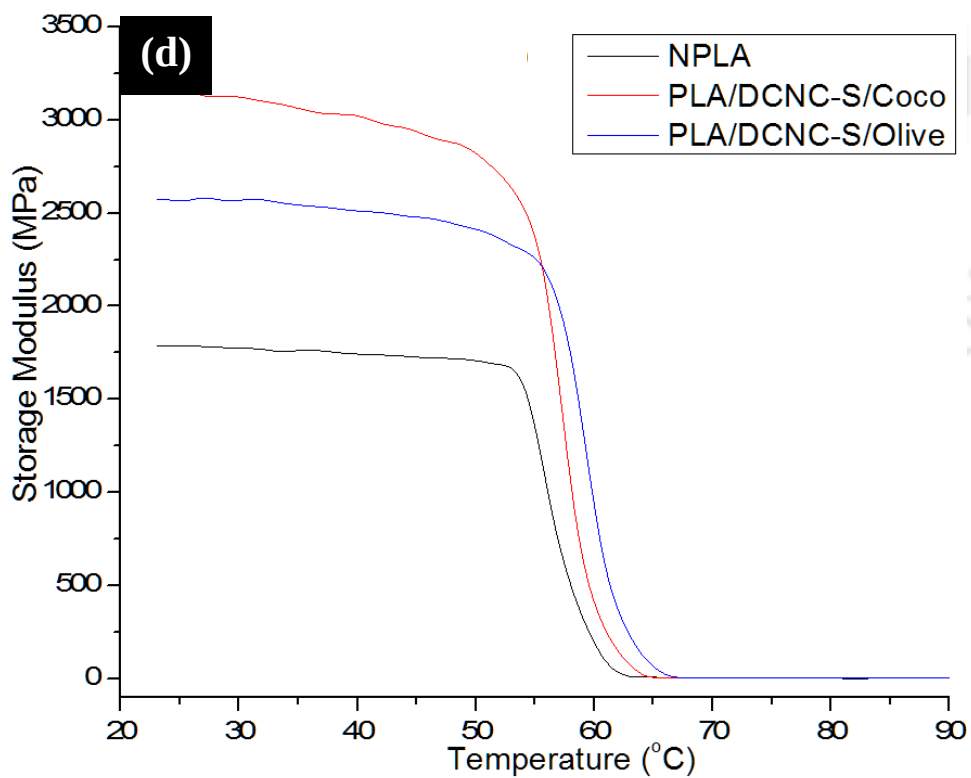
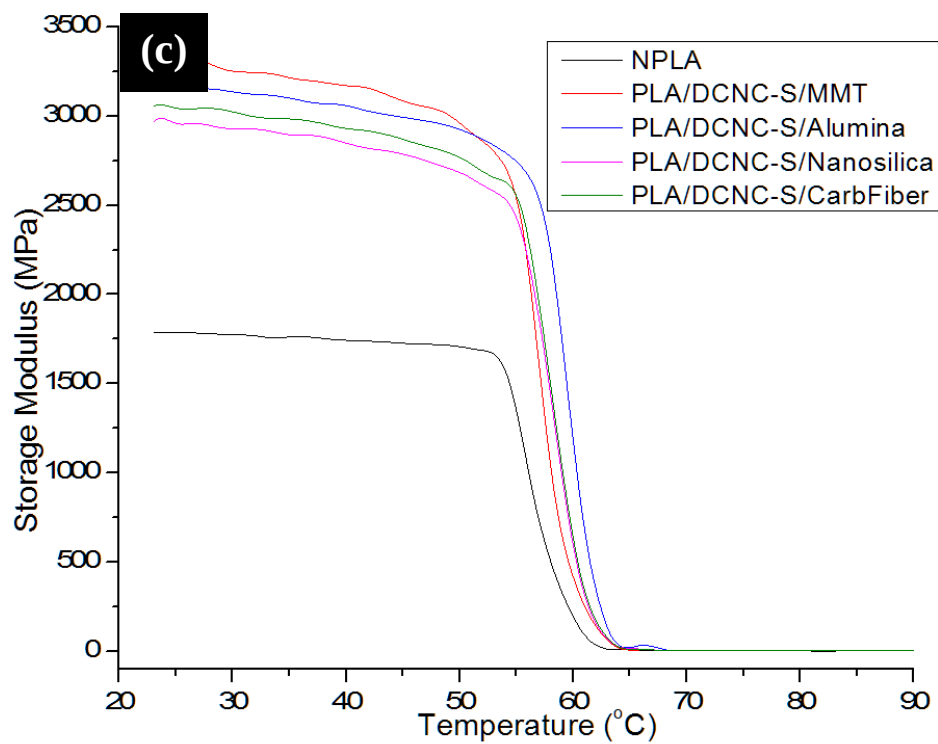


Figure 6.18: DMA of reactively extruded PLA films with (a) acid derived CNCs, (b) inorganic/organic nanofillers (c) biopolymers, and (d) vegetable oils.

Table 6.5: Comparison of thermo-mechanical properties of reactively extruded PLA/CNC nanocomposites fabricated with various compatibilizers

Samples	Storage Modulus E'(MPa) @46°C	Glass Transition Temp. (T _g)(°C)	Storage Modulus E'(MPa) @85°C	DL (µm)	C _{FE}
Neat PLA extruded	2050	65.2	3.08	4596	-
<i>Different Acid derived CNCs</i>					
PLA/DCNC-S-1p	2203	68	1.26	3916	2.62
PLA/DCNC-S-2p	2649	66.4	2.08	3602	1.91
PLA/DCNC-CI-1p	2472	67.0	1.89	4207	1.96
PLA/DCNC-CI-2p	3016	66.6	2.44	4102	1.85
<i>Biopolymers</i>					
PLA/DCNCS/Cellulose	3182	66.6	2.68	4119	1.77
PLA/DCNC-S/Starch	3007	66.7	2.44	4241	1.85
<i>Inorganic/Organic Nanofillers</i>					
PLA/DCNC-S/MMT	3082	65.9	3.61	4295	1.28
PLA/DCNC-S/Alumina	2991	68.1	1.91	4248	2.35
PLA/DCNCS/Nanosilica	2784	66.2	3.57	4189	1.17
PLA/DCNCS/CarbFiber	2866	66.4	3.39	4149	1.27
<i>Vegetable Oils</i>					
PLA/DCNC-S/Coco	2933	66.1	2.82	4441	1.56
PLA/DCNC-S/Olive	2476	66.9	2.38	4068	1.56

(where T_g is glass transition temperature, E' is storage modulus, DL is the elongation and C_{FE} is filler effectiveness coefficient respectively)

- Barrier Properties of Reactively Extruded PLA/CNC films in presence of Compatibilizers**

PLA/CNC films fabricated through a single step reactive extrusion are easily processible with inherent biodegradable and non-toxic characteristics which could potentially be used for packaging applications, especially for storage of food products. For efficient preservation of food items, films with simultaneously improved water vapor and oxygen barrier properties are desirable, which remains a major challenge especially with the incorporation of hydrophilic

bio-nanofillers. The effectiveness of the reactive extrusion approach (with different compatibilizers), are evaluated from the WVTR studies conducted at 37.8°C, 95% R.H. and OTR investigated over a varied temperature range of 15-45°C, 0% R.H. In an effort to understand the molecular mechanism governing the increased resistance to oxygen (OP) and water vapor permeability (WVP) several parameters such as diffusivity, solubility and activation energy of OP are predicted (for the various added compatibilizers). The solubility coefficient (S) is a chemical interaction parameter which predicts the degree of affinity of water vapor or oxygen molecules traversing through PLA matrix, whereas, diffusion coefficient (D) measures the degree of tortuosity or resistance provided by CNCs or compatibilizers to the diffusing moieties [272]. The neat PLA fabricated through extrusion shows WVP value of 1.157 g.mm/m².day.kPa which matches with the value reported in literature[17]. Introduction of different acid derived CNCs, leads to reduction in the WVP by ~42% for both PLA/DCNC- S-1p and PLA/DCNC-S-2p respectively (Table 6.6). Evaluation of barrier parameters such as D showed significant reduction from 0.857 to 0.346 m²/day, however, S almost remained unchanged or increased to 1.891 from 1.35 g/m³.kPa (for case of PLA/DCNC-Cl-2p). This suggests that PLA graft CNCs formed during reactive extrusion helps in improving dispersion of hydrophilic CNCs (as also evident from C_{FE} values which also shows improved reinforcement effect) resulting in more tortuous pathway for permeation of water molecules. Although the addition of biopolymers such as cellulose or starch results in lower water uptake and lowered S but the higher D led to an increase in WVP (even higher than PLA). Grafting of higher M_w PLA chains onto cellulose or starch during reactive extrusion led to increased hydrophobicity which prevents the easy wetting by water molecules. Therefore, higher WVP is possibly due to presence of amorphous segments from cellulose or starch which

provides easy diffusivity of water molecules thereby increasing the D values. Incorporation of vegetable oils which contains high weight fractions of saturated and unsaturated fatty acid chains, further, increases the hydrophobicity of fabricated nanocomposites. It is probably because these oils facilitates branching or cross-linking of high M_w PLA chains onto CNCs surface (as discussed previously), forming an encapsulated gel-like domains which are interconnected through hydrophobic vegetable oil networks (as shown in Scheme 6.3(vi)) inside the polymeric matrix. Presence of such phenomenon leads to drastic decrease in water uptake and S values for PLA/DCNC-S/Coco and PLA/DCNC-S/Olive. However, D values increases since the tortuosity effect induced by vegetable oils is almost negligible. Addition of non-reacting inorganic/organic nanofillers such as nanosilica or carbon fibers, which are known to have good compatibility with PLA which results in improved gel fraction yield alongwith higher grafting efficiency, shows significantly lowered water uptake and S values, which subsequently decreased the WVP by ~22%. For case of PLA/DCP/MMT-1p and PLA/DCP/Alumina-1p, both degree of grafting as well as M_w are lowered which results in higher WVP values (comparable to PLA). Therefore, through the reactive extrusion approach hydrophilic fillers such as CNCs can effectively be modified with hydrophobic PLA chains thereby reducing the WVP values of prepared nanocomposites.

The oxygen barrier property of reactively extruded films were studied at various temperatures to understand the mechanism governing the permeation of O_2 molecules using several predicted parameters such as diffusivity, solubility and activation energy. The PLA films processed through twin-screw pilot plant extruder shows OP of ~1.06 ml.mm/m².day.kPa, which is in line with the values reported literature[17]. Addition of the acid derived CNCs (both CNC-SO₄ and CNC-Cl), shows a drop in the OP values by 20%

and ~40% for PLA/DCNC-S-2p and PLA/DCNC-Cl-2p respectively (Table 6.7). CNC-SO₄, due to its higher aspect ratio provides more tortuous pathway to permeation of oxygen molecules compared to CNC-Cl (with lower aspect ratio), which results in lowered D values for PLA/DCNC-S films. However, CNC-Cl underwent improved dispersion due to higher grafting efficiency and formation of branched or cross-linked PLA chains of high M_w (as discussed in previous sections) onto the surface which significantly reduces S values. The reduction in S was more pronounced than the increase in D for the case of CNC-Cl, which thereby lead to significant decrease in OP values. The biopolymers namely cellulose and starch also shows substantial decline in D values (compared to S) suggesting that reduction in OP by ~36 and ~12% respectively, is predominantly a tortuous effect. Similarly, for the case of chemically non-reacting nanofillers lowered D values suggests that MMT, alumina, nanosilica and CNF created a resistive pathway to the permeation of O₂ molecules (similar behavior was observed in the case of WVP). Presence of such barrier networks decreased the OP by ~23%, 27%, 31% and 36% for PLA/DCNC-S/MMT, PLA/DCNC-S/Alumina, PLA/DCNC-S/Nanosilica and PLA/DCNC-S/CarbFiber respectively. Introduction of vegetable oils during reactive extrusion, which are known to have plasticizing properties, did not show any tortuous effect and as expected led to higher D values. Interestingly, significant decrease in the S values are observed for both coconut and olive oil as compatibilizers by ~63% and 41% respectively, which nullified the effect of D and subsequently led to decline in OP by ~58 and ~50% respectively. As discussed earlier, hydrophobic vegetable oils acts as dispersion agents for the high M_w PLA graft CNC micro-gels, which decreases the affinity of the oxygen molecules with polymeric matrix, thereby, preventing easy permeation of O₂ molecules (Scheme 6.3). To further understand the stability of the percolated networks for the different

compatibilizers added, OP studies were carried out over a varied temperature range (15-45°C) and activation energy of permeation (E_p) are calculated. Reactively extruded PLA shows comparatively higher E_p of ~24.3 kJ/mol, with respect to solution casted films. As discussed in the previous section 5.2.1 and 5.2.2, CNCs are known to form a percolated network in PLA matrix which resists easy permeation of O₂ molecules thereby leading to increased E_p values. CNC-SO₄, with higher aspect ratio shows enhanced E_p of ~40 kJ/mol (at ~1 and 2wt. % loadings) compared to E_p of ~24 and 34 kJ/mol (at ~1 and 2wt. % loadings respectively) for CNC-Cl. For the case of inorganic/organic nanofillers and biopolymers, E_p almost remains unchanged suggesting that reduction in OP for both the cases is predominantly due to decrease in D caused by increase in tortuosity of the diffusion path [430]. However, for vegetable oils as compatibilizers, the decrease in S effect was responsible for the drop in OP values, thereby leading to significantly higher E_p of ~38 and 48 kJ/mol for PLA/DCNC-S/Coco and PLA/DCNC-S/Olive respectively. Therefore, it could be inferred that the proposed reactive extrusion approach provides a single- step processing of PLA/CNC films with easy recyclability and improved physio- chemical characteristics and barrier properties for possible food packaging applications.

Table 6.6: Calculation of the Water vapor permeability (WVP) with their calculated parameters.

Samples	Permeability (g.mm/m ² .day.kPa)	Water Uptake (%)	Solubility (g/m ³ .kPa)	Diffusivity (m ² /day)
Neat PLA extruded	1.157	0.55	1.350	0.857
<i>Different Acid derived CNCs</i>				
PLA/CNC-S-1p	1.292	0.50	1.228	1.052
PLA/CNC-Cl-1p	0.841	0.46	1.129	0.744
PLA/DCNC-S-1p	0.672	0.38	0.933	0.720
PLA/DCNC-S-2p	0.790	0.47	1.154	0.684
PLA/DCNC-Cl-1p	0.765	0.41	1.007	0.759
PLA/DCNC-Cl-2p	0.655	0.77	1.891	0.346
<i>Biopolymers</i>				
PLA/DCNC-S/Cellulose	1.300	0.43	1.056	1.231
PLA/DCNC-S/Starch	1.170	0.34	0.835	1.140
<i>Inorganic/Organic Nanofillers</i>				
PLA/DCNC-S/MMT	1.340	0.49	1.203	1.113
PLA/DCNC-S/Alumina	1.130	0.53	1.301	0.868
PLA/DCNC-S/Nanosilica	0.928	0.20	0.491	1.887
PLA/DCNC-S/CarbFiber	0.900	0.16	0.392	2.295
<i>Vegetable Oils</i>				
PLA/DCNC-S/Coco	0.851	0.30	0.736	1.156
PLA/DCNC-S/Olive	0.963	0.13	0.319	3.018

Table 6.7: Calculation of the Oxygen Permeability (OP) with their calculated parameters (Solubility and Diffusivity values).

Samples	Permeability (ml.mm/m ² .day.kPa) (x 10 ³)	Solubility (cm ³ /cm ³ .Pa) (x 10 ⁻³)	Diffusivity (m ² /sec) (x 10 ⁻¹²)
Neat PLA extruded	1.06	4.78	2.56
<i>Different Acid derived CNCs</i>			
PLA/DCNC-S-1p	0.867	5.63	1.78
PLA/DCNC-S-2p	0.855	5.21	1.65
PLA/DCNC-Cl-1p	0.963	5.11	2.18
PLA/DCNC-Cl-2p	0.650	3.97	1.90
<i>Biopolymers</i>			
PLA/DCNC-S/Cellulose	0.682	5.17	1.53
PLA/DCNC-S/Starch	0.939	6.89	1.58
<i>Inorganic/Organic Nanofillers</i>			
PLA/DCNC-S/MMT	0.816	5.64	1.67
PLA/DCNC-S/Alumina	0.777	5.72	1.57
PLA/DCNC-S/Nanosilica	0.729	6.17	1.37
PLA/DCNC-S/CarbFiber	0.678	6.27	1.25
<i>Vegetable Oils</i>			
PLA/DCNC-S/Coco	0.448	1.79	2.89
PLA/DCNC-S/Olive	0.530	2.81	2.19

- **Shelf-life and Migration Studies of Reactively Extruded PLA/CNC films**

The reactively extruded PLA/CNC films show both improved oxygen as well as water vapor barrier properties. However, their effectiveness as real food packaging material needs to be estimated by studying the shelf-life of preserved food items. Two different types of food items, a nonperishable product such as edible oils and a perishable product such as dairy based beverages are selected for shelf-life study (Figure 6.19). The edible oils are generally sensitive to O₂ environment and gradually undergoes lipid oxidation with increased exposure to moisture and air. It has been assumed in the studies that selected food items deteriorates in quality or undergoes chemical or physical changes because of sensitivity to oxygen or moisture conditions only, and other environmental or microbial effects are neglected. The shelf-life of

the edible oils using PLA packages were found to be ~50 days (considering the effect of lipid oxidation), which matches with the previously reported literature [431]. Introduction of different acid derived CNCs, increases the shelf-life of edible oils to ~79 days for case of PLA/DCNC-S and to ~86 days for PLA/DCNC-CI based films. The shelf-life of edible oils can intrinsically be tuned from 67-91 days through incorporation of different biopolymers and inorganic/organic nanofillers. PLA/DCNC-S/Coco and PLA/DCNC-S/Olive based packages shows significantly higher shelf-life of ~102 days and 151 days respectively, which is almost ~2 to 3 times higher compared to neat PLA based packages (Figure 6.19(a)). Storage of easily perishable food items such as dairy based beverages requires stringent packaging conditions and applications of bio-based PLA packages for long term stability is a challenging task. The perishable food products such as milk or dairy based beverages, are generally stored in refrigerated conditions (10-15°C) to prevent early deterioration. However, in this study the shelf-life prediction for milk based products have been carried out for items stored at 23°C, since the OP values (at ~15°C) for some of the reactively extruded PLA/CNC films are so low that they are not detectable with the instrument. Shelf-life of the dairy based beverages are found to be only ~7 days using neat PLA films (Figure 6.19(b)). The storage stability increases to ~10 days on incorporation of the different compatibilizers such as starch or carbon fibers into PLA matrix through reactive processing. Interestingly, PLA/DCNC-S/Coco and PLA/DCNC-S/Olive films shows significantly higher shelf-life of ~12 and 17 days respectively, for preservation of dairy based beverages (Figure 6.19(b)). Therefore, reactively extruded PLA/CNC films with different added compatibilizers, can be effectively used for

food packaging applications at commercial scale for preservation of oil-based and dairy-based beverages for a maximum of ~5 months and ~2 weeks respectively.

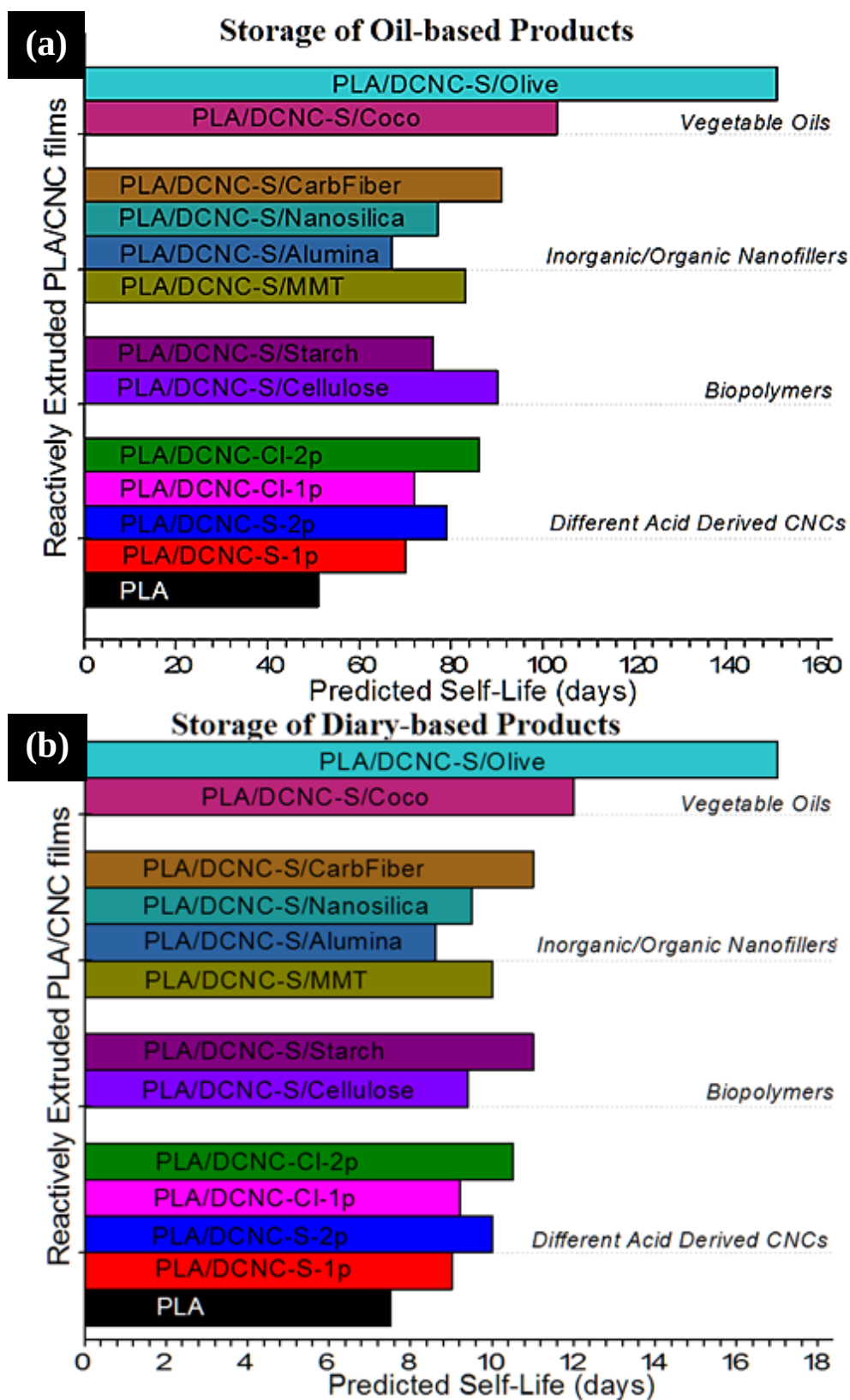


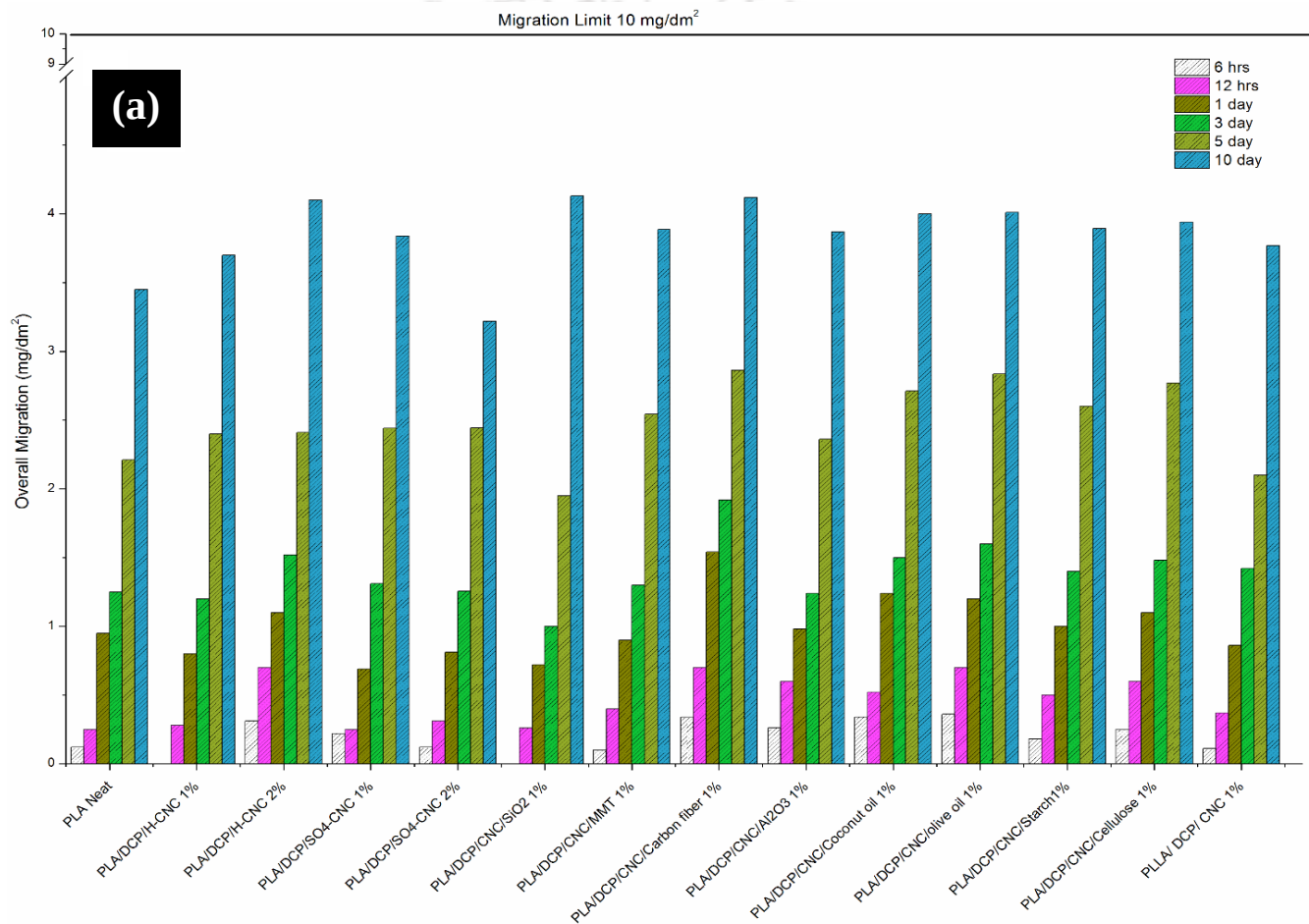


Figure 6.19: The predicted shelf-life for the (a) oil-based products (especially rapeseed oil) and (b) dairy based products using the PLA nanocellulose based composite films produced with the different compatibilizers. (c) Molded plates from reactively extruded PLA/CNC films for storage of food products with different compatibilizers (i) PLA/DCNC-S-1p, (ii) PLA/DCNC-S/Carbfiber, (iii) PLA/DCNC-S/Cellulose and (iv) PLA/DCNC-S/Olive Oil.

Reactively extruded PLA/CNC films having improved thermo-mechanical, barrier properties and enhanced shelf-life for storage of wide variety of food products, alongwith their excellent processability makes it an ideal candidate for packaging applications. Moreover, reactive extrusion using DCP radical initiators is itself an ecofriendly process and fabricated PLA/CNC films are also biodegradable, recyclable, non-toxic and environmental-friendly in nature. However, such sustainable packages with various added compatibilizers can only be used for food packages, if it follows the standard migration limits as established by the current regulations. In case of reactively extruded PLA/CNC films, potentially three different components namely (i) PLA chains, (ii) CNCs and (iii) compatibilizers can effectively undergo migration from films surface to the food materials in contact. During reactive extrusion PLA chains grafted onto CNCs undergo branching or cross-linking to form percolated gel-like network of high M_w , which is expected to impede the migration of both the simulants and

compatibilizers when kept in contact with food products. The effect of chemically interacting or non-interacting compatibilizers on overall migration of PLA/CNC films are studied in presence of the different food simulants namely acetic acid (3%v/v), ethanol (15%v/v) and water (Figure 6.20(a), (b) and (c)). For an initial period of ~3days, the overall migration values of the PLA/CNC films are considerably low even in the presence of different compatibilizers. However, after a time period of 3 to 5 days the overall migration values shoots up exponentially for all the three food simulants. The food simulant, acetic acid (3%v/v) shows higher overall migration values ($\sim 4 \text{ mg/dm}^2$), especially for non-reacting compatibilizers such as PLA/DCNC-S/Nanosilica, PLA/DCNC-S/Carb fiber and vegetable oils such as PLA/DCNC-S/Coco or PLA/DCNC-S/Olive due to their leaching effects (acetic acid also have higher solubility with oils). These films also show similar behavior of higher overall migration values for ethanol ($\sim 3.0 \text{ mg/dm}^2$) and water ($\sim 3.8 \text{ mg/dm}^2$) as food simulants (Figure 6.20(b) & (c)). The inorganic/organic nanofillers are well dispersed in PLA due to their improved compatibility and do not have any chemical interaction sites with the PLA graft CNC gels, because of which it could easily undergo migration when in contact with food simulants for prolonged period of time (Figure 6.20(a)). Several factors such as degradation of PLA chains in the presence of food simulants, migration of low M_w chains into simulants, lowering of T_g and formation of higher fractions of crystallites can lead to significant variations in overall migration values (with the different compatibilizers). Interestingly, overall migration values for all the reactively extruded PLA/CNC films in presence of different compatibilizers for all three food simulants are well under the standard limits of $\sim 10 \text{ mg/dm}^2$ set by the existing legislation (Commission Directive, 2002/72/EC) (Figure 6.20 (a-c)). This confirms that different compatibilizers could successfully be incorporated during reactive extrusion process

to fabricate PLA/CNC films with improved physio-chemical, structural and barrier properties. Therefore, our study provides an excellent opportunity to develop PLA/CNC based biodegradable films through a single-step reactive extrusion process, which is an industrially viable and eco-friendly approach of processing films for potential food packaging applications with non-toxic, recyclable and ecologically sustainable characteristics.



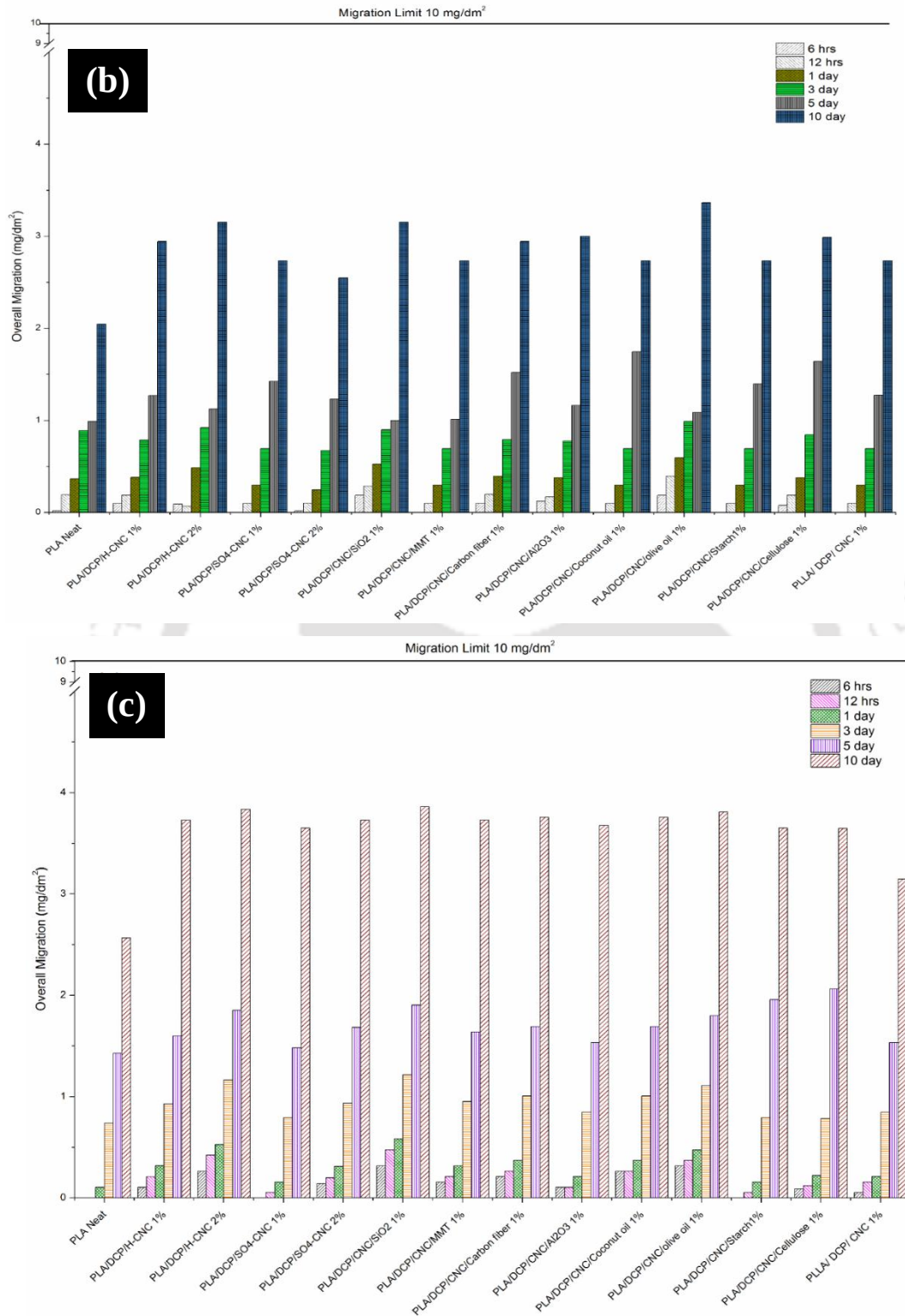


Figure 6.20: Migration studies of reactively extruded PLA/CNC nanocomposites films using different food simulants (a) Acetic Acid (b) ethanol and (c) water respectively.

6.3 CONCLUSION

This chapter primarily focuses on improving the processability and recyclability of polylactic acid (PLA) based cellulose nanocrystal (CNC) films, through industrially viable extrusion overcoming the technical challenges associate with traditional processes. The single step reactive extrusion process for fabrication of thermally stable PLA-g-CNC nanocomposites using dicumyl peroxide as crosslinking agent provides an opportunity for their efficient recycling, without causing any significant breakage in the molecular structure of PLA. NMR and FTIR spectroscopy studies showed that amorphous PLA chains graft on CNC surface through C-C bonds formation, the degree of grafting of which depends upon the type of compatibilizers used. PLA chains graft onto CNCs, formed cross-linked gel-like structures of high molecular weight ($M_w \sim 150\text{--}245\text{kDa}$), with varying grafting efficiency (14-67%) or gel-fraction yield (16-69%), depending on the type of compatibilizers used. In comparison to traditional process, PLA/CNC films processed through our approach are transparent and possess improved processability, dimensional stability, reduced thermal or shear induced degradation of either CNCs or PLA chains. Interestingly, the reactively extruded PLA/CNC films show reduced necking behavior/morphological instabilities, as evident from the normal stress rheological studies and measured parameters such as improved necking or thickness swell ratio and decreased film inhomogeneity index values. Furthermore, the reactively fabricated PLA/CNC films show enhanced thermo-mechanical responses and the added compatibilizers acted as strong reinforcing agents, due to formation of C-C crosslinks which acts as an efficient transfer of modulus of CNCs to PLA matrix. The detailed investigations of OP and WVP show that different compatibilizers induce tortuous diffusion path which comparatively decreases the diffusivity (D) for case of acid-derived CNCs, biopolymers and

inorganic/organic nanofillers. In contrast, reduction in solubility (S) is more predominant in case of vegetable oils as compatibilizers. The predicted shelf-lives for storage of oil-based and dairy-based beverage products using the reactively extruded PLA/CNC films are found to be maximum of ~5 months and ~2 weeks respectively, which are significantly improved compared to neat PLA films. Migration studies in the presence of different food simulants shows that existence of relatively larger fractions of grafted or branched high M_w PLA chains diminishes the mobility of added compatibilizers and all the reactively extruded PLA/CNC films successfully qualified the standard limits set by the legislations. Moreover, these reactively extruded PLA based CNC films (with different compatibilizers) are biodegradable, non-toxic in nature and can be easily recycled without any significant deterioration in properties. Therefore, this study provides an industrially viable approach of fabricating sustainable and biodegradable PLA based CNC packages with improved processability, dimensional stability alongwith enhanced physio-chemical, structural and barrier properties for potential packaging applications at the commercial scale.

Fabrication and Characterization of Magnetic Cellulose Nanocrystals and Applications in Sustainable Chemical Processes

Motivation

Due to the presence of abundant surface hydroxyl groups on cellulose nanocrystals (CNCs), their surface can be decorated with various species of iron nanoparticles to introduce magneto-responsive behavior for wide range of applications. The inherently non-toxic and biocompatible CNCs can be strategically modified to incorporate autonomous self-propulsion behaviour for targeted drug delivery applications. Transformation of the abundantly available renewable resources into bionanocatalysts for potential bioremediation and conversion of chemical moieties with high turnover frequency provides a novel approach of recycling ‘waste into wealth’. Owing to their distinct rod-like morphology, CNCs have the potential to be used as templates for fabrication of graphene nanoscrolls, which are known to be good hydrogen storage nanomaterials. Sustainable and environmental-friendly routes for modification of CNCs will lead to the development of high performance nanocomposites with potential applications in various chemical processes.

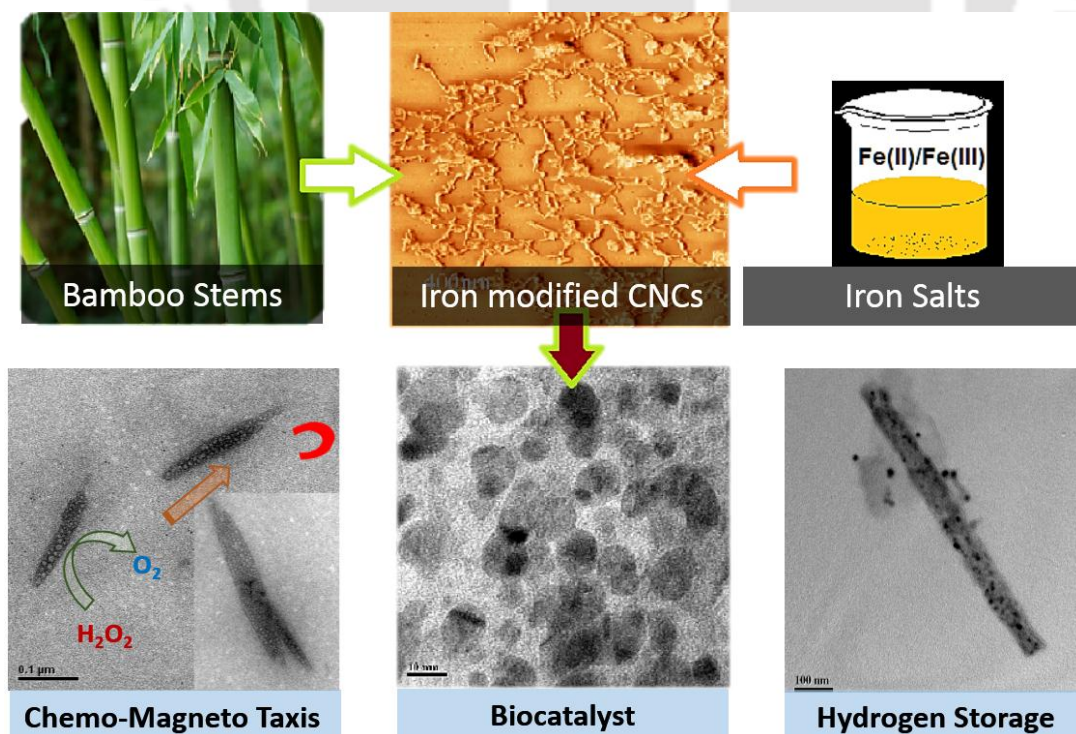
The work in this chapter originated from:

- Dhar, P., Kumar, A., & Katiyar, V. (2015). Fabrication of cellulose nanocrystal supported stable Fe (0) nanoparticles: a sustainable catalyst for dye reduction, organic conversion and chemo-magnetic propulsion. *Cellulose*, 22(6), 3755-3771.
- Dhar, P., Gaur, S., Chakroborty, G., Kumar, A., & Katiyar, V. “Cellulose Nanocrystal based templates for fabrication of Graphene nanoscrolls with high hydrogen storage capacity: An experimental and molecular dynamics study.” (in process of submission).

Abstract

This chapter focuses on the development of surface-modified CNCs derived from bamboo pulp with various forms of iron precursors and metallic nanoparticles through sustainable routes. High content of available hydroxyl groups on CNC surfaces are utilized as anchor points for the simultaneous reduction, precipitation and stabilization of metallic nanoparticles. These CNC-supported, zerovalent iron nanoparticles (Fe-CNC) can catalyze the degradation of methylene blue (MB) and the hydrogenation of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP), which makes them a potentially active and nontoxic biocatalyst. Interestingly, Fe-CNCs exhibit autonomous motion in the presence of peroxide fuel, whose trajectories could be externally controlled under both magnetic field and pH gradient. CNCs modified with iron-oxide nanoparticles (Fe_2O_3 -CNC), and catalytically active moieties such as palladium (Pd) and platinum (Pt) nanoparticles, can fold graphene sheets to form graphene nanoscrolls with tunable morphological dimensions and encapsulated metallic nanoparticles, through a template-based approach. This chapter discusses novel routes for the fabrication of CNC-based high performance value-added nanomaterials with potential applications in biocatalysis, targeted drug delivery, bioremediation and hydrogen storage devices.

Graphical Abstract



7.1 INTRODUCTION

Cellulose is the most abundantly available renewable biopolymer which, either in its pure form or as derivatives, has found potential application in electronics as sensor material [30], pharmaceutical [5], cosmetic, and packaging industries [4]. Recent developments in cellulose-based nanotechnology, especially fabrication of rod like crystalline cellulose nanocrystals (CNCs) through controlled acid hydrolysis, has generated a great deal of interest among researchers [4]. CNCs usually consist of glucose repeating units (10-100 units), each unit having six hydroxyl groups, which provides enhanced functionality and reactivity of the CNC surface towards chemical moieties [4]. The nanoscale dimension coupled with the high surface reactivity makes CNCs a novel green structural template for deposition of metal nanoparticles [432]. Synthesis of such biopolymer supported noble metal nanoparticles has been shown to improve the dispersibility, reduce the coagulation/agglomeration related problems and drastically enhance the stability of colloidal suspensions of metallic nanoparticles [433]. Till date, several reports have been published on the synthesis of metal nanoparticles using CNCs as a reducing template. Benaissi et al. [434] first reported the fabrication of platinum nanoparticles (PtNPs) using CNCs as reducing agent in the presence of supercritical carbon dioxide. Rezayat et al. [435] synthesized palladium nanoparticles (PdNPs) with CNCs acting as both reducing agent and supporting substrate in the presence of subcritical and supercritical carbon dioxide. Detailed investigation by this research group revealed that the abundant hydroxyl groups on the surface of CNCs provided a stable surface for the reduction of the metal ions, for e.g. platinum and silver. Further, they fabricated carbon supported PtNPs by simply removing the CNCs through calcination, which showed high electrocatalytic activity [436]. On a similar note, Yang et al. [232] fabricated silver nanoparticles by simply reducing

the nitrate salt of silver under hydrothermal conditions for 2 hours. Cirtiu et al. [437] incorporated PdNPs uniformly deposited on the cellulosic surface through reduction with hydrogen under pressurized conditions. Recently, Dong et al. [36] reported fabrication of silver nanoparticles within highly dispersed bacterial cellulose nanofibers based hydrogels, synthesized in situ without any application of reducing agents. Several other metal nanoparticles such as gold [438], [439], selenium [234], titanium [238], [237], nickel [233] etc. have been synthesized using CNC as precursor which acted as both reducing and stabilizing agent [236]. Green synthesis of such CNC based metal nanoparticles has potential application as biocatalyst for efficient degradation of pollutants and conversion of chemicals, with improved reusability.

In the last few decades, zerovalent iron (ZVI) nanoparticles have generated great interest among researchers in academia and industry, as these nanoparticles have the potential to reduce pollutants and disinfect water contaminants. ZVI nanoparticles have large specific surface area, high surface reactivity and low cost [440]. Their nontoxicity allows them to be directly injected at the site of contamination [441]. However, there still persist several drawbacks of ZVI, such as the tendency to coagulate, leading to reduction in surface area, and rapid formation of oxide layer, leading to drastic reduction in activity [442]. To overcome these limitations, several approaches have been proposed such as entrapment of ZVI in polymers, stabilizers and supported porous materials. Such methods lead to improved stability but also result in drastic reduction in reactivity due to less availability of reactive sites [443]. However, there are very few reports [444], [445] on the application of “reagentless” approach and utilization of renewable materials as green reducing agent and stabilizer for the fabrication of ZVI.

Along with the ZVI nanoparticles, two distinct varieties of iron oxide nanoparticles exist (with +2 and +3 valence) namely maghemite and magnetite. Modification of the surface of CNCs with such iron oxide nanoparticles (Fe_2O_3 -modified CNCs are discussed in this chapter and Fe_3O_4 -modified CNCs in the next Chapter) leads to the introduction of the magneto- responsive behaviour in the CNCs. Due to their rod-like morphology, CNCs have been widely used as templates for fabrication of hollow metallic nanorods [235] or micro- beads/micro- capsules through self-assembly routes [446]. CNCs are generally hydrophilic in nature and precipitation of the iron oxide nanoparticles on its surface increases the hydrophobicity, leading to the formation of distinct regions on the CNC surface having opposing functionalities and interaction behavior, mimicking Janus materials. As discussed in previous section, wide variety of metallic nanoparticles have been adsorbed onto the surface of CNCs for the formation of template structures. However, modification of CNCs with graphene for the fabrication of carbon-nanotube-like structures have not been previously reported. Covalent functionalization of such multi-responsive magnetic CNCs with nanomaterials such as graphene, leads to incorporation of multi-functional properties. Formation of Janus graphene have been reported in literature which are an interesting material with asymmetric functional groups on opposite sides that have potential applications as self- propelled motors, sensors or as building blocks for super-structures. Therefore, through strategic chemical modifications of magneto-responsive CNCs with other nanomaterials it is possible to use it as structure- directing agent as well as introduce remarkably interesting stimuli-responsive properties as present in Janus-like materials.

Formation of graphene nanoscrolls (GNS) through self-assembly of graphene sheets which rolls up onto the surface of the iron nanorods have been recently reported through

simulation studies [447]. The rolling of graphene sheets into scrolls is an energy intensive process with a threshold energy barrier of ~ 1000 J/mol, which can be overcome through the introduction of external force or stimulus. Once this energy barrier is overcome, the rolling of graphene sheets self-propagates due to the presence of π - π interactions [448]. The fabrication of such GNS takes place either from corner or end, through rolling of single graphene sheet which results in excellent conductivity and higher accessible surface area in comparison to single walled nanotubes. Presence of concentric graphene layers in GNS makes it potentially viable for a wide range of applications such as energy storage (as supercapacitors), hydrogen storage, catalysis etc. the efficiency of which can be finely tuned depending on the inter-layer spacing and dimensions of GNS.

Despite the unique properties of GNS, very few reports comprising both experimental and simulation studies have been reported. GNS can be classified into two types depending on the fabrication process followed: (i) template-based approach through utilization of carbon nanotubes, nanodroplets and nanoparticles, and (ii) application of external forces such as ultrasonication, microwave sparks, thermal processing, electric fields and solvents [447]. The dimensions of fabricated GNS through the above-mentioned methodologies varies widely with diameter in the range of ~ 20 – 600 nm and lengths of ~ 0.7 – $10\mu\text{m}$, which can be controlled through experimental reaction conditions, dimensions of the initial precursor graphene flakes and its surface chemistry [449]. Fabrication of GNS through utilization of rod-like CNCs as templates, coupled with magnetic and electrostatic interaction between Fe_2O_3 nanoparticles and graphene, has not been reported till now. Presence of both the external stimuli forces acting on the surface of graphene sheets is expected to have a synergistic effect which would probably lead to improved scrolling efficiency with reduced defects compared to other methods.

Moreover, through the template-based approach it is also possible to incorporate other catalytically active nanoparticles inside the GNS (at desired loadings), depending on the targeted applications. The variation in inherent characteristics of CNCs (the initial precursor) in terms of structural and morphological behaviour (depending on the cellulose polymorph, hydrolyzing acid etc.) also provides an opportunity to tune the physicochemical properties of GNS. The rolling of graphene sheets into GNS in the presence of magneto-responsive CNCs is a complex phenomenon, and its mechanism has been investigated in the present work through experimental and simulation studies, as discussed in the subsequent sections 7.2.5 and 7.2.6 respectively.

In this study, the surface of CNCs has been strategically modified with two variants of iron precursors, ZVI (Fe^0) and maghemite (Fe_2O_3) nanoparticles and studied for their potential applications in various chemical processes such as degradation of dye, catalytic conversion of chemical moieties, self-propagating autonomous nanobots for targeted drug delivery and hydrogen gas adsorption capacity. In the first case (modification with ZVI), we report green and single step process for the fabrication of ZVI-adsorbed CNCs (Fe-CNCs), in which the precursor CNCs act both as reducing as well as stabilizing agents. This innovative approach reduces the usage of harmful reducing agents and with the abundant resources of cellulose available, provides an attractive cost effective option for large scale fabrication of CNC- supported ZVI. Moreover, the fabricated ZVI showed enhanced stability and reduction in formation of oxide layer due to the corrosion inhibitory activity of the CNCs. It is noteworthy to mention that the synthesized ZVI nanoparticles show high catalytic activity towards the rapid degradation of the model pollutant dye methylene blue (MB) and the conversion of toxic 4- nitrophenol (4-NP) to nontoxic products. Further, the synthesized

CNC- supported ZVI showed the interesting phenomenon of self-propulsion under the influence of pH gradient and magnetic field. Such self-propelled CNCs, reported for the first time here, will be an ideal candidate for drug delivery and design of biomedical devices because of their nontoxicity and biocompatibility. In the second case, we utilize the Fe_2O_3 -CNCs as templates for scrolling up graphene sheets onto its surface followed by calcination of CNC to form the GNS. The effect of variation in morphology of initial CNC precursor on the morphological dimensions of GNS have been studied. The mechanism of scrolling up of graphene sheets into GNS has been investigated in detail, and the experimental observations are supported by molecular dynamics simulation. Furthermore, we encapsulated two different varieties of catalytically active Pt and Pd nanoparticles in the GNS, and studied their effect on the hydrogen gas adsorption capacity of the GNS. Therefore, this chapter discusses the development of magneto-responsive CNCs with different iron nanoparticles adsorbed on the CNC surface, for a wide range of potentially high performance applications, with an aim to convert the renewable biomass to value-added products.

7.2 RESULTS AND DISCUSSIONS

7.2.1 *Fabrication and Characterization of Cellulose Nanocrystal-Supported Zerovalent Iron (Fe-CNCs)*

CNCs were isolated from pretreated bamboo biomass source through sulphuric acid hydrolysis. The synthesized CNCs had an average length of 350 ± 50 nm, a width of 20 ± 5 nm, with aspect ratio of ~ 20 (obtained from AFM micrograph shown in Figure 7.1(a)). FESEM micrographs (Figure 7.1(b)) show the uniformly dispersed but agglomerated freeze-dried CNCs. The agglomeration is due to strong intermolecular hydrogen bonding interactions between CNCs. The TEM micrographs (Figure 7.1(c)) reveal that the CNCs have length and

diameter in the range 50–80 nm and 10–20 nm respectively. The fabricated CNCs had a crystallinity index (C.I.) of 89.8% and BET surface area of 5.154 m²/g. The CNC-supported ZVI nanoparticles (Fe-CNCs) were synthesized by dispersing CNC solution (30 and 70 wt. %) dropwise into iron (III) chloride salt solution through vigorous magnetic stirring under an inert atmosphere. A uniformly stabilized colloidal dispersion of CNCs was obtained immediately, even at high CNC concentrations. The driving force for such phenomenon is the increased interaction between the positively charged metal salt and the negatively charged CNCs due to the presence of sulfate groups on CNC surface. Iron salts have higher binding constants with hydroxyl/carboxyl groups of cellulose, and hence eliminated water molecules from interfibrils of CNCs [450]. This led to enhanced interaction of metal ions with hydroxyl/carboxyl groups and reduction in the distance between interfibrils, resulting in better adhesion and dispersion. Such interaction in iron-CNC colloidal dispersion was confirmed through FTIR studies (Figure 7.2) which showed characteristic peaks at 1738–1750 cm⁻¹ and split bands in 1608–1615 cm⁻¹ range as reported by Dong et al. [450]. The intensity of characteristic peaks (1750 cm⁻¹ and 1610 cm⁻¹) decreases with the reaction time, which possibly represents the reduction of iron salt and formation of stable ZVI nanoparticles. The peaks at 1750 and 1610 cm⁻¹ correspond to interaction of –OCO– groups with the metal salts and the asymmetric –OCO– stretching vibration respectively [36]. The broad band centered around 3500 cm⁻¹ represents the stretching vibrations of bonded hydroxyl groups [451] and the low intensity sharp peak at ~3750 cm⁻¹ represents free hydroxyl groups of cellulose [450]. Moreover, in line with the reported literature, the peaks at ~3750 cm⁻¹ in CNCs shifted to ~3730 cm⁻¹ after the reaction with the ferric salts. Further on addition of iron salt and its subsequent reaction, this broad peak disappeared and two new peaks appeared at 3130 and 3020 cm⁻¹. The peaks at

~ 1429 and $\sim 2910\text{ cm}^{-1}$ in CNCs, which correspond to symmetric CH_2 bending and stretching vibrations respectively [302][53], shifted to 1410 and 2889 cm^{-1} on reacting with iron salts after 4 hours. Similarly, the peak corresponding to ferric chloride at 414 cm^{-1} [452] also decreased in intensity after 2 and 4 hours of reaction time. This is probably due to the adhesion or formation of ZVI nanoparticles on the hydroxyl groups present on the surface of CNCs. Therefore, the decrease in intensities of characteristic peaks with the reaction time and appearance of new peaks (at 3130 and 3020 cm^{-1}), is probably due to the interaction of CNCs with the iron salt, which represents the reduction process and formation of stable ZVI nanoparticles. Further, molecular modelling studies [453] have shown that iron cations have very high displaced solvation volumes and binding energy values. The 3d valence states of the iron easily hybridizes with the 2p valence states of oxygen (hydroxyl) to form strong coordination-covalent bonding [453].

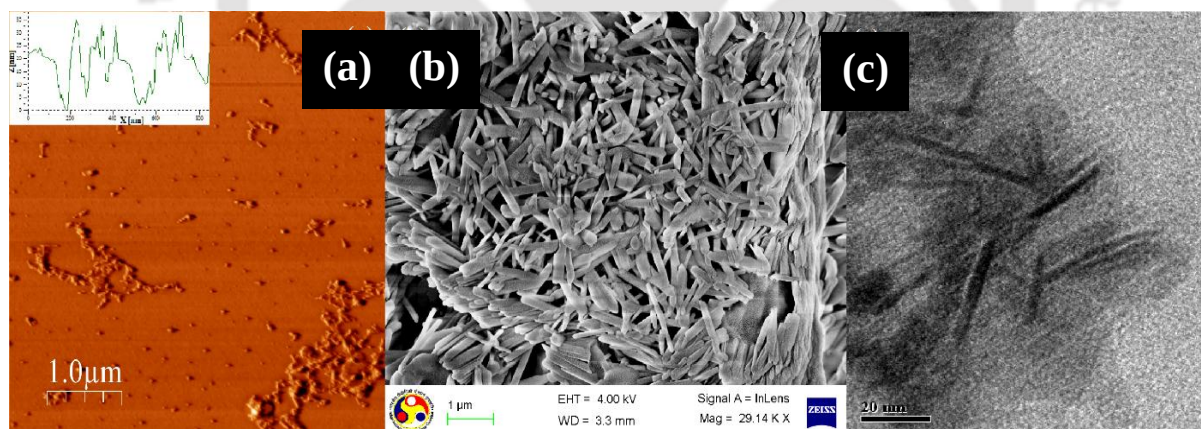


Figure 7.1: (a) AFM, (b) FESEM and (c) TEM micrographs of laboratory-synthesized CNCs used as precursor for the synthesis of Fe-CNCs.

After mixing and mechanically agitating the iron-CNC colloidal suspension (30 and 70 wt. %) the suspension immediately turned light greenish in color. The suspension was kept under inert gas flow for 4 hours at 80°C . The greenish color gradually changed to black color

after a few hours, suggesting the complete conversion of the iron salt to ZVI nanoparticles. The Fe-CNCs were then immediately separated by applying magnetic field, and washed with ethanol and vacuum dried to minimize oxidation.

The morphological investigation of the reaction product has been carried out using TEM (for both 30 and 70 wt. % CNC solution), and compared with the pristine CNC template. Figure 7.3(a) presents the TEM image of the ZVI nanoparticles synthesized using 30 wt. % CNCs along with the corresponding particle size distribution (Figure 7.3(a')). The ZVI nanoparticles are uniformly dispersed with diameters in the range of 3-9 nm, and are nearly spherical in shape. However, tiny ZVI nanoparticles are also observed, displaying a very narrow size distribution in the range of 1.6-3.8 nm (Figures 7.3(b) and (b')). TEM images at higher magnification showed that both types of ZVI nanoparticles maintained their distinct spherical morphology. High resolution transmission electron microscopy (HRTEM) and SAED patterns show crystalline structure of the ZVI nanoparticles (Figures 7.3(c) and (d)). ZVI clearly shows co-directionally oriented lattice fringes of 0.211nm, representing the single crystalline nature of synthesized nanoparticles. Electron diffraction ring patterns confirmed the presence of crystalline ZVI nanoparticles having bcc type Fe crystallites, as evidenced from the presence of (110) and (200) type diffused ring patterns. Synthesis of ZVI nanoparticles with uniform size distribution and tiny dimensions has been reported in literature previously. Huang et al. [454] reported synthesis of highly monodisperse ZVI nanoparticles with average diameter of 6.0 ± 1.3 nm using palladium ion seeds and polyacrylic acid as dispersing agent. Farrell et al. [455] demonstrated that controlled thermal decomposition of iron carbonyl yields monodisperse ZVI of diameter 7.0 ± 0.8 nm. However, very few studies [456] on the synthesis of ZVI using “green” biopolymer-based nanomaterials with simultaneous reducing and

dispersing activity, and with uniform size distribution have been reported. Electron micrographs in Figures 7.3(a) and (b) show the formation of large fraction of highly dispersed spherical ZVI nanoparticles which are usually formed under strong reducing conditions due to the hydroxyl functional groups available on CNC surface (30 wt. %). It is noteworthy to mention here that addition of 70 wt. % CNC solution leads to the formation of rice grain morphology of CNCs supported ZVI (see Figures 7.4(a) and (b)). The size of the ZVI nanoparticles formed on the surface of CNCs (70 wt. %), shown in Figure 7.4(c), was found to be in the range of 11–23 nm. On increasing the concentration of the reducing agent (i.e., CNCs), it is possible that the ZVI nanoparticles are attaching to the CNC surface through hydrogen bonding or electrostatic interaction with the oxygen atoms present in the hydroxyl groups. The EDX spectra and mapped FESEM image of CNC supported ZVI (see Figures 7.4(d) and (e)) show the presence of iron, carbon, oxygen, chlorine and trace amount of sulphur, confirming the presence of sulphuric acid hydrolyzed CNCs. The presence of chlorine could be attributed to the formation of the ammonium chloride as by-product during the fabrication process of CNCs supported ZVI. This is also supported by the FTIR spectra in Figure 7.2 which show the presence of peaks at $\sim 1190\text{ cm}^{-1}$ and $\sim 1410\text{ cm}^{-1}$ corresponding to ammonium chloride. We also found sparsely dispersed submicron polyhedrons with octahedral geometry resembling fcc-Fe lattice structures (prism like morphology) (see Figures 7.5(a) and (b)), which might have formed when the reduction precursors (30 wt. % CNC) became weak leading to the formation of aggregated structures. Under such conditions, the thermodynamic equilibrium leads to the formation of fcc-Fe nanoparticles as reported in literature [457]. When active sites of CNCs (30 wt. %) are already capped/interacting with ZVI, the reducing activity of hydroxyl groups is reduced significantly which leads to the formation of icosahedral

structures. Fabrication of different morphologies of iron nanoparticles using plant extracts as reducing agent have been reported in literature [445]. It has been observed that at higher loading fraction of CNCs (70 wt. %) in Fe^{3+} salt solution, the abundant hydroxyl groups completely reduced the salts into ZVI nanoparticles which were adsorbed on CNC surface. TEM micrographs in Figure 7.4(c) show the rice grain-like morphology of CNCs, with their surface modified by the spherical ZVI nanoparticles agglomerated into bundles. Therefore, it can be concluded that, depending on the applications the morphology of ZVI nanoparticles can be tuned by varying the weight fraction of reducing CNC precursor.

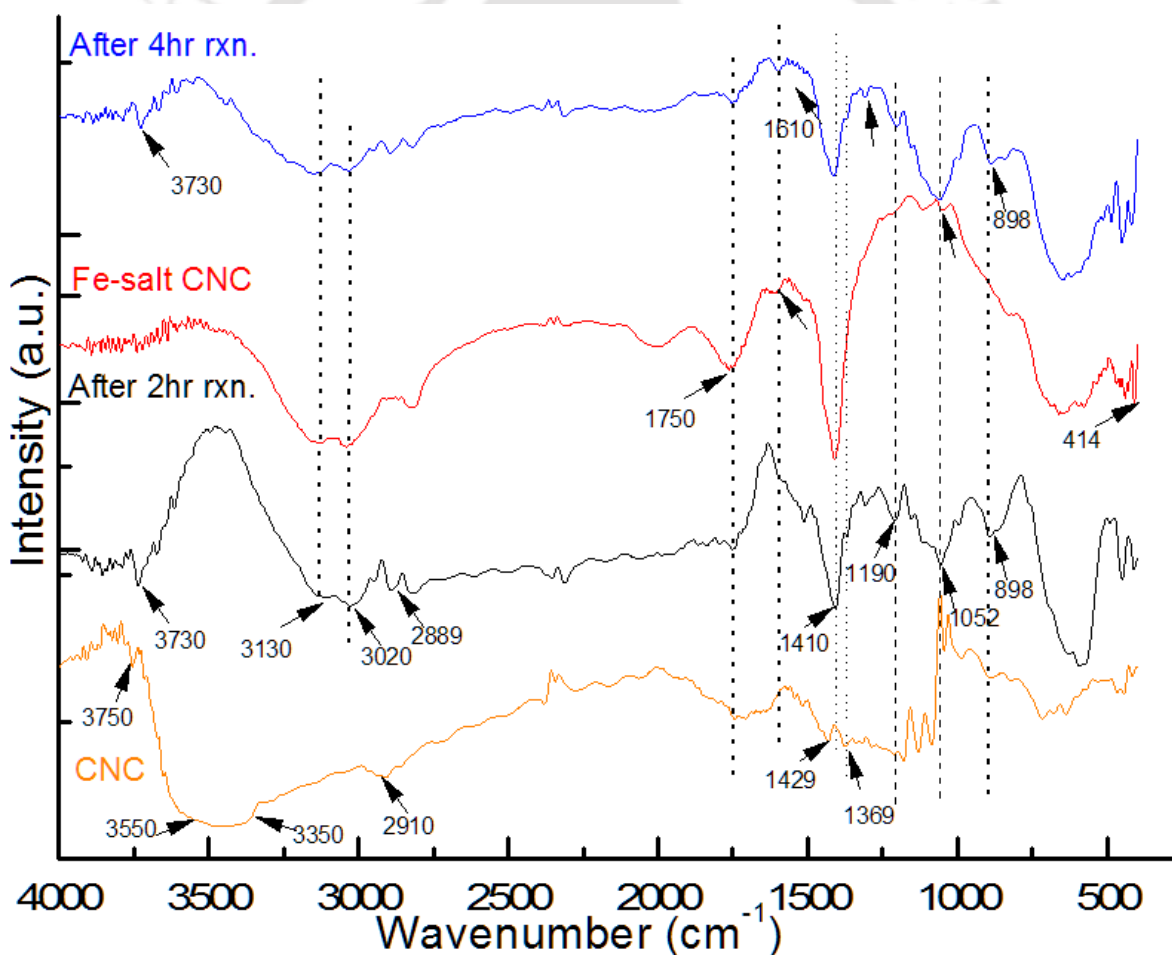


Figure 7.2: FTIR spectra of the CNC and iron-CNC colloidal solution at different time intervals (magnetically stirred at 1000 rpm and 90°C).

Crystallographic studies of ZVI using XRD showed single, distinct, highly crystalline peak at 44.9° for 70 wt. % CNCs supported ZVI, which is a fingerprint of the bcc lattice structure of iron nanoparticles (Figure 7.6 (a)). The XRD diffractogram of the CNC shows peaks positioned at 14.5° ($1\bar{1}0$), 16.2° (110) and 22.4° (200) respectively (Figure 7.6(a)). The XRD pattern for CNCs supported ZVI in Figure 7.6(a) clearly shows the presence of cellulose peak at 22.8° which is or magnetic ($\text{Cu}_{0.5}\text{Co}_{0.5}\text{Fe}_2\text{O}_4$) ferrite cellulose nanocrystal consistent with the cellulose I crystal structure peak as reported [458].

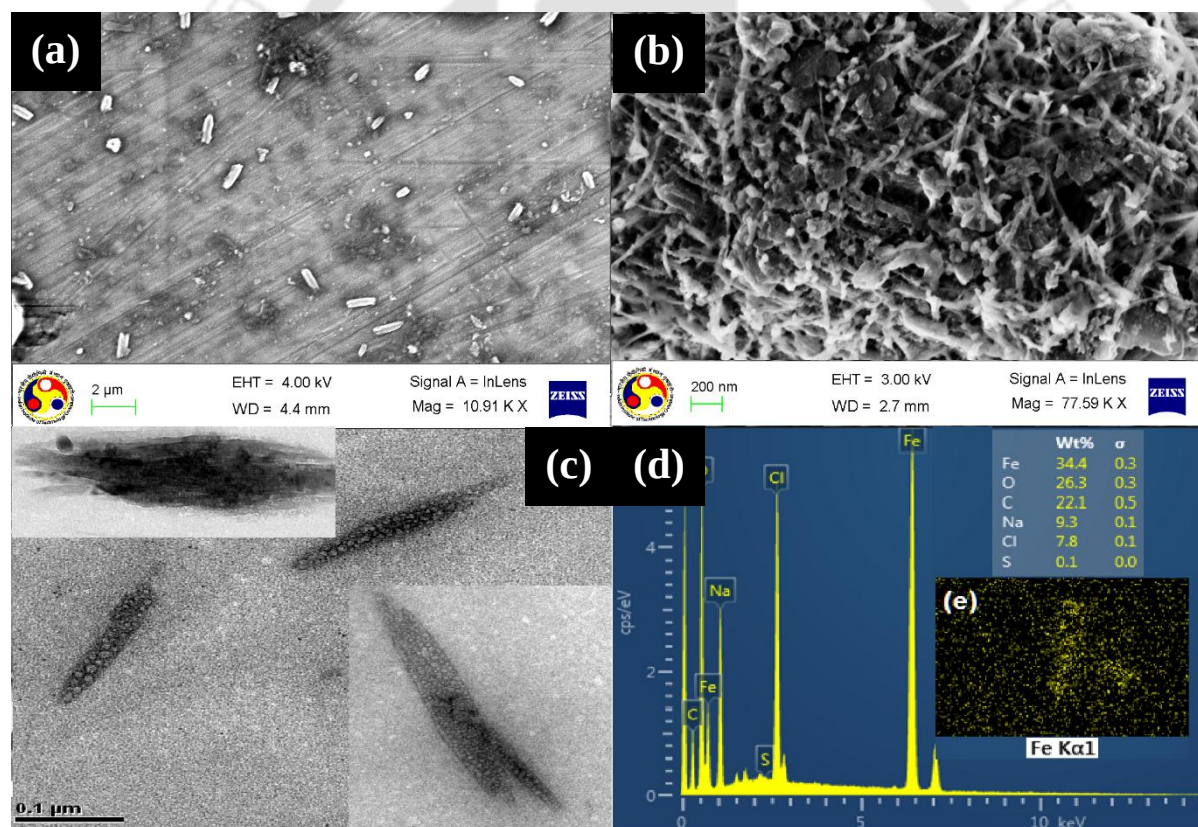


Figure 7.3: (a) TEM image of CNCs supported ZVI (at 30 wt. % CNC) and (a') their size distribution, (b) TEM image of narrow size CNCs supported ZVI (at 30 wt. % CNC) and (b') their size distribution, (c) HRTEM image showing representative planes, and (d) SAED pattern.

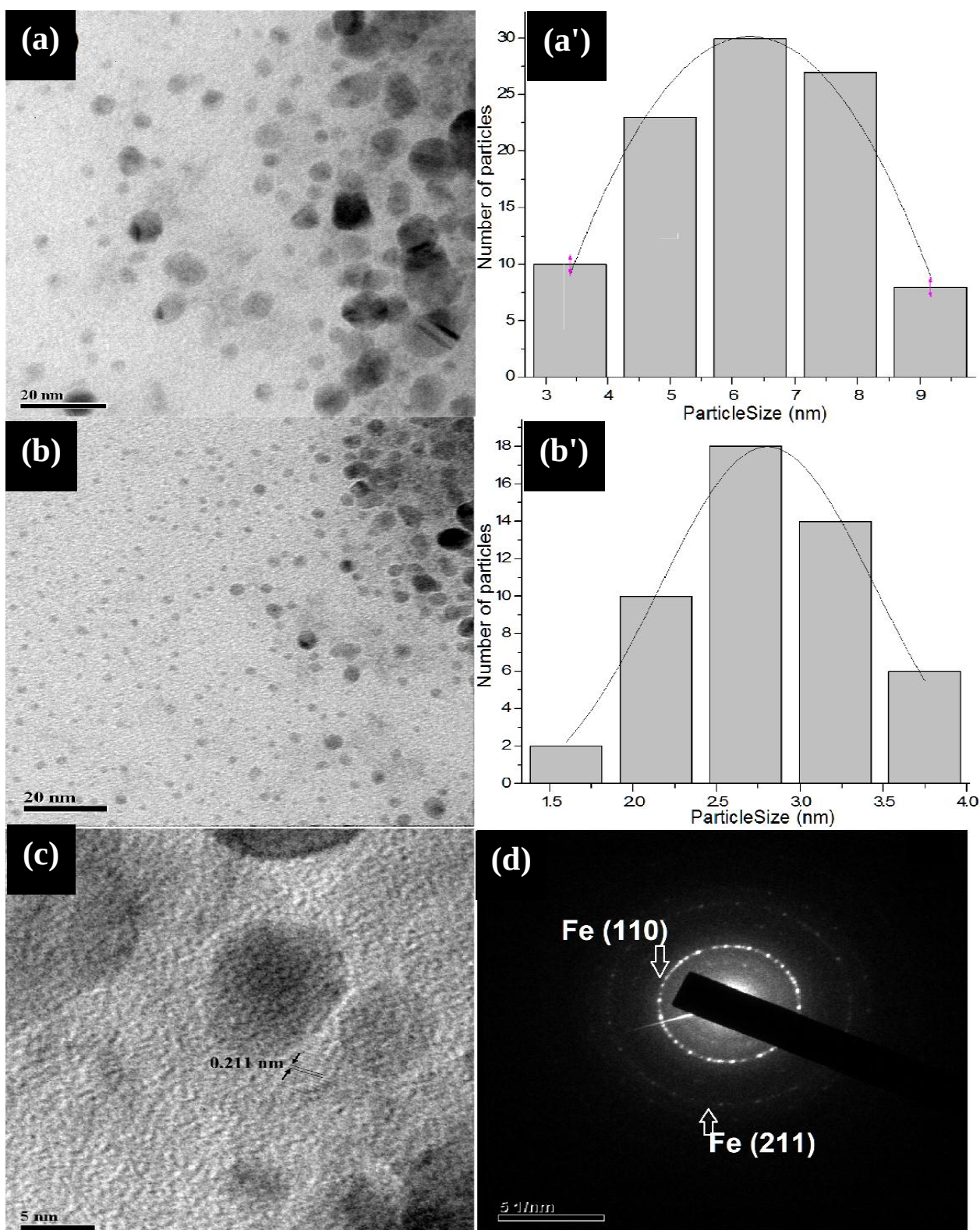


Figure 7.4: (a) & (b) FESEM and (c) TEM images of CNCs supported ZVI (at 70 wt. % CNC).

(d) & (e) EDX spectra and representative mapped image of iron on selected region.

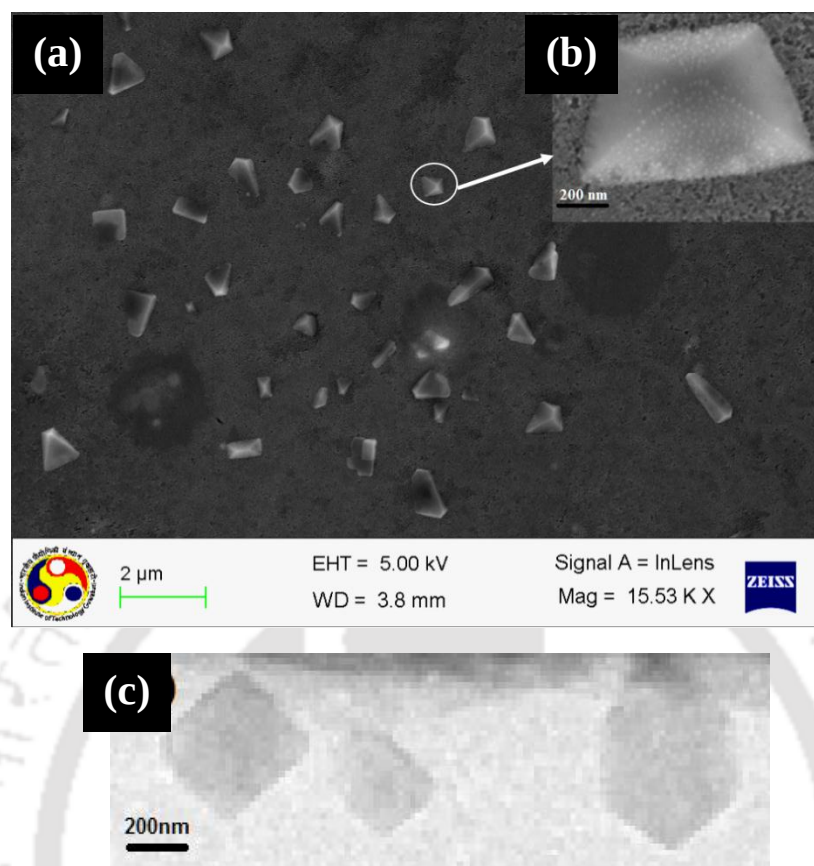
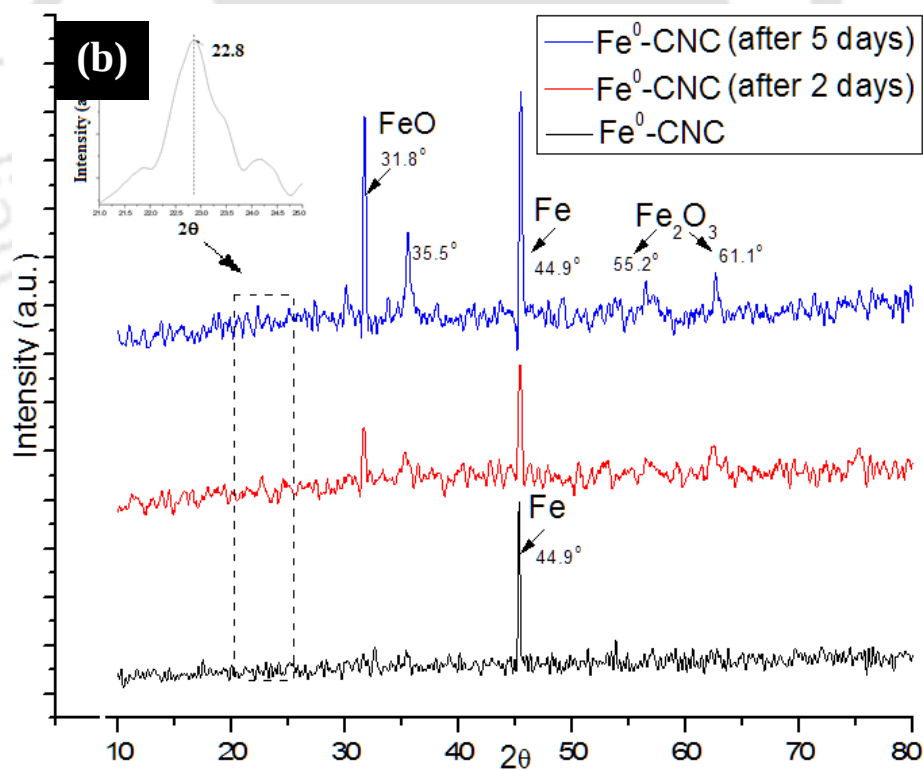
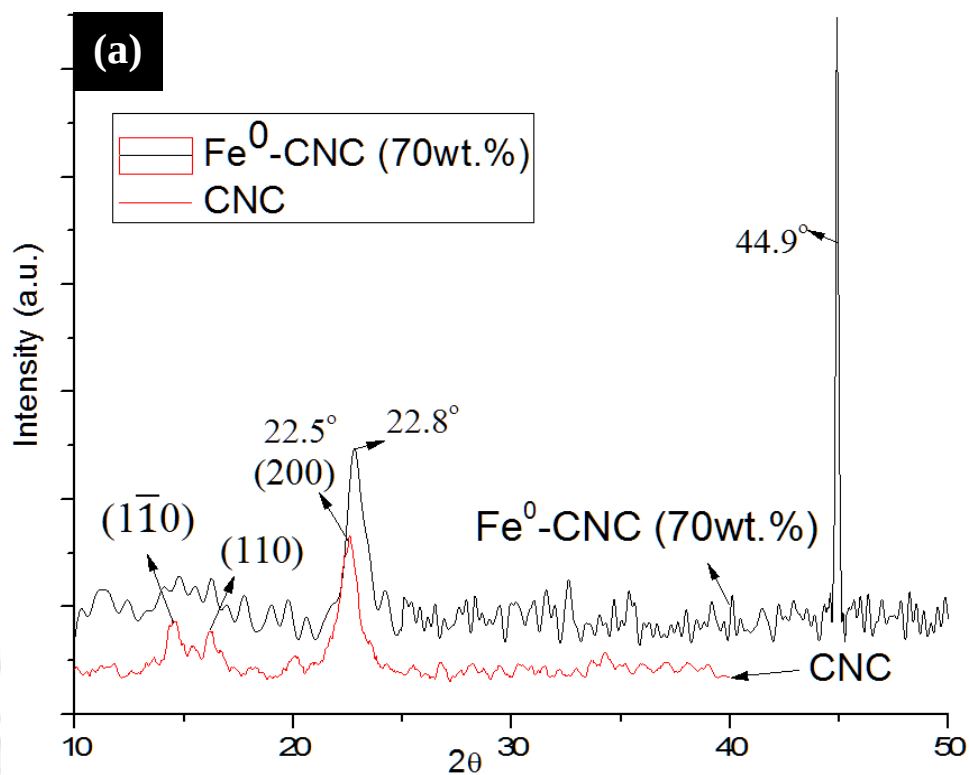


Figure 7.5: (a) FESEM image of octahedral fcc-Fe nanoparticles (prism like morphology in inset (b)), (c) TEM image of the octahedral fcc-Fe nanoparticles.

The minimum crystallite size of ZVI (corresponding to the zero iron peak at 44.9°) for Fe^0 -CNC (70 wt. %) was computed as 21 nm (using Scherrer equation) which is consistent with the size of Fe^0 nanoparticle (in the range of 11–23 nm) calculated from TEM image in Figure 7.4(c). The ZVI nanoparticles supported on CNCs were found to be highly stable and resistant to corrosion as compared to nascent ZVI nanoparticles. The dark brown color of CNCs supported ZVI remained unchanged even after days of exposure under ambient conditions. UV-Vis spectroscopy analysis of CNCs supported ZVI (dispersed in water) was carried out before and after exposure to air (for 7 days) to understand the oxidation process.

Consistent with values reported in literature [459], ZVI showed the characteristic peaks at 245 and 350 nm, which confirms the presence of pure Fe nanoparticles (Figure 7.6(d)). However, after exposure to air for 7 days CNCs supported ZVI nanoparticles showed peaks at 369 and 487 nm, indicating the presence of iron oxides. Further, XRD studies were carried out to understand the formation of oxide layers on CNCs supported ZVI (Figure 7.6(b)). It shows the distinct crystalline peak of the zerovalent iron along with the oxide layer peaks which appear after two days of exposure. The peaks at 31.8° and 35.5° correspond to the formation of FeO, and low intensity peaks at 55.2° and 61.1° represent the initiation process for the formation of Fe₂O₃. The presence of XRD peak at 44.9° shows that ZVI nanoparticles still retain their zerovalent properties, with a thin oxide layer which prevents its oxidation. Such high stability of CNCs supported ZVI could be explained by the corrosion inhibition properties of CNCs. Recent studies by Garner et al. [460], have shown that charged CNCs, especially the sodium form, act as potential corrosion inhibitors for ferrous metals as they form a coating around the ionic components of metal surface and prevent electrochemical reduction. Similar ionic interactions at the nanoscale between the CNC surface and ZVI, prevents the rapid formation of oxide layer. Therefore, from both XRD and UV-Vis spectroscopy studies, it could be concluded that apart from acting as a reducing agent and support during ZVI nanoparticle synthesis, CNCs act as a corrosion inhibitor for the synthesized ZVI nanoparticles.



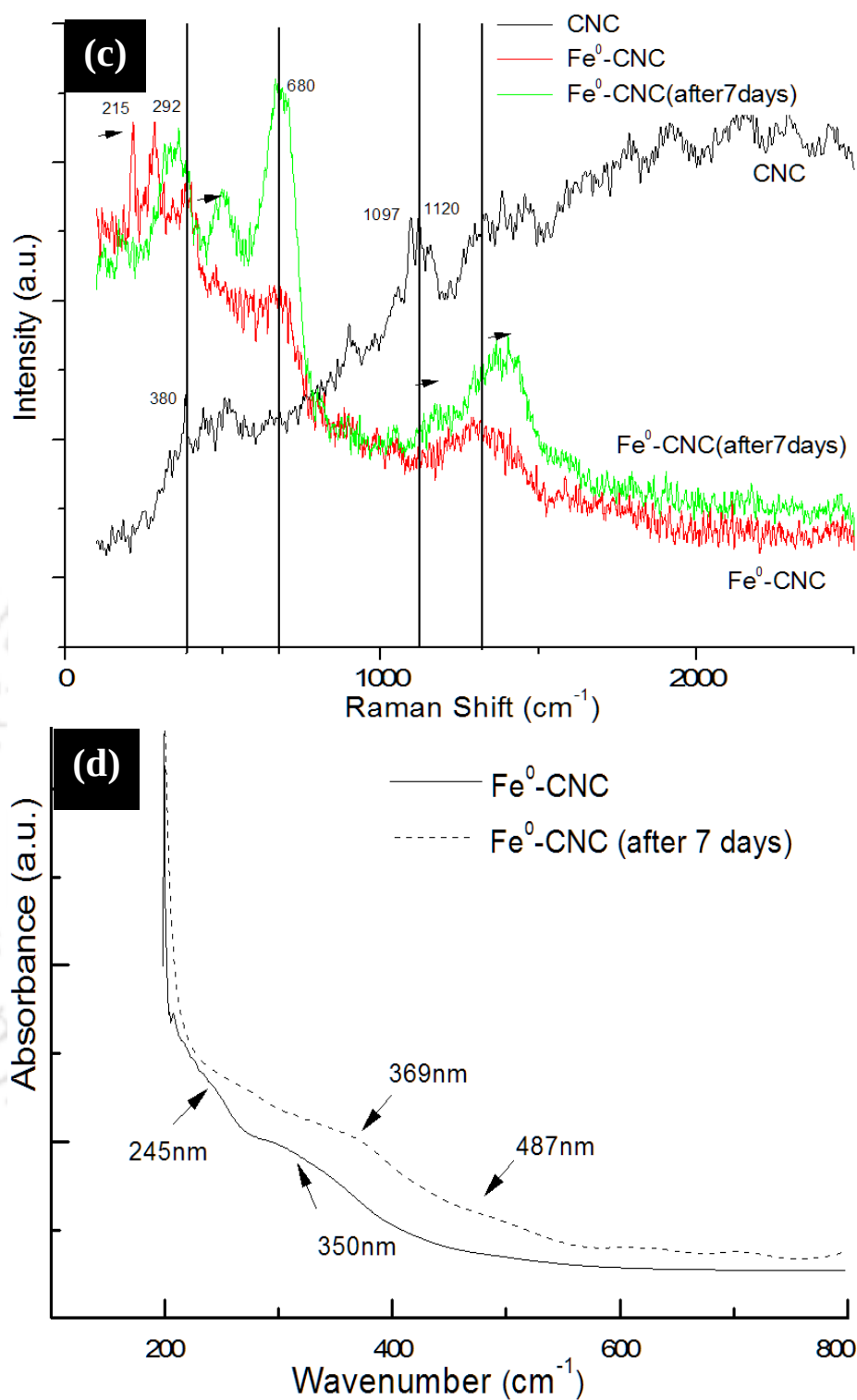


Figure 7.6: XRD patterns of (a) CNCs and CNCs supported ZVI (70 wt. % CNC), (b) after exposure for 2 and 5 days (with inset shows CNC peak at 22.8°), (c) Raman spectra and (d) UV/VIS absorption spectra of CNCs supported ZVI nanoparticles dispersed in water before (solid) and after exposure for 7 days (dashed).

In addition to oxidation stability, physical observation of the CNCs supported ZVI suspension in water showed enhanced dispersion stability with very little aggregation over time. The increased stability of the suspension (1 wt. %) was characterized by an increase in the surface potential or zeta potential of ZVI nanoparticles. As per literature studies, the ZVI nanoparticles tend to agglomerate due to low surface charge, usually below 10 mV at neutral pH [461]. In general, the zeta potential of a suspension must be between +30mV to -30mV to form an effective metastable suspension. The CNCs supported ZVI have a negative zeta potential (at neutral pH) of -35 mV, which leads to the formation of a stable dispersion. Such stable suspension of ZVI could be explained on the basis of zeta potential of the supporting CNC precursor (-55 mV) [321]. CNCs usually form a stable suspension due to the presence of intermolecular repulsive forces arising from the sulfate charge groups. The presence of sulfate and hydroxyl groups on CNCs decreases the overall surface charge of synthesized ZVI nanoparticles, thus stabilizing them and preventing their aggregation. The negatively charged CNCs supported ZVI would have enhanced mobility in groundwater, and thus have potential application in waste water remediation.

The chemical composition of the synthesized ZVI was studied using Raman and FTIR spectroscopy, to understand the interactions with the supporting CNCs. CNC precursor showed the characteristic Raman peaks (Figure 7.6 (c)) at 380, 1097 and 1120 cm^{-1} [462]. The CNCs supported ZVI nanoparticles showed the characteristic peaks of ZVI at ~ 215 and ~ 292 cm^{-1} consistent with values reported in literature [463], and a peak at 380 cm^{-1} showing the presence of CNCs. The presence of CNC peak at 380 cm^{-1} indicates that the reduction of ZVI took place only on the surface hydroxyl groups of CNCs, and there was no significant change in the bulk structure of CNCs [462]. FTIR study also showed the characteristic peak for the ZVI

nanoparticles at 1052 cm^{-1} (Figure 7.2). The peak corresponding to the sulfate group of CNCs at $\sim 1369\text{ cm}^{-1}$ remains unaltered in case of CNC-iron salt solution (after 2 and 4 hr of reaction), which played a significant role in increasing the stability and overall surface charge of synthesized ZVI. The shift and change in the intensity of the -OH stretching after formation of ZVI clearly shows interaction of the iron nanoparticles with surface hydroxyl groups of CNCs. Similar type of phenomenon has been reported by Horzum et al. [456] on the interaction of iron nanoparticles with chitosan fibers. However, the band due to crystalline cellulose was missing suggesting that the overall crystallinity of CNCs might have decreased. Comparison of FTIR spectra shows that the distinctive peaks of crystalline cellulose at ~ 1427 and 898 cm^{-1} significantly decreased in intensity after the formation of ZVI [464]. The overall decrease in crystallinity of cellulose might have occurred due to treatment of CNCs at high temperatures in the presence of sodium hydroxide/ammonia, or disorientation of cellulose nanofibrils due to the formation of highly crystalline ZVI. After 7 days under ambient conditions, the Raman spectra of ZVI nanoparticles showed new representative peaks at 680 and 1389 cm^{-1} (Figure 7.6 (c)), whose intensity increased with time, representing the formation of oxide layer on ZVI. As discussed above, CNC acts as a corrosion inhibitor towards formation of oxide layer on ZVI. From the Raman and FTIR studies, it can be concluded that surface hydroxyl groups on CNCs act as strong reducing agent, and play an important role in stabilizing the ZVI.

7.2.2 Applications of Cellulose Nanocrystal-supported Zerovalent Iron (Fe-CNCs):

Bioremediation and Sustainable Bionanocatalysts

Negatively charged CNC-supported ZVI have potential application in the bioremediation of wastewater. To measure the effectiveness of these nanoparticles in wastewater remediation,

degradation of methylene blue dye (MB) and conversion of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) in the presence of CNC-supported ZVI was studied. The degradation of MB was visually examined as blue colored solution turned transparent, and spectroscopically monitored through continuous decline in absorbance at 665 nm [465]. With addition of small amount (10 mg) of CNC-supported ZVI, the dye degraded immediately, which indicates that the fabricated ZVIs are highly effective in catalyzing MB degradation. Figures 7.7(a) and (b) show the degradation profiles of two different concentrations of MB dye (600 and 400 μM respectively) with respect to time. With increase in contact time, a gradual reduction in the intensity of the 660 nm peak can be observed. Gradual decolorization of MB with time was also observed visually. The corresponding rate constants were obtained from plots shown in Figure 7.7(c). The rate constants measured were 0.01361 and 0.0205 min^{-1} for the degradation of 600 and 400 μM MB dye respectively. This decolorization is possibly due to the reduction of MB by oxy radicals generated by ZVI [465] along with the adsorption by negatively charged CNCs. However, the rate of MB degradation through hydroxyl radicals is an instantaneous and fast process compared to adsorption. The degraded products were analyzed with GCMS (Figures 7.7(d)) which showed complete mineralization of the three nitrogen containing groups in the MB molecule, along with the formation of phenol, catechol, hydroxyhydroquinone and many other hydroxylates, as reported in literature [466].

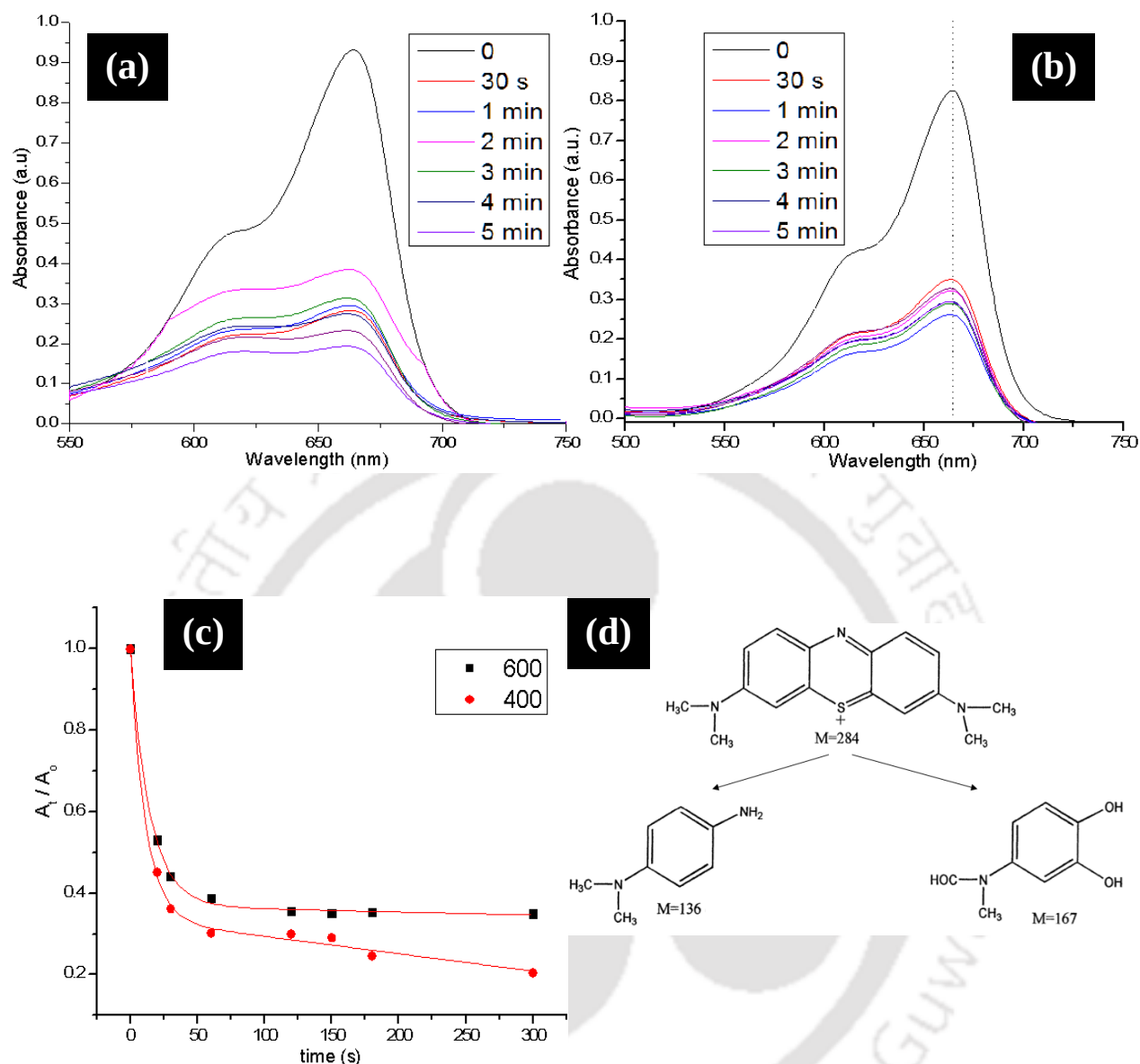


Figure 7.7: (a) & (b) UV-Vis spectra of the reduction of MB (600 and 400 μM respectively), catalyzed with CNCs supported ZVI, and (c) plot of A_t/A_0 against time with different initial dye concentrations (600 and 400 μM). (d) Plausible degraded products of methylene blue using CNCs supported ZVI, observed from GCMS studies.

The catalytic effect of CNCs supported ZVI on redox reactions was studied with 4-NP as the model compound. The reaction was extremely fast with quick degradation of the yellow colored 4-NP to transparent colorless 4-AP. With time the characteristic peak of 4-NP at

400 nm gradually diminished and a new peak at 300 nm corresponding to 4-AP is formed [467]. Figure 7.8(a) shows the degradation profile of 4-NP and rate of formation of 4-AP with time which follows first order kinetics. The degradation products of 4-NP were monitored through GCMS measurement which showed the formation of 4-AP and other byproducts as reported in literature [468]. The higher rate of hydroxyl radical generation led to further degradation of 4-AP to hydroquinone, p-benzoquinone and ring opening organic compounds, as reported in literature. Such high catalytic activity is due to the high surface area ($18.4 \text{ m}^2/\text{g}$) and surface reactivity of the CNCs supported ZVI.

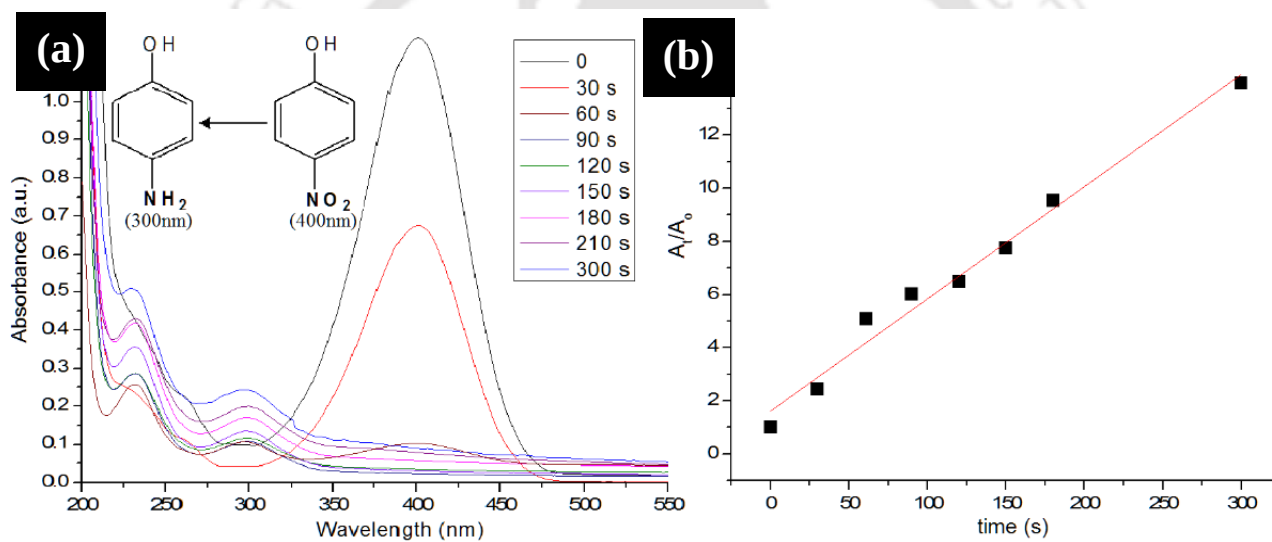


Figure 7.8: (a) UV-Vis spectra of the conversion of 4-NP to 4-AP, and (b) plot of A_t/A_0 for formation of 4-AP (peak at 300 nm) against time.

7.2.3 Applications of Cellulose Nanocrystal-supported Zerovalent Iron (Fe-CNCs):

Self-propagating autonomous nanobots for targeted drug delivery.

The work highlights another application of CNCs supported ZVI, where it can act as a chemo-motorized vehicle. To the best of our knowledge, this is the first time that controlled propulsion of CNCs supported ZVI is being reported. We have fabricated motorized CNCs, which can move under the influence of chemically induced pH gradient and magnetic field. In

presence of CNCs supported ZVI, hydrogen peroxide fuel would undergo catalytic decomposition asymmetrically on CNC surface leading to vigorous production of oxygen gas bubbles. Since the ZVI is non-uniformly grafted on the CNC surface, it creates a local concentration of vigorously ejecting oxygen bubbles, which creates a force imbalance along the particle surface. Recently such observation has been reported with polymer micro beads decorated with zerovalent iron nanoparticles, which showed both pH directed and external magnetic field responsive motion [469], [470]. The imbalance of such forces along the surface leads to random undirected motion of the CNCs. However, such undirected motion can be externally controlled through the application of magnetic field and pH gradient, which may help in targeted drug delivery application. pH directed migration is carried out by introducing a pH gradient in the fuel bath, which led to slow uniform motion of CNCs in the low pH region and subsequent accelerated motion towards higher pH region. Figures 7.9(a), (b) and (c) show the representative motion of a selected microparticle (final positions marked by circles with solid boundary) in three different case studies. In all the experiments, small microparticles of CNCs supported ZVI were selected ($100 \pm 20 \mu\text{m}$), which are actually lumps of nanoparticles as shown in Figure 7.10 (a), (b) and (c). The microparticles were placed in a petri dish filled with 10% (v/v) H_2O_2 aqueous solution acting as fuel for the propulsion of CNCs supported ZVI. We studied the motion of the CNCs supported ZVI by measuring the average and instantaneous speed of the CNC microparticles at various magnetic field strengths, different pH and in the presence of pH gradient (Figure 7.9).

Figure 7.11(a) shows that on increasing the magnetic field strength at a constant pH (~ 4), the CNCs supported ZVI attained higher velocity, with the maximum speed reaching as high as $\sim 7 \text{ mm/s}$ (~ 35 body lengths of motor per second). This is because the ZVI nanoparticles

usually show very high induced magnetization, which led to propulsion of CNCs supported ZVI at such high velocity. However, the rate of increase in speed decreased with increasing magnetic field, which might be due to the frictional resistance and buoyancy force offered by the fluid to the moving CNCs. In the second case studied, on increasing the pH of the H_2O_2 fuel at a constant magnetic field of 25 mT, the velocity of the CNC motors increased steadily, reaching a maximum value of $\sim 5\text{mm/s}$ (~ 25 body lengths of motor per second) (Figure 7.11(b)). With increase in pH, the rate of decomposition of the H_2O_2 fuel to O_2 molecules increases exponentially, which creates a local gradient of oxygen bubbles around the surface of the CNC microparticles.

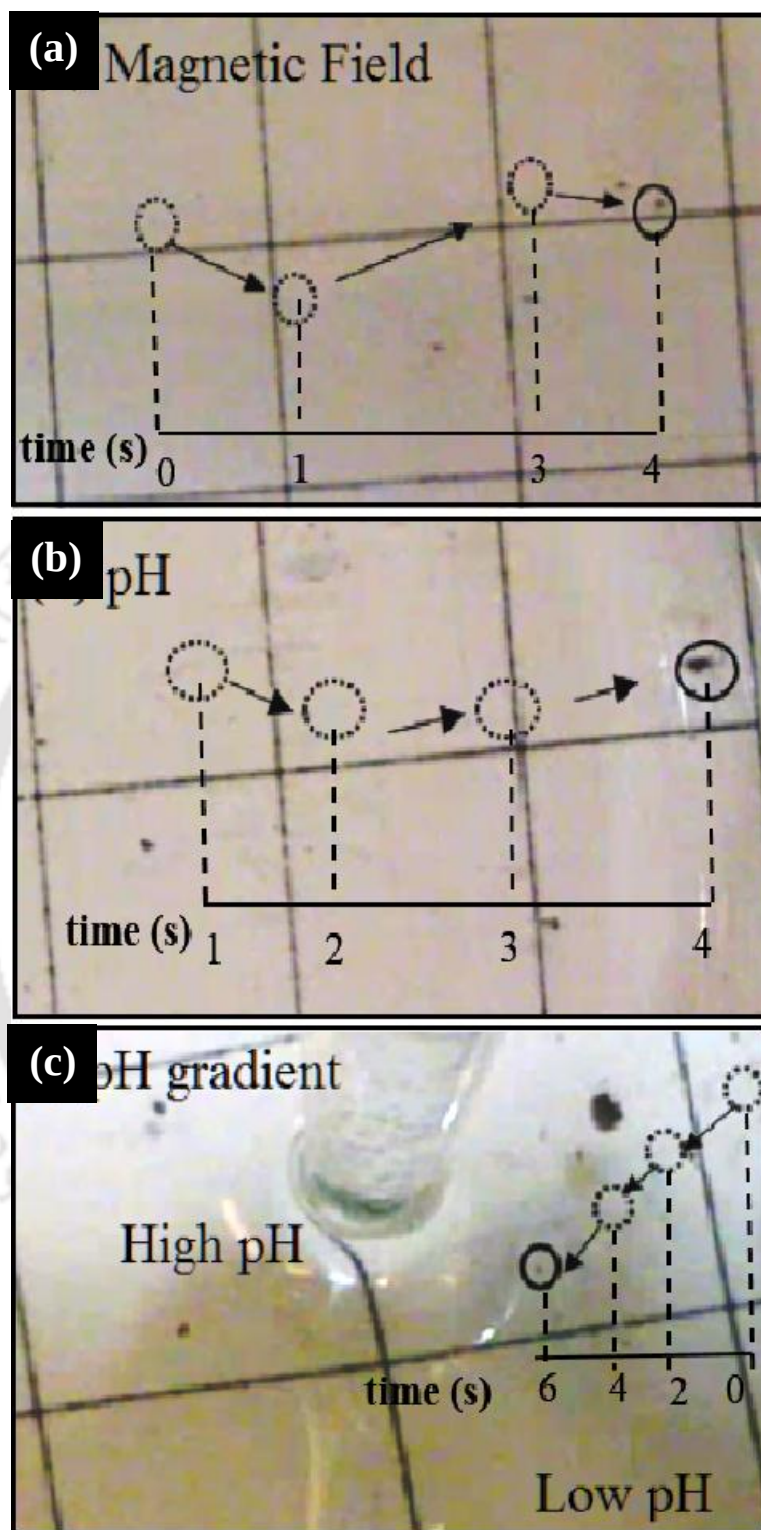


Figure 7.9: (a) Effect of magnetic field and (b) pH on motion of CNC motors (magnetotaxis).

(c) Self propulsion of CNC motors under pH gradient (pH taxis).

At higher pH, the gradient of oxygen bubbles increases, which propels the CNCs at very high speed. As this process is governed by the reaction rate of decomposition of H_2O_2 fuel, the effect of buoyancy and frictional resistance from the fluid was less pronounced. For the third case, a pH gradient was introduced in the H_2O_2 fuel reservoir, which led to the autonomous motion of CNCs supported ZVI towards the higher pH region in the absence of any external forces such as magnetic field (Figure 7.11(c)). The pH gradient around the CNCs supported ZVI created a homogenous local imbalance, which led to propulsion at a very uniform speed. However, as the CNC motors approached the high pH source (i.e., the burette) the velocity increased exponentially. Figure 7.11(c) shows that at large distance from the burette (4–7 mm), the CNC motors were moving at a uniform speed of $\sim 1 \pm 0.2$ mm/s, due to the uniform pH gradient around the surface of CNCs. Among all the three cases reported above, application of pH gradient led to the most precise remote regulation of CNC micromotors at uniform speed. Thus, it was found that the speed of these CNCs supported ZVI motors can be finely tuned and their motion can be externally controlled by altering the pH gradient and application of magnetic field. The three case studies discussed here underline the potential of the CNCs supported ZVI for the design of nano-machinery that may find application in the delivery of drugs, biomolecules, proteins or enzymes to the targeted cells.

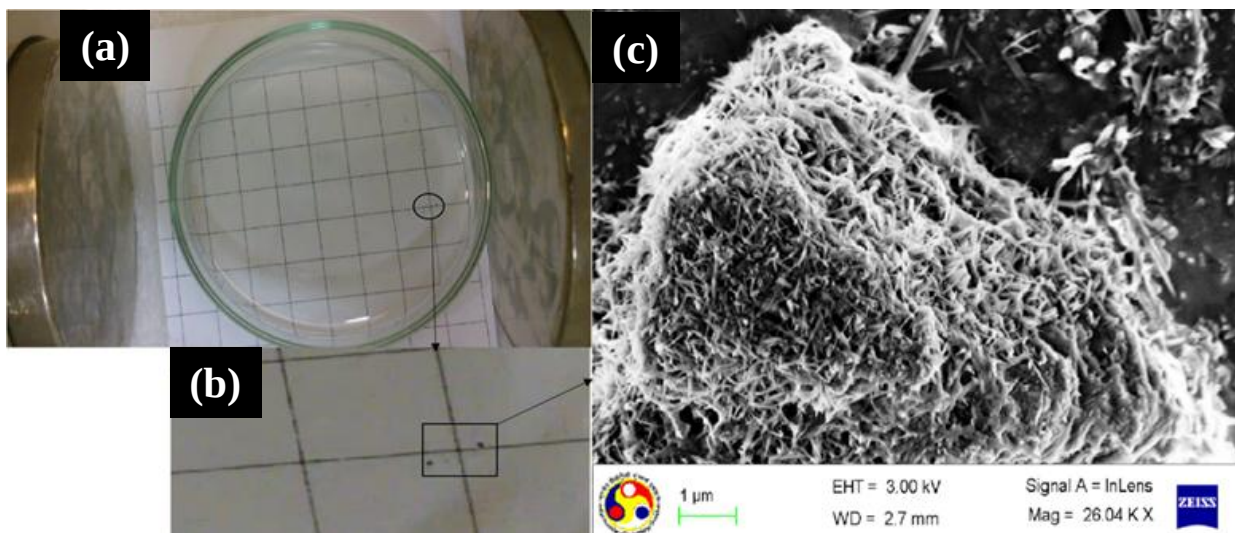
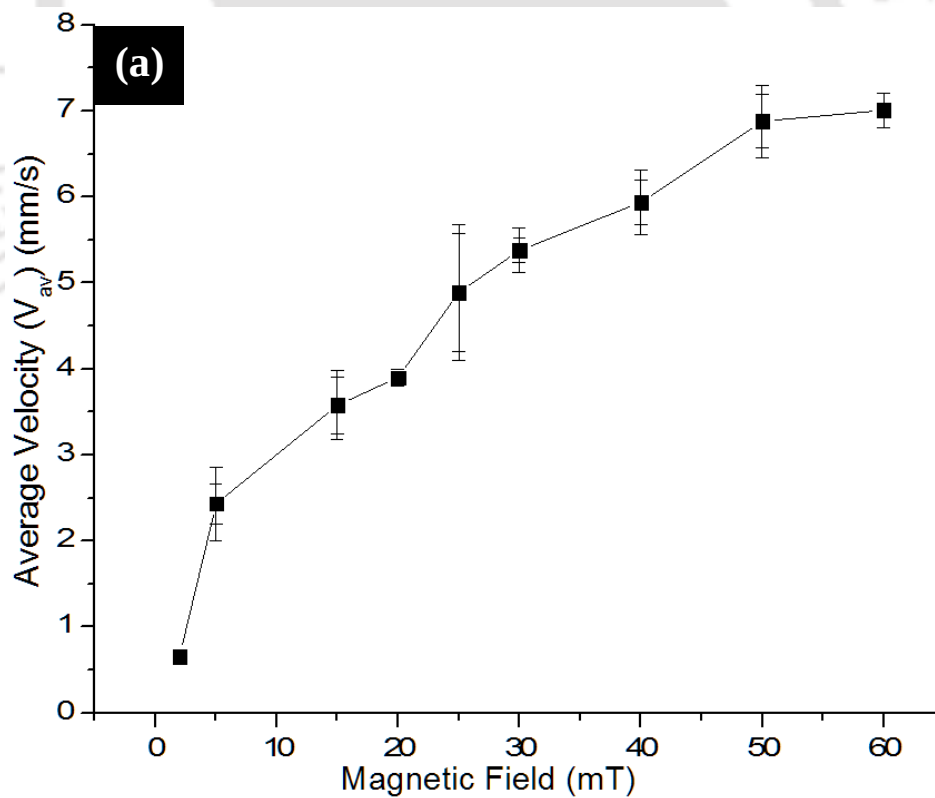


Figure 7.10: (a, b) Selected microparticles of size $100 \pm 20 \mu\text{m}$ for experiments, (c) FESEM image shows that selected microparticles are agglomerated Fe-CNCs.



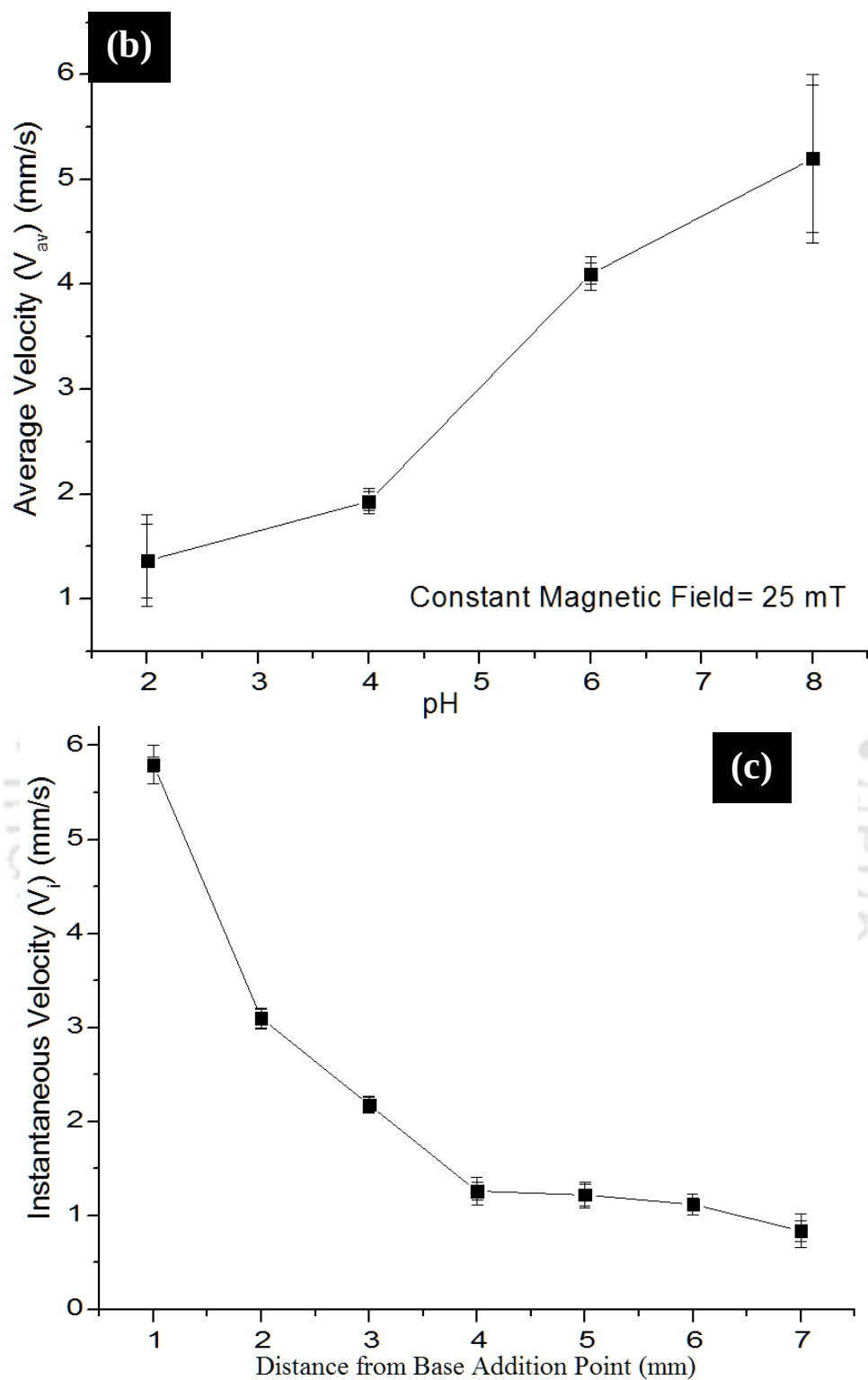


Figure 7.11: (a) Effect of magnetic field, (b) Effect of pH on the average speed of CNC motors in presence of H_2O_2 fuel. (c) Change in instantaneous velocity of CNC motors, as they migrate towards burette (higher pH) in the presence of pH gradient.

7.2.4 Fabrication and Characterization of Iron Oxide Nanoparticle-modified CNCs

The CNCs fabricated from pretreated bamboo pulp after sulphuric acid hydrolysis were selected as template for the precipitation of Fe_2O_3 nanoparticles in the presence of reducing agent. After the reduction reactions, smooth rod-like surface of CNCs were modified with spherical Fe_2O_3 nanoparticles. This leads to alteration in the morphological dimensions of CNCs with an average length of $745 \pm 63 \text{ nm}$ and diameter of $56 \pm 8 \text{ nm}$, where the distinct rod-like morphology of CNCs is prevalent with an aspect ratio of ~ 13 (as shown in Figure 7.12 (a)). The spherical Fe_2O_3 nanoparticles of size $\sim 45\text{--}56 \text{ nm}$ get anchored onto CNCs due to strong electrostatic interaction between the positively charged metal ions and negatively charged hydroxyl groups on the surface of CNCs.

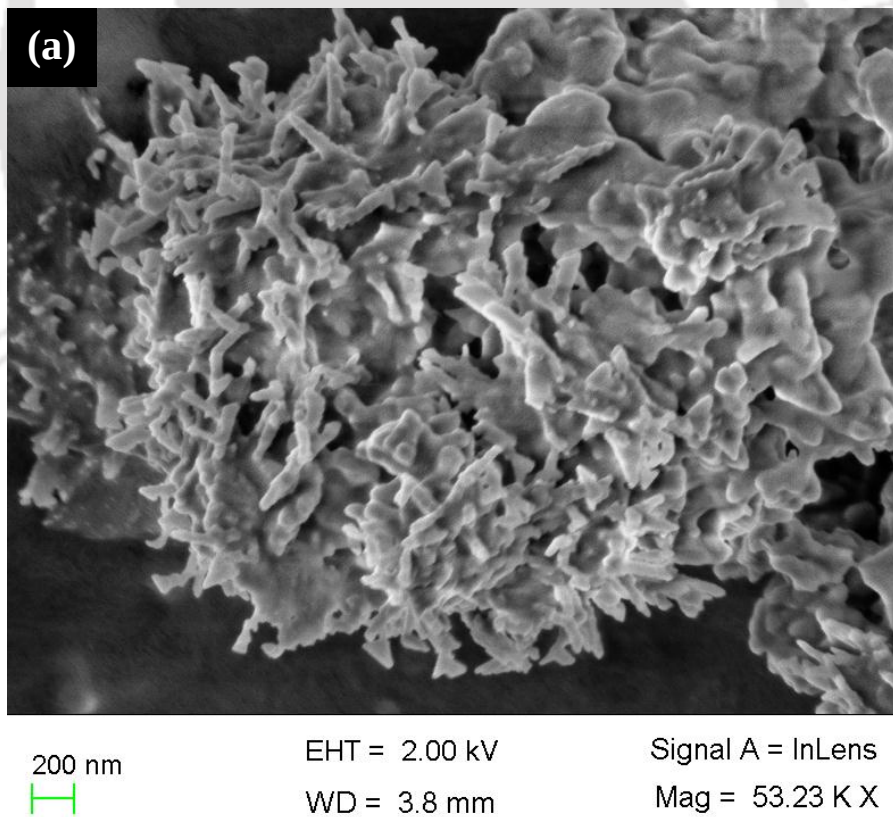
XRD diffractograms of $\text{Fe}_2\text{O}_3\text{-CNC}$ shows the presence of diffraction peaks at $2\theta = 30.4^\circ$, 35.8° , 43.2° , 53.7° , 57.4° and 63.0° which correspond to the crystal planes of Fe_2O_3 nanoparticles at (220), (311), (400), (422), (511) and (440) respectively [471]. The small intensity peaks obtained at $2\theta = 14.8^\circ$, 16.5° and 22.5° correspond to $(\bar{1}10)$, (110) and (002) planes in the cellulose I crystal structure of CNCs, which remains unaltered during the precipitation process. The minimum crystallite size of the Fe_2O_3 nanoparticles adsorbed onto CNCs are calculated to be $\sim 52 \text{ nm}$ (using Scherrer equation corresponding to the diffraction peak at $2\theta = 35.8^\circ$), which falls within the size range calculated from FESEM micrographs. Therefore, from the crystallographic studies it can be confirmed that Fe_2O_3 nanoparticles of uniform size distribution can be adsorbed onto CNCs through simple precipitation of iron salts in the presence of reducing agents.

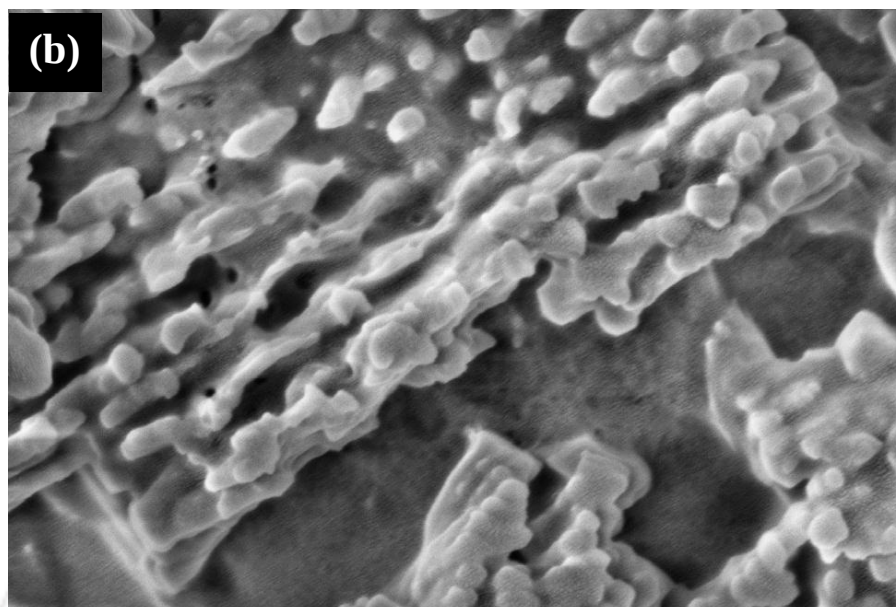
The fraction of Fe_2O_3 nanoparticles incorporated onto the surface of CNCs is determined through TGA and EDX analysis of the $\text{Fe}_2\text{O}_3\text{-CNC}$ (as shown in Figures 7.12(d) and (e)). TGA

thermograms show that CNC undergoes slight weight loss in the range of $\sim 100\text{--}200^\circ\text{C}$ corresponding to the removal of moisture and sulphate groups present on the CNC surface, followed by the complete degradation of cellulose in range of $\sim 250\text{--}500^\circ\text{C}$. Incorporation of Fe_2O_3 nanoparticles on the surface of CNCs leads to improved thermal stability of CNCs with the reduction in weight by only $\sim 20\text{--}25\%$ in the temperature range of $150\text{--}400^\circ\text{C}$. This is probably due to incorporation of the Fe_2O_3 nanoparticles, which generally have high thermal stability and remain unaltered up to $\sim 600^\circ\text{C}$. The percentage fraction of Fe_2O_3 nanoparticles present on the surface of CNCs was determined from the difference in percentage weight loss of CNC and $\text{Fe}_2\text{O}_3\text{-CNC}$ thermograms measured at $\sim 500^\circ\text{C}$. From TGA measurements, it was observed that about $\sim 35\text{ wt\%}$ of Fe_2O_3 nanoparticles are incorporated on the surface of CNCs. Further, the compositional analysis of $\text{Fe}_2\text{O}_3\text{-CNC}$ through EDX spectroscopy also confirms the presence of $\sim 38\text{ wt\%}$ of Fe along with $\sim 37\text{ wt\%}$ of O and $\sim 24\text{ wt\%}$ of C respectively. Introduction of such high content of Fe is probably due to the improved interaction between the hydroxyl groups of CNCs with the iron salts, which enhances the nucleating sites for growth of Fe_2O_3 nanoparticles during precipitation reactions.

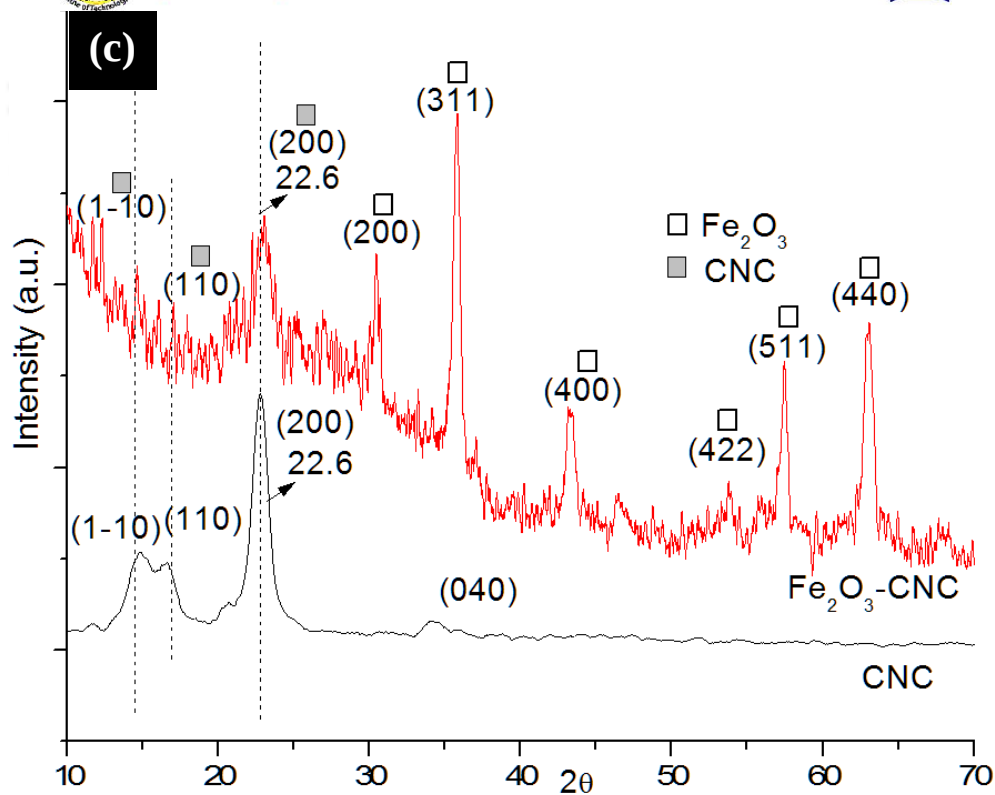
The surface modification of CNCs with such high content of Fe_2O_3 nanoparticles results in the introduction of the magneto-responsive behaviour, which has been evaluated through VSM studies measured at 298K , as shown in Figure 7.12(f). VSM curves show that the $\text{Fe}_2\text{O}_3\text{-CNCs}$ have high magnetization values of $\sim 43\text{ emu/g}$. Such high magnetic moment is probably due to the presence of high fractions of Fe_2O_3 nanoparticles ($\sim 35\text{ wt. \%}$) compactly adsorbed on the surface of CNCs, which leads to introduction of ferromagnetic behaviour. Fabrication of such catalytically active ferromagnetic nanocrystals from naturally-derived biomass resources in addition to the interesting properties of non-toxicity and biocompatibility

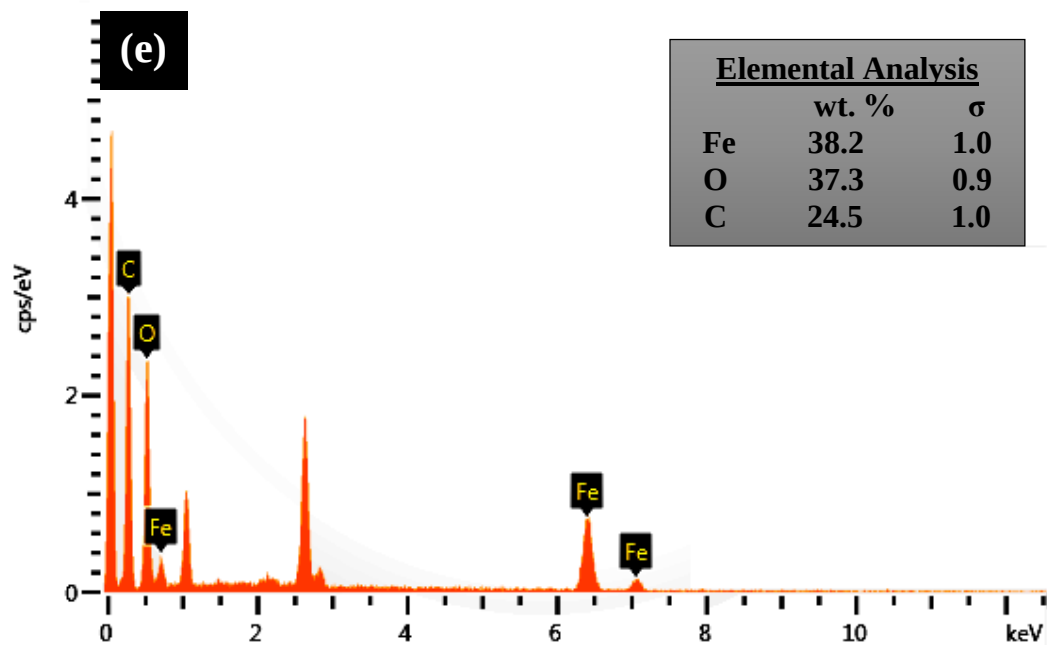
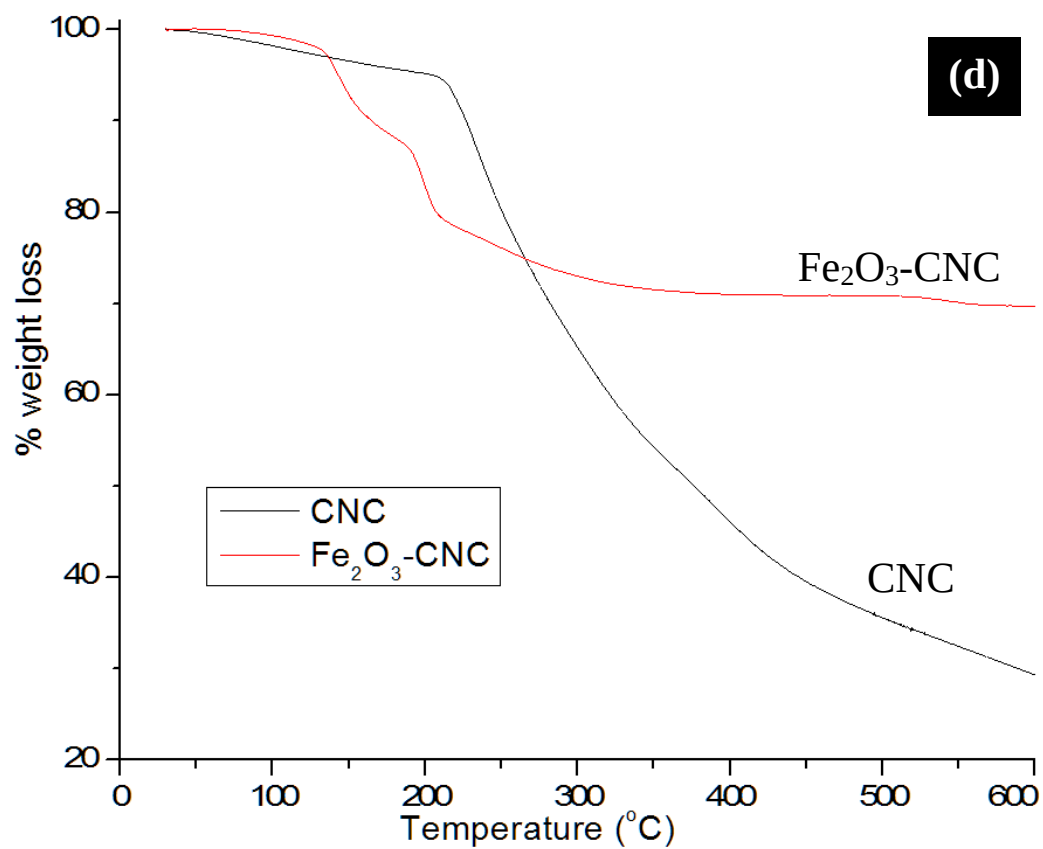
finds potential applications in fields related to biomedical engineering and healthcare products. Moreover, presence of such high magnetic moment maghemite nanoparticles along with the distinct rod-like morphology of Fe_2O_3 -CNC provides a unique opportunity to scroll up graphene sheets with the Fe_2O_3 -CNC surface acting as a structural template; this studies have been discussed in subsequent section 7.2.5.





300 nm EHT = 2.00 kV Signal A = InLens
WD = 3.8 mm Mag = 119.77 K X ZEISS





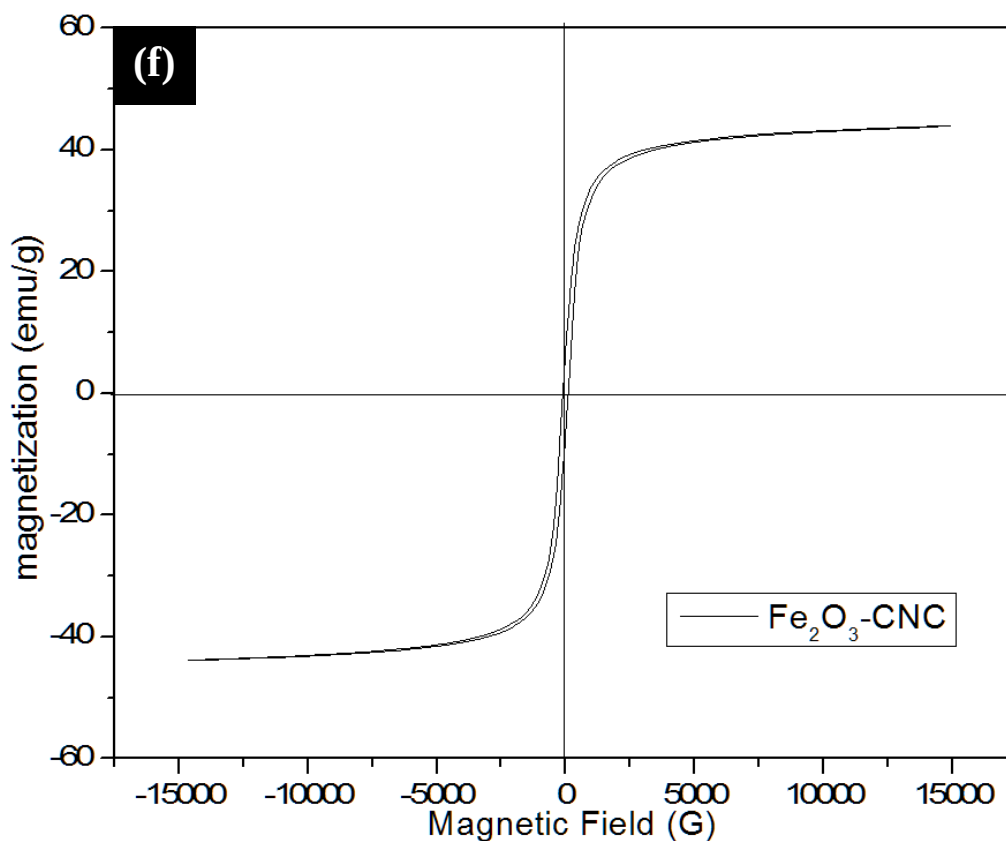
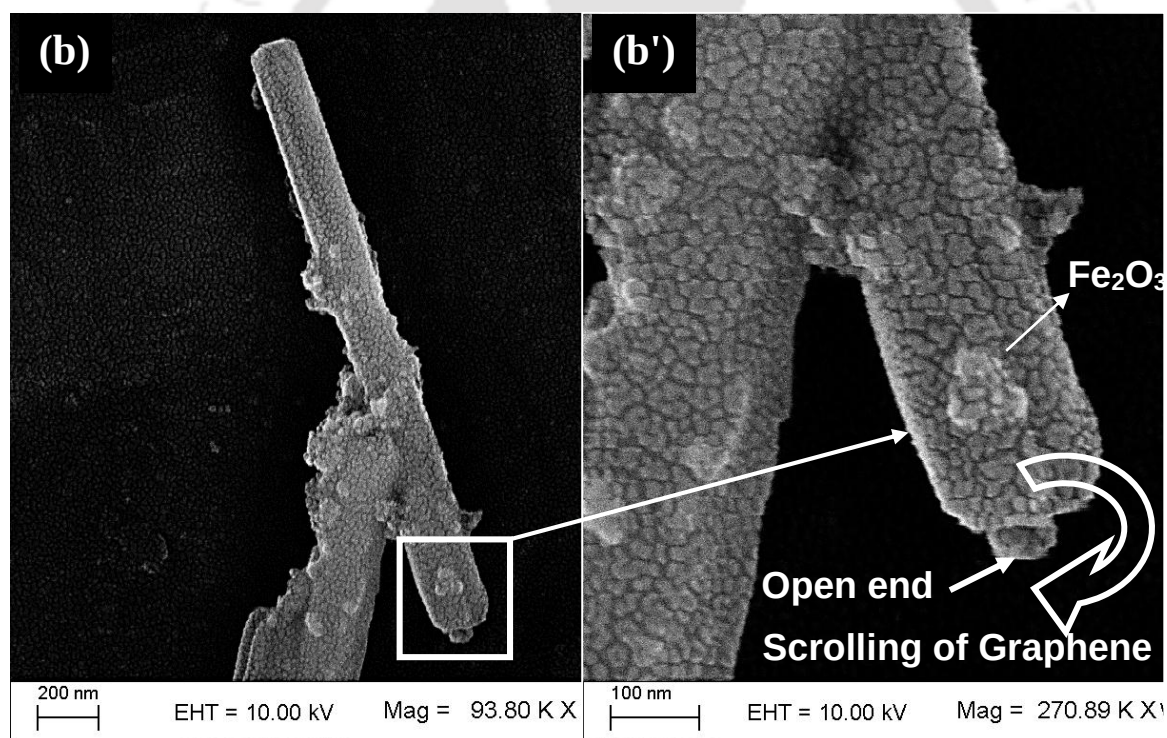
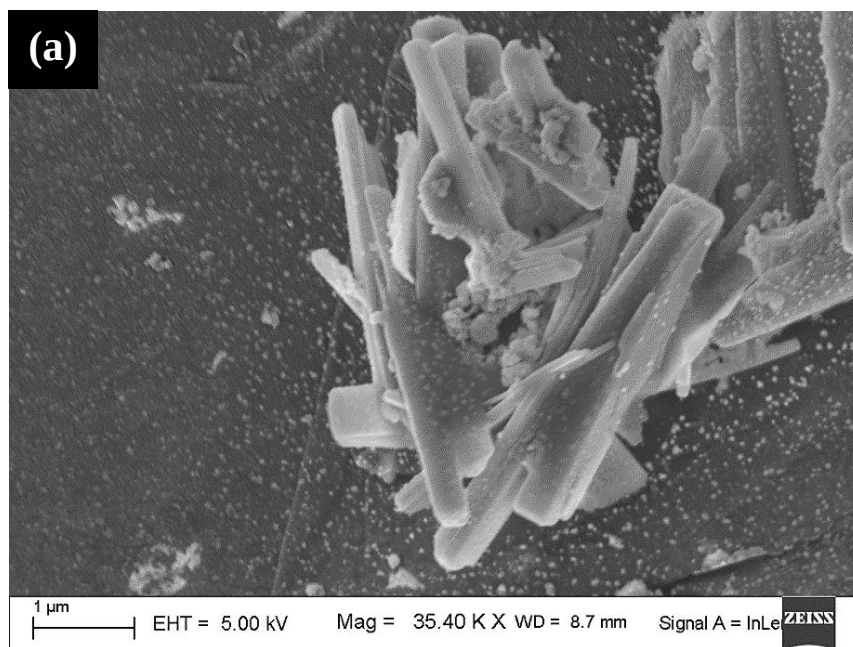


Figure 7.12: FESEM micrographs of the fabricated (a) $\text{Fe}_2\text{O}_3\text{-CNC}$ through a single step precipitation method, (b) high resolution micrographs of $\text{Fe}_2\text{O}_3\text{-CNC}$ (at a resolution of $\sim 100\text{KX}$) which shows the presence of spherical Fe_2O_3 nanoparticles adsorbed onto CNCs, (c) XRD patterns of $\text{Fe}_2\text{O}_3\text{-CNC}$ with the crystal planes of Fe_2O_3 and CNC marked in white and black squares respectively, (d) TGA profiles for the $\text{Fe}_2\text{O}_3\text{-CNC}$ and CNCs respectively, (e) EDX spectra of $\text{Fe}_2\text{O}_3\text{-CNC}$ with the respective composition of elements (in wt. %) shown in the table (inset) and (f) Magnetic hysteresis loop for the fabricated $\text{Fe}_2\text{O}_3\text{-CNC}$ measured at 298K.

7.2.5 Fabrication of Graphene Nanoscrolls using Fe_2O_3 -CNC as Templates (Fe_2O_3 -NS)

The Fe_2O_3 -CNCs fabricated have high degree of the maghemite nanoparticles decorated on their surface with loading as high as ~ 35 wt%, which introduces high magnetic moment of ~ 43 emu/g to the CNCs. The high magnetic moment along with the distinct rod-like morphology of CNCs makes them an ideal candidate for fabrication of GNS. The epoxidation of Fe_2O_3 -CNCs surface leads to improved interaction with the partially oxidized GRO through covalent coupling which results in the nucleation of CNCs on the surface of graphene sheets. According to earlier reported studies [472], the introduction of the maghemite clusters on the graphene sheets reduces the energy barrier for the initial rolling as well as the formation energy for the complete scrolling of sheets into GNS. Therefore, adherence of high magnetic moment Fe_2O_3 -CNCs to the graphene sheets results in the reduction in energy barrier to initiate the scrolling phenomenon. Moreover, due to the distinct rod-like morphology of CNCs, preferential rolling of graphene sheets takes place on Fe_2O_3 -CNCs, with the graphene sheets acquiring the morphology as well as the dimensions of the initial CNC substrate. Furthermore, magnetic spin-spin interactions are observed when two neighbouring high magnetic moment Fe_2O_3 -CNCs interacts with each other (opposite spins) which further decreases the scrolling energy [472]. Therefore, introduction of Fe_2O_3 -CNCs is thermodynamically favourable process for formation of GNS which is governed by several parameters such as density of maghemite nanoparticles, magnetic moment, morphology and dimension of structural template.



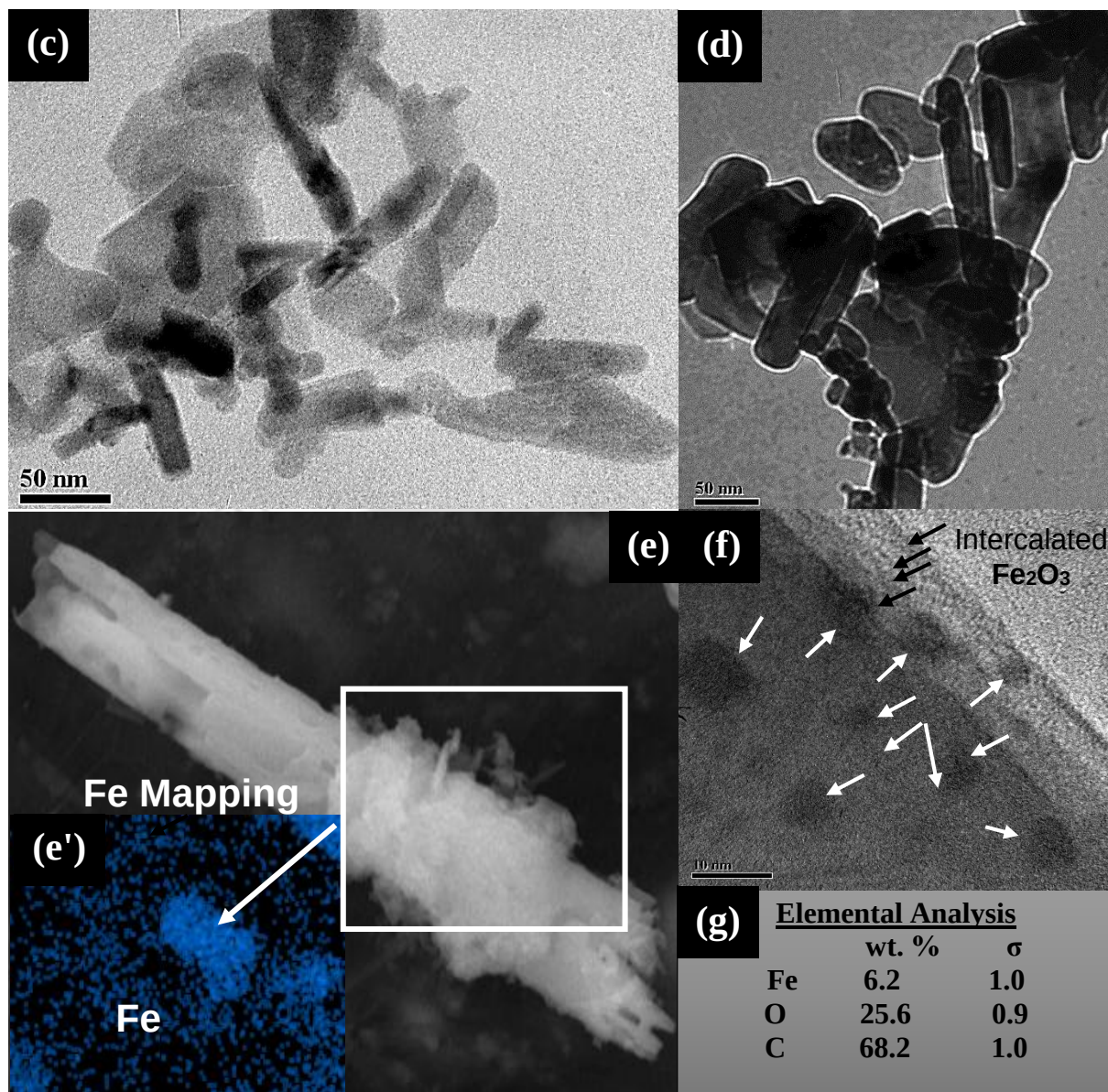


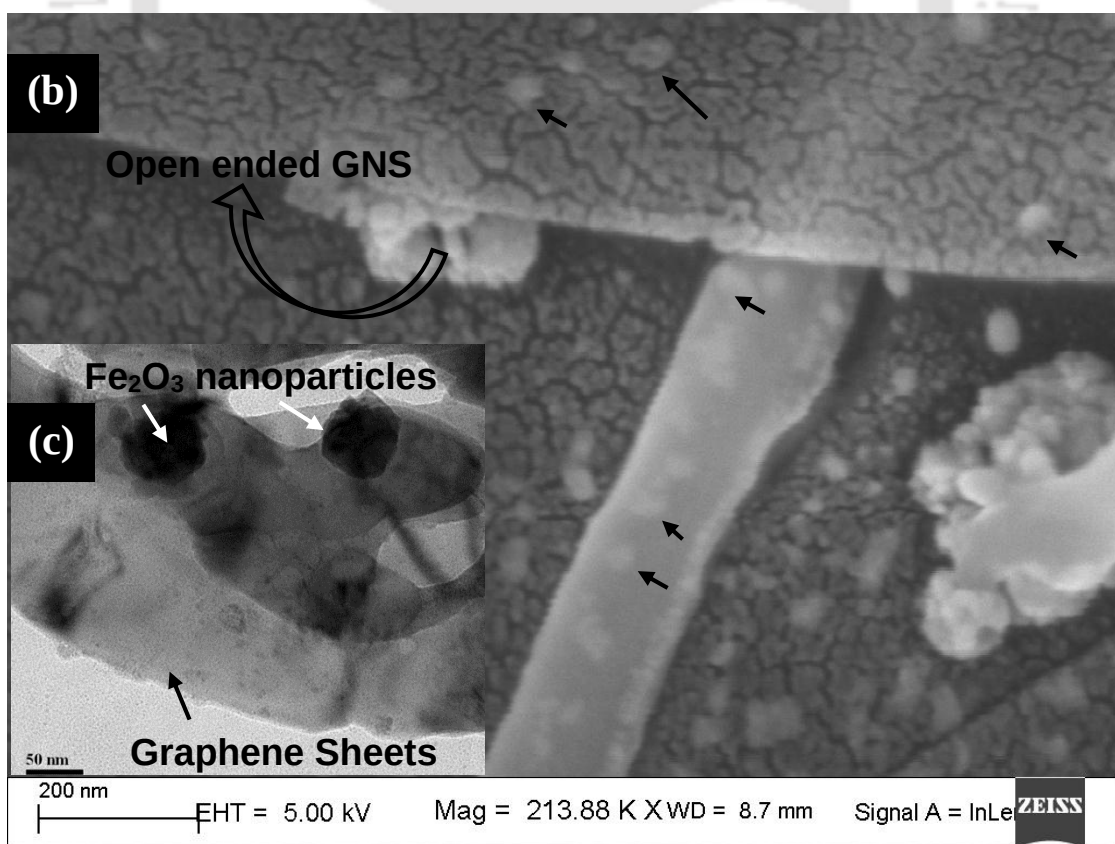
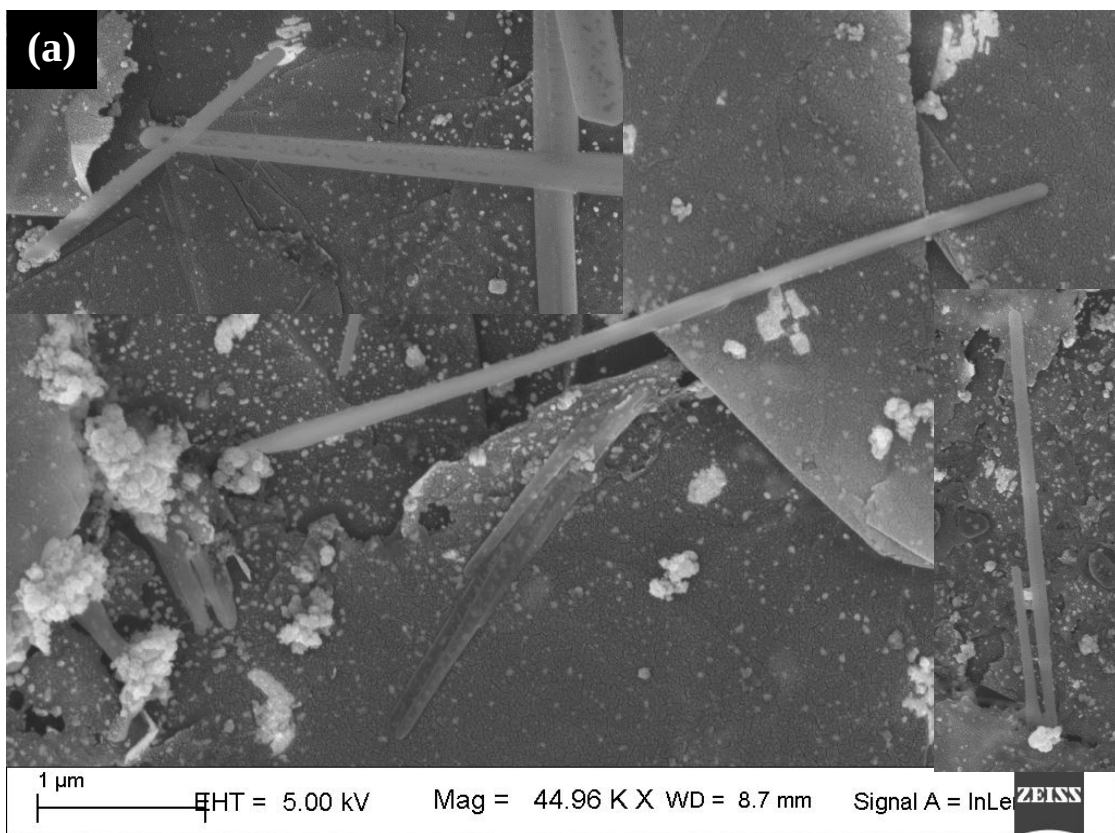
Figure 7.13: (a) FESEM images of the Fe₂O₃-NS fabricated using low aspect ratio Fe₂O₃-CNCs, (b) and (b') FESEM micrographs of a Fe₂O₃-NS (at magnification ~230KX), (c) and (d) TEM micrographs of low aspect ratio Fe₂O₃-NS, (e) and (e') EDX map of selected region in FESEM micrographs for Fe₂O₃-NS encapsulated with Fe₂O₃ nanoparticles (f) TEM image of an edge of Fe₂O₃-NS which shows number of graphene layers (marked with black arrows) and encapsulated Fe₂O₃ nanoparticles (marked with white arrows) and (g) compositional analysis of the Fe₂O₃-NS.

In this study, GNS is prepared using two different aspect ratios of the Fe_2O_3 -CNCs templates, to understand the effect of the initial CNC substrate on the properties of the fabricated GNS. Fabrication of GNS with tunable morphology, especially with variable diameter/length, along with a variety of encapsulated metallic nanoparticles provides a novel approach to tune the physicochemical properties as well as widen the potential applications of GNS in various fields. Figure 7.13(a) shows the FESEM micrographs of several Fe_2O_3 -NS fabricated using low aspect ratio Fe_2O_3 -CNCs as templates, which have been removed after the calcination process. The fabricated Fe_2O_3 -NS are found to be of length $\sim 750 \pm 300$ nm and diameter $\sim 174 \pm 50$ nm. In comparison to the initial precursor Fe_2O_3 -CNCs, the fabricated Fe_2O_3 -NS have higher morphological dimensions. This suggests that during the epoxidation process, more than one Fe_2O_3 -CNC adhere to the surface of a graphene sheet to initiate the scrolling of graphene sheets into Fe_2O_3 -NS. High resolution FESEM micrographs (Figure 7.13(b) and (b')) of a single Fe_2O_3 -NS shows the presence of Fe_2O_3 nanoparticles encapsulated in the core of GNS (bright spot in FESEM micrograph). The edge of the single Fe_2O_3 -NS shows the scrolling of a single layer of graphene sheet which rolls to form a GNS with open ends. Figure 7.13(c) and (d) show several Fe_2O_3 -NS formed with the low aspect ratio Fe_2O_3 -CNCs as templates. The high resolution TEM (HR-TEM) micrographs of an edge of the Fe_2O_3 -NS shows the presence of several layered graphene sheets (marked with black arrows) with the Fe_2O_3 nanoparticles adhered to their surface as well as present in the core of the GNS (marked with white arrows). The TEM micrographs suggest that there are multiple layers of rolled graphene sheets present in the Fe_2O_3 -NS, with the Fe_2O_3 nanoparticles intercalated in between the layers of the rolled sheets (as marked in white arrows in Figure 7.13(f)). From Raman spectroscopy (spectra shown in Fig. 7.15(b) and discussed in subsequent

section), the high intensity of the D and G band also suggests the presence of a single layer of graphene sheet rolled into Fe₂O₃-NS. The EDX mapped image of a single Fe₂O₃-NS (Fig. 7.13(e')) shows the presence of the Fe₂O₃ nanoparticles in its core with a composition of ~6.2 wt. % Fe present in the selected region (Fig. 7.13(g)). Therefore, the morphological analysis confirms that the low aspect ratio Fe₂O₃-NS can be successfully fabricated using Fe₂O₃-CNCs as templates, with Fe₂O₃ nanoparticles encapsulated in the core of the nanoscroll.

Utilization of high aspect ratio Fe₂O₃-CNC also leads to the formation of the Fe₂O₃-NS with altered morphological dimensions as shown in Figure 7.14(a) and (b). From the FESEM and TEM micrographs, several high aspect ratio Fe₂O₃-NS are observed with a length of $\sim 4.5 \pm 1.2 \mu\text{m}$ and diameter of $0.37 \pm 0.21 \mu\text{m}$. The fabricated high aspect ratio Fe₂O₃-NS have inherited the structural properties of the initial Fe₂O₃-CNCs template. High resolution FESEM micrograph in Figure 7.14(b), at a resolution of $\sim 210\text{KX}$, shows the presence of Fe₂O₃ nanoparticles in the core of GNS (white spots marked with black arrows) along with the presence of the open ends suggesting the hollow nature of the Fe₂O₃-NS. Figure 7.14 (c) shows the TEM micrograph with Fe₂O₃ nanoparticles wrapped within a thin layer of a graphene sheet, which can enhance the catalytic activity of the encapsulated metallic nanoparticles. The compositional analysis (see Figure 7.14(e)) shows that a single Fe₂O₃-NS contains about ~15 wt. % of Fe₂O₃ nanoparticles. The higher Fe loading in high aspect ratio Fe₂O₃-NS compared to that in the low aspect ratio Fe₂O₃-NS is probably due to adsorption of higher fractions of Fe₂O₃-CNCs onto graphene sheets which are responsible for the formation of GNS. Moreover, the HR-TEM micrographs in case of the high aspect ratio Fe₂O₃-NS show the presence of higher number of layers of graphene sheets compared to low aspect ratio Fe₂O₃-NS. This can be attributed to the presence of higher loading fractions of Fe₂O₃

nanoparticles present in the inner cores of Fe₂O₃-NS, which have substantially decreased the graphene scrolling energy, thereby enhancing the number of layers of graphene sheets rolled on it. Therefore, the number of graphene layers scrolled into GNS can be intrinsically tuned by varying the density of the maghemite nanoparticles as well as the magnetic moment of Fe₂O₃-CNCs. The HR-TEM image in Figure 7.14(g) shows the presence of crystal lattice fringes with the interlayer spacing of ~0.35 nm representing the (200) crystal plane of graphene sheets. Presence of the high intensity peak in XRD (see Figure 7.15(a)) as well as the lattice fringes confirm the presence of graphene sheets in the Fe₂O₃-NS. Therefore, from the morphological analysis of the two types of Fe₂O₃-NS having different aspect ratios, it can be inferred that the morphological dimensions and density of the scrolled graphene sheets can be remotely controlled by simply tuning the structural and physical properties of the initial CNC precursor, which provides a facile approach of fabricating Fe₂O₃-NS depending on the targeted applications.



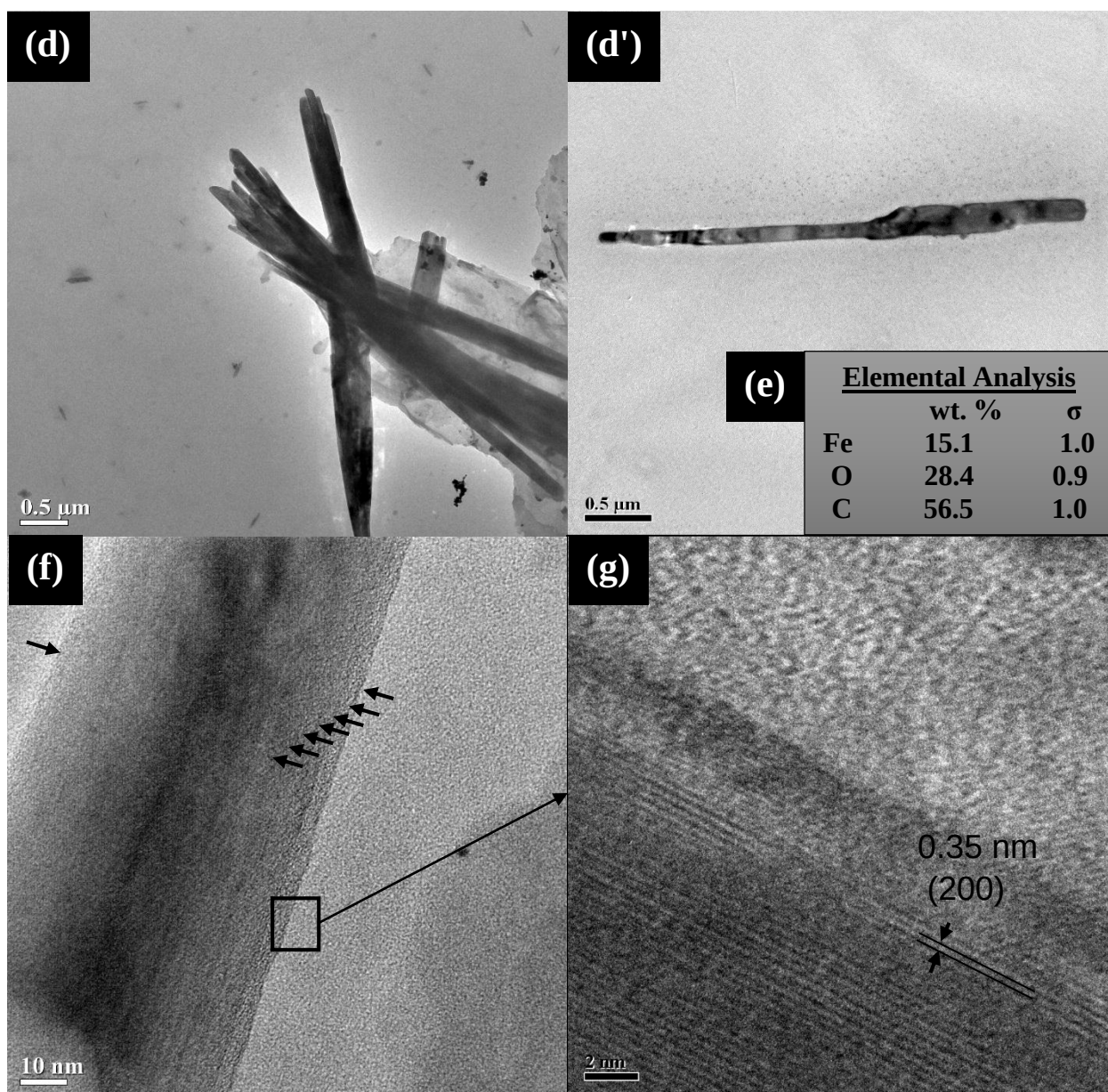


Figure 7.14: (a) FESEM micrographs of Fe_2O_3 -NS fabricated using high aspect ratio Fe_2O_3 -CNCs as templates. (b) High resolution FESEM (at $\sim 210\text{KX}$) (c) TEM micrograph shows wrapping of Fe_2O_3 nanoparticles with graphene, (d) and (d') TEM micrographs of Fe_2O_3 -NS, (e) elemental composition, (f) HRTEM image of Fe_2O_3 -NS with multiple layers of graphene on it (with the black arrows). (g) HRTEM image of selected region, (f) with lattice fringes $\sim 0.35\text{ nm}$ corresponding to (200) plane of graphene.

Figure 7.15(a), shows the XRD diffractograms of GNS formed using different aspect ratios of Fe₂O₃-CNCs as initial structural templates. All the diffractograms show the presence of peak at $2\theta = 26.5^\circ$ corresponding to graphene representing the (002) crystal planes. The XRD pattern for Fe₂O₃-NS (with high aspect ratio) shows the presence of the distinctive peaks at $2\theta = 30.4^\circ, 35.6^\circ, 43.2^\circ, 54.5^\circ, 57.3^\circ$ and 63.1° corresponding to the (220), (311), (400), (422), (511) and (440) crystal planes of the Fe₂O₃ nanoparticles respectively, which confirms their presence in the inner core of GNS. However, the diffractogram of Fe₂O₃-NS (with low aspect ratio) shows the presence of low intensity peaks at $2\theta = 43.2^\circ$ and 62.5° representing the (400) and (440) crystal planes of Fe₂O₃ nanoparticles respectively. Such low intensity XRD peaks of Fe₂O₃, in the case of low aspect ratio Fe₂O₃-NS, is probably due to the presence of low fractions of encapsulated Fe₂O₃ nanoparticles (~6.2 wt%) along with the presence of multi-layered graphene layers covering their surface, which restricts the detection. Therefore, the XRD diffractograms of both high and low aspect ratio GNS confirm the presence of scrolled graphene sheets with Fe₂O₃ nanoparticles encapsulated in the core.

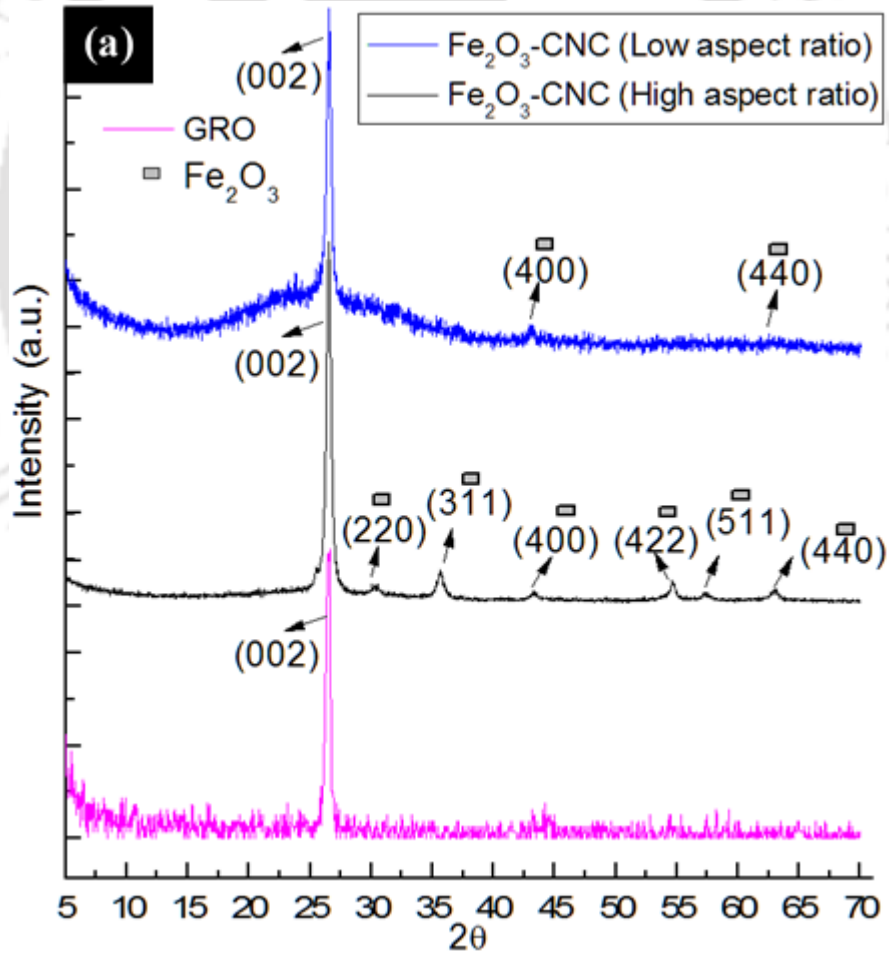
The Raman spectra for GNS formed with Fe₂O₃-CNCs as structural templates show distinct variation on changing the aspect ratio of the initial precursor (as shown in Figure 7.15 (b)). The Raman spectrum of partially oxidized GRO sheets shows a G-band at $\sim 1582\text{cm}^{-1}$ and the D- band at $\sim 1354\text{ cm}^{-1}$ with an I_D/I_G ratio of ~ 0.97 . The epoxidation of the Fe₂O₃-CNCs followed by its reaction with the oxidized GRO leads to ring opening reaction which results in adhesion of the Fe₂O₃-CNCs on the graphene substrate. As discussed in earlier section, the introduction of maghemite nanoparticles within such close proximity of graphene sheets is a necessary step to initiate the nanoscroll formation process which occurs through π - π interactions and magnetic interactions. Such epoxidized forms of Fe₂O₃ nanoparticles attached

to the inner core of GNS (after calcination to remove the CNCs) through –epoxy cross-linkages leads to the formation of open-ended GNS with more defects (increased sp^3 hybridization). This is evident from the values of the I_D/I_G ratio of the Fe_2O_3 -NS which increased to ~ 1.01 . The aspect ratio of Fe_2O_3 -CNCs affects the morphology of GNS, which also leads to significant alteration in Raman spectra with an I_D/I_G ratio of ~ 1.03 for the GNS obtained from the low aspect ratio Fe_2O_3 -CNCs and ~ 1.01 for that from the high aspect ratio Fe_2O_3 -CNCs. The Raman spectroscopy studies confirm that GNS with multiple scrolling of graphene sheets and tunable morphological dimensions can be fabricated by simple tuning of the aspect ratio of the initial precursor.

7.2.6 Mechanism of Formation of Graphene Nanoscrolls using Fe_2O_3 -CNCs as Templates.

In order to understand the mechanism and driving forces responsible for the formation of GNS over Fe_2O_3 -CNCs substrate, molecular simulation studies were carried out and corroborated with the experimental results. Molecular dynamics simulation studies were conducted using representative Fe_2O_3 -CNC and graphene models to gain more insight into the phenomenon of nanoscroll formation. As a typical representation, a molecular model of Fe_2O_3 -CNC with similar aspect ratio (~ 13), magnetic performance and loading fractions of Fe_2O_3 nanoparticles (~ 35 wt%) to experimental Fe_2O_3 -CNC was prepared, followed by its geometry optimization to obtain energy minimized configuration (Figure 7.16 (a)). The Fe_2O_3 -CNC was placed at the centre of a graphene sheet (with length $\sim 116.2\text{\AA}$ and width $\sim 58.1\text{\AA}$) along the axis of the Fe_2O_3 -CNC. On geometry optimization of the structure, the Fe_2O_3 -CNC approaches the surface of graphene sheet because of the presence of favourable attractive forces between the two structures. Thereafter, using the Materials Studio 7.0 software package, molecular dynamics simulation was carried out on the optimized structure over a time

period of ~ 400 ps to observe the conformational changes as well as to understand the effect of the non-bonded (van der Waals (vdW) and electrostatic) interactions between the graphene sheets and Fe_2O_3 -CNC. Initially at $t = 0$ ps, the Fe_2O_3 -CNC is at the centre of the single layer graphene sheet which starts to form discontinuous wrinkled structures up to $t = 100$ ps. It is also evident from the total energy and potential energy plots in Figure 7.17, which show a drop up to $t = 20$ ps due to improved interactions and thereafter remain almost constant (fluctuation without drift) and steady till $t = 200$ ps.



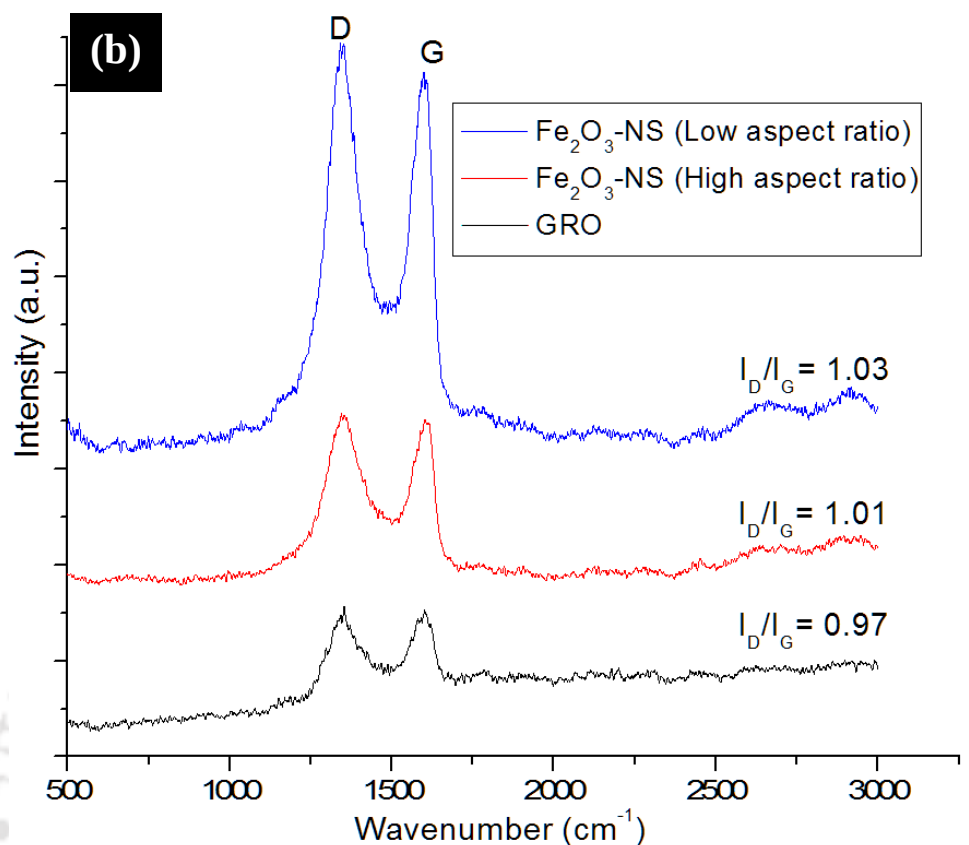


Figure 7.15: (a) XRD diffractograms and (b) Raman spectra of the Fe_2O_3 -NS fabricated using two different aspect ratios of Fe_2O_3 -CNCs as the structure directing templates.

After a time period of $t = 200$ ps, the initial curling of graphene sheets starts to occur on the Fe_2O_3 -CNC surface. The initiation of curling of graphene sheet is also evident from the FESEM micrographs (in Figure 7.16 (b)) which shows the formation of the twisted morphology along the ends of the Fe_2O_3 -CNCs (marked in blue arrow) adhered to the surface of the GRO. In the next 100 ps, the graphene sheet starts to roll on the surface of Fe_2O_3 -CNC from one end (left side first) and tries to adjust to obtain the best possible stable configuration (corresponding experimental FESEM micrographs shown in Figure 7.16 (c) and (d)). It is observed that the graphene layers slides over the Fe_2O_3 -CNC slowly during which the total

and potential energy remains almost constant (see figure 7.17). The rolling of the graphene sheets during this stage is evident from the FESEM micrographs (marked with blue arrow in Figure 7.16 (e)) which shows partially scrolled graphene layer sheets onto Fe₂O₃-CNC surface. At around $t = 300$ ps, the curved but relatively planar graphene sheet starts rolling over the CNC to form a one-dimensional tubular graphene nanoscroll due to strong interaction between the graphene sheet and the Fe₂O₃-CNC. The transition is also evident from the sharp drop in the potential as well as total energy values (as shown in Figure 7.17) during the time of ~ 320 – 350 ps, which suggests that the system has overcome the kinetic barrier and undergone significant structural alteration to attain a lower energy state. During this state the graphene sheet completely covers the surface of Fe₂O₃-CNC and acquires the structural configuration of the rod-like Fe₂O₃-CNC (corresponding experimental FESEM micrographs shown in Figure 7.16 (f) and (g)). Due to presence of high loadings of Fe₂O₃ nanoparticles and magnetic moment of Fe₂O₃-CNC, the graphene sheet continues to undergo further scrolling as evident from the rolled edges at $t = 375$ ps and $t = 400$ ps. Eventually, after a simulation run of ~ 400 ps, a structure resembling a nanoscroll is formed with the encapsulated Fe₂O₃-CNC in its core. In experimental fabrication, the CNCs are removed from the nanoscrolls through a controlled calcination process, which further aids in scrolling of graphene sheets into perfect nanoscrolls containing multiple layers, mimicking the morphological dimensions of the CNC template and encapsulating the catalytically active metallic nanoparticles present in the initial substrate.

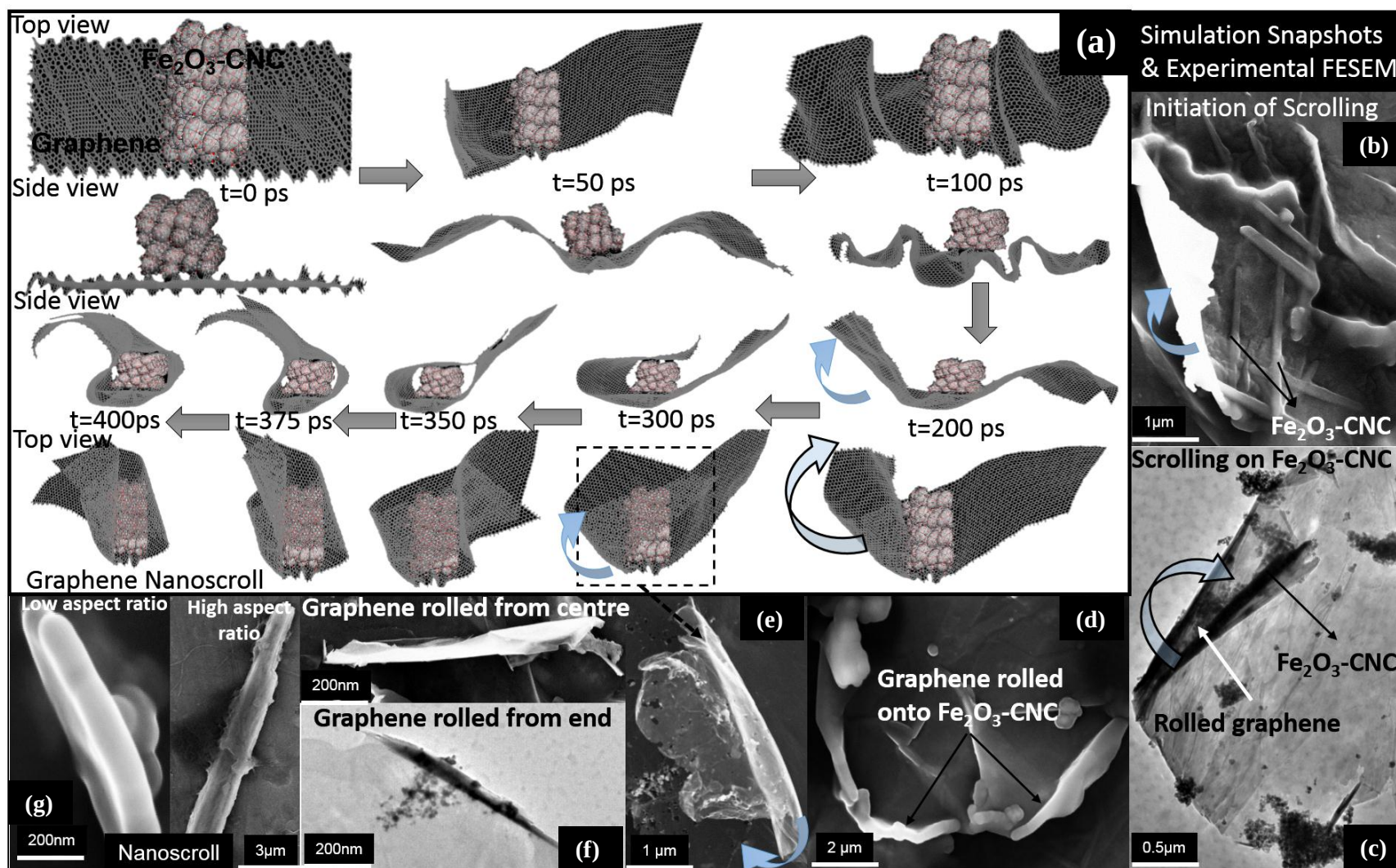


Figure 7.16: Snapshots of dynamic self-assembly of graphene on Fe_2O_3 -CNCs (a) from molecular dynamics simulations, and experimental evidence from (b)–(g) electron micrographs, at different time-scales and stages.

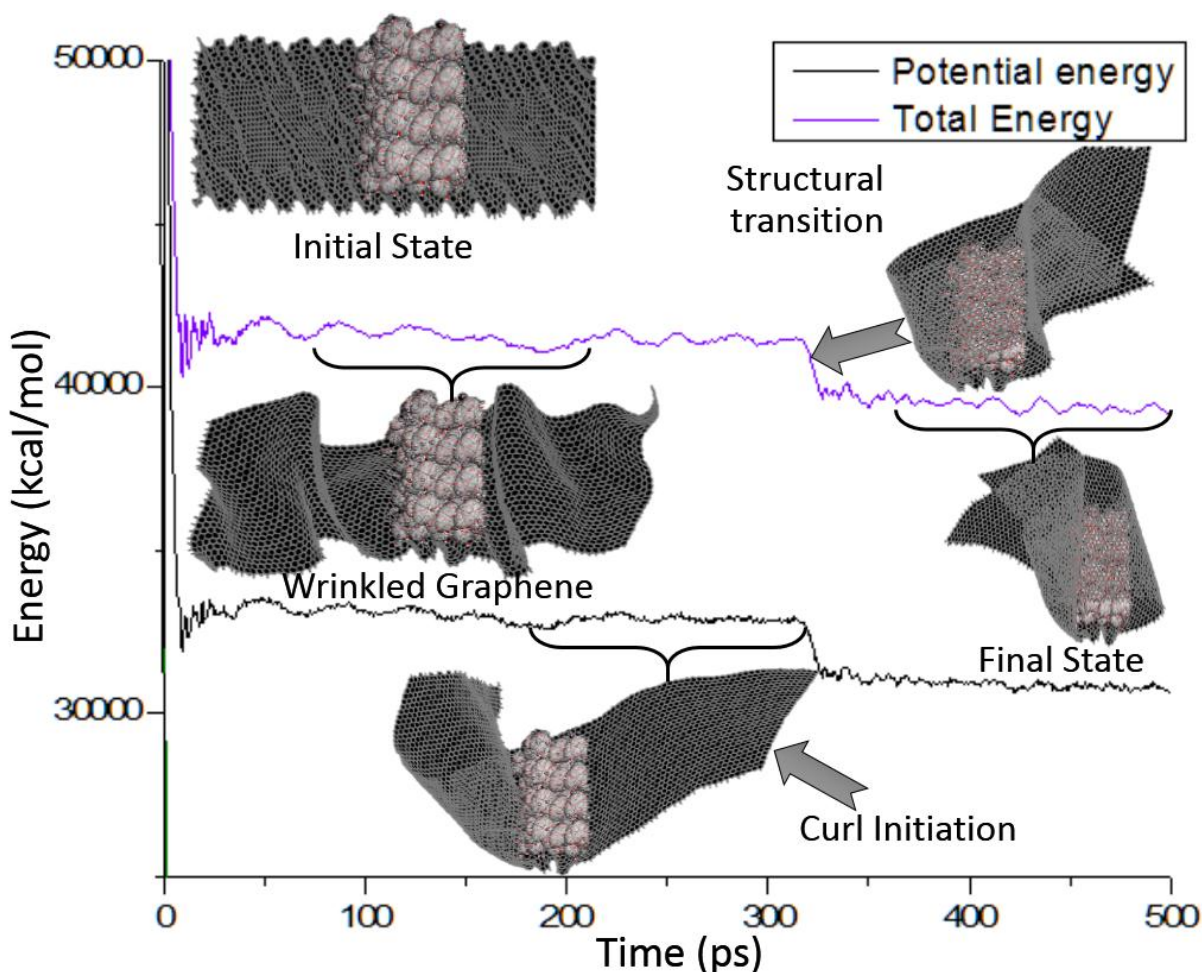


Figure 7.17: Evolution of the total and potential energy during the molecular dynamics simulation of Fe_2O_3 -NS formation using Fe_2O_3 -CNCs as templates, with the conformational changes at different times also shown.

7.2.7 Fabrication and Characterization of Fe_2O_3 -CNCs Chemically Modified with Pd and Pt Nanoparticles ($\text{Pt/Pd-Fe}_2\text{O}_3$ -CNCs)

The spherical Pd and Pt nanoparticles are precipitated onto Fe_2O_3 -CNCs in the presence of reducing agents, which leads to uniform dispersion of the metal nanoparticles on the surface of Fe_2O_3 -CNCs. Figures 7.18(a) and (c) show the FESEM micrographs of Pd- Fe_2O_3 -CNCs and Pt- Fe_2O_3 -CNCs which have distinct fiber-like morphology with the Pd and Pt nanoparticles adsorbed on the surface, as shown by the dark circles. High resolution FESEM

micrograph with magnification $>125,000\times$ (Figure 7.18(b)) shows the presence of the distinct spherical nanoparticles (bright spots marked with black arrows) which are in the size range of 25–30 nm due to the presence of agglomerates. Due to introduction of lower loading of the palladium and platinum salts, the adsorbed spherical Pd and Pt nanoparticles are uniformly distributed on the surface of Fe_2O_3 -CNCs in the form of small patches. Both the Pd- Fe_2O_3 - CNC and Pt- Fe_2O_3 -CNCs did not show any significant variation in terms of morphological dimensions and distribution of the adsorbed metallic nanoparticles. Due to the high contrast of metallic Pt/Pd nanoparticles, they are clearly visible in the TEM micrographs (Figure 7.18(e), (f)) as dark dots in comparison to the CNC substrate which are not clearly visible in the background due to their low contrast [435]. However, the rod-like alignment for both Pt/Pd and Fe_2O_3 nanoparticles confirms the presence of CNCs as substrate; otherwise, the nanoparticles would have been randomly distributed in the micrographs. TEM micrographs also reveal the similar fiber-like morphology of CNC substrate on which the spherical Pd and Pt nanoparticles are adsorbed, with dimensions in the range of 4–9 nm and 6–11 nm respectively.

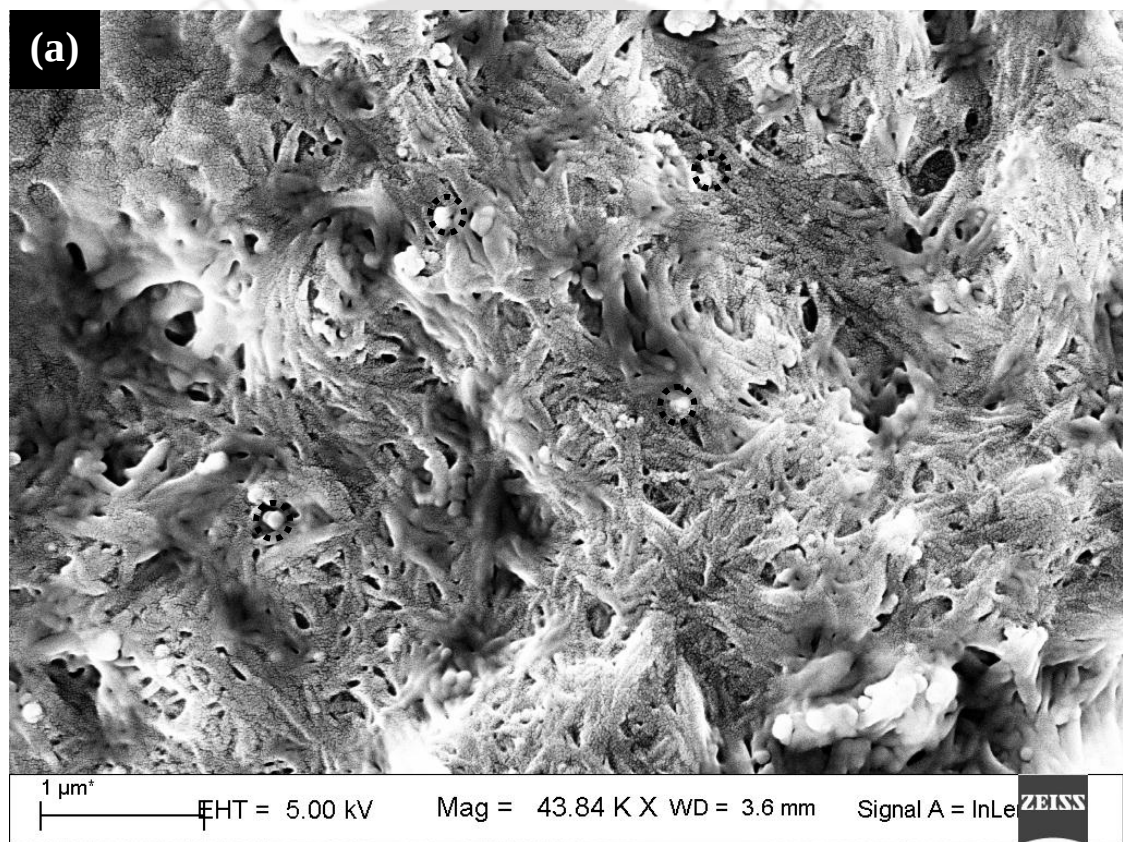
Figure 7.18(d) shows the XRD diffractogram of the CNCs with well resolved peaks at $2\theta = 14.7^\circ, 16.5^\circ, 22.6^\circ$ and 34.0° which correspond to the crystallographic planes (101), (10 $\bar{1}$), (002) and (040) respectively. The diffraction patterns for the fabricated nanocomposites of Pd- Fe_2O_3 -CNC and Pt- Fe_2O_3 -CNCs show presence of peaks corresponding to CNCs, Fe_2O_3 nanoparticles along with the Pd and Pt nanoparticles distinctively. Both Pd- Fe_2O_3 -CNC and Pt- Fe_2O_3 -CNCs show the presence of peaks corresponding to cellulose I crystal structure, which remains unaltered after the precipitation reactions. The diffractogram of Pd- Fe_2O_3 -CNC shows the presence of peaks at $2\theta = 14.7^\circ, 16.5^\circ, 22.6^\circ$ and 34.0° corresponding to the CNC

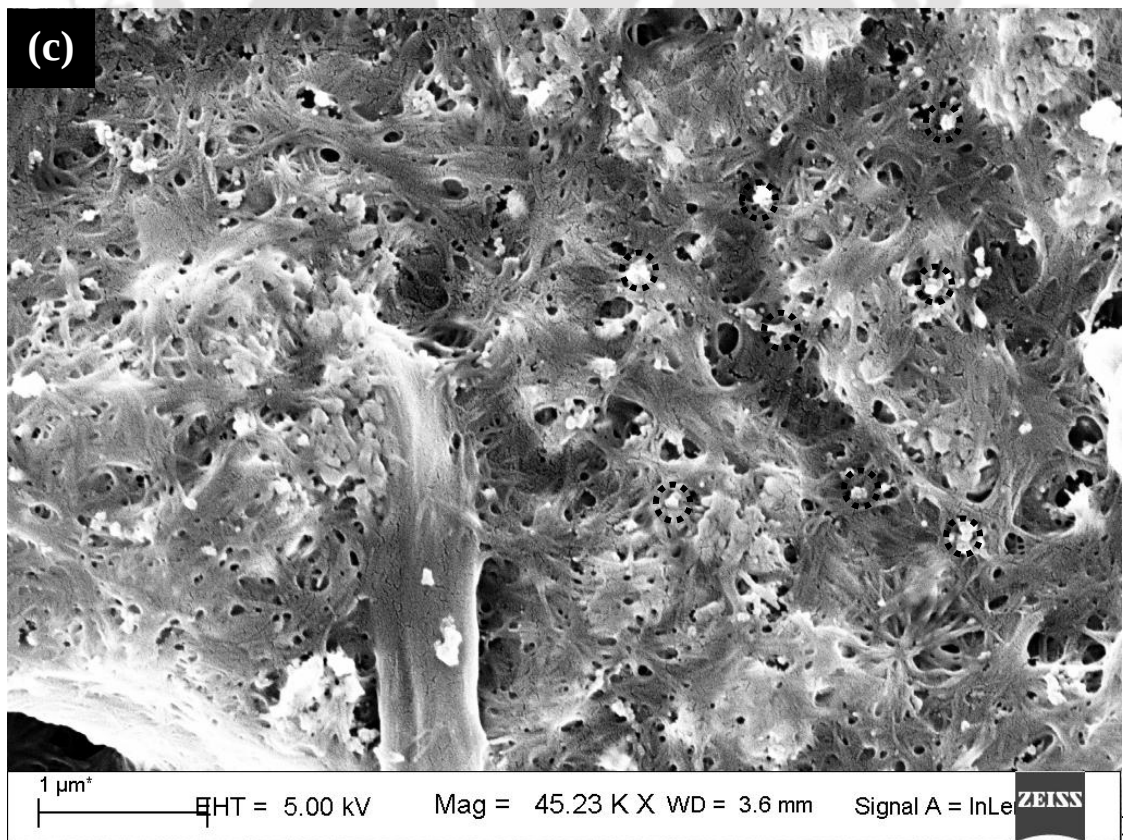
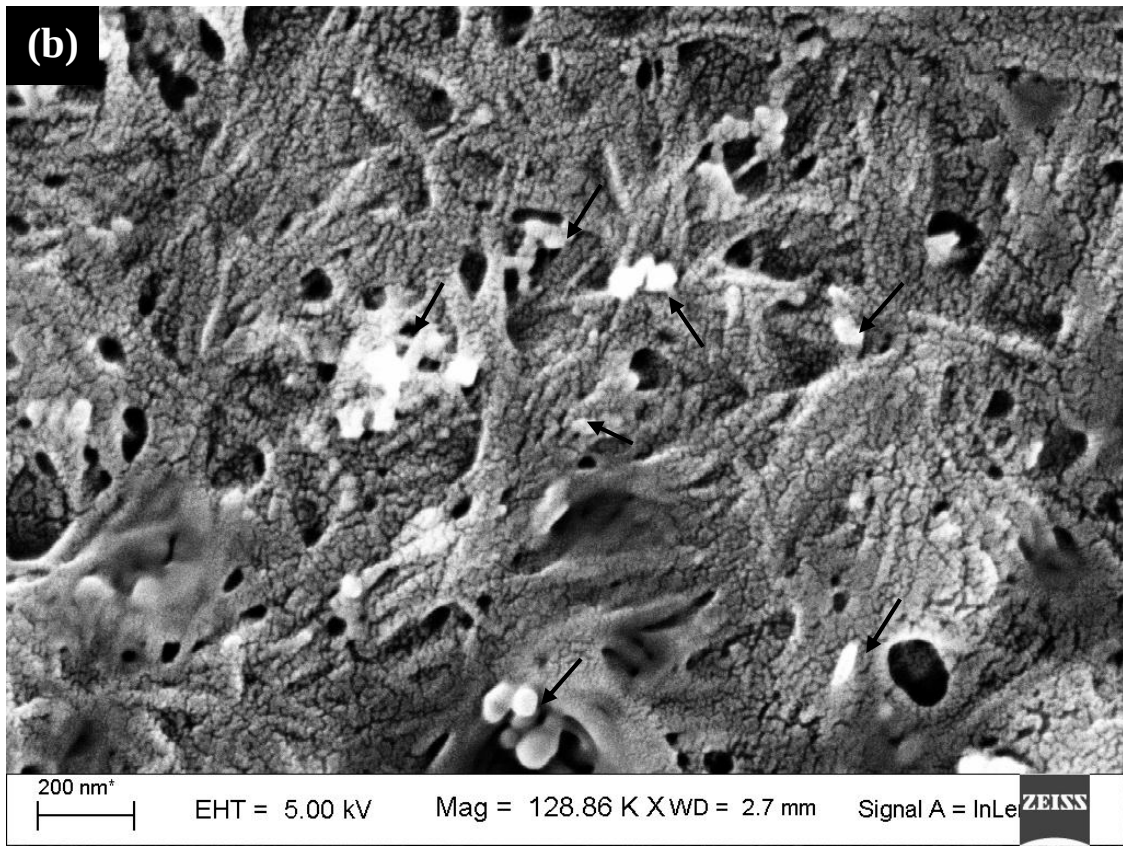
crystal planes (101), (10 $\bar{1}$), (002) and (040) respectively. Peaks at $2\Theta = 39.9^\circ$, 46.4° and 68.3° represent the (111), (200) and (220) crystal planes of Pd nanoparticles respectively, and the low intensity peaks at $2\Theta = 35.3^\circ$ and 43.5° suggests the presence of the Fe₂O₃ nanoparticles with (311) and (400) crystallographic planes respectively. Similarly, for Pt-Fe₂O₃-CNCs, XRD diffractograms show presence of peaks at $2\Theta = 14.9^\circ$, 16.6° , 22.7° and 34.2° representing the CNC crystal planes (101), (10 $\bar{1}$), (002) and (040) respectively. Peaks at $2\Theta = 39.8^\circ$, 46.5° and 68.2° signify the (111), (200) and (220) crystal planes of Pt nanoparticles respectively, and the low intensity peaks at $2\Theta = 35.1^\circ$ and 43.8° correspond to (311) and (400) crystallographic planes of the Fe₂O₃ nanoparticles. The minimum crystallite sizes of the Pd and Pt nanoparticles adsorbed on the surface of CNCs (calculated by Scherrer equation) are found to be ~ 5.35 nm and 7.35 nm respectively (corresponding to the peak at $2\Theta = 39.8^\circ$ (111)), which is consistent with the measured nanoparticle dimensions from the corresponding TEM micrographs. From the XRD diffractograms, it is interesting to observe that the peaks corresponding to Fe₂O₃ crystal planes underwent substantial reduction in intensity, but for CNCs the peak intensities almost remained unaltered. This suggests that precipitation of the Pd/Pt nanoparticles probably took place onto the Fe₂O₃ nanoparticles present in Fe₂O₃-CNCs rather than the backbone of the CNCs which remains intact. This is probably due to the absence of hydroxyl groups of CNCs in the Fe₂O₃-CNCs which are already occupied by the adsorbed Fe₂O₃ nanoparticles at very high loading fractions (~ 35 wt%). Due to the absence of hydroxyl functionality, the precipitation of Pd/Pt salts is governed by the Fe₂O₃ nanoparticles which provide the sites for adsorption of metal salts followed by their reduction, which is an electrochemically favourable process due to lower standard reduction potential [454,473]. To confirm further that the reduction of Pt and Pd nanoparticles took place on the surface of Fe₂O₃-CNCs in case of

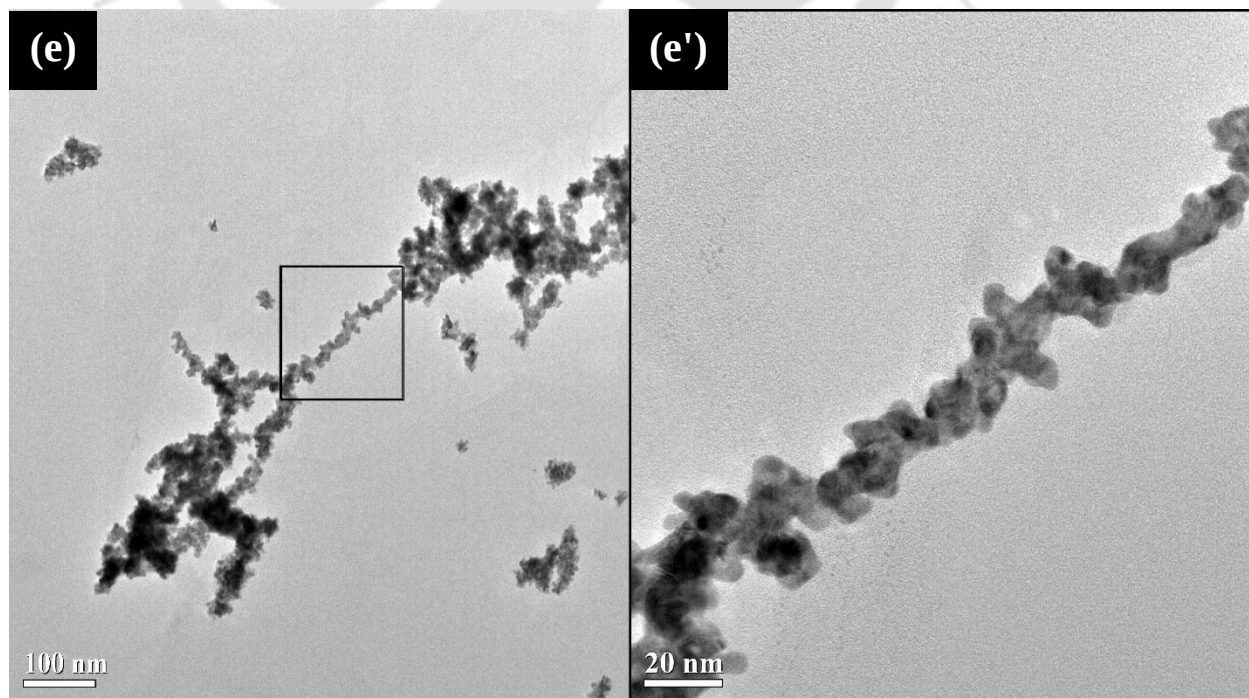
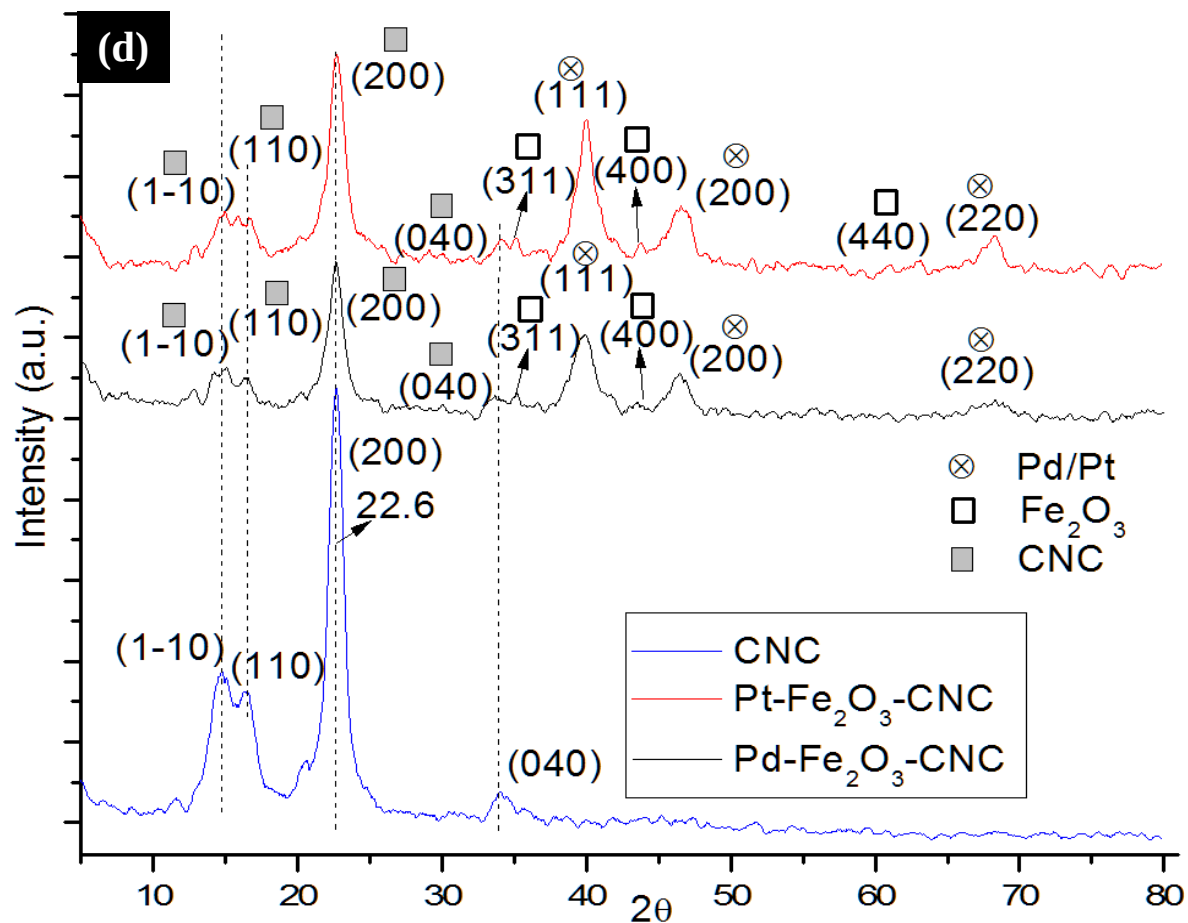
Pd- Fe_2O_3 -CNCs and Pt- Fe_2O_3 -CNCs, the crystal planes observed in high resolution-TEM (HR-TEM) micrographs are studied (Figure 7.18(g), (h)). The HR-TEM image for the Pd- Fe_2O_3 -CNCs shows the presence of crystal lattice fringes with interlayer spacing of ~ 0.200 nm and ~ 0.222 nm corresponding to (111) and (200) planes of Pd nanoparticles, which are especially present in the outer layers. The inner dark regions show the presence of lattice plane (311) for Fe_2O_3 nanoparticles with inter-layer spacing of ~ 0.250 nm, as also evident from the XRD diffractogram which shows a small intensity peak at $\sim 35.2^\circ$ (311). Similarly for the case of Pt- Fe_2O_3 -CNCs, the (111) crystal planes of Pt nanoparticles are observed at the edges and (311) crystal lattice of Fe_2O_3 nanoparticles in the inner region, with an interlayer spacing of 0.230 nm and 0.250 nm respectively. For both Pd/Pt- Fe_2O_3 -CNCs, HRTEM micrographs show the presence of crossover of lattice fringes which suggest that Pt/Pd nanoparticles have successfully adhered to the surface of Fe_2O_3 nanoparticles. Therefore, during the chemical reduction of the $\text{PdCl}_2/\text{PtCl}_2$ salts, the Pt/Pd nanoparticles coalesce onto the surface of the Fe_2O_3 nanoparticles due to improved electrochemical interactions. Similar observation of fabricating nickel nanoparticles onto the surface of ZVI nanoparticles was studied by Li et al. [473] in which the ZVIs acted as adsorbent as well as reductant simultaneously. Thus, from the crystallographic studies it can be concluded that both Pd/Pt nanoparticles can be supported on the surface of Fe_2O_3 -CNCs. The supported Pd/Pt nanoparticles have uniform size distribution and form a bimetallic structure with the Fe_2O_3 particles.

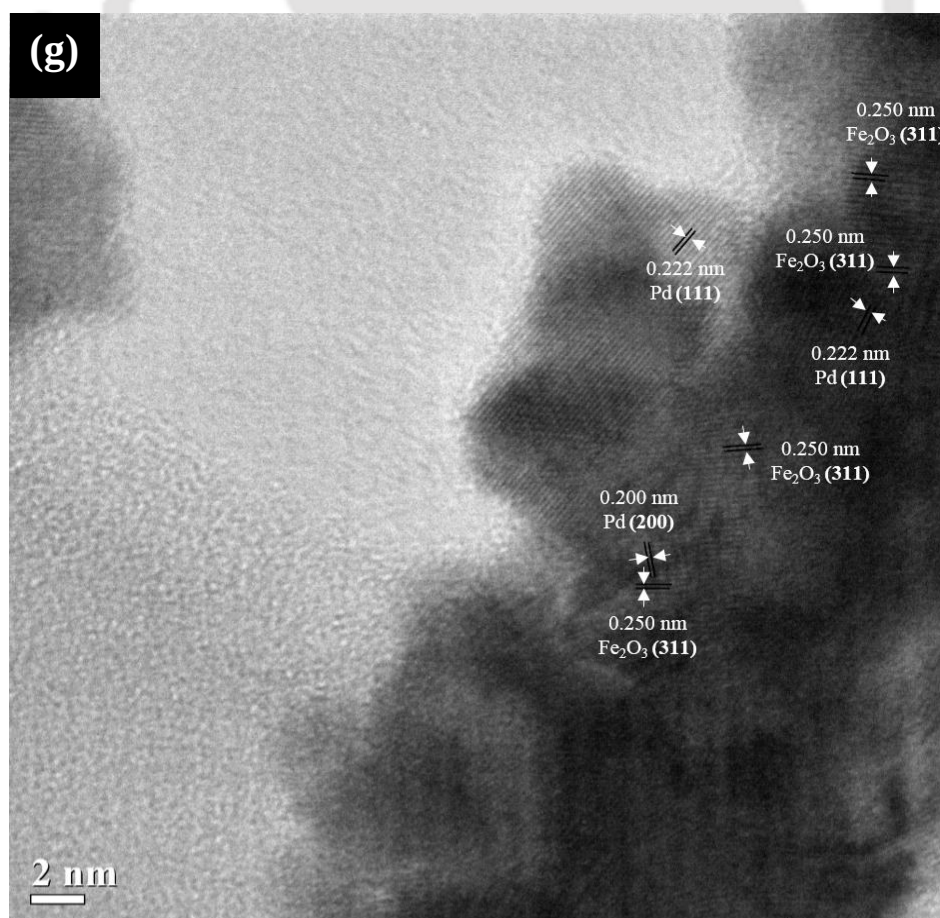
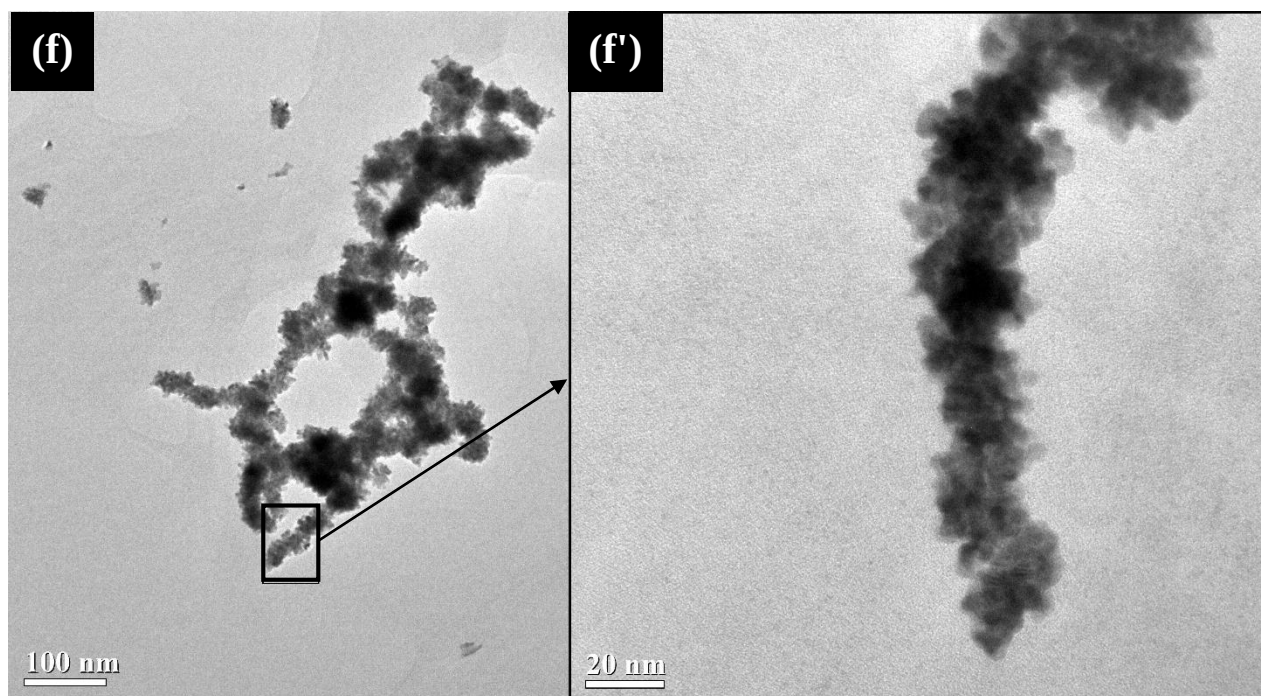
Figure 7.18(j) shows the VSM plots for magneto-responsive Fe_2O_3 -CNCs modified with the catalytically active Pd and Pt nanoparticles. Due to incorporation of the non-magnetic Pt and Pd species, the magnetization values decreased to ~ 26.4 and 19.4 emu/g for the Pd- Fe_2O_3 -CNCs and Pt- Fe_2O_3 -CNCs respectively. The slight variation in the magnetization

values is probably due to the difference in size of Pt and Pd nanoparticles adsorbed on the CNCs. Both Pd-Fe₂O₃-CNCs and Pt-Fe₂O₃-CNCs shows the presence of ferromagnetic behaviour prevalent in the initial Fe₂O₃-CNCs precursor. However, magnetization values of Pd-Fe₂O₃-CNCs and Pt-Fe₂O₃-CNCs are sufficient to cause graphene sheets to roll up around their distinct fiber/rod-like structure. The graphene nanoscrolls thus formed contain encapsulated metallic nanoparticles, as discussed in the next section.









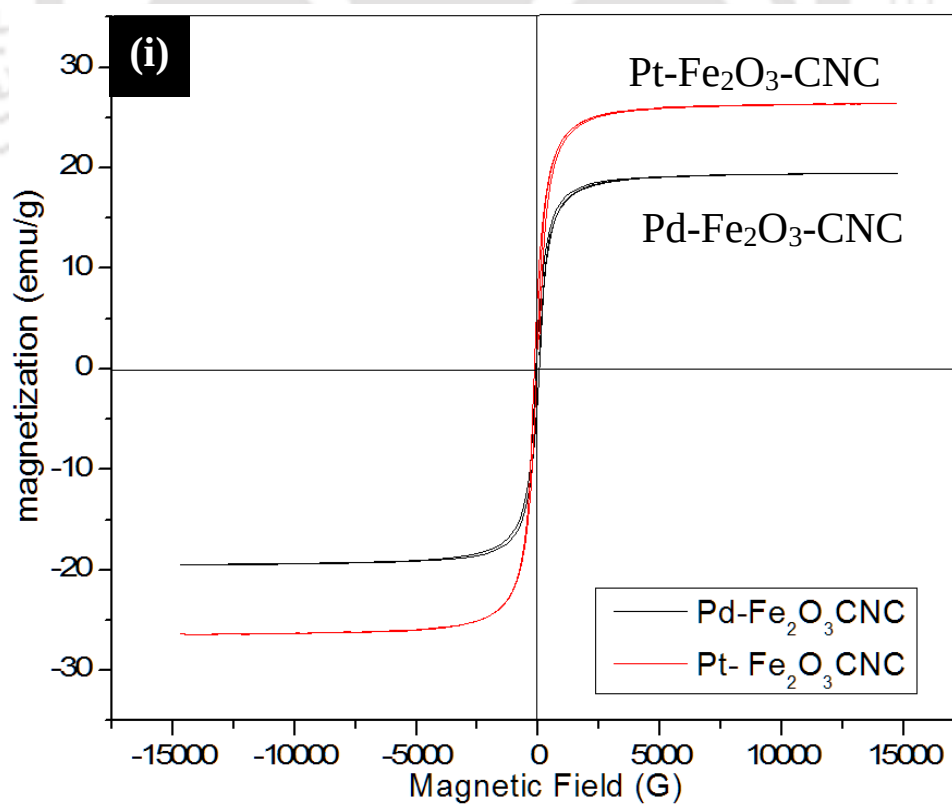
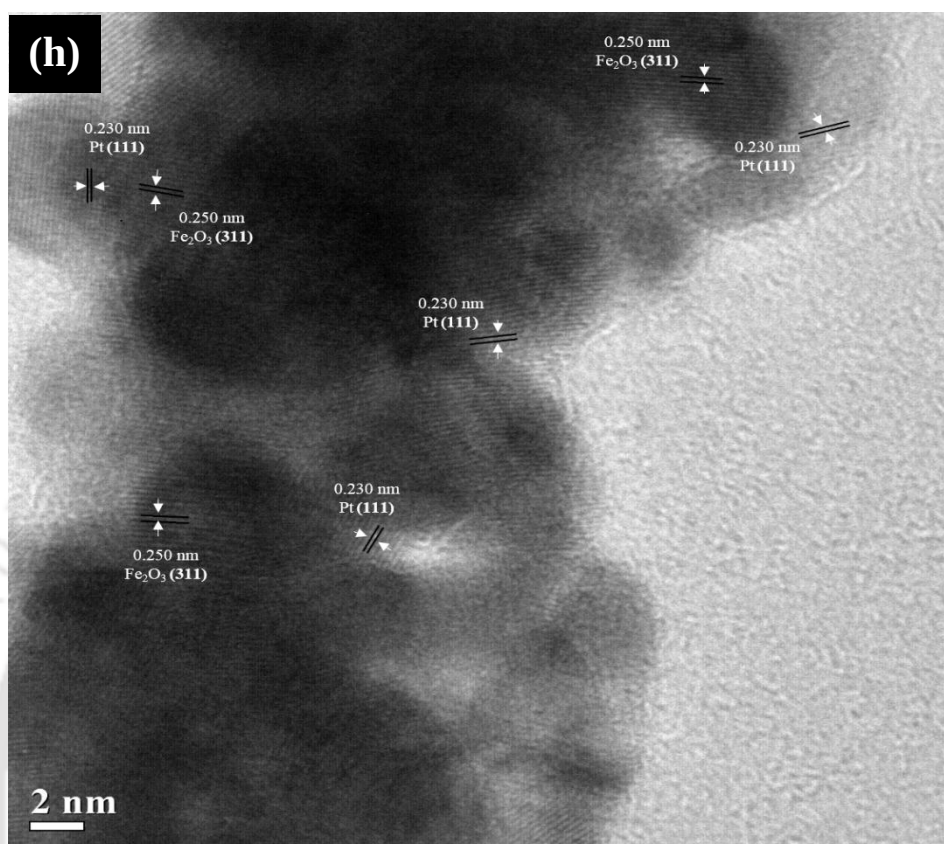


Figure 7.18: FESEM micrographs of the fabricated (a) Pd-Fe₂O₃-CNC with (b) high resolution micrographs of Pd-Fe₂O₃-CNC (at a resolution of ~125KX) which shows the presence of fine spherical Pd nanoparticles adsorbed onto Fe₂O₃-CNCs, (c) FESEM micrographs of Pt-Fe₂O₃-CNC, (d) XRD patterns of Pd-Fe₂O₃-CNC, Pt-Fe₂O₃-CNC and CNC. TEM micrographs of (e)Pt-Fe₂O₃-CNC and (f)Pd- Fe₂O₃-CNC with the inset (e') and (f') showing the high resolution micrographs for the selected region in rectangular block which shows the presence of spherical Pd and Pt nanoparticles adsorbed onto CNCs. HRTEM micrographs of (g) Pd- Fe₂O₃-CNC and (h) Pt-Fe₂O₃-CNC with their respective crystal planes of Pd and Fe₂O₃ marked in white and (i) Magnetic hysteresis loop for the fabricated Pd- Fe₂O₃-CNC and Pt-Fe₂O₃-CNC.

7.2.8 Fabrication of Graphene Nanoscrolls with Pd-Fe₂O₃-CNC and Pt-Fe₂O₃-CNC as Templates

Pd-Fe₂O₃-CNC and Pt-Fe₂O₃-CNC with their high magnetic moment (~26.4 and 19.4 emu/g respectively) and distinct rod/fiber-like morphology are used as templates for fabrication of GNS. In earlier reported studies, incorporation of palladium oxide nanoparticles on graphene sheets did not show any rolling behaviour [472]. This is probably due to the absence of magnetic character as well as structure directing agents, which prevents the graphene sheets to overcome the threshold potential required to initiate the scrolling phenomenon. In the present study, Pd-Fe₂O₃-CNC and Pt-Fe₂O₃-CNC with high magnetic moment are modified with epoxy functional groups to improve their interaction with the oxidized graphene sheets, which leads to covalent coupling. It has been observed that the separation distance between the graphene and adsorbed nanoparticles is a key factor in

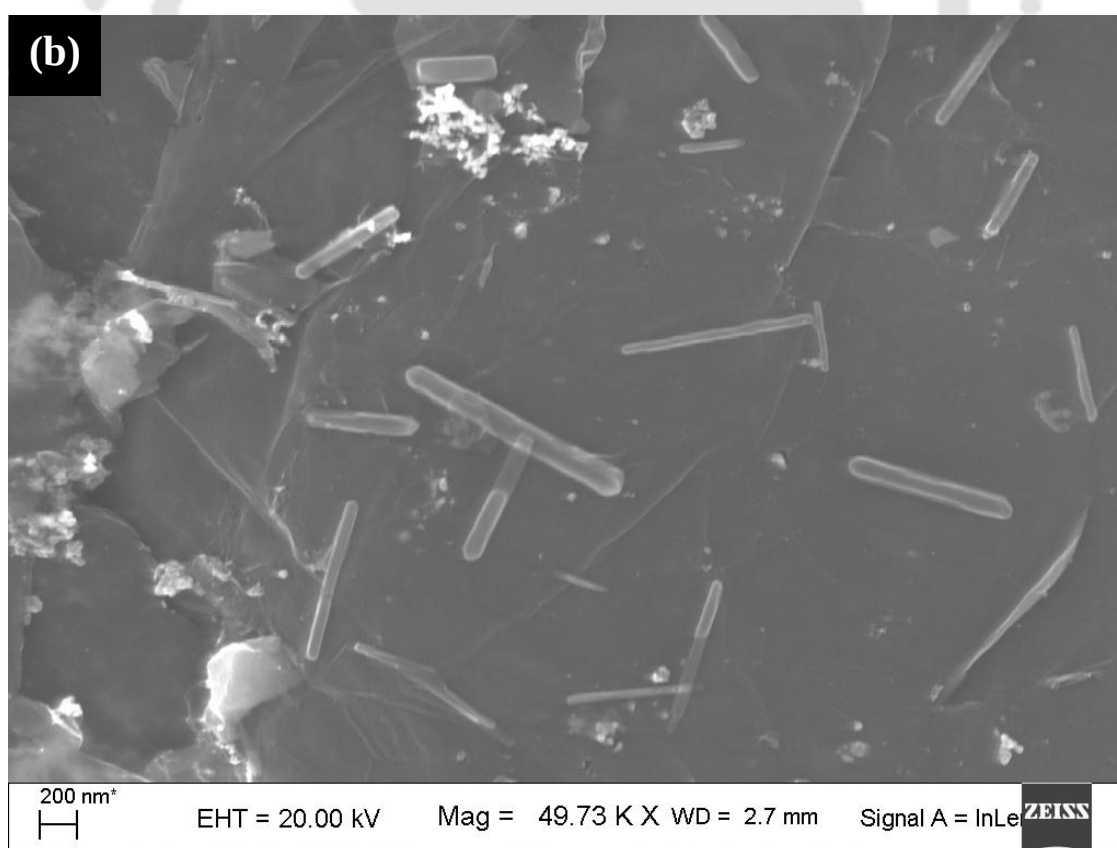
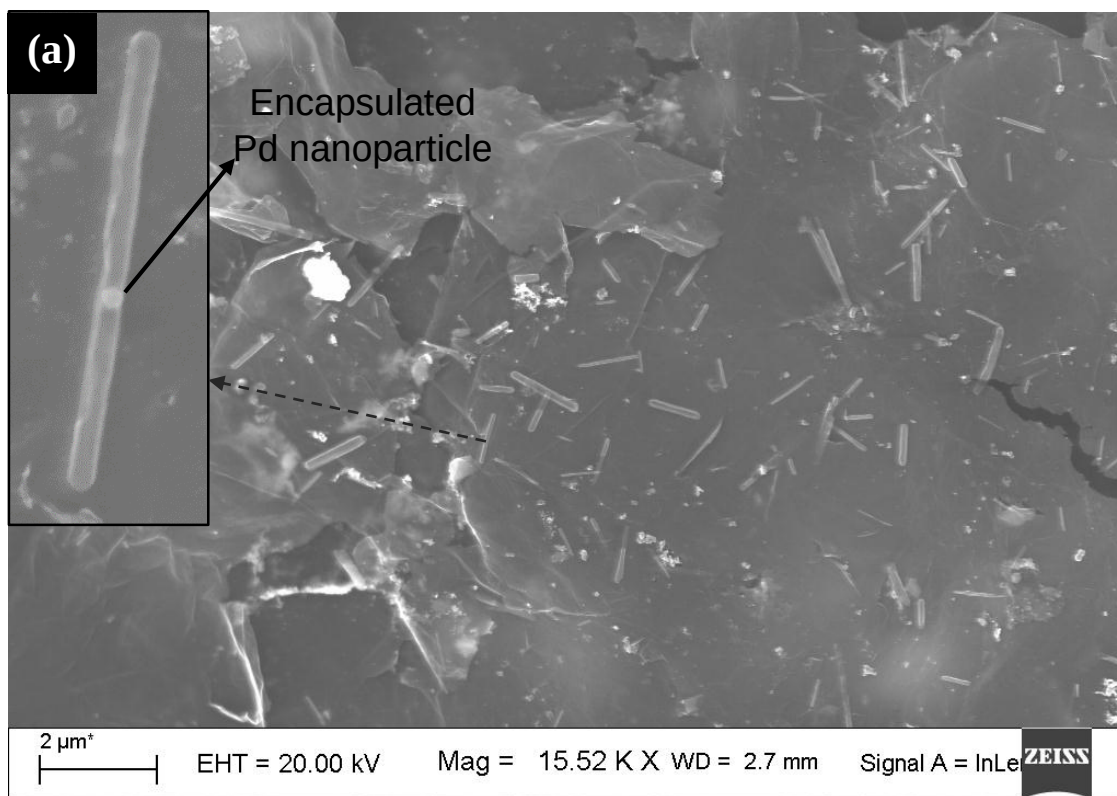
initiating the scrolling process which is governed by the van der Waals force of interaction [472]. In this study, the graphene sheets are brought in close proximity to Pt/Pd-Fe₂O₃-CNC through epoxidation based crosslinking between the two systems. . In addition, the strong magnetic moment of Pt/Pd-Fe₂O₃-CNC helps in overcoming the energy barrier, thereby initiating the scrolling up of graphene sheets onto the CNCs. Once the scrolling process is complete, the inner core containing CNCs is removed through controlled calcination process ensuring that the morphology of the GNS remains intact. The removal of CNCs from Pd- Fe₂O₃-CNC and Pt-Fe₂O₃-CNC leads to the encapsulation of catalytically active Pd and Pt nanoparticles inside the core of GNS, which significantly alters the structural, morphological and physicochemical properties.

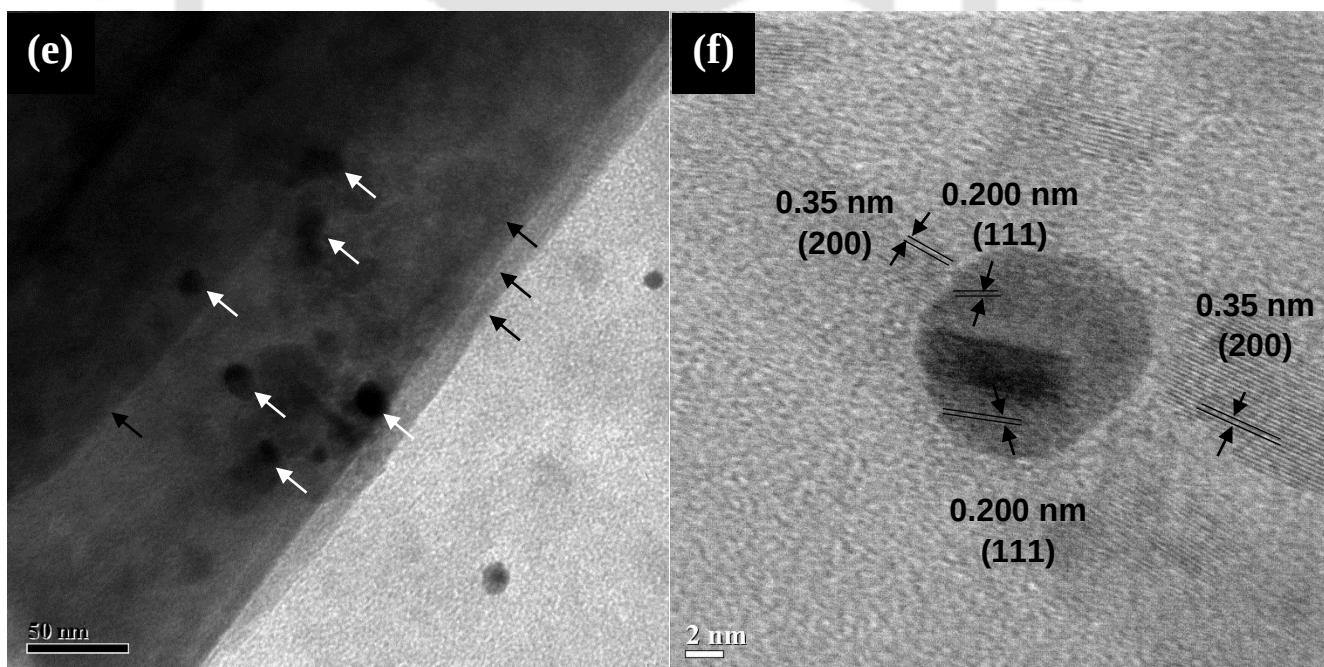
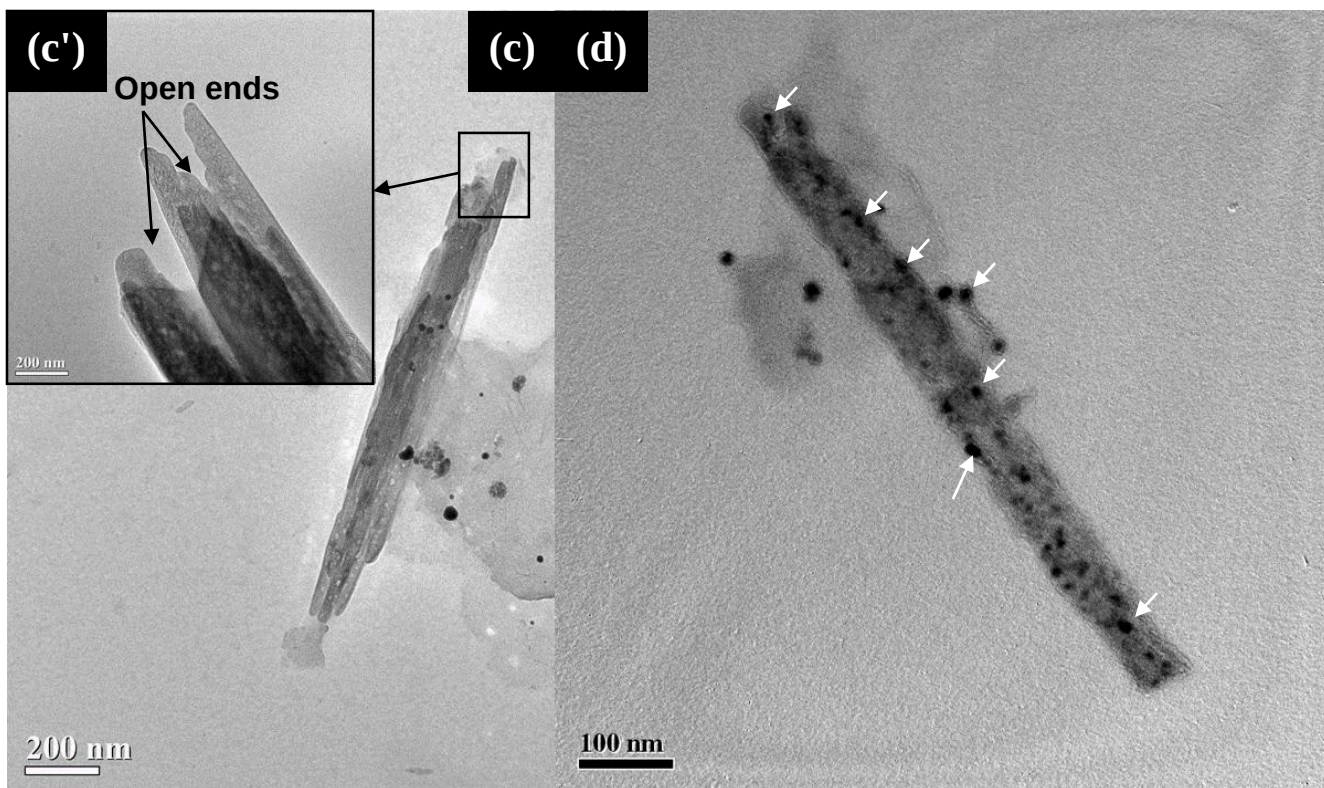
Fabrication of GNS with the incorporation of different varieties of catalytically active nanomaterials such as Pd and Pt nanoparticles leads to their increased efficiency along with improved activity. Therefore, we report a facile approach of encapsulating the metallic nanoparticles in the core of GNS through a template-based approach in which the type of nanoparticles incorporated can be tuned, depending on the particular application. Figure 7.19(a) and (b) show the uniformly distributed hollow Pd-Fe₂O₃-NS which suggests the complete removal of the CNC from the inner core. The inset shows the high resolution micrograph of the single Pd-Fe₂O₃-NS (selected region in Figure 7.19(a)) which shows the presence of spherical Pd nanoparticles at the centre of the nanoscrolls. Similarly, the TEM micrographs of a single Pd-Fe₂O₃-NS (in Figure 7.19(c) and (d)) show the presence of uniformly distributed Pd nanoparticles as black dots along the length of nanoscrolls (marked with white arrows). From the FESEM micrographs, the dimensions of Pd-Fe₂O₃-NS are found to be $\sim 710 \pm 110$ nm (length) and $\sim 70 \pm 22$ nm (diameter). The distinct rod-like morphology of

CNCs is retained in Pd-Fe₂O₃-NS even after the removal of CNCs through calcination. The dimensions of Pd-Fe₂O₃-NS are comparable to those of Fe₂O₃-NS which suggests that the morphology of structural template is the governing factor in tuning the dimensions of the fabricated nanoscrolls. Figure 7.19(e) displays the presence of multiple graphene layers scrolled (marked in black arrows) in Pd-Fe₂O₃-NS, with encapsulated Pd nanoparticles (marked in white arrows). HRTEM micrograph (in Figure 7.19(f)) shows the presence of the crystal lattice planes with inter-layer spacing of ~ 0.350 and 0.200 nm corresponding to the (200) and (111) crystal planes for graphene and Pd nanoparticles respectively. The crossover of the crystal lattice planes at the edges of the Pd nanoparticles confirms its encapsulation within the graphene sheets. Further, the line EDX spectra shows the presence of both Pd and Fe in the selected region (marked with black line). Interestingly, EDX-line spectra shows the presence of higher intensity peaks for Pd element in line with the Fe element (but with comparatively lower intensity). This is consistent with the XRD analysis of Pd-Fe₂O₃-CNC which suggests precipitation of Pd nanoparticle on Fe₂O₃ surface. Further, the encapsulation of Pd nanoparticles within the GNS is confirmed through compositional analysis of Pd-Fe₂O₃-NS, which shows the presence of ~ 1.8 wt% Pd and 0.7 wt% Fe.

Similarly, Pt-Fe₂O₃-NS are fabricated by using of Pt-Fe₂O₃-CNC as template followed by controlled calcination to remove CNCs and form nanoscrolls with encapsulated Pt nanoparticle, as shown in Figure 7.20(a) and (b). FESEM micrographs display the presence of hollow Pt-Fe₂O₃-NS nanoscrolls with the Pt nanoparticle encapsulated in between the rolled graphene sheets. The inset of Figure 7.20 (a) shows the presence of spherical Pt nanoparticle (seen as bright spot) incorporated at the centre of the nanoscrolls. Morphological analysis shows that the fabricated Pt-Fe₂O₃-NS are of length $\sim 810 \pm 90$ nm and diameter $\sim 95 \pm 20$ nm

respectively, which is comparable to the dimensions of $\text{Fe}_2\text{O}_3\text{-NS}$ and $\text{Pd-Fe}_2\text{O}_3\text{-NS}$. TEM micrographs shown in Figures 7.20(c) and (d) display several hollow $\text{Pt-Fe}_2\text{O}_3\text{-NS}$ with the Pt nanoparticles present at the core and the rolled graphene sheets forming an open-ended morphology (shown in the inset of Figure 7.20 (d')). HRTEM micrographs further suggest that Pt nanoparticles are also distributed/intercalated between the adjacent layers of the graphene nanoscroll. The presence of lattice fringes with interlayer spacing of $\sim 0.35\text{nm}$ represent the (200) crystal plane, confirming that the nanoscrolls are made up of rolled graphene sheets. Further, EDX-mapped section (of selected white box) shows the uniform distribution of Pt nanoparticles in the graphene nanoscrolls with an elemental composition of $\sim 1.6\text{ wt. \%}$ (Figures 7.20(g) and (g')). Therefore, the graphene nanoscrolls can be efficiently fabricated through the utilization of $\text{PtPd-Fe}_2\text{O}_3\text{-CNC}$ as templates in which the percentage loading of Pt/Pd content can be tuned depending on the desired activity of the catalyst.





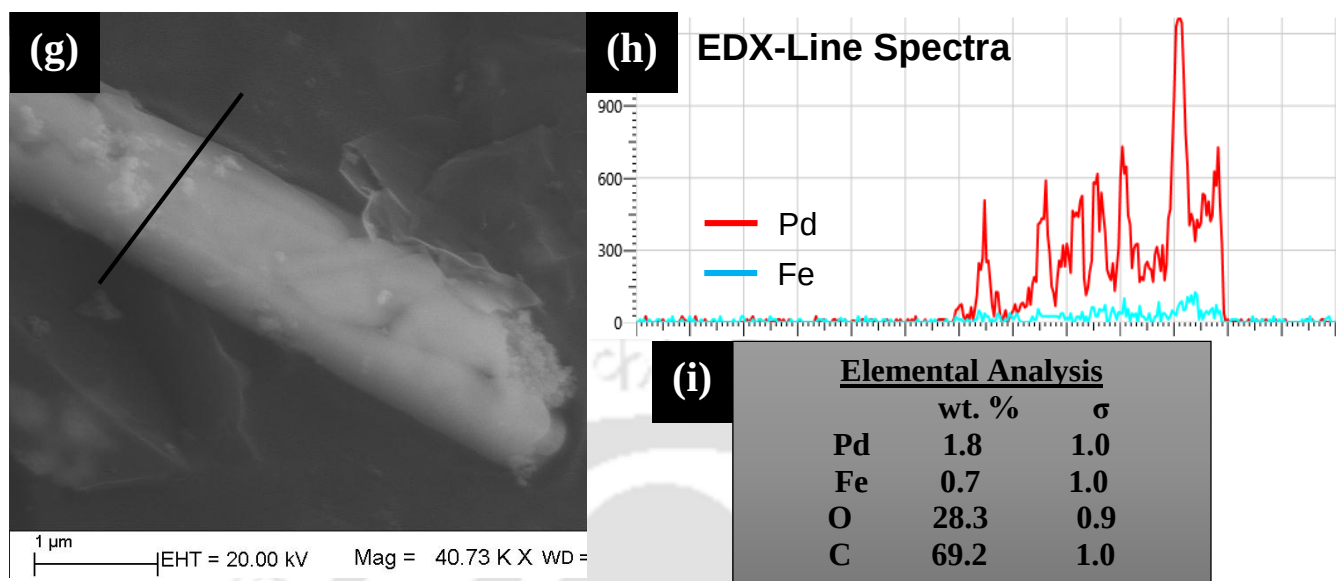
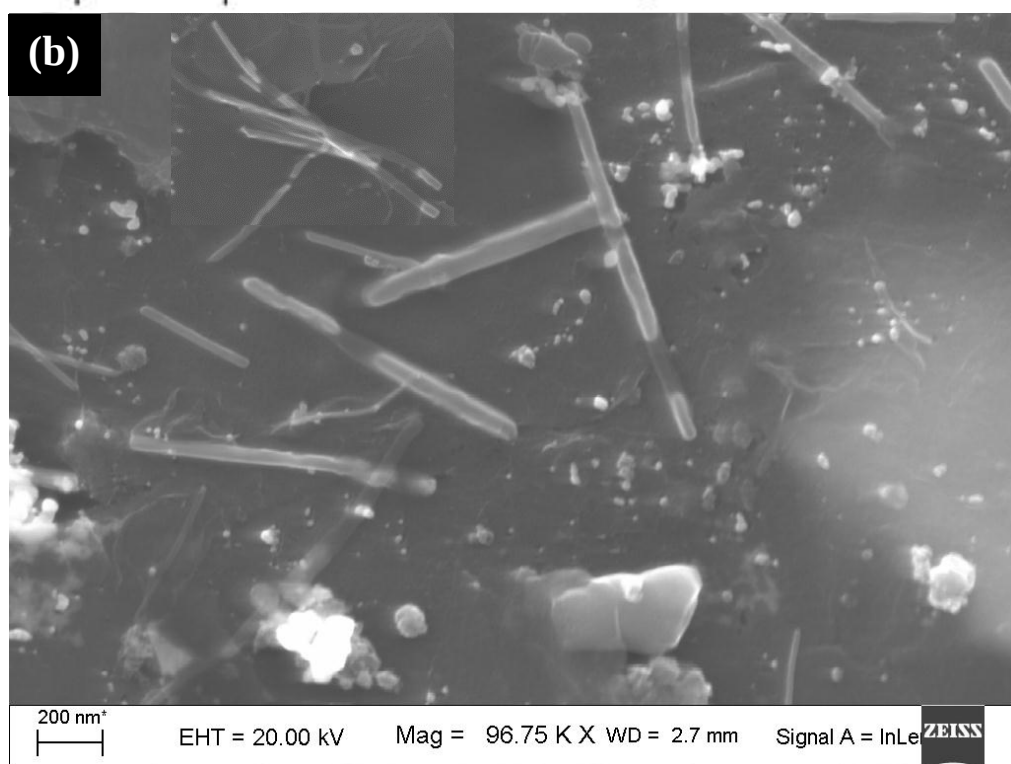
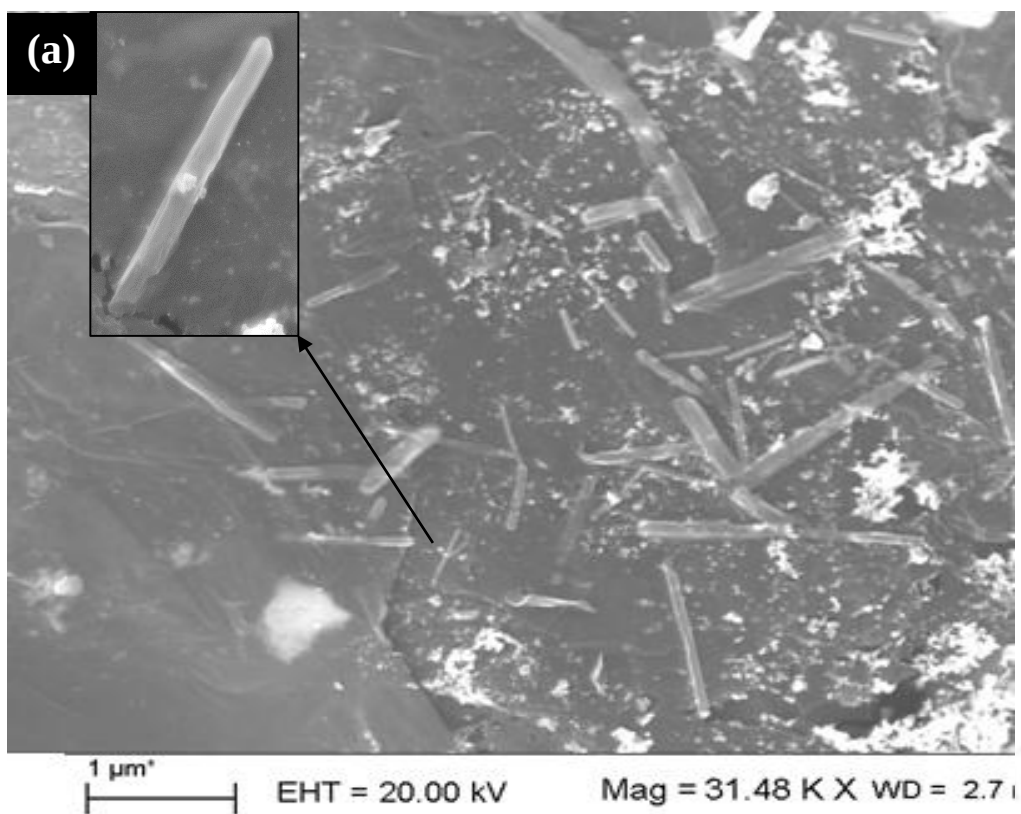


Figure 7.19: (a) FESEM micrographs of the fabricated Pd-Fe₂O₃-NS (with the inset showing a single GNS with the encapsulated Pd nanoparticle at its centre), (b) Pd-Fe₂O₃-NS FESEM micrograph at higher resolution, (c) and (d) TEM micrographs of the Pd-Fe₂O₃-NS with the encapsulated Pd nanoparticles in it (marked with white arrows) (c') Inset displays the magnified open end of the Pd-Fe₂O₃-NS (marked with rectangular box), (e) TEM micrograph of an edge of Pd-Fe₂O₃-NS which shows the presence of multiple layers of rolled graphene sheets (marked with black arrows) with the Pd nanoparticles (marked with white arrows) encapsulated within its core and between the layers. (f) HRTEM micrographs of Pd-Fe₂O₃-NS illustrating the presence of lattice fringes of Pd nanoparticles and graphene, (g) and (h) FESEM-EDX line-spectra of the selected region (marked in black line) showing the distribution of the Pd and Fe on the Pd-Fe₂O₃-NS, and (i) compositional analysis of the elements in the Pd-Fe₂O₃-NS.



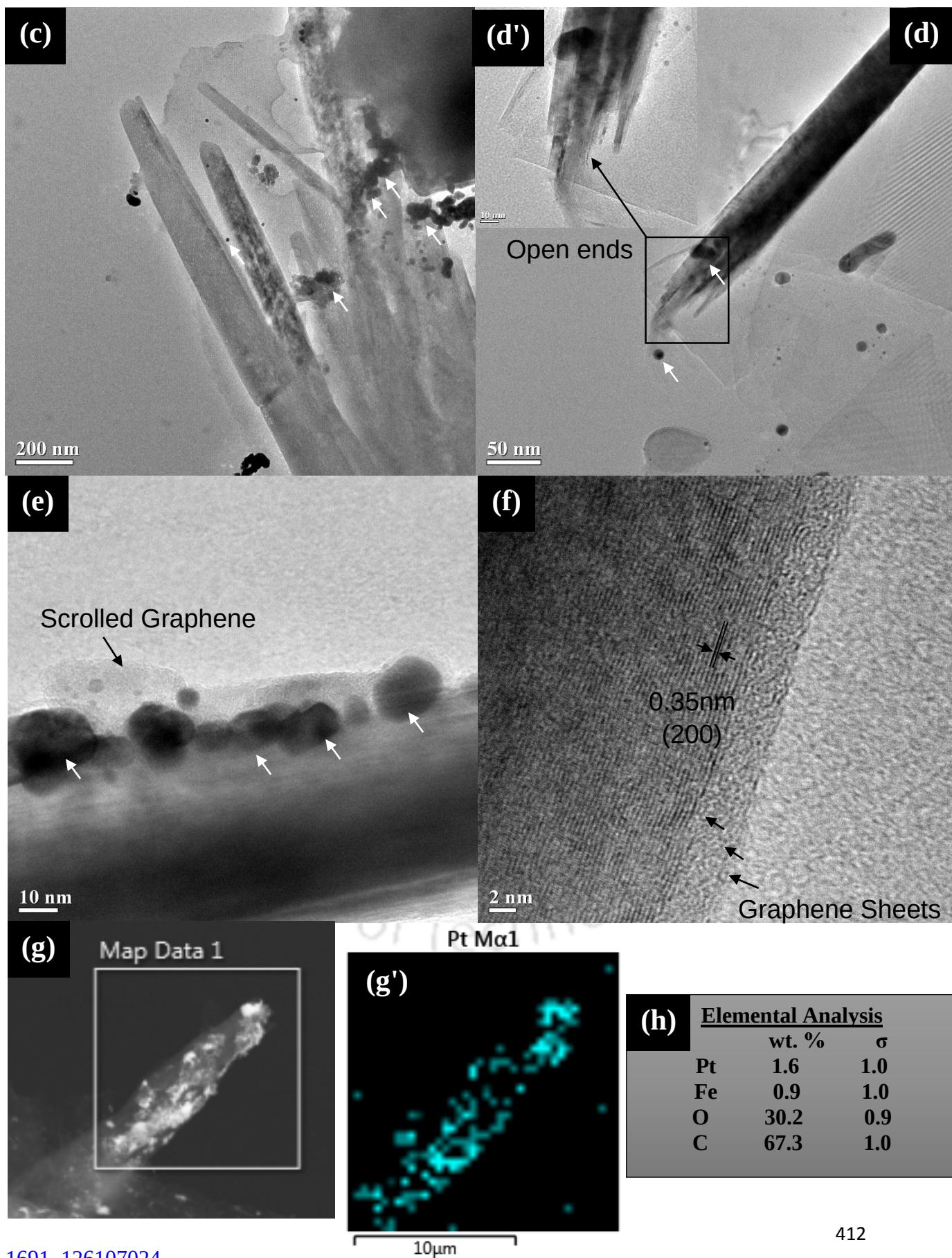


Figure 7.20: (a) FESEM micrographs of the fabricated Pt-Fe₂O₃-NS (with the inset showing a single GNS with the encapsulated Pt nanoparticle at its centre), (b) Pt-Fe₂O₃-NS FESEM micrograph at higher resolution, (c) and (d) TEM micrographs of the Pt-Fe₂O₃-NS with the encapsulated Pt nanoparticles in it (marked with white arrows) (d') Inset displays the magnified open end of the Pt-Fe₂O₃-NS (marked with rectangular box), (e) TEM micrograph of an edge of Pt-Fe₂O₃-NS which shows the graphene sheets rolled (marked with black arrows) with the Pt nanoparticles (marked with white arrows) encapsulated within its core and between layers. (f) HRTEM micrographs of Pd-Fe₂O₃-NS illustrating the presence of lattice fringes of graphene, (g) and (g') FESEM-EDX mapping of the selected region (marked in white box) showing the distribution of Pt on Pt- Fe₂O₃- NS, and (h) compositional analysis of the elements in the Pt- Fe₂O₃- NS.

Figure 7.21, shows the XRD diffractograms of GNS with the encapsulated Pd and Pt nanoparticles in its core along with the Fe₂O₃ nanoparticles. The GRO sheets show a sharp peak at $2\theta = 26.6^\circ$ corresponding to the (002) planes of the layered graphene sheets. However, the peak at $2\theta = 26.6^\circ$ significantly broadens upon the formation of Pt/Pd-Fe₂O₃-NS which is possibly due to scrolling of graphene sheets with intercalated metallic nanoparticles between its layers. For the case of Pd-Fe₂O₃-NS, the peak at $2\theta = 26.6^\circ$ (002) corresponding to GRO shifts to 25.5° and is considerably broadened. This is probably due to the infiltration of Pd and Pt nanoparticles with different crystallite sizes (5.3 and 7.3 nm respectively) between the layers of the GNS resulting in increased inter-layer spacing between the rolled graphene sheets. Such alteration of the (002) diffraction peak of GNS was also observed by Xu et al. [448] on

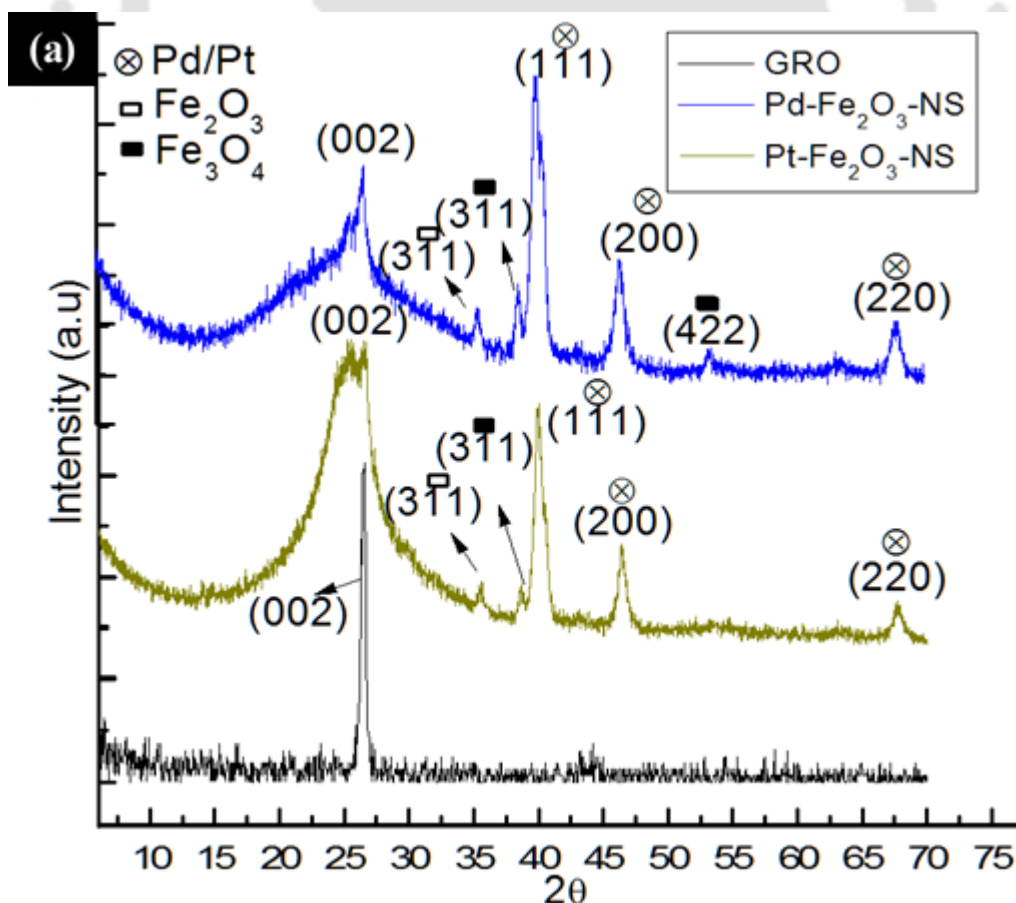
intercalation of different solvent followed by swelling and drying. The diffractograms also show the presence of the peaks corresponding to the Pd/Pt nanoparticles along with the Fe_2O_3 nanoparticles and Fe_3O_4 nanoparticles (which might have formed during the processing steps). Pd- Fe_2O_3 -NS shows the presence of peaks at $2\theta = 40.0^\circ$, 46.5° and 67.8° representing the (111), (200) and (220) crystal planes of Pd nanoparticles, peaks at $2\theta = 35.5^\circ$ corresponding to the (311) crystal of Fe_2O_3 nanoparticles, and low intensity peaks at $2\theta = 38.6^\circ$ and 53.4° designating the (311) and (422) crystal planes of Fe_3O_4 nanoparticles. Similarly, the diffractogram of Pt- Fe_2O_3 -NS shows the presence of peaks at $2\theta = 39.9^\circ$, 46.3° and 67.9° representing the (111), (200) and (220) crystal planes of Pt nanoparticles, peaks at $2\theta = 35.6^\circ$ corresponding to the (311) crystal of Fe_2O_3 nanoparticles and a small peak at $2\theta = 38.6^\circ$ designating the (311) crystal planes of Fe_3O_4 nanoparticles. The absence of any peak corresponding to the crystal planes of the CNCs suggests complete removal of the CNCs during the calcination process. The formation of Fe_3O_4 nanoparticles may have occurred during the calcination process, and their presence is evident from the small intensity peaks in the diffractograms. Due to the prolonged time period for calcination, it was observed that large fractions of Fe_3O_4 nanoparticles are formed in GNS, the fraction of which can be tuned through controlled calcination process. The crystallite sizes of Pd and Pt nanoparticles (calculated using Scherrer equation) encapsulated within the GNS are found to ~ 8.0 nm and 10.7 nm respectively which match well with the dimensions of Pd/Pt nanoparticles measured from TEM micrographs for Pd/Pt- Fe_2O_3 -NS. Interestingly, the crystallite size of Pd and Pt nanoparticles falls within the range of Pt/Pd nanoparticles adsorbed on Pd/Pt- Fe_2O_3 -CNC. This suggests that the Pd/Pt nanoparticles adsorbed on the surface of CNCs can be effectively transferred to the inner core of the GNS without any significant agglomeration or accumulation of nanoparticles.

From the crystallographic studies, it is observed that the proposed methodology for fabrication of GNS can efficiently utilize the CNCs as templates for the encapsulation of a wide variety of metallic nanoparticles inside the GNS core, with the choice of nanoparticle depending on the targeted applications.

Figure 7.21(b) shows the Raman spectra of the oxidized graphene and the GNS with encapsulated Pd and Pt nanoparticles in its core (Pd/Pt-Fe₂O₃-NS). The Raman spectra of oxidized graphene shows a G-band at $\sim 1582\text{ cm}^{-1}$ which arises due to hexagonal vibrations and the D-band at $\sim 1354\text{ cm}^{-1}$ which corresponds to defects and sp^3 hybridized bonds. The D band occurs due to symmetry breaking or disordered arrangement of carbon especially at the edges of graphene planes in the sp^2 hybridization state. The I_D/I_G ratio provides an efficient tool to understand the degree of covalent coupling of Pd/Pt-Fe₂O₃-CNC with the GRO sheets as well as π - π interactions with the graphene sheets. It was observed that the I_D/I_G for the oxidized graphene sheets is ~ 0.97 , which increased to ~ 1.01 and 1.03 for the Pd-Fe₂O₃-NS and Pt- Fe₂O₃- NS respectively. The increased I_D/I_G ratio is attributed to the formation of new defects as well as sp^3 hybridization which might have occurred during epoxidation of graphene sheets with the Pd/Pt-Fe₂O₃-CNC. On the removal of CNCs through calcination, Pt/Pd- Fe₂O₃ nanoparticles remain attached to the inner-core of GNS. Moreover, the increased broadness and intensity of the G band in case of Pd/Pt-Fe₂O₃-NS is probably due to scrolling up of the graphene sheets into multiple layers, which increases the elastic strain on it. Therefore, from the Raman spectroscopy studies it can be concluded that high quality GNS are formed around the Pd/Pt-Fe₂O₃-CNCs templates, which is possible due to increased π - π stacking as well as due to the presence of structure directing templates.

7.2.8 Potential Applications of the Graphene Nanoscrolls for Hydrogen Adsorption Study.

The fabricated GNS with tunable morphology and doped with various transition metal precursors such as Pd and Pt have been used to evaluate their hydrogen adsorption capacity. It is expected that the nanoscale hollow cylindrical channels present in the GNS with uniform dimensions will provide a favourable route for hydrogen transport and storage. Moreover, the interlayer spacing in the scrolled sheets of multiple GNS layers is larger than the molecular kinetic diameter of hydrogen molecules. This will provide easy entrapment of the hydrogen molecules in the pores or interlayer spacing of scrolled graphene sheets through physisorption mechanism. Most of the studies reported in literature on the adsorption of hydrogen in the graphene nanoscrolls are based on molecular dynamics simulation.



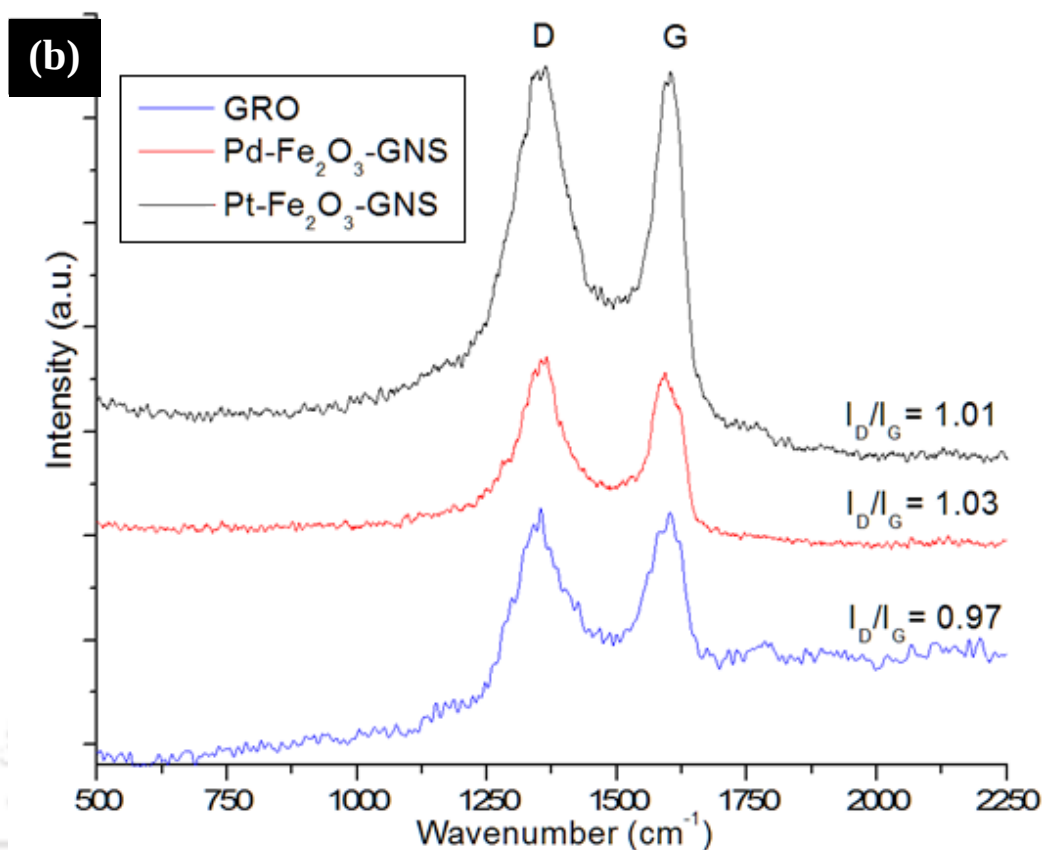


Figure 7.21: (a) XRD diffractograms and (b) Raman spectra of Pt-Fe₂O₃-NS and Pd-Fe₂O₃-NS fabricated using catalytically active Pt-Fe₂O₃-CNCs and Pd-Fe₂O₃-CNCs as the structure directing templates.

In a recent study, predicted through molecular simulations that utilization of the fluorinated graphene nanoscrolls would lead to hydrogen uptake of ~7 wt% at 77K, 25 bar and ~0.4 wt% at 298K, 25 bar [474]. Similarly, introduction of lithium as a dopant into carbon nanoscrolls leads to enhanced hydrogen uptake of ~3.5 wt% at 293K, 50 bar as predicted by classical Monte Carlo (MC) simulations [449]. Organic pillared graphite structures show enhanced hydrogen adsorption of ~4 wt% at 300K, 100 bar as studied through grand canonical MC simulations. To the best of our knowledge, no reports are available in literature on the hydrogen adsorption capacity of GNS measured through experimental studies. Most of the

experimental studies reported on hydrogen adsorption are based on graphene or single/multi-walled carbon nanotube structures. The maximum hydrogen uptake reported in experimental studies using such carbon-based nanostructures are relatively lower in comparison to the predicted values in GNS from molecular simulation studies. Utilization of reduced graphene sheets shows a hydrogen uptake of ~ 2 wt% at 77 K, 50 bar and ~ 0.025 wt% at 300K, 20 bar. Decoration of the graphene sheets with iron metal leads to increase in hydrogen uptake to 2.16 wt% at 77K, 50 bar and ~ 0.10 wt% at 300K, 20 bar [475]. The above-mentioned graphene-based nanomaterials generally have very high surface area in the range of 159- 2139 m^2/g which is responsible for the improved hydrogen capacity. Therefore, inspired by the simulation studies reporting high hydrogen uptake by the graphene nanoscrolls, and hydrogen adsorption capacity of graphene nanostructures, we studied the hydrogen adsorption behaviour of the fabricated graphene nanoscrolls, and the effect of the incorporation of the Pt/Pd nanoparticles on hydrogen adsorption.

Figure 7.22 shows the hydrogen uptake capacity of graphene and graphene nanoscrolls with encapsulated Pt and Pd nanoparticles (Pt- Fe_2O_3 -NS and Pd- Fe_2O_3 -NS) in their core. The graphene shows an adsorption capacity of ~ 0.08 wt% measured at 273K and 20 bar which is comparable to the data reported in literature. Introduction of Pd and Pt nanoparticles inside the core of graphene scrolls leads to considerable increase in the hydrogen adsorption capacity, with ~ 0.17 and ~ 0.14 wt% adsorption loading observed for Pt- Fe_2O_3 -NS and Pd- Fe_2O_3 -NS respectively, measured at 273K and 20 bar. The adsorption isotherms typically show a physical adsorption profile, indicating that the hydrogen gets adsorbed inside the interlayer spacing of GNS through physisorption routes. However, the measured BET surface areas for Pt- Fe_2O_3 - NS and Pd- Fe_2O_3 -NS are 20.5 and 21.3 m^2/g respectively, which are comparatively

lower in comparison to the initial graphene precursor with a surface area of $\sim 36.3 \text{ m}^2/\text{g}$. Therefore, the improvement in hydrogen adsorption capacity of Pt-Fe₂O₃-NS and Pd-Fe₂O₃-NS even with very low surface areas, is probably due to the presence of the nanochannels in GNS with encapsulated Pt/Pd nanoparticles which could adsorb hydrogen through spill over mechanism. The presence of highly dispersed Pd/Pt nanoparticles in the core as well as interlayer spacing of the GNS acts as a physical bridge, which increases the chemisorption of the hydrogen molecules. Moreover, after the adsorption of hydrogen species, the presence of multi-layered graphene scrolls acts as barrier to the diffusion of the hydrogen molecules out from the noble metal surface. Therefore, it can be concluded that the formation of scrolled graphene layers with encapsulated Pd/Pt nanoparticles through the utilization of bio-based nanomaterials as templates, provides a novel approach to develop hydrogen storage devices operational at relatively feasible conditions (relatively low pressure $\sim 20 \text{ bar}$ and high temperature $\sim 0^\circ \text{C}$).

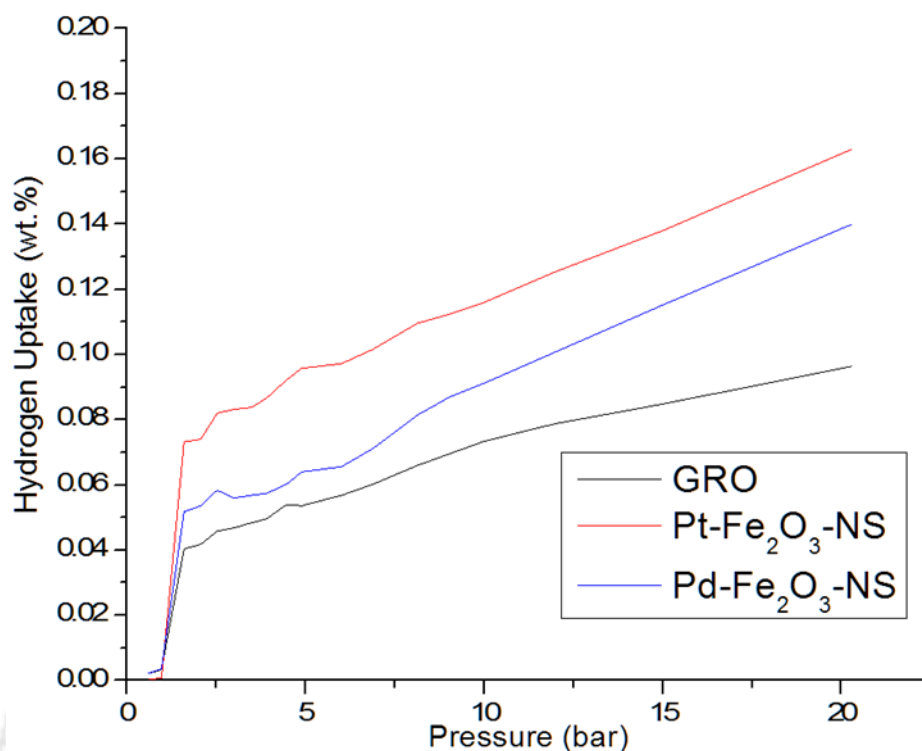


Figure 7.22: Hydrogen adsorption isotherms of Pt-Fe₂O₃-NS and Pd-Fe₂O₃-NS in comparison with the initial graphene precursor, measured at 273K and over a pressure range of 1–20 bar.

7.3 CONCLUSION

We report a novel approach of synthesizing various nanoparticles such as the zero-valent iron nanoparticles, iron oxide nanoparticles and catalytically active metallic nanoparticles on CNC substrate derived from renewable biomass resources (bamboo pulp). The abundant hydroxyl functionality of CNCs acts as both support and reducing/precipitation sites for the metallic nanoparticles, which further increases the dispersion stability of the nanoparticles. In the case of Fe⁰-CNC, CNCs also act as corrosion inhibitor, which enhances the zero state life of supported ZVI. Fe⁰-CNCs show high catalytic activity towards redox reactions such as the conversion of 4-NP to 4-AP as well as towards the degradation of dyes

such as methylene blue. This makes these Fe-CNCs cost effective biobased catalysts that can be easily fabricated for large scale industrial processes. These supported nanoparticles are negatively charged with high dispersion stability which could help overcome the drawbacks of aggregation and reduced mobility, usually associated with nascent ZVI. Moreover, the Fe^0 -CNCs show the unique behavior of self-propulsion in the presence of pH gradient which can be controlled externally through the application of magnetic field. In the presence of variable magnetic field and pH, the CNC motors attained speeds as high as ~ 35 body lengths per second. CNCs modified with Fe_2O_3 nanoparticle and catalytically active moieties such as Pd and Pt nanoparticles can also find potential application as structural templates. Due to their high magnetic moment as well as distinct rod-like morphology, the Fe_2O_3 -CNC, Pt- Fe_2O_3 -CNC and Pd- Fe_2O_3 -CNC can be effectively used for fabrication of graphene nanoscrolls with tunable morphological dimensions. Further, through controlled calcination, graphene nanoscrolls encapsulating the catalytically active Pd and Pt nanoparticles can be fabricated which display high hydrogen storage capacity. The formation of GNS using Fe_2O_3 -CNC as templates is further studied through simulation studies, which suggest that the scrolling of graphene sheets is thermodynamically favourable, and driven by the strong attractive interactions between the metal nanoparticles and the graphene sheet. Therefore, we conclude that CNCs can be readily modified with iron and catalytically active metallic nanoparticles through novel surface modification strategies. The modified CNCs are well suited for various high performance applications such as sensing and delivery of biomolecules through chemo-magneto-taxis, sustainable bionanocatalysts and hydrogen storage devices.



Magnetic Cellulose Nanocrystal-based Anisotropic Polylactic Acid Nanocomposites

Motivation

Cellulose Nanocrystals (CNCs) are usually helical-shaped rod-like nanoparticles with cellulose chains stacked parallelly into monoclinic crystal structure, which leads to incorporation of anisotropic structural behaviour (higher elastic modulus along parallel direction, $E_{||} \sim 110\text{--}220\text{GPa}$ in comparison to perpendicular direction, $E_{\perp} \sim 2\text{--}50\text{GPa}$). Due to their high hydroxyl functionality, the surface of CNCs can be decorated with iron-oxide nanoparticles, which introduces magneto-responsive stimuli behaviour. The orientation and distribution of these reinforcing bio-nanoparticles through non-invasive technique (application of low magnetic field) are key to enabling effective reinforcement and incorporating anisotropic mechanical, thermal, electrical, magnetic and hyperthermia characteristics, when dispersed in polymeric systems. Structural biological composites/artificial biomedical scaffolds can be manufactured using such non-toxic, biodegradable and bio-compatible nanocomposites by accurately controlling the orientation of anisotropic nano-sized building blocks, reinforcing the material either in a specific directions or in multiple directions for potential applications in medical diagnostics and tissue engineering.

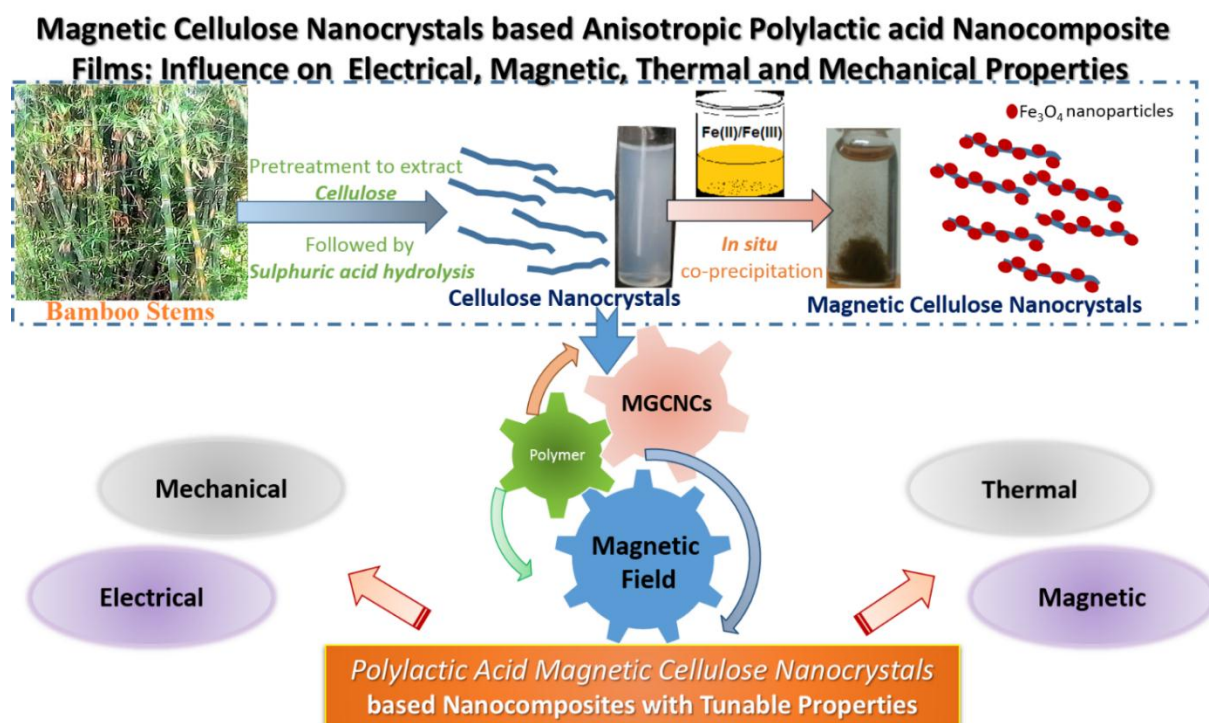
The work in this chapter is published as:

- Dhar, P., Kumar, A., & Katiyar, V. (2016). Magnetic Cellulose Nanocrystal Based Anisotropic Polylactic Acid Nanocomposite Films: Influence on Electrical, Magnetic, Thermal, and Mechanical Properties. *ACS Applied Materials & Interfaces*, 8(28), 18393-18409.

Abstract

The chapter discusses on the fabrication of magnetic cellulose nanocrystals (MGCNCs) and its nanocomposites through directional alignment of MGCNC-based building blocks in presence of low magnetic fields which introduces anisotropic characteristics in processed films. Magnetic field assisted directional alignment of MGCNCs led to the incorporation of anisotropic mechanical, thermal and electrical properties in the fabricated PLA/MGCNC nanocomposites. Alignment of MGCNCs led to the improvement in the percentage crystallinity and with absence of cold crystallization phenomenon, finds potential applications in polymer processing in presence of magnetic field. Further, its anisotropically induced electrical and magnetic properties are desirable for fabricating self-biased electronics products. The processed films interestingly show hyperthermia behaviour, i.e. the ability to heat-up in the presence of alternating magnetic fields, up to a maximum temperature of $\sim 50^{\circ}\text{C}$. Therefore, the current study provides a novel non-invasive approach of orienting non-toxic bio-derived CNCs in the presence of low magnetic fields, with potential applications in manufacturing three dimensional composites with microstructural features comparable to biological materials for high performance engineering applications.

Graphical Abstract



8.1 INTRODUCTION

Cellulose nanocrystals (CNCs) are the most promising bio-nanoparticles of 21st century, because of their numerous unique properties which make them potentially applicable in diverse fields of scientific and technological advancements. CNCs are the crystalline domains of cellulose with rod-like morphology, generally obtained from lignocellulosic renewable resources by controlled acid hydrolysis process [476]. High surface area, tunable aspect ratio, chemical functionality, anisotropic mechanical properties, self-ordering behavior, biodegradability and most importantly non-toxicity, are some of the appealing properties of CNCs[4]. Recently, studies regarding the growth of organic/inorganic nanostructures (especially nanoparticles [437] and nanotubes [238]) on the CNCs as templates, are attracting considerable interest due to the potential applications in electronics and energy storage materials [436]. The abundant hydroxyl groups on the surface of negatively charged CNCs act as both nucleating sites and reducing agent [477] for the growth of the metal nanoparticles. Till date, several reports of CNC-supported metal nanoparticles such as Au [439], Pt [434], Ag [236], Pd [437], Fe [477] etc., have been reported in literature through green chemistry route, having potential applications as high performance conductive polymer nanocomposites, biocatalysts for pollutant remediation [477] and electro-catalytic activity [436]. Moreover, fabrication of such CNC-supported metal nanoparticles have been shown to improve the dispersion quality [433], chemical stability and solve the problems related to coagulation/agglomeration of the nascent metal nanoparticles.

In this regard, biopolymer-supported iron nanoparticles are environment-friendly non-toxic systems which exhibit excellent electrical, magnetic and hyperthermic properties [478] for potential applications as sensor materials [465], medical diagnostics [479], electromagnetic

wave shielding [480], drug delivery for cancer therapy [481] and as catalyst for remediation of hazardous pollutants [477]. However, there are few reports available on the fabrication of CNC-supported iron nanoparticles in the literature. Chen et al. [482] synthesized Fe₃O₄- immobilized SiO₂-coated CNCs, with β -cyclodextrin grafted on their surface for adsorption of model pharmaceutical compounds. The hybrid nanocomposites had core-shell structure with high magnetization values and better thermal stability than CNCs along with high adsorption capacity. Subsequently, the Fe₃O₄ supported CNC nanoparticles were coated onto the filter paper at a loading concentration of 14.75 g/m² for potential application as anti-static packaging materials[483]. Using cobalt salt as another metal precursor, Garcia et al. [484] fabricated cobalt ferrite nanotubes using bacterial cellulose nanofibers as template with Co-Fe nanoparticles of dimension 9–15 nm impregnated on their surface. Similarly, Tian et al. [458] used Cu-Co ferrite salts to synthesize *in situ* Cu_{0.5}Co_{0.5}Fe₂O₄ of size 13.5nm supported onto CNC surface via hydrothermal reaction approach. Such Co-Fe impregnated CNCs were incorporated into polyvinyl alcohol fibers through electrospinning which showed high magneto-responsive behavior along with hyperthermia properties for potential biomedical applications [485]. Interestingly, in another study [50] papain enzyme was immobilized onto the magnetic CNCs through coprecipitation-crosslinking techniques, which showed enhanced enzyme-substrate activity for dipeptide biosynthesis. The Fe₃O₄ based magnetic CNCs showed enhanced enzyme immobilization capacity, with improved thermal, storage stability and recyclability [50],[53]. Recently, cellulose nanofibers were decorated with iron oxide nanoparticles at a loading of ~60 wt. % through *in situ* hydrolysis of metal precursors and casted into low density flexible magnetic membranes. The fabricated membranes were

mechanically strong and tough (~ 5 GPa stiffness) with high magnetic moment ($\sim 70 \text{ Am}^2/\text{kg}$) which make them ideal for fabrication of ultrathin prototype loud speakers [48].

CNCs are helically-shaped anisotropic nanoparticles perpendicular to their axis, with diamagnetic susceptibility behavior which makes them align perpendicularly in presence of magnetic field [26],[26]. Anisotropic behavior originates from the parallel stacking of the cellulose chains in the monoclinic crystalline structure along its axis, which leads to higher elastic modulus in parallel direction ($E_{\parallel} = 110\text{-}220 \text{ GPa}$) compared to perpendicular direction of CNC ($E_{\perp} = 2\text{-}50 \text{ GPa}$)[297]. Such inherent properties of the CNCs could be tuned into the polymer nanocomposites during processing, for fabrication of high performance CNC reinforced composites with anisotropically tunable optical, thermal and mechanical properties for targeted engineering applications. Several studies on directional orientation of CNCs using different processing techniques such as electro-spinning [212], dry [486] or wet spinning [487], application of magnetic and electric fields [488], shear-induced deformation [489], drawing of films during casting [490] or combination of such techniques have been reported. However, there remain several challenges in these processes. For example, magnetic field-based orientation requires very high field strength [491] and it orients only the mesophasic chiral nematic CNC domains and not the individual CNC rods [492]. In case of shear induced and the drawing processes, individual CNCs could be oriented, however once the tensile/shear forces have been relaxed the alignment of CNCs disappears with time [246]. Although wet or dry spinning are viable approaches, but they have been mostly used to orient CNCs in hydrophilic polymers with which CNCs are more compatible. Hydrophilic polymers are more susceptible to moisture and humidity conditions and hence hydrophobic polymers find wider applications in packaging, electronics etc., for day-to-day life usages. To the best of our

knowledge, no reports are available till date in literature on the fabrication of CNC-reinforced oriented hydrophobic polymeric nanocomposites with anisotropic properties under the application of very low magnetic field.

In the present work, we have fabricated CNCs adsorbed with Fe_3O_4 nanoparticles (MGCNCs) through *in situ* coprecipitation approach using iron salts and CNC derived from bamboo as precursor. Subsequently, the MGCNCs were dispersed into poly lactic acid (PLA) and aligned in the nanocomposites through the application of directionally tunable magnetic field. Due to rod-like morphology with high aspect ratio, magnetically susceptible MGCNCs could be easily aligned in polymeric matrix through application of low magnetic field. It is expected that synergistic effect of two different nanofillers (Fe_3O_4 nanoparticles and CNCs) in PLA/MGCNC nanocomposites will result in improved dispersion of CNCs in the polymeric matrix. Furthermore, effect of directional alignment of nanofillers in the presence of magnetic field has been demonstrated to improve mechanical, thermal, electrical and magnetic properties of nanocomposites films. The orientation and distribution of reinforcing bio-nanoparticles through this non-invasive technique (magnetic field) are key to enable effective reinforcement and incorporate anisotropic characteristics in artificial polymeric systems in three dimensions [493]. Structural biological composites/ artificial biomedical scaffolds (e.g. knee joints, flexible bones) can be manufactured using such non-toxic, biodegradable and bio-compatible nanocomposites by accurately controlling the orientation of anisotropic nano-sized building blocks, reinforcing the material in specific directions to multiple directions for potential applications in medical diagnostics and tissue engineering.

8.2 RESULTS AND DISCUSSIONS

8.2.1 Fabrication and Characterization of Magnetic Cellulose Nanocrystals

Fe_3O_4 nanoparticles of almost monodisperse size distribution were uniformly decorated on the surface of CNCs (MGCNCs) through a simple one step co-precipitation method. CNCs fabricated from the pretreated bamboo pulp after acid hydrolysis had smooth rod-like morphology with an average length of 735 ± 85 nm and diameter of 37 ± 8 nm (aspect ratio ~ 20) (Figure 8.1(a)). After co-precipitation reaction, spherical-shaped iron nanoparticles were anchored on the surface of CNCs, forming a densely packed structure with caterpillar-like morphology (Figure 8.1(b),(g')). The fabricated MGCNCs have modified dimensions (length of 770 ± 75 nm and diameter of 51 ± 5 nm) compared to CNCs. The increase in dimensions of MGCNCs is probably due to the precipitation of iron nanoparticles on their surface which led to the new aspect ratio of ~ 15 . High resolution FESEM images (with magnification $>500,000$ X, Figure 8.1(b')) shows that spherical-shaped Fe_3O_4 nanoparticles (size distribution of ~ 18 –30 nm) covered the whole surface of CNCs uniformly (with the absence of nascent Fe_3O_4 nanoparticles) and had strong interaction which prevented the separation of nanoparticles even under intense sonication.

XRD diffractogram for CNCs showed well resolved peaks at $2\theta = 14.4^\circ$, 16.2° , 22.5° and 34.5° corresponding to the crystallographic planes (101), $(10\bar{1})$, (002) and (040) (Figure 8.1(c)), representing the cellulose I crystal structure[494]. However, the XRD pattern for the MGCNCs shows presence of relatively new diffraction peaks at $2\theta = 33.1^\circ$, 35.6° , 49.5° , 54.1° , 62.4° and 64.0° which is consistent with the (220), (311), (422), (511), (440) and (531) reflections of Fe_3O_4 nanoparticles[495] (Figure 8.1(c)). The small peak at $2\theta \sim 22.2^\circ$ probably corresponds to the (002) plane of cellulose I, which confirms the presence of the crystalline

CNCs in the system. However, the absence of the other CNC peaks could be due to the difference in the atomic scattering factors as well as the co-precipitation reaction in presence of ammonia (at $\sim 90^{\circ}\text{C}$) which might reduce the crystallinity of CNCs, thereby decreasing the diffraction intensities in the hybrids significantly[496]. The minimum crystallite size for the Fe_3O_4 nanoparticles anchored on the CNC surface in MGCNCs was measured as ~ 28 nm, corresponding to the diffraction peaks at $2\theta = 33.1^{\circ}$ (220) and 35.6° (311) respectively. The dimension of the Fe_3O_4 nanoparticles (measured from XRD studies) is consistent with the size range ~ 20 - 35 nm calculated from the FESEM-based morphological studies. Therefore, crystallographic studies confirm that the MGCNCs hybrids retained the cellulose I crystal structure along with the presence of the uniformly distributed Fe_3O_4 nanoparticles encapsulated on their surface.

The chemical composition of the Fe_3O_4 nanoparticle-modified MGCNCs and CNCs were further investigated and compared using FTIR spectroscopy (Figure 8.1(d)). CNCs have abundant hydroxyl functional groups on their surface which impart them a negative charge. Addition of the positively charged iron salt solutions ($\text{Fe}^{+2}/\text{Fe}^{+3}$) led to strong electrostatic interaction with the negatively charge hydroxyl groups of CNCs forming a uniformly dispersed iron-CNC solution [477]. Subsequently, co-precipitation of the iron salts in the presence of aqueous ammonia led to formation of the Fe_3O_4 nanoparticles on the hydroxyl groups of the CNCs surface. FTIR spectra of CNCs showed representative peaks at ~ 3280 , 2908 , 1647 , 1427 , 1319 , 1208 , 1163 , 1035 and 894 cm^{-1} corresponding to $-\text{OH}$ stretching, $-\text{CH}$ stretching, $-\text{OH}$ bending, $-\text{CH}_2$ bending, $-\text{CH}_2$ wagging, $\text{C}-\text{OH}$ bending in plane at C-6, $-\text{C}-\text{O}-\text{C}$ asymmetric stretching of β -glucosidic linkage, $\text{C}-\text{OH}$ stretching in plane at C-6 and $-\text{C}-\text{O}-\text{C}$ asymmetric bending of β -glucosidic linkage respectively [494]. MGCNCs showed the

characteristic peaks of cellulose at 2908, 1427, 1319, 1208 and 894 cm^{-1} representing the $-\text{CH}$ stretching, $-\text{CH}_2$ bending, $-\text{CH}_2$ wagging, $\text{C}-\text{OH}$ bending in plane at C-6, $-\text{C}-\text{O}-\text{C}$ asymmetric stretching of β - glucosidic linkage and $-\text{C}-\text{O}-\text{C}$ asymmetric bending of β - glucosidic linkages respectively, albeit with low intensities due to the immobilization of Fe_3O_4 nanoparticles on the CNC surface. The shoulder peak corresponding to free hydroxyl groups of CNC at region $\sim 3280 \text{ cm}^{-1}$, was found to be absent and peak at 1647 cm^{-1} was found to be shifted to 1652 cm^{-1} in the MGCNC hybrid systems. This is probably due to the formation of the Fe_3O_4 nanoparticles on the hydroxyl surface of CNCs during the co-precipitation reaction, which leads to the encapsulation of the nascent hydroxyl groups in CNCs. Further, the spectra for MGCNCs shows the presence of the new peak at $\sim 620 \text{ cm}^{-1}$ and a shoulder peak around $\sim 700 \text{ cm}^{-1}$ which represents the stretching and bending vibrations of the $\text{Fe}-\text{O}$ confirming the formation of the iron oxide nanoparticles [477]. Raman spectroscopic studies were also carried out to confirm the formation of Fe_3O_4 nanoparticles on the hydroxyl sites of CNCs (Figure 8.1(e)). CNCs show characteristic Raman peaks at 380, 898, 1098 and 1120 cm^{-1} which correspond to the surface hydroxyl groups, $\text{C}-\text{OH}$ bending at C-6 and the $\text{C}-\text{O}-\text{C}$ stretching and bending bands present in CNCs respectively [477] (Figure 8.1(e)). The sharp and intense peaks at 219, 283 and 670 cm^{-1} in case of MGCNCs confirm the presence of iron oxide nanoparticles. Similar to FTIR spectroscopic studies, Raman spectra also showed that the precipitation of iron oxide nanoparticles onto CNCs led to disappearance of the peak at 898 cm^{-1} corresponding to $\text{C}-\text{OH}$ bending at C-6 and shift in the $\text{C}-\text{O}-\text{C}$ stretching and bending bands to 1260 cm^{-1} . Further, presence of the peak at 380 cm^{-1} in the MGCNC hybrid system confirms that the iron oxide nanoparticles nucleated mostly on the hydroxyl groups of CNCs present on its surface, without altering the bulk structure and crystalline nature of the

CNCs [497]. Therefore, the chemical compositional analysis of MGCNCs using FTIR and Raman spectroscopic studies confirms the formation of Fe_3O_4 nanoparticles during the precipitation reaction which are strongly bound to the surface hydroxyl groups of CNCs.

The TGA thermographs in Figure 8.1(f) show the thermal behavior of the CNCs and the modified Fe_3O_4 nanoparticle-based MGCNCs. The degradation profile of the CNCs usually consists of two steps: firstly in range of $\sim 100\text{--}200^\circ\text{C}$ corresponding to the removal of the moisture and sulphate groups present on the CNC surface and secondly, in range of $\sim 250\text{--}500^\circ\text{C}$ which represents the degradation of cellulose [477]. MGCNCs showed high thermal stability in comparison to the nascent CNCs, with only 4 wt.% weight loss in the range of $\sim 100\text{--}200^\circ\text{C}$ and 10 wt. % at $\sim 200\text{--}500^\circ\text{C}$. The degradation profile of MGCNCs also showed two-step process similar to the precursor CNC substrate and the nascent Fe_3O_4 nanoparticles. The increase in the thermal stability is probably due to the successful incorporation of the Fe_3O_4 nanoparticles on the hydroxyl surface groups of CNCs, which are thermally stable at 600°C in inert conditions [498]. The weight content of the Fe_3O_4 nanoparticles precipitated on the CNC surface in the MGCNC hybrid system was calculated from the difference in percentage weight loss for the two systems (CNC and MGCNC) at $\sim 500^\circ\text{C}$. It was estimated that through the single-step co-precipitation technique, MGCNCs were incorporated with ~ 51 wt. % content of the Fe_3O_4 nanoparticles on the CNC surface. Compositional analysis of MGCNCs using EDX spectroscopic studies confirmed the presence of Fe, C and O elements (Figure 8.1(g)). Consistent with the TGA studies, it was estimated from EDX spectra that ~ 54 wt.% of Fe content is present which corresponds to the Fe_3O_4 nanoparticles that are adsorbed onto CNCs. Similar studies on incorporation of such high loading (> 50 wt. %) of the inorganic CoFe_2O_4 nanoparticles on the surface of cellulose

nanofibers through *in situ* precipitation reactions has been demonstrated by Galland et al. [48]. Decoration of CNCs with such high content of Fe₃O₄ nanoparticles is probably due to the abundant source of hydroxyl groups on their surface which leads to enhanced interaction with the metal ions and act as a nucleating sites for condensation /growth of nanoparticles during wet precipitation reactions.

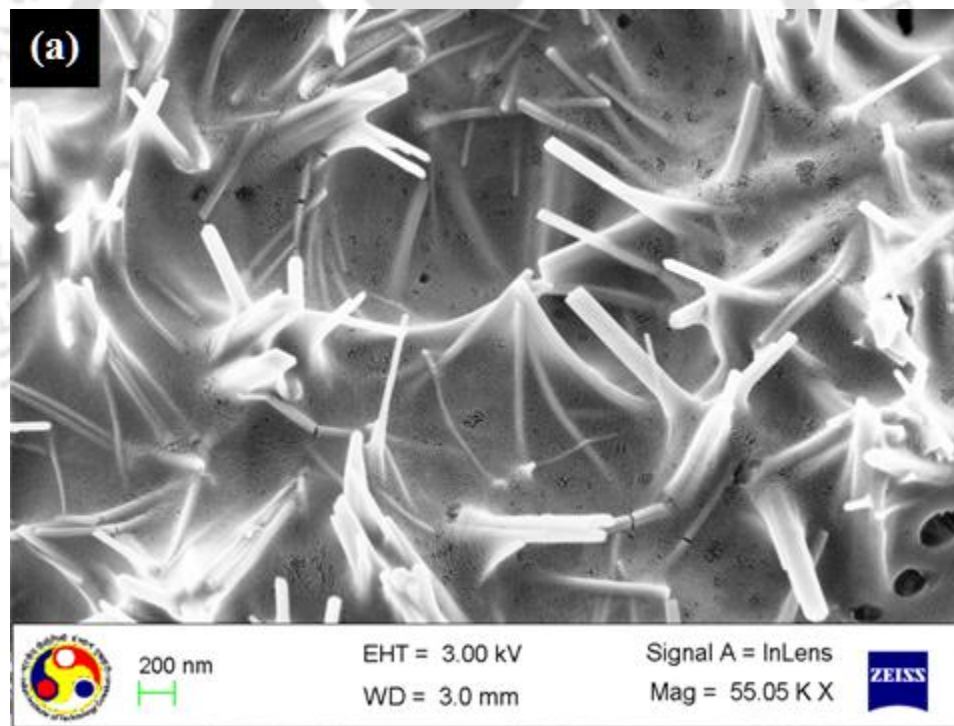
Surface modification of the CNCs with high fractions of Fe₃O₄ nanoparticles led to incorporation of the magneto-responsive behavior, which were evaluated from VSM studies at 298K (Figure 8.1(h)). The VSM curves for the MGCNCs hybrids showed very low coercivity without any distinct hysteresis loop and absence of remanence confirming the presence of the superparamagnetic Fe₃O₄ nanoparticles in the system. Because of the superparamagnetic nature, MGCNCs have very high magnetization values of ~50.5emu/g, which is comparable with the previously reported values in literature[482]. This is also due to the high loading fractions and densely packed Fe₃O₄ nanoparticles (18–30 nm) on the surface of CNCs which contributed to the high magnetic properties along with the superparamagnetic behavior[499]. Formation of such superparamagnetic nanocrystals derived from renewable resources with non-toxic properties has potential application in the field of medical sciences as novel bio- ferrofluids for drug delivery vehicles, inducing hyperthermic effects in cells and as MRI agents [499]. Immobilization of the iron based inorganic nanoparticles on the surface of the hydrophilic CNCs led to enhanced compatibilization with the hydrophobic polymeric systems as well as tunable orientation in the presence of low magnetic field. Therefore, the fabricated MGCNCs were dispersed into the PLA through a simple sonication based approach and their orientations were directionally tuned by application of the magnetic field to form PLA-based

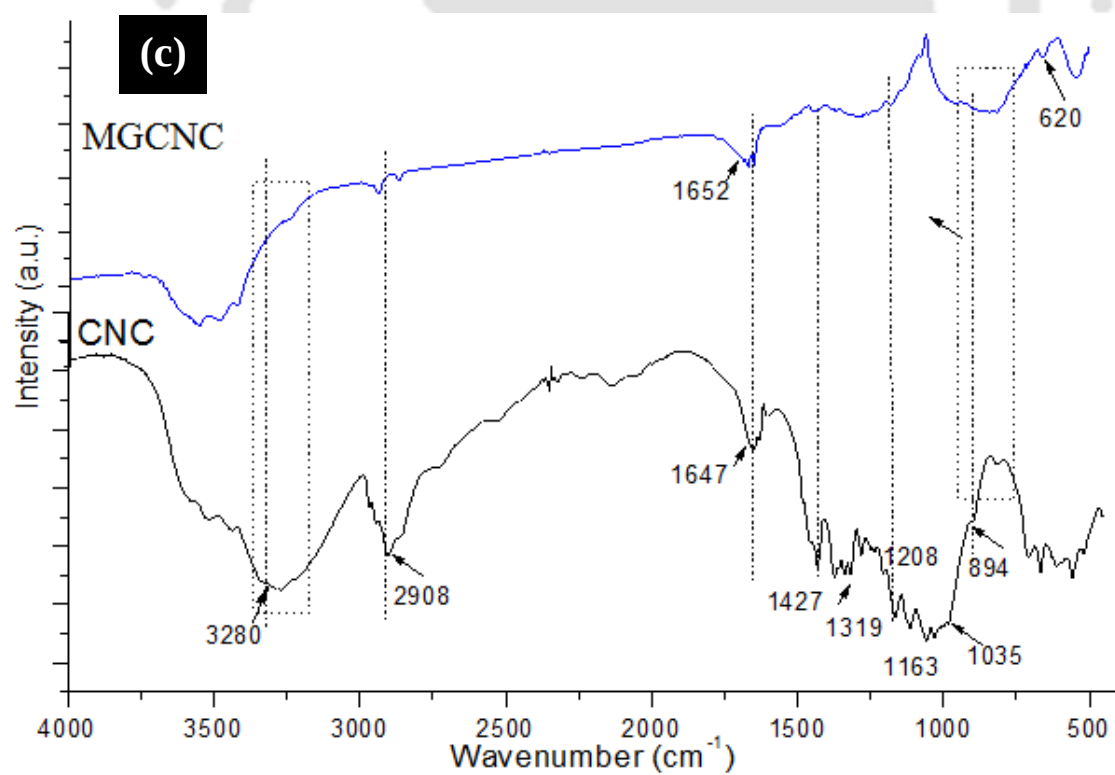
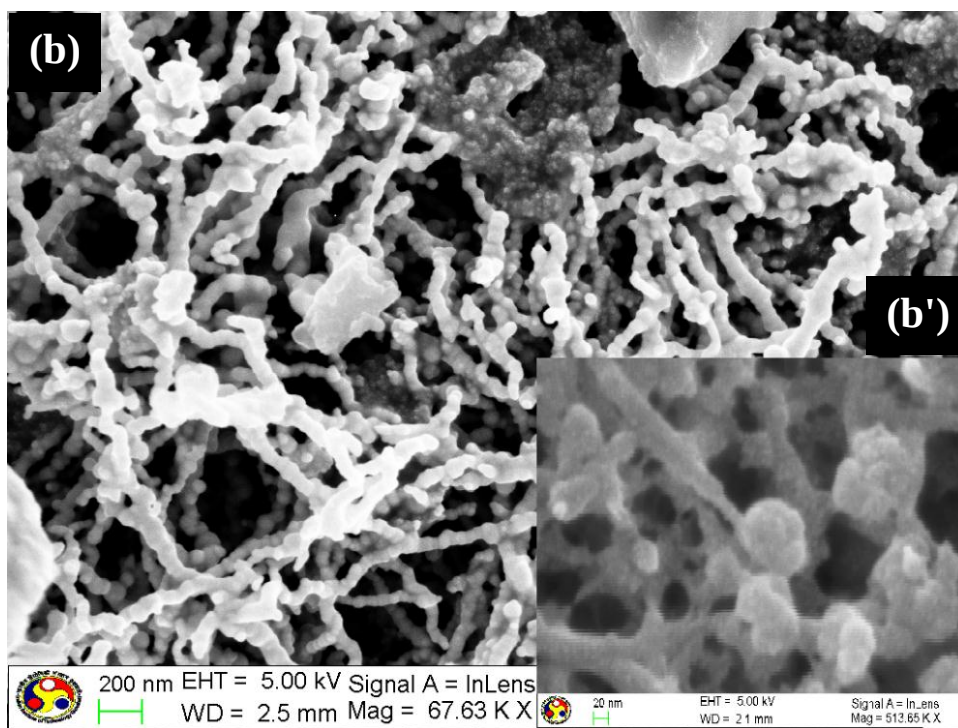
nanocomposites with anisotropic properties, which are characterized and discussed in the subsequent sections.

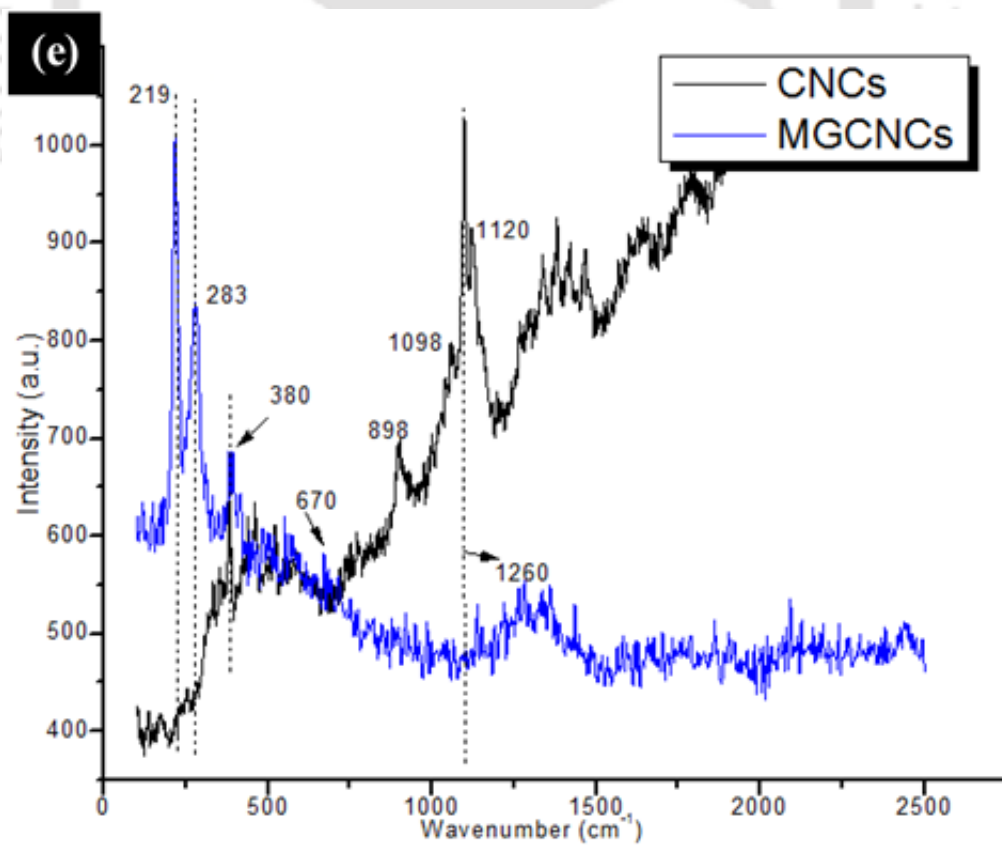
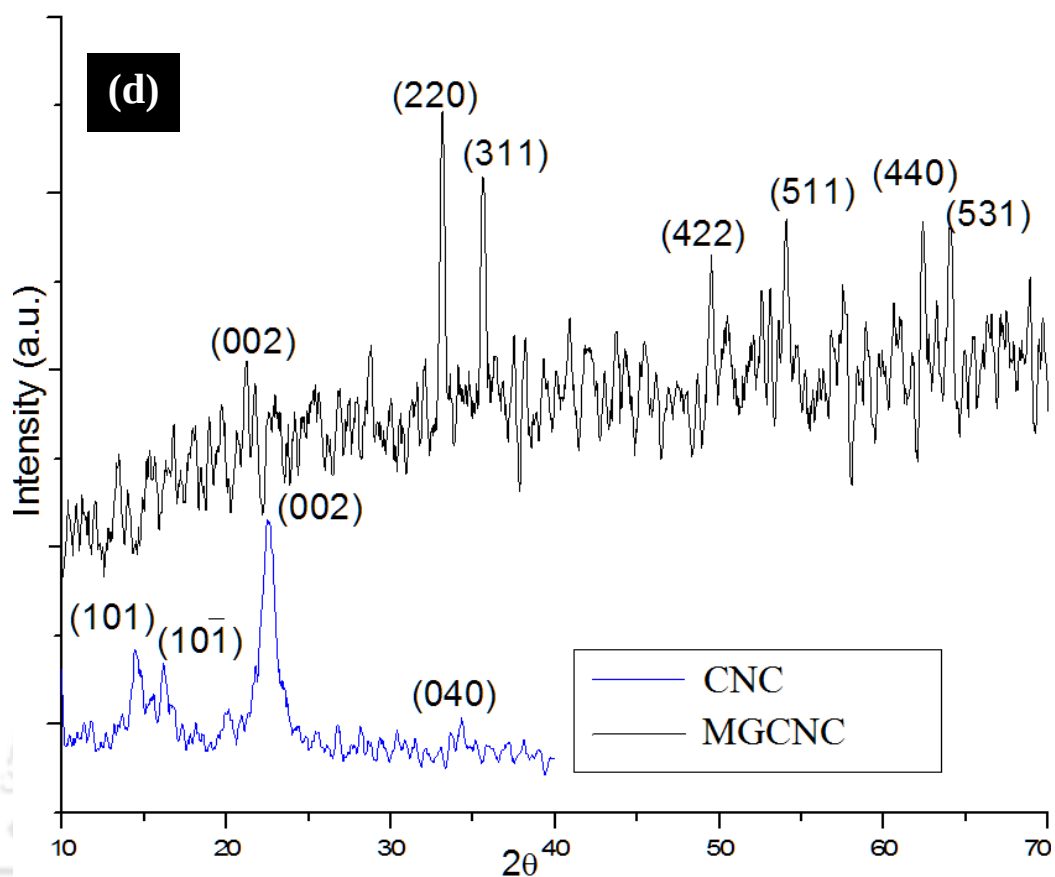
8.2.2 Fabrication and Characterization of Magnetically oriented PLA-based MGCNC Nanocomposites

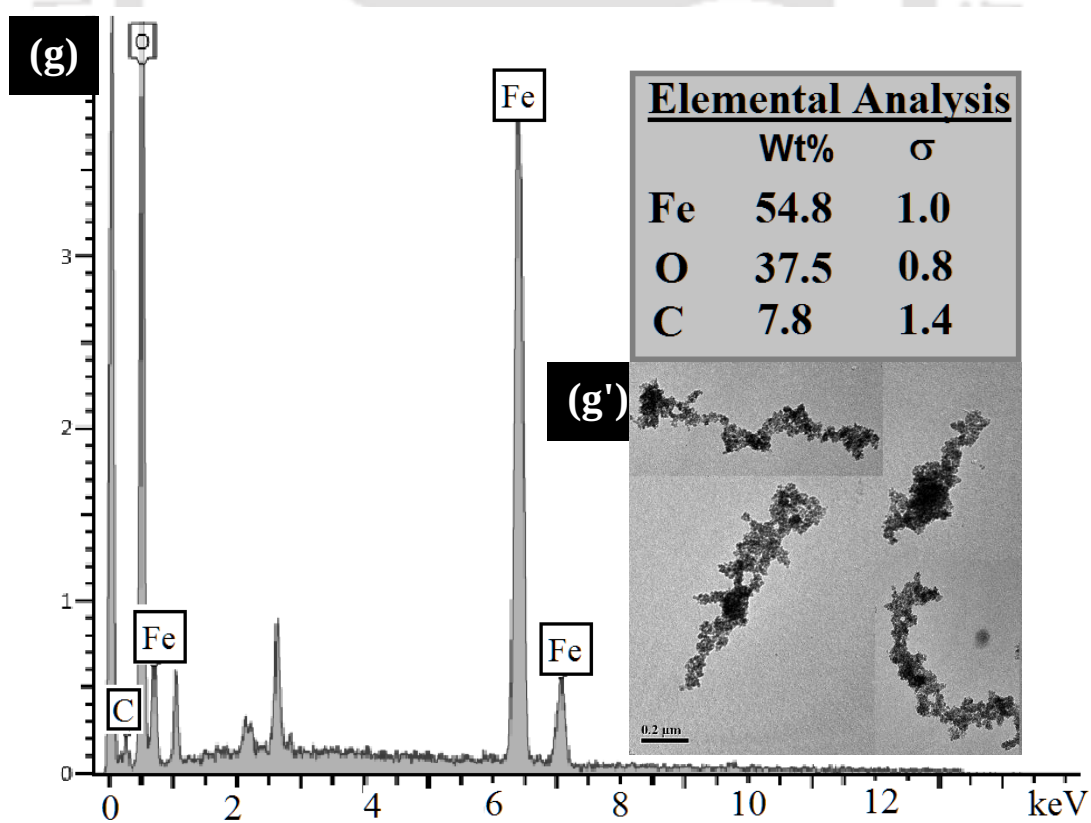
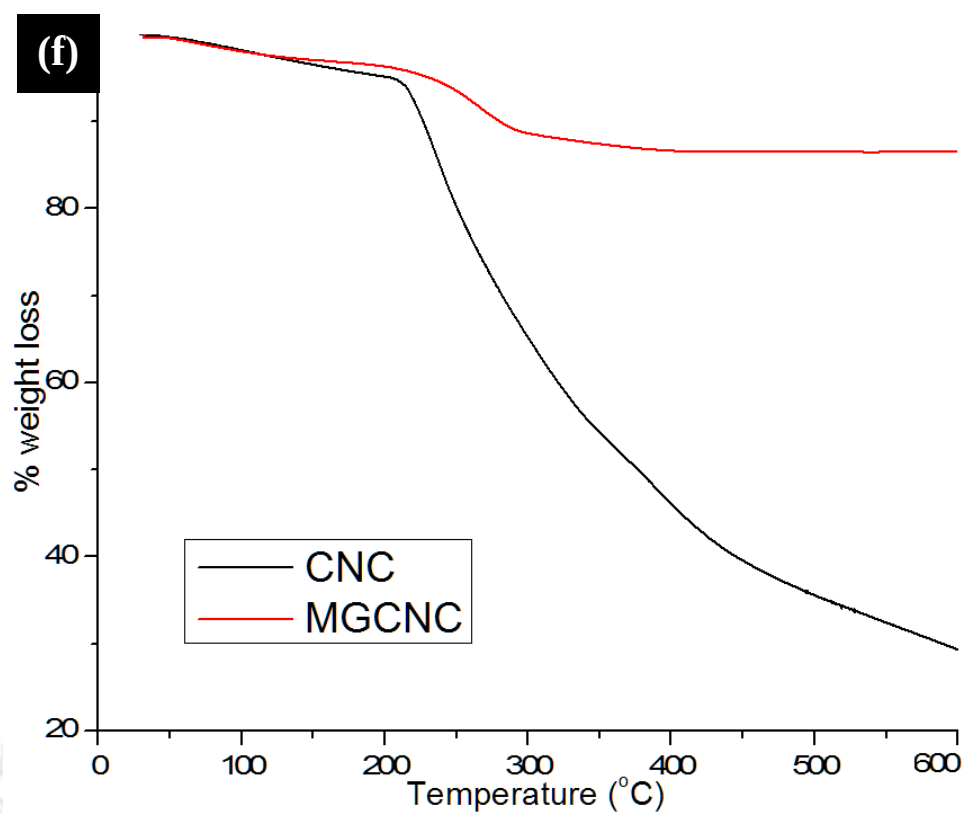
MGCNCs, after surface modification with the Fe_3O_4 nanoparticles, formed uniform dispersion in the PLA-dissolved chloroform solution under mild sonication. On the other hand, the unmodified hydrophilic CNCs are very difficult to disperse in the organic solvent even in the presence of intense sonication [500]. Therefore, modifying the CNCs surface with inorganic molecules provides a novel approach of dispersing the CNCs into hydrophobic polymeric systems to fabricate uniformly dispersed nanocomposites [501]. The PLA/MGCNCs suspension after sonication was poured in the Teflon plates and oriented in parallel and perpendicular direction by changing the orientation of magnetic field as per the setup shown in Figure 8.2. The optimum magnetic field required to orient the MGCNCs in the polymeric matrix depends upon several factors such as viscosity of the polymer matrix, density of the polymer as well as the nanoparticles, morphology and magnetic moment of the fabricated MGCNCs. The effective alignment of the MGCNCs in the PLA matrix could only be obtained when the disordered state of nanoparticles arising due to Brownian motion and shear forces acting from the polymer chains are balanced by the momentum gain from the application of the directional magnetic field [502]. Therefore, we theoretically predicted the minimum magnetic field required to orient the MGCNCs in the PLA matrix taking into consideration the effect of the magnetic force, viscous force and gravitational torque. The magnetic torque, gravitational torque and viscous torque acting on the MGCNCs were calculated as per the model equations mentioned by Jiao et al. [502] and solved by torque

balance equation to predict the minimum magnetic field. As the MGCNCs had circular shaped Fe_3O_4 nanoparticles precipitated on their surface forming a caterpillar-like morphology, it was assumed to be elliptically-shaped for solving the model equations. It was calculated for the PLA/MGCNC system that a minimum magnetic field of $\sim 50\text{--}60\text{mT}$ is required, which confirms that a very low magnetic field can be applied to effectively orient the MGCNCs in PLA matrix. Therefore, in this work, magnetically oriented PLA/MGCNC films are fabricated under a constant magnetic field ($\sim 50\text{--}60\text{mT}$), by varying the MGCNC loading from 1– 3 wt. % and their anisotropic properties were studied.









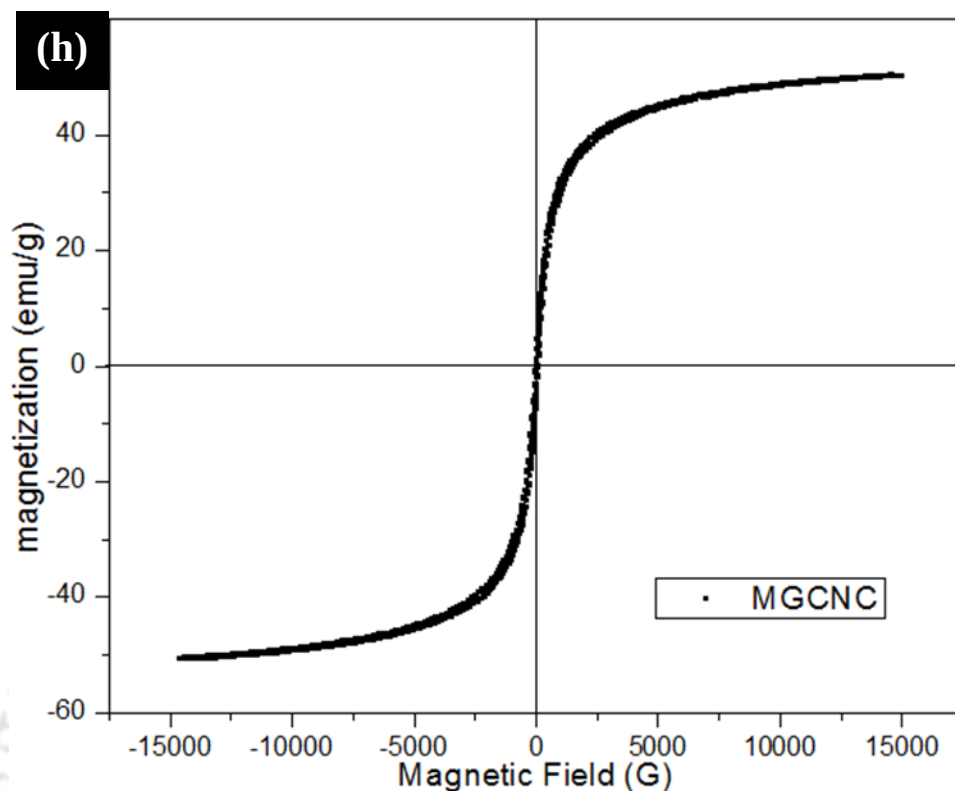


Figure 8.1: FESEM micrographs of the fabricated (a) CNCs isolated from pre-treated bamboo pulp through sulphuric acid hydrolysis and (b) MGCNCs obtained through a simple one step co-precipitation method (inset (b') shows the high resolution micrographs of MGCNCs at a resolution of $\sim 500\text{KX}$). (c) Comparison of the FTIR spectra of the MGCNCs and CNCs. (d) XRD patterns for the MGCNCs and CNCs with their respective planes marked in brackets. (e) Comparison of the Raman spectra of the MGCNCs and CNCs. (f) TGA profiles for the MGCNCs and CNCs respectively, (g) EDX spectra of MGCNCs with the respective composition of elements (in wt.%) shown in the table (inset), (g') TEM micrographs of MGCNCs and (h) Magnetic hysteresis loop for the fabricated MGCNCs measured at 298K.

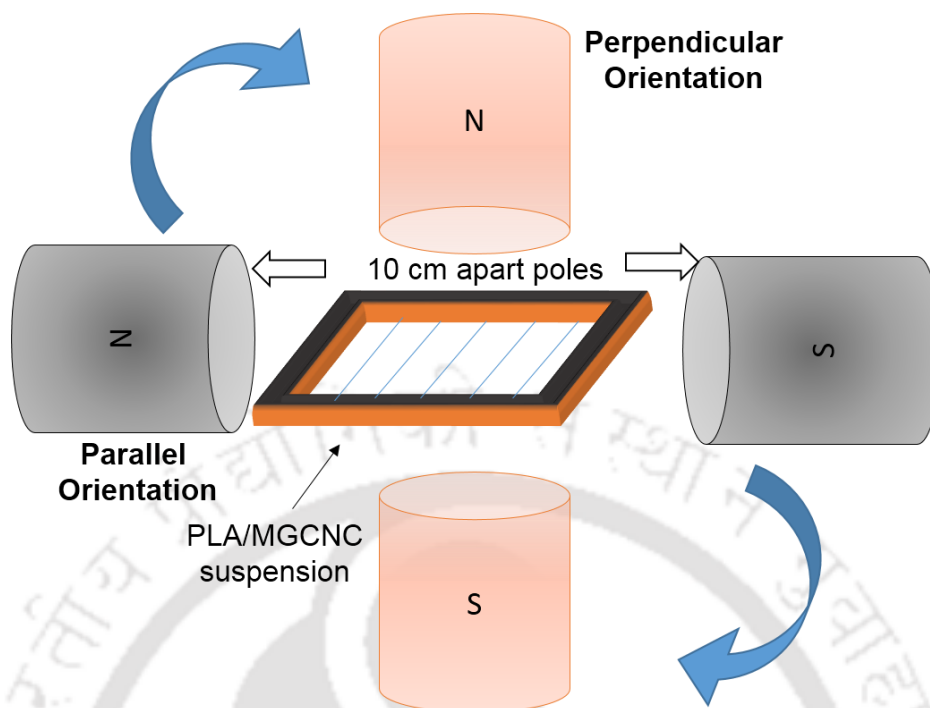


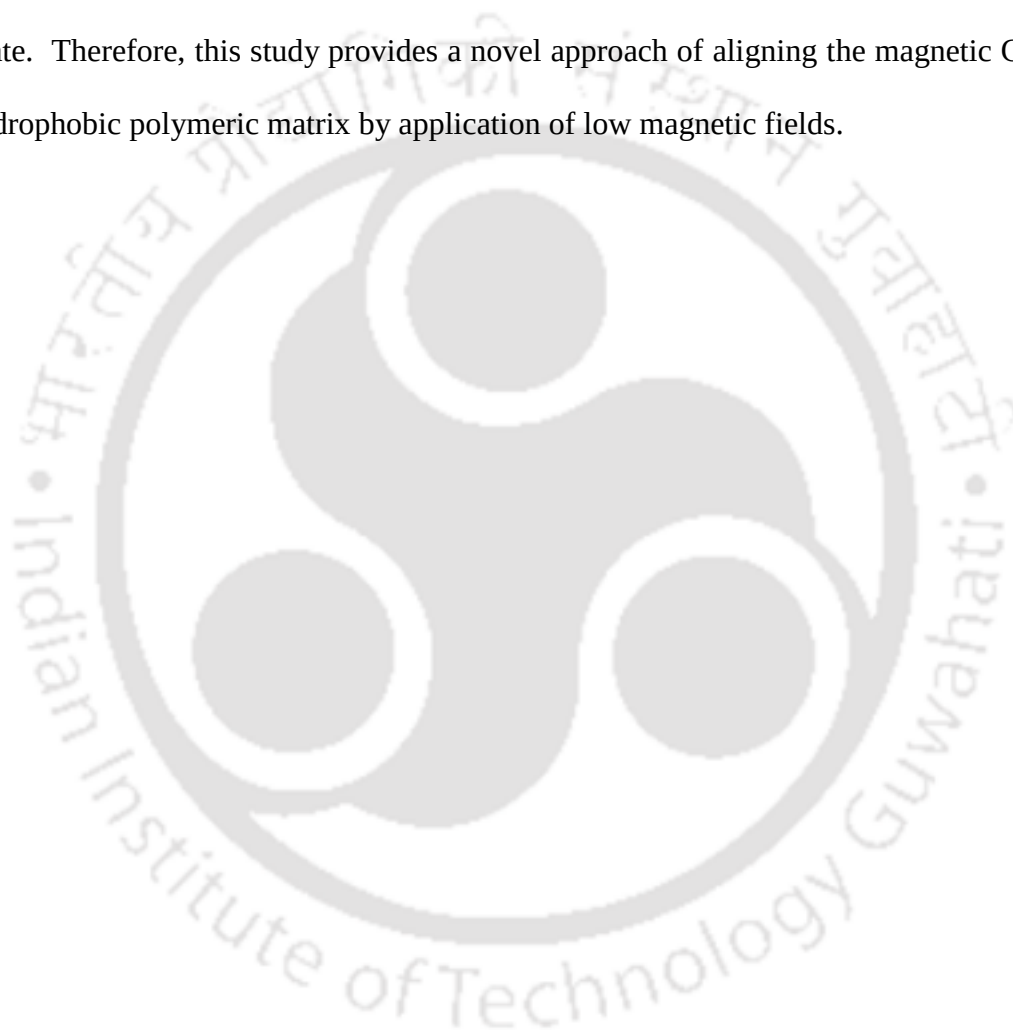
Figure 8.2: Setup with directionally tunable electromagnets for fabricating PLA/MGCNC nanocomposites under parallel and perpendicular orientations.

The alignment of MGCNCs in the PLA matrix (both parallel and perpendicular) was confirmed from the morphological analysis of the FESEM micrographs (Figure 8.3). In the absence of magnetic field, the MGCNCs were randomly dispersed in the PLA matrix without any alignment features (Figure 8.3(a)). However, on application of low magnetic field (~ 50 mT) in parallel direction, the MGCNCs were found to orient in the direction of the applied magnetic field (Figure 8.3(b)). The white parallel lines in the FESEM micrographs are due to the presence of high atomic weight metallic iron in contrast to the black background of PLA matrix which confirms the orientation. Further, the alignment was confirmed from the mapped images of Fe in the PLA/MGCNC matrix using EDX analysis (Figure 8.3(b')). It shows the presence of yellow colored parallel lines running through the PLA matrix which indicates that MGCNCs have oriented successfully in the PLA matrix. Similarly, in case of orientation in perpendicular direction, FESEM micrographs show the presence of white dots and EDX

mapping shows dotted yellow spots which represent the iron capped end tips of the MGCNCs (Figure 8.3(c) and (c')). Therefore, to further confirm the perpendicular alignment, we carried out the FESEM-EDX mapping of the cross section of PMGCNC1p $\perp$ films (Figure 8.3(c'')). It shows the presence of parallelly orientated MGCNCs (parallel purple lines) which confirms that PLA/MGCNC was successfully fabricated with alignment of MGCNCs in perpendicular direction (inset Figure 8.3(c'')). Figures 8.3(a)–(c) also show the schematic representations of the fabricated PLA/MGCNC films at 1 wt. % loading with unaligned, parallel and perpendicular orientations. From visual examination, it was found that the alignment of MGCNCs showed significant variation in their transparency levels, with the PMGCNC1p $\perp$ being more transparent, followed by the PMGCNC1p $\parallel$ and PMGCNC1p uno. Further, the order parameter 'S' was predicted from the FESEM micrographs to determine the degree of effective alignment of MGCNCs in the PLA matrix on application of magnetic field in parallel (S=1) or perpendicular (S=-1) directions [503]. S values are found to be ~0.81, -0.76 and 0.18 for the parallel, perpendicular and unoriented PLA/MGCNC films respectively. Therefore, from the FESEM-EDX micrographs and predicted order parameter 'S' values, confirms the presence of perpendicular or parallel alignment of MGCNCs in PLA matrix.

The magnetic field was applied continuously during the drying of films and it is expected that shear based viscous forces on the MGCNCs will increase as the viscosity of the polymer melt increases with the removal of the solvent (chloroform). However, the presence of the oriented structures in the dried solid films confirms that the effect of magnetic force-based alignment is dominant enough and viscous forces from the polymer matrix have negligible influence on the MGCNC orientations. It was found that MGCNCs are magnetically susceptible with the presence of dipolar magnetism behavior with both north and south poles

at their ends, which helps in their anisotropy in the presence of strong magnetic fields ($\sim 17\text{--}28\text{T}$) (technically difficult process to scale-up) which moreover leads to the alignment of the chiral nematic phase only [504][505]. However, it is technically challenging to generate such high magnetic field and most of the studies reporting the alignment of CNCs are with water-based aqueous solvents with which aligned cellulose-based nanocomposites are difficult to fabricate. Therefore, this study provides a novel approach of aligning the magnetic CNCs in the hydrophobic polymeric matrix by application of low magnetic fields.



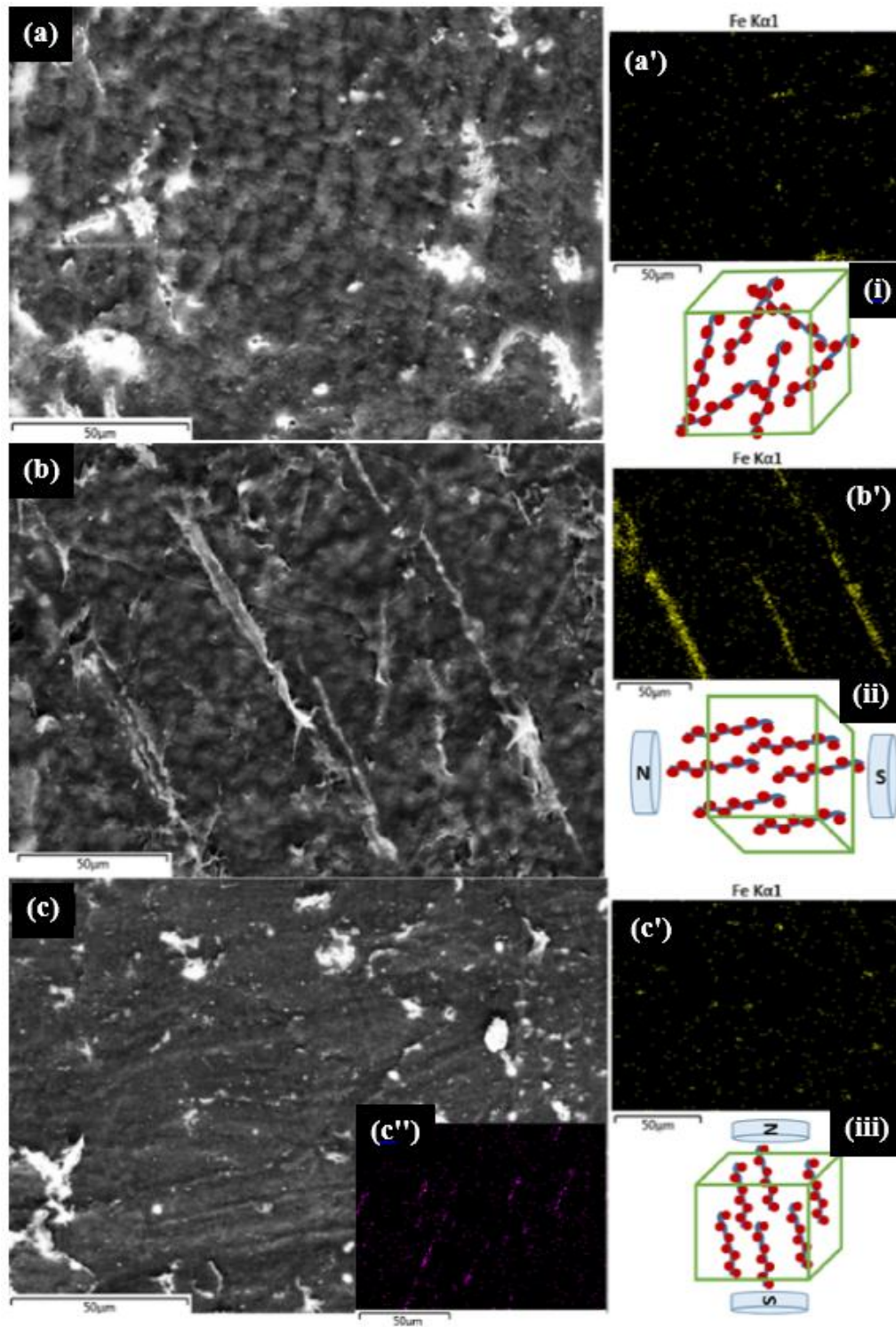


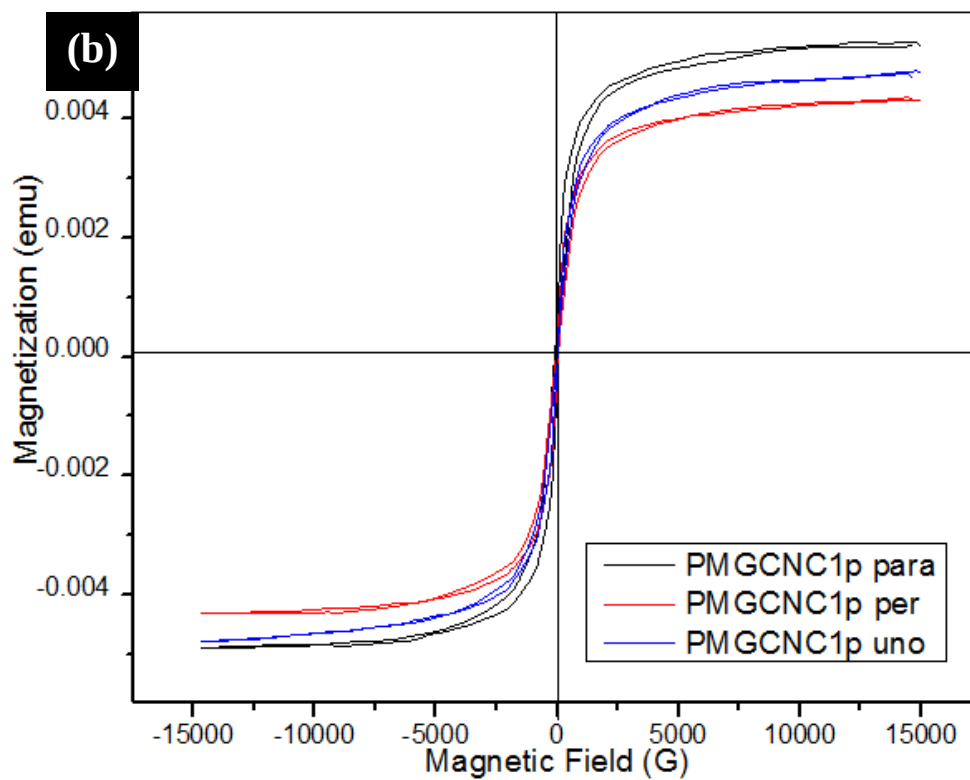
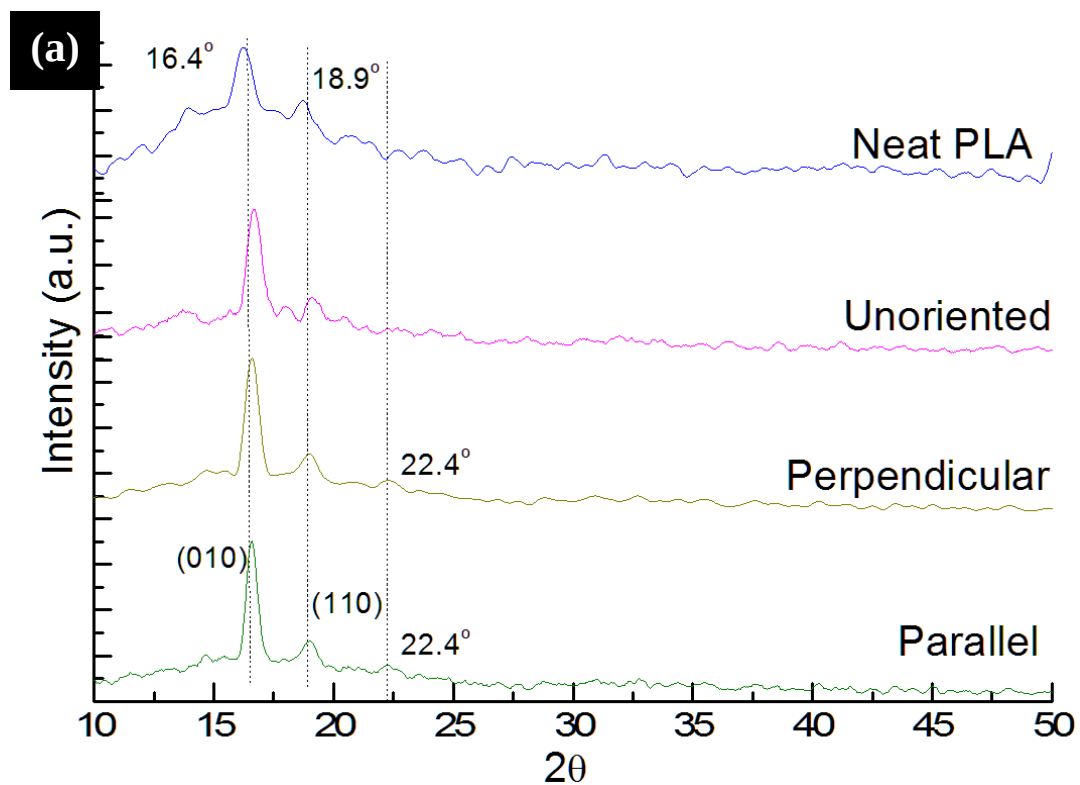
Figure 8.3: (a) FESEM micrographs of PLA/MGCNC nanocomposites with 1wt. % MGCNC loadings at (a) unoriented (b) parallel and (c) perpendicular orientations in presence of low magnetic field. The EDX mapped images of (a') parallel, (b') unoriented and (c') perpendicular alignment of the MGCNCs with the yellow lines representing the presence of iron in the nanocomposites. (c'') Inset of Figure 2 (c) shows the EDX mapped image of the cross-section of the perpendicularly oriented PLA/MGCNC nanocomposites with the parallel purple lines representing the presence of iron in the nanocomposites. Schematic illustrations of probable orientation of the MGCNCs in the PLA matrix are shown for (i) parallel, (ii) unoriented and (iii) perpendicular alignment

The effect of MGCNC alignment on the crystalline properties of PLA was studied using XRD analysis (as shown in Figure 8.4(a)). PLA is a semi-crystalline polymer which is evident from the amorphous hump along with the presence of crystalline peaks at 16.3° and 18.8° , corresponding to α -PLLA crystallites with (010) and (110/200) planes [506]. Incorporation of the MGCNCs into the PLA matrix led to slight increase in the intensity of the peak at $2\theta=16.3^\circ$, along with the presence of the amorphous hump. However, on orientation of the MGCNCs in PLA matrix (for parallel and perpendicular conditions), the XRD pattern showed sharp increase in the intensity of the peaks at $2\theta=16.3^\circ$ (010) and 18.9° (110). XRD diffractograms of the oriented PLA/MGCNC films also showed presence of a peak at $2\theta=22.4^\circ$ (200) corresponding to the cellulose I crystal structure [494], which is absent in the unoriented samples. However, the peaks representing the Fe_3O_4 nanoparticles at $2\theta=33.1^\circ$ (220) and 35.6° (311) was present only in the parallel orientation of PMGCNC1p|| samples. The presence of the intense peaks of MGCNCs in the oriented samples is probably

due to the increase in concentration of planes along which the MGCNCs are aligned, which satisfies the Bragg's equation and leads to proportional increase in intensity [502]. However, in the absence of magnetic field, the MGCNCs are randomly oriented and there is no regular arrangement of planes along which the Bragg's equation will be satisfied. The Fe_3O_4 nanoparticles are known to be compatible with the hydrophobic PLA which leads to their uniform dispersion in the nanocomposites [507]. On application of low magnetic field it is expected that along the orientation of the MGCNCs, the PLA chains adhered on the surface of MGCNCs also get aligned in the direction of magnetic field. This is evident from the XRD diffractograms of the PLA/MGCNCs (for the case of perpendicular and parallel orientation) which shows increase in the intensity of peaks corresponding to $2\theta = 16.3^\circ$ (010) and 18.9° (110). Although there was no significant change in the XRD patterns but the increase in the intensity due to alignment of MGCNCs suggests increase in the overall crystallinity of the nanocomposites[508]. It was calculated that the crystallinity of the PLA/MGCNC nanocomposites was $\sim 55\%$, which increased to $\sim 62\%$ on perpendicular alignment and to $\sim 66\%$ on parallel alignment at 1 wt. % loading of MGCNCs. Therefore, it could be concluded that the overall crystallinity of the polymer nanocomposites could be tuned by simply orienting the nanofillers in the presence of directional magnetic field during their processing.

The chemical structural analysis of the neat PLA and PLA/MGCNC films was carried out through FTIR spectroscopy as shown in Figure 8.4(c). PLA showed characteristic FTIR peaks at 1748, 1452, 1362, 1180, 1128, 1078, 1042 and 954 cm^{-1} representing C=O stretching, CH_3 asymmetric and symmetric vibrations, C–O–C asymmetric stretching, C–OH side group vibrations, C–O vibrations –CH stretching and C–C vibrations respectively [509]. Incorporation of MGCNCs led to the shift of the C=O stretching peak from 1748 to 1742 cm^{-1} .

¹ along with increase in the intensity of the peaks at 2854 and 2924 cm^{-1} corresponding to asymmetric and symmetric $-\text{CH}_2$ vibrations. The shift in the $\text{C}=\text{O}$ stretching is probably due to the presence of the hydrogen bonding between the PLA matrix and MGCNCs and the increase in intensity (~ 2854 and 2924 cm^{-1} peaks) is due to the incorporation of CNCs which leads to increase in the concentrations of the $-\text{CH}$ groups in the nanocomposites. Moreover, the presence of new peaks at 1712 and 1035 cm^{-1} corresponds to the $-\text{C}-\text{OH}$ asymmetric bending, $-\text{C}-\text{O}$ stretching at C6 of cellulose and the broad hump at $\sim 802 \text{ cm}^{-1}$ corresponds to the iron oxide nanoparticles, confirming the presence of the MGCNCs in the nanocomposites. However, it may be noted that, as expected, alignment (parallel and perpendicular) of the MGCNCs in the PLA matrix did not lead to any shift in the FTIR spectrum.



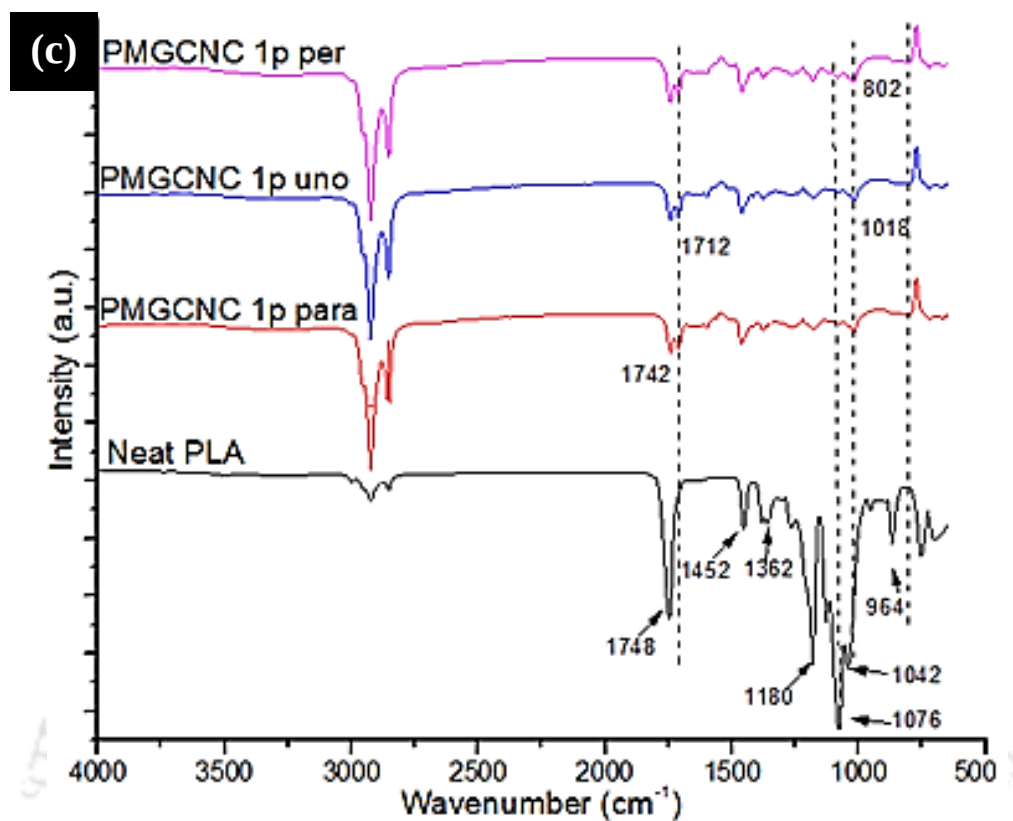


Figure 8.4: (a) Comparison of the XRD diffractographs for the neat PLA and magnetically oriented and unoriented PLA/MGCNC samples at 1wt. % MGCNC loadings. (b) Magnetic hysteresis loop for the PLA/MGCNC samples at 1wt. % MGCNC loadings under unoriented, parallel and perpendicular alignments measured in-plane at 298K and (c) FTIR spectrographs for the neat PLA and magnetically oriented and unoriented PLA/MGCNC samples at 1wt. % MGCNC loadings.

The orientation of the MGCNCs in the PLA matrix led to magneto-anisotropic properties in the fabricated nanocomposites, which were analyzed by VSM studies (Figure 8.4(b)). The VSM curves for the PLA/MGCNC nanocomposites had identical shape and characteristics as MGCNCs (powder), suggesting that the nanoparticles had retained its intrinsic properties in the nanocomposites [510]. Orientation of nanocomposites showed significant variation in the

magnetic properties such as coercivity (H_c), saturation magnetization (M_s) and remnant magnetization (M_r). As per Figure 8.4(b), PMGCNC1p $\parallel$ had higher magnetization (M_s) value than that of PMGCNC1p $\perp$. It is probably because of the parallel orientation of the MGCNCs (in plane), due to which magnetic dipoles are generated along the direction of the applied magnetic field (parallel) subsequently leading to increased magnetic moment[511]. However, the PMGCNC1p $\perp$ showed even lower M_s values than the PMGCNC1p uno, probably because of the perpendicularly oriented MGCNCs (out of plane), due to which the magnetic dipole moment generated is perpendicular to the applied magnetic field leading to generation of lower magnetic moments and M_s values. Further, the magnetic remanence (M_r) value of randomly oriented PMGCNC1p uno was found to be $\sim 267.3 \times 10^{-6}$ emu, which increased to 612.1 and 348.8×10^{-6} emu for the PMGCNC1p $\parallel$ and PMGCNC1p $\perp$ films respectively. The higher M_r values confirms the presence of magnetically oriented MGCNCs, which simultaneously led to significant increase in the squareness of the hysteresis loop from 55.3×10^{-3} (PMGCNC1p uno) to 0.1177 (PMGCNC1p $\parallel$) [498]. The degree of the alignment of the nanofillers in the polymer matrix can be evaluated from several parameters such as the ratio of remnant to saturation magnetization (M_r/M_s) as well as the coercivity values (H_c)[511]. In case of PMGCNC1p $\parallel$ the M_r/M_s ratio was ~ 0.12 , which was higher than that for PMGCNC1p $\perp$, ~ 0.07 (in plane), 0.10 (out plane), and PMGCNC1p uno, ~ 0.05 . Also a large difference in the H_c values was found for oriented PMGCNC1p $\perp$ ~ 51.5 and PMGCNC1p $\parallel$ ~ 54.5 samples in comparison to the unoriented PMGCNC1p uno samples ~ 35.2 . This suggests that larger fractions of the MGCNCs are aligned in case of parallel direction of the applied magnetic field (in line with the predicted order parameter 'S' values from FESEM micrographs), which leads to magnetic anisotropy behavior in the fabricated PLA/MGCNC nanocomposites. Also, the increase in the

magnetization values for the oriented samples could be attributed to the formation of self-assembled columnar structures in the PLA matrix under the magnetic field (as also evident from the FESEM-EDX analysis). The size of such assemblies is expected to depend upon the strength and direction of magnetic field, and weight fractions of the nanofillers, which subsequently will affect the polymeric properties. Therefore, it could be concluded that simple tuning of the magnitude and direction of the low magnetic field during polymer processing would lead to incorporation of magnetic anisotropy behavior in nanocomposites for self-biased magnetic applications.

8.2.3 Anisotropic PLA-based MGCNCs Nanocomposites: Structural Properties

The magnetic field-based alignment of the MGCNCs (in perpendicular and parallel directions) led to incorporation of the anisotropic mechanical behavior in the nanocomposites at different weight fractions of MGCNCs as shown in Figure 8.5(a), (b) and (c). The anisotropic mechanical behavior in nanocomposites is not only due to the rod-like morphology of CNCs but also due to their high inherent Young's modulus (YM) of 110-220 GPa along axial and 2-50GPa in transverse direction. The nascent PLA has a YM of 0.54 GPa, tensile strength (TS) of ~22 MPa and stretched to ~20% elongation before failure, in line with the earlier reported studies [494]. It is noteworthy to mention here that the mechanical strength of the PLA is very much dependent on the selection of processing technique through which the films are prepared. Melt extruded films can yield higher tensile strength than solution casted films[512]. However, in the current studies, we are demonstrating the change in properties of PLA by incorporation of MGCNCs in PLA using solution-cum-evaporation technique. Incorporation of the MGCNCs into PLA matrix without the application of any magnetic field led to improvement in the TS by ~23, 73 and 120% at 1, 2 and 3 wt. % loading respectively. However, as per our

previously reported study [494], such significant improvements in the TS were found to be absent in case of unmodified CNCs. The significant improvement in TS is probably due to the improved dispersion of the Fe_3O_4 nanoparticle-modified CNCs due to better compatibility with the PLA matrix. As suggested from FTIR studies, this surface modification approach led to improved hydrogen bonding with PLA matrix, which act as bridge towards transfer of applied load to CNCs in the nanocomposites which results in the improvement in the mechanical properties. Therefore, the adsorbed Fe_3O_4 nanoparticles onto CNCs not only led to fusion of magneto-responsive behavior into CNCs but also symbiotically acted as better dispersing agent, due to which they could be aligned in PLA matrix through application of low magnetic field. Orientation of MGCNCs in the parallel direction into PLA matrix led to the improvement of TS and % elongation by ~ 70 and 240% at 1 wt. % MGCNC loadings (in comparison to the unoriented nanocomposites at similar loading fractions). However, orienting the MGCNCs (~ 1 wt. % loading) in perpendicular direction led to improvement in TS and % elongation by ~ 58 and 172% respectively (compared to PMGCNC1p uno). It could be observed that the parallel orientation of MGCNCs led to comparatively greater improvement in TS and % elongation compared to the perpendicular alignment. This is probably due to the presence of ordered arrangement of the parallelly oriented MGCNCs in PLA along the tensile direction, so that it can withstand higher stress and undergo higher strain along with swift matrix failure. In addition, the higher modulus of CNCs along the axial direction (110–220 GPa) [297] could also be responsible for the improvement in mechanical properties along parallel direction. However, when MGCNCs are oriented in the perpendicular direction, the decrease in % elongation is probably due to the restricted motion of the vertically oriented CNCs in the PLA matrix when the tensile load is applied perpendicular to it (as shown in Figure 8.3(c)). Also,

CNCs have a comparatively lower transverse modulus of 2–50 GPa [297], which further leads to decrease in the tensile strength for the perpendicularly oriented MGCNCs. Presence of such anisotropic properties in the nanocomposites suggests that the surface modification of the CNCs with Fe_3O_4 nanoparticles did not alter the inherent characteristic properties of the CNCs. As the MGCNC loading is increased, it tends to get more oriented along the magnetic field direction due to generation of the higher magnetic moment at higher MGCNC concentration in the polymeric matrix, leading to enhanced mechanical reinforcement effects. It is predominant from (Figure 8.5(c)) the fact that both the TS and YM tends to increase almost linearly with the increased MGCNCs loadings (~5 wt. %). It was found that at higher MGCNC loading (~3 wt. %), the % elongation and TS improved by ~101 and 44% respectively in case of PMGCNC3p $\parallel$ and by ~73 and 26% respectively in case of PMGCNC3p $\perp$ compared to PMGCNC3p uno . However, decrement in the % elongation and TS is probably due to agglomeration of the MGCNCs in the PLA matrix, leading to the formation of porosity and uneven dimensions of MGCNCs [486], which hindered the swift failure of the polymer matrix. Presence of such agglomeration decreases the polymer-CNC interfacial interaction through hydrogen bonding (as suggested from FTIR studies), thereby hindering the reinforcement effect of CNCs. It was found that the YM of the oriented samples improved by ~27 and 116% at 1 and 3 wt. % respectively in case of parallel orientation and by ~11 and 105% at 1 and 3 wt. % respectively in case of perpendicular orientation. The lower YM values for the magnetically oriented samples compared to unoriented samples are probably due to the improvement in the elongation behavior, which leads to the formation of more flexible films. The improved TS and % elongation values suggest that alignment of MGCNCs could lead to

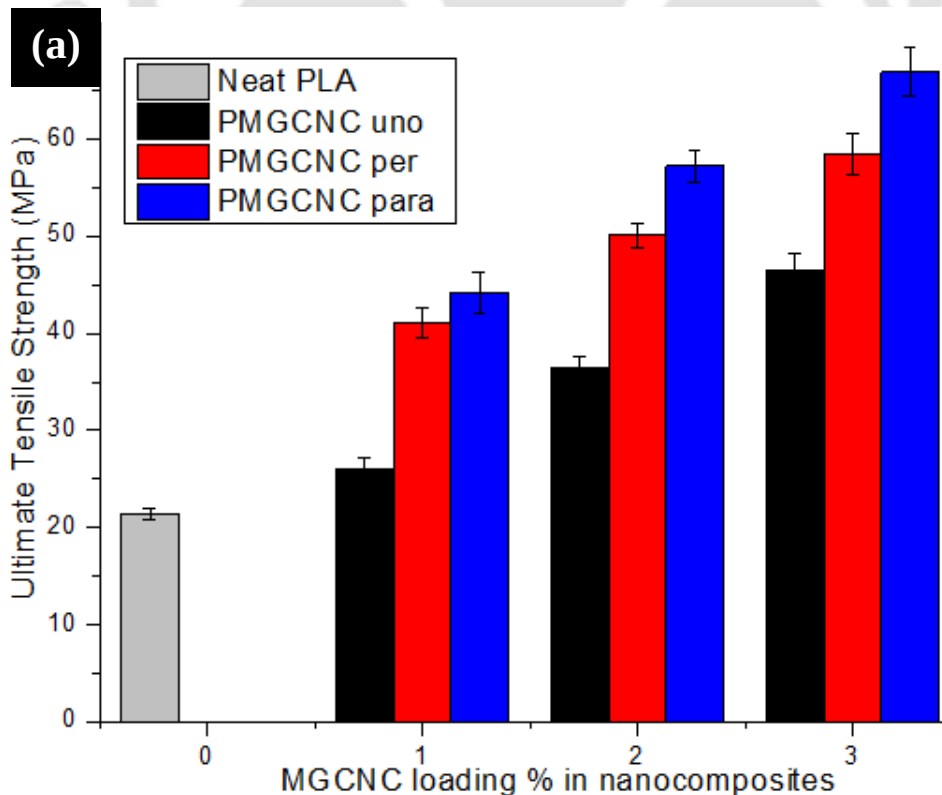
effective stress transfer of CNCs to polymer matrix and incorporate anisotropic mechanical behavior into the nanocomposites.

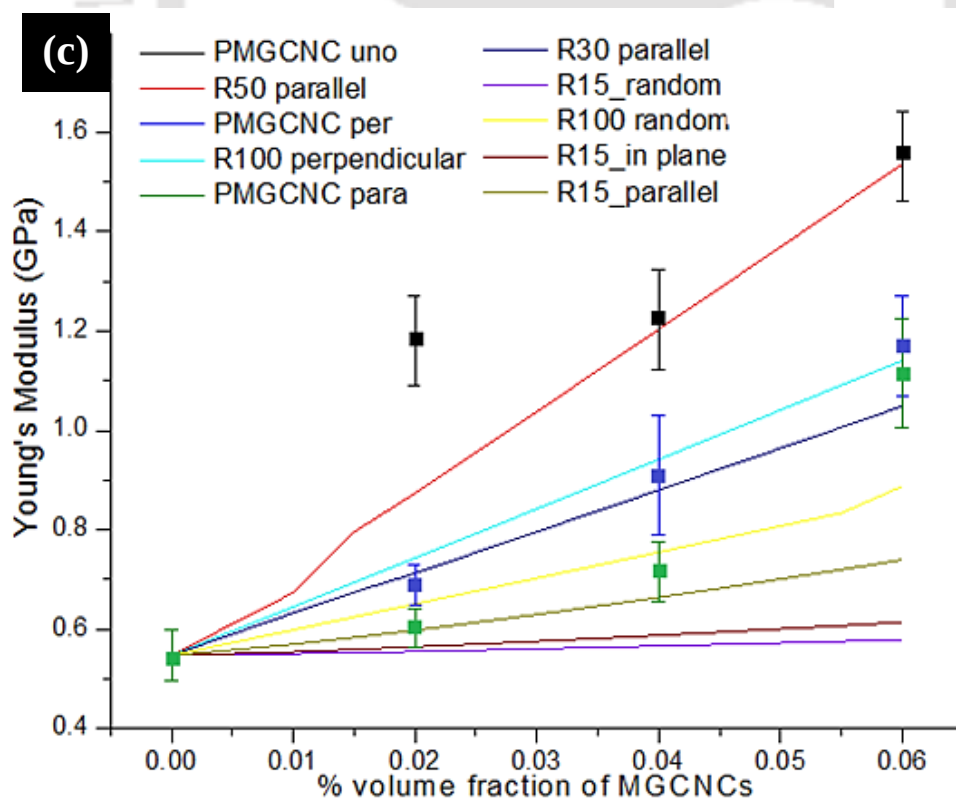
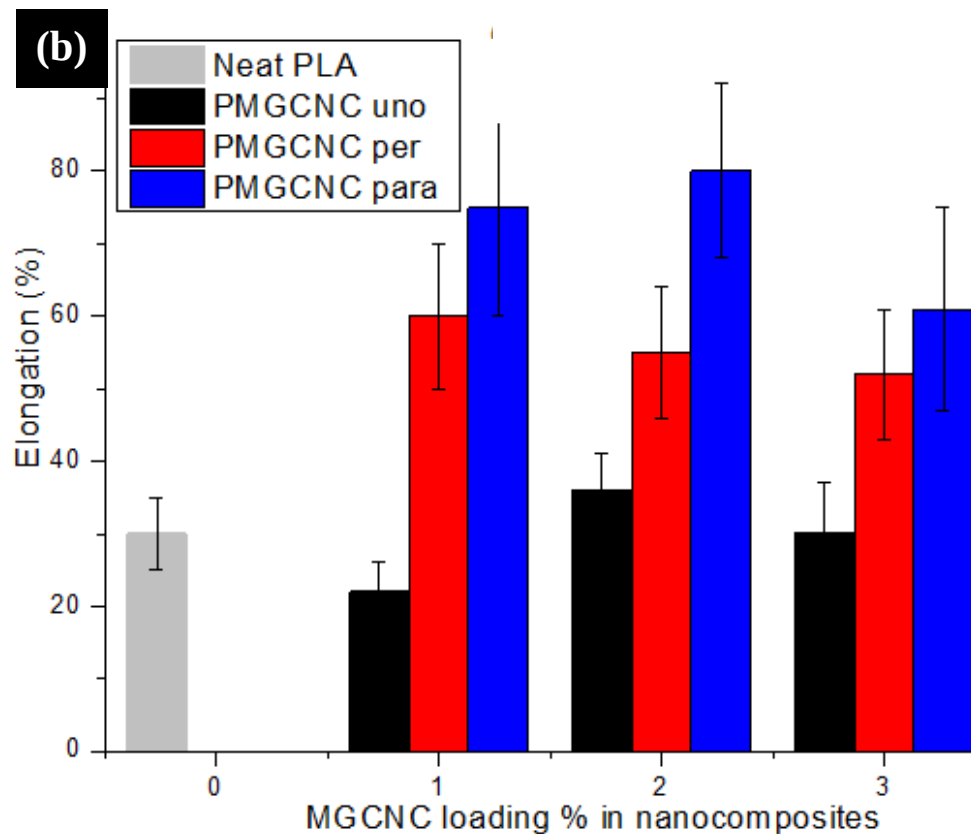
To further confirm the effective alignment of the MGCNCs in the polymeric matrix the experimental Young's modulus was compared with the predicted modulus from the Cox and Halpin-Tsai models which takes into consideration the effect of the parallel and perpendicular alignment of the nano-fillers. It was found that the experimental modulus of the parallelly oriented MGCNCs at lower volume fractions (~ 2 wt.% loading) fitted perfectly with the Cox and Halpin-Tsai models at the aspect ratio (R) of the $R=15$ predicted experimentally from the FESEM micrographs (Figure 8.1a). However, at higher loading fractions (~ 3 wt. %) the predicted modulus fits well with the parallelly oriented nanofillers at a higher aspect ratio of $R=30$ (from Cox model) and $R=40$ (from Halpin-Tsai model). As evident from the FESEM micrographs (Figure 8.3), MGCNCs, in presence of parallel magnetic field, self-assemble into columnar structures leading to formation of nano-fillers with higher aspect ratio which will subsequently have higher reinforcing efficiency. However, it is very difficult to calculate the effective aspect ratio of the MGCNCs when dispersed in PLA matrix under parallel orientation of magnetic field, due to its higher magnetic moment and problems of agglomeration prevalent at such high loading fractions. For the perpendicular/in-plane alignment of the MGCNCs, the effective aspect ratio of MGCNCs in the PLA matrix at which it fitted with the experimentally calculated modulus (interestingly in ~ 3 wt.% range of MGCNC loadings) further increased to $R=100$ (from Cox models). Such drastic increase in the aspect ratio is probably due to the end-to-end alignment of the MGCNCs inside the PLA matrix under the application of perpendicular magnetic field leading to rapid increase in the length while keeping the diameter almost unchanged. But for the unoriented samples, both Cox and Halpin-Tsai models were incapable

in predicting the composite modulus, probably due to agglomerations of MGCNCs thereby decreasing the % elongation significantly. The effective prediction of composite modulus (albeit at high R values) for the parallel and perpendicularly oriented PLA/MGCNC nanocomposites using both models supports the hypothesis that complete alignment of the MGCNCs has occurred in the polymer matrix in presence of magnetic field. From the Cox and Halpin-Tsai models, it was found that the experimentally calculated modulus fitted with the theoretically predicted modulus for an upper limit of $R=100$ and lower limit of $R=15$, indicating that the dispersion of CNCs and magnetic alignment played an important role in reinforcing efficiency of nanocomposites. Therefore, from this study it could be concluded that the effective alignment of MGCNCs in the polymer matrix by application of the directionally tunable magnetic field, could lead to the fabrication of the high performance biopolymer-based composites.

8.2.4 Anisotropic PLA-based MGCNCs Nanocomposites: Thermo-Mechanical behavior

Dynamic mechanical analysis of the nanocomposites is carried out to study the effect of alignment of nanofillers on the thermo-mechanical behavior, i.e., the influence on the storage (E') and loss modulus (E'') of the nanocomposites with 1wt. % MGCNC loadings, over a wide temperature range of 25–90 °C at 1Hz frequency (Figure 8.5(d)). It was observed that incorporation of the MGCNCs in absence of magnetic field led to improvement in the E' values by almost two times (at 3 wt. % loadings) in comparison to the neat PLA matrix (Table1). Moreover, the glass transition temperature (T_g) also improved significantly (by $\sim 13.6^\circ\text{C}$) in comparison to neat PLA at 3 wt. % MGCNC loadings. Improvement in T_g is probably due to the formation of the hydrogen bonds between the PLA matrix and Fe_3O_4 nanoparticles adsorbed on the CNCs in MGCNCs (as suggested from FTIR studies) which restricted the polymer chain motion, thereby decreasing the loss modulus at higher temperature ranges.





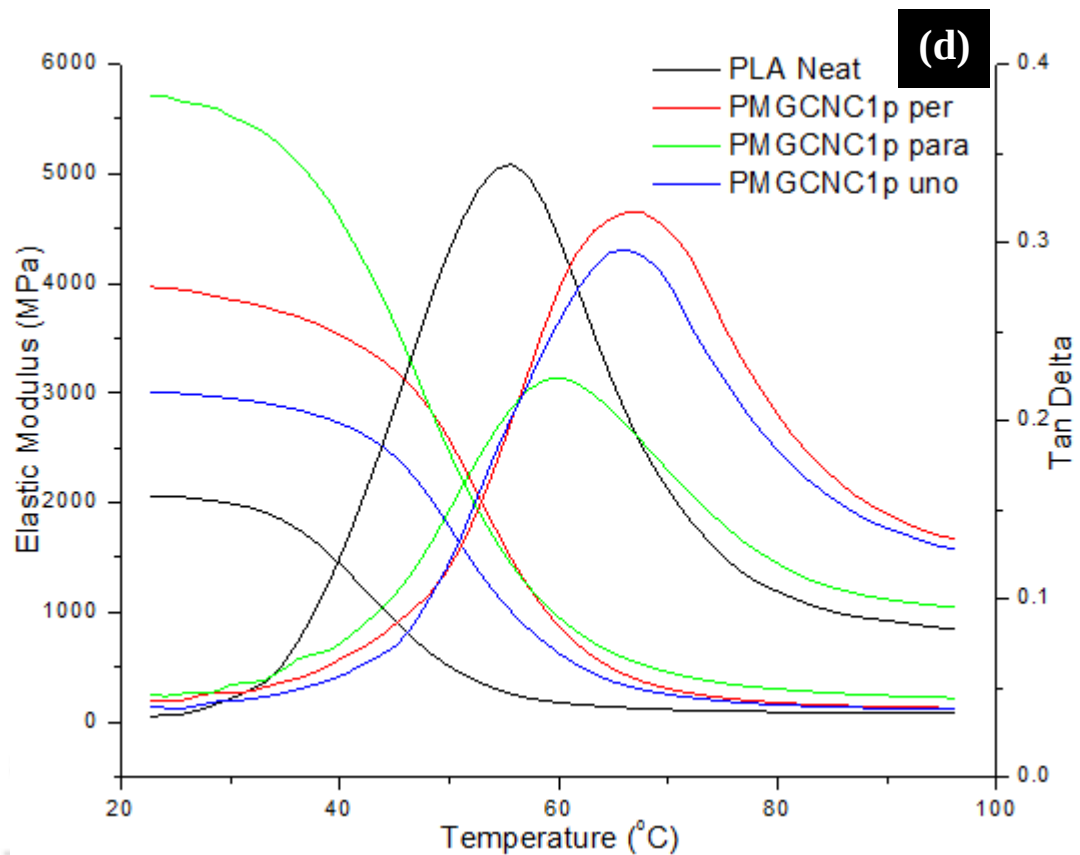


Figure 8.5: (a) Tensile strength (b) percentage elongation for the unoriented, parallel and perpendicular aligned PLA/MGCNC nanocomposites at 1-3 wt. % MGCNC loadings. (c) Comparison of the experimentally calculated Young's modulus and theoretically predicted modulus from modified Cox (marked in solid lines) and Halpin-Tsai (HT) (marked in dashed lines) models at different aspect ratio (R) and (d) Dynamic mechanical analysis of the neat PLA and magnetically oriented samples showing the effect of alignment on the elastic modulus and the tan delta of the nanocomposites at constant 1wt. % MGCNC loadings.

The effect of alignment (parallel or perpendicular) on the degree of reinforcing efficiency in the nanocomposites over the varied temperature range was predicted from the filler effectiveness coefficient (C_{FE}),

$$C_{FE} = \left(\frac{E'_g}{E'_r} \right)_{comp} / \left(\frac{E'_g}{E'_r} \right)_{matrix} \quad (8.1)$$

where E'_g and E'_r represents the storage modulus of the polymer matrix and nanocomposites measured at glassy (46°C) and rubbery (85°C) state of the polymers [513]. It is generally considered that lower the C_{FE} value better the reinforcing capability of the nanofillers in the polymer nanocomposites. From Table1, it was found that the C_{FE} values followed a trend of $(C_{FE})_{uno} > (C_{FE})_{\perp} > (C_{FE})_{para}$ at different loading fractions of MGCNCs. This suggest that parallel and perpendicular alignment of MGCNCs in PLA matrix led to better reinforcing efficiency, subsequently leading to improvement in mechanical properties over the wide temperature range compared to the unoriented samples. Comparison of the storage modulus at glassy and rubbery state for the magnetically aligned nanocomposites at constant 1 wt. % MGCNC loading (Figure 8.5(d)) showed significant variation confirming the presence of the anisotropic thermo-mechanical behavior in the nanocomposites. E'_g for parallely and perpendicularly oriented samples were found to be ~1.8 and 1.3 times higher than the unoriented samples. Interestingly, similar behavior was observed in the nanocomposites at the rubbery state with E'_r almost ~2.3 and 1.3 times higher than the unoriented samples. This confirms that the alignment of MGCNCs in polymer matrix is not disturbed at temperatures higher than the T_g of the PLA even in the presence of tensile load. As discussed in previous sections, such improvement in the storage modulus values (in parallel and perpendicular direction) is due to inherent anisotropic properties of CNCs which have axial modulus of ~110–220GPa and

transverse modulus of 2–50GPa. Moreover, the increase in the aspect ratio of the MGCNCs on alignment in perpendicular or parallel direction inside the PLA matrix (from R=15 to R=30-100) may also contribute to improvement in the E' values. However, comparison of the T_g values for the magnetically oriented nanocomposites showed a slight increase in case of perpendicular alignment compared to the parallel and unoriented samples. The T_g improved significantly from 55.4°C for the neat PLA to 70.2°C for the perpendicular aligned PMGCNC3p $\perp$ samples. Improvement in the T_g values for the unoriented and the perpendicularly oriented samples is probably due to presence of the entanglements of MGCNCs which restricted the deformation of the polymer chains under the direction of the tensile load. However, such entanglements are expected to be absent in case of the parallelly oriented samples and with the application of tensile load in same direction, the polymer chains underwent higher elongation at higher temperature leading to steep decrease in the storage modulus in the rubbery region[514]. Therefore, the parallelly oriented samples had lower T_g compared to the perpendicularly and unoriented samples. Consequently, it could be concluded that the alignment of the MGCNCs in the PLA matrix over a wide temperature range led to anisotropic thermo-mechanical behavior of the fabricated nanocomposites along with tunable glass transition properties.

8.2.5 Anisotropic PLA-based MGCNCs nanocomposites: Thermal and Crystallization

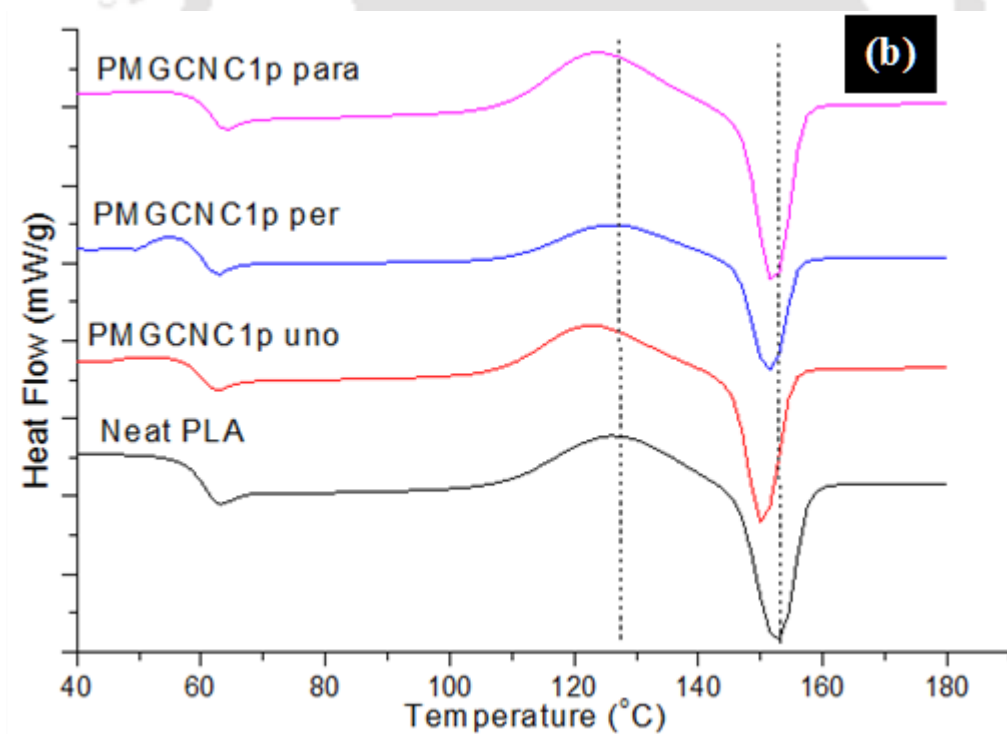
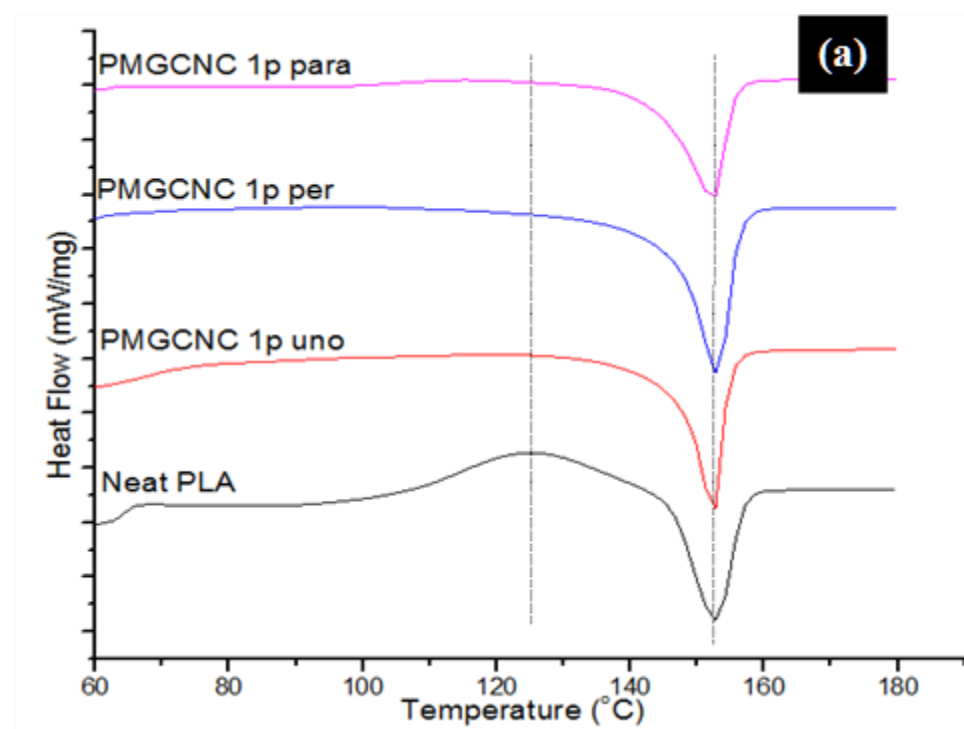
Properties

It is expected that magnetically tuning the orientation of MGCNCs in the PLA matrix would lead to preferential orientation of the polymer chains surrounding its surface thereby bringing in changes in the thermal properties of the nanocomposites. As discussed in previous section 4.2.6, it has been found that biopolymers like polyhydroxybutyrate could form a thin adsorbed layer on the surface of CNCs probably due to enhanced hydrogen bonding with the hydroxyl groups of CNCs. Moreover, in case of MGCNCs containing iron oxide nanoparticles, it is well known that interaction of polymers with the metal oxide nanoparticles is more pronounced [515].

Table 8.1: Dynamic Mechanical Analysis of the PLA/MGCNC samples for the unoriented, parallel and perpendicularly aligned samples as a function of 1-3 wt. % MGCNC loadings. The storage modulus were measured at the glassy (46°C) and rubbery (85°C) state of the nanocomposites.

Sample	Storage Modulus E'(MPa) @46°C	Glass Transition Temp. (T _g) (°C)	Storage Modulus E'(MPa) @85°C	C _{FE}
Neat PLA	1844	55.4	87	-
PMGCNC1p uno	2881	66.2	110	1.236
PMGCNC1p ∥	5253	60.2	262	0.946
PMGCNC1p ⊥	3739	67.7	151	1.168
PMGCNC2p uno	2897	68.1	114	1.188
PMGCNC2p ∥	5933	60.5	291	0.962
PMGCNC2p ⊥	5320	69.3	262	0.958
PMGCNC3p uno	3182	69.0	131	1.15
PMGCNC3p ∥	5026	61.8	302	0.8
PMGCNC3p ⊥	4519	70.2	214	0.996

Further, the FTIR studies of the PLA/MGCNC nanocomposites showed the presence of enhanced hydrogen bonding thereby leading to better compatibility and dispersion of MGCNCs in PLA matrix. This probably led to the adsorption of the chains of PLA onto the surface of MGCNCs, thereby forming a thin layer of polymer wrapped on its surface. Presence of such improved interaction led to significant improvement in the mechanical properties due to the strong reinforcing behavior and decrease in the percentage crystallinity. Similar observation was also reported by Kim et al. [516], where polyethylene oxide chains were adsorbed on the surface of the iron nanoparticle-coated carbon nanotubes thereby improving the structural properties of this nanocomposite. It is expected that in case of magnetically oriented MGCNCs, the adsorbed layer of PLA chains would also preferentially orient in the direction of the magnetic field thereby incorporating the anisotropic thermal properties in the fabricated nanocomposites. DSC studies were carried out for the magnetically oriented samples for the first and second heating cycles, in which the anisotropic behavior of the nanocomposites is expected to be predominant in case of first heating cycle only.



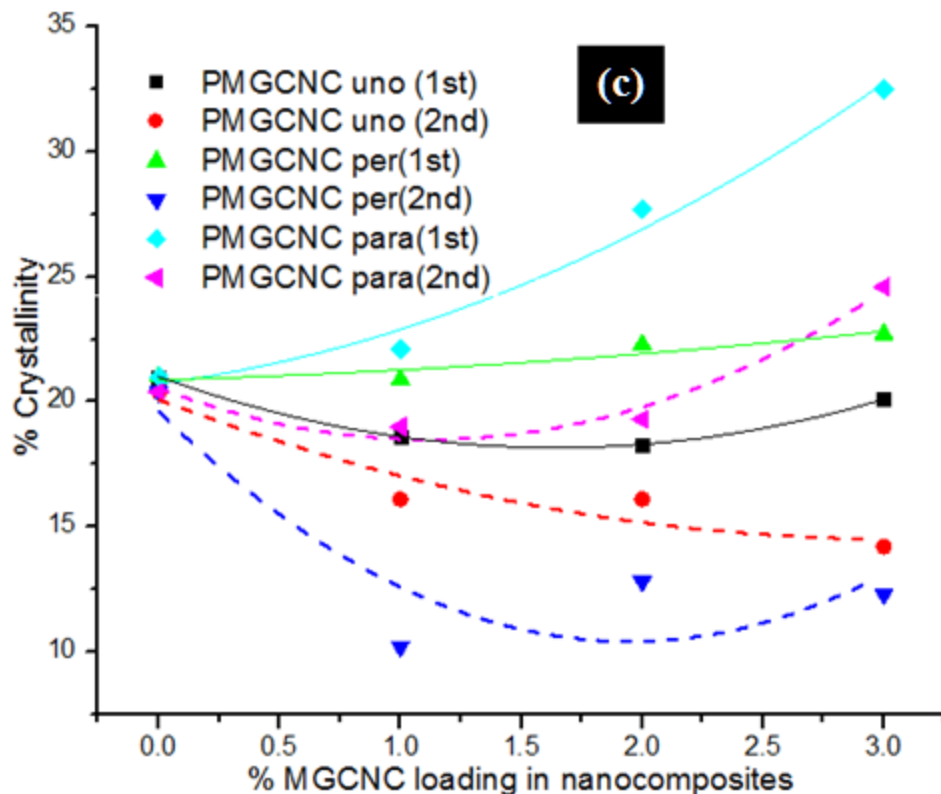


Figure 8.6: (a) First heating and (b) second heating cycles of the DSC for the neat and magnetically oriented samples of PLA at 1wt. % MGCNC loadings. (c) The percentage crystallinity of the PLA/MGCNC nanocomposites for the oriented (parallel and perpendicular) and unoriented samples as function of the 1-3 wt. % MGCNC loadings. (1st heating cycle represented by continuous lines and 2nd heating cycles represented by dotted lines).

After the first heating cycle and subsequent melting of the nanocomposites, the orientation of MGCNCs is expected to get disordered, leading to the absence of anisotropic properties in the nanocomposites. The effect of MGCNC loading and orientation in presence of magnetic field has negligible effect on the melting behavior (first heating cycle of DSC thermographs) of the nanocomposites. However, it is found that the crystallization temperature (T_c) of the

unoriented samples decreases on incorporation of MGCNCs, which is in-line with the previously reported studies [517]. For the case of first heating cycle, the percentage crystallinity (X_c^a) of the unoriented nanocomposites decreased by $\sim 3\%$ and T_c decreased by $\sim 11^\circ\text{C}$ at 1 wt. % MGCNCs loadings compared to neat PLA. Interestingly, in case of magnetically oriented samples, both parallel and perpendicular aligned nanocomposites did not show any crystallization peaks in the first heating cycle (for all MGCNCs loadings) (Figure 8.6(a)). This is probably due to the preferential alignment of the polymer chains in the magnetically oriented samples which requires more activation energy of folding to crystallize in the form of spherulites at the crystallization temperature. During the DSC studies at a heating rate of $10^\circ\text{C}/\text{min}$, the time interval at which the oriented PLA chains could gain enough thermal energy to overcome the energy barrier to crystallize, the chains experience melting at which point the effect of orientation as well as the presence of crystallites disappears. Hence, there was complete absence of the T_c peak in the oriented samples which subsequently led to significant improvement in the percentage crystallinity values of the nanocomposites. Figure 8.6(c), shows the effect of MGCNC loadings and the first (continuous lines) and second (dotted lines) heating cycles on the % crystallinity values of the nanocomposites. The parallelly oriented samples showed an exponential improvement in the crystallinity values whereas the perpendicular oriented sample had linear improvement in the crystallinity values at different MGCNC loadings. It was found that the maximum improvement in the percentage crystallinity was obtained for the parallelly oriented samples with an enhancement by $\sim 11\%$ for PMGCNC3p|| samples (Table 8.2). For the perpendicularly oriented samples, the percentage crystallinity improved by $\sim 4\%$ at 3 wt. % MGCNC loading. As discussed in the previous section, the presence of the polymer chain entanglements was minimum in case of parallelly

oriented samples which subsequently led to more preferential alignment in the presence of magnetic field thereby having comparatively higher $\%X_c^a$ values. However, in the second heating cycles where the effect of MGCNC alignment was absent, the variation in the T_c and T_m values due to anisotropy was not found (Figure 8.6(b)). The T_c as well as X_c^b values were found to decrease at 1–3 wt. % MGCNC loadings and under all orientations (both parallel and perpendicular), similar to that of the unoriented samples. Therefore, it was found that magnetically orienting the direction of the MGCNC nanofillers significantly altered the thermal properties, especially the crystallization behavior of polymer nanocomposites which has potential application in industrial scale processing of the polymers especially through extrusion-based approaches.

Table 8.2: Thermal and crystallization properties of the unoriented and aligned (parallel or perpendicular) samples at different loadings of MGCNCs (1-3wt. %).

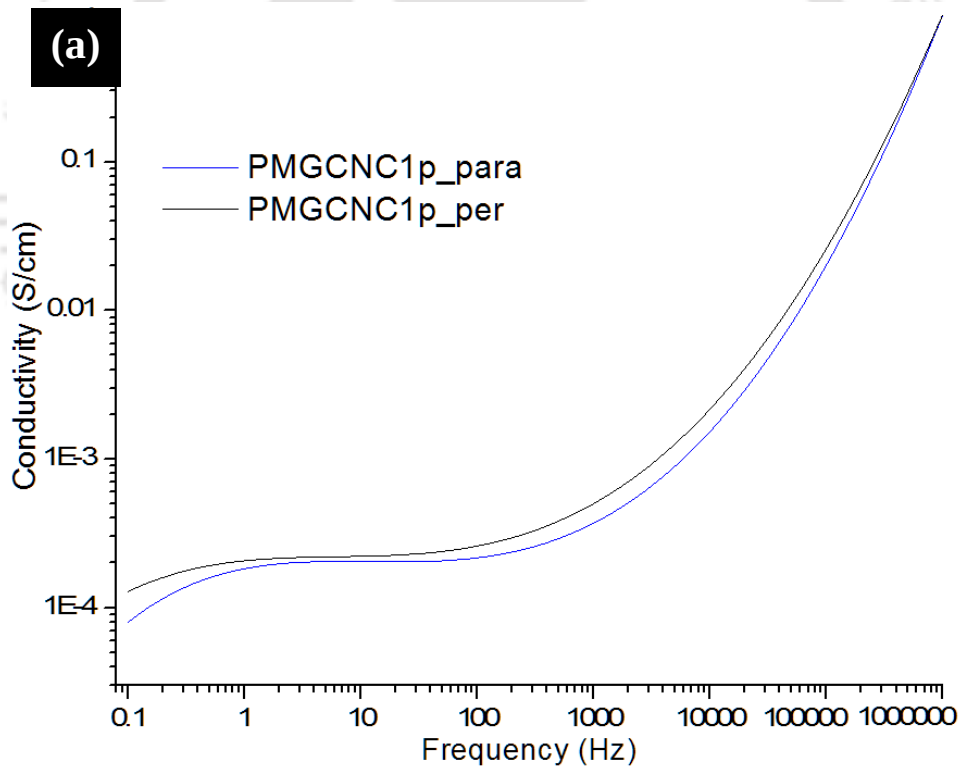
Sample	First Heating					Second Heating				
	T_c^a (°C)	ΔH_c^a (J/g)	T_m^a (°C)	ΔH_m^a (J/g)	X_c^a (%)	T_c^b (°C)	ΔH_c^b (J/g)	T_m^b (°C)	ΔH_m^b (J/g)	X_c^b (%)
Neat PLA	125.0	16.15	152.6	-19.6	21.0	126.1	11.78	152.7	-19.03	20.4
PMGCNC 1p uno	115.2	2.45	151.8	-17.3	18.6	123.8	11.90	151.3	-15.04	16.1
PMGCNC 1p $\parallel$	-	-	152.5	-20.6	22.1	126.0	15.03	150.3	-17.69	19.0
PMGCNC 1p $\perp$	-	-	152.7	-19.5	20.9	126.7	5.80	151.8	-9.52	10.2
PMGCNC 2p uno	115.9	2.61	152.3	-17.1	18.3	122.4	12.89	150.4	-14.99	16.1
PMGCNC 2p $\parallel$	-	-	152.4	-25.8	27.7	122.1	15.78	150.6	-18.00	19.3
PMGCNC 2p $\perp$	-	-	152.8	-20.8	22.3	126.3	7.84	151.3	-11.95	12.8
PMGCNC 3p uno	119.2	6.63	151.3	-18.7	20.1	121.2	18.22	150.0	-13.28	14.2
PMGCNC 3p $\parallel$	-	-	152.8	-30.3	32.5	123.2	27.05	152.1	-22.89	24.6
PMGCNC 3p $\perp$	-	-	152.1	-21.2	22.7	124.3	13.7	150.8	-11.5	12.3

(T_m^a , T_m^b = melting temperature at first and second heating cycles, ΔH_m^a , ΔH_m^b = enthalpy of crystallization at first and second heating cycles. X_c^a , X_c^b = % crystallinity at first and second heating cycles, T_c^a , T_c^b = crystallization temperature at first and second heating cycles.)

8.2.6 Anisotropic PLA-based MGCNCs Nanocomposites: Electrical Conductivity Studies

The effect of alignment of the MGCNCs on the electrical conductivity properties of the fabricated PLA/MGCNC nanocomposites was analysed over a frequency range of 0.1Hz–1MHz (as shown in Figure 8.7(a)). Incorporation of the MGCNCs with such high content of adsorbed Fe_3O_4 metal nanoparticles (~50% by mass) led to improvement in the electrical conducting properties of nanocomposites with increased nanofiller loading. From Figure 8.7(a), it was found that alignment of the MGCNCs in the parallel direction led to significant improvement in the electrical conductivity followed by the perpendicular and unoriented samples. The high conductivity for the aligned samples suggests that anisotropic conductive behavior could be incorporated in the nanocomposites through alignment of MGCNCs in the presence of low magnetic field. In case of the parallelly aligned samples, it is expected that the ends of the MGCNCs are interconnected forming columnar structures in the nanocomposites (as evident from the FESEM micrographs shown in Figure 8.3(a)) which leads to enhanced electron transport [518]. It was found that at lower frequency range the electrical conductivity was found to be independent of the frequency. Therefore, the effect on the electrical conductivity of the nanocomposites at various MGCNC loadings and different alignments (parallel and perpendicular) was compared at a constant frequency of 1Hz (as shown in Figure 8.7(b)). As expected, with increased MGCNC loadings, the Fe_3O_4 nanoparticle content increased in the nanocomposites thereby improving the conductivity values. The ratio of the electrical conductivity for parallel and perpendicular oriented nanocomposites could be used as a tool to predict the effectiveness of degree of alignment of MGCNCs in the nanocomposites [516]. It was found that the ratio was 1.62, 3 and 1.23 at 1, 2 and 3 wt. % MGCNC loadings respectively. The ratio of the electrical conductivity for the parallel and perpendicular oriented

nanocomposites was found to decrease at higher MGCNC loadings (~3 wt.%). As discussed in the earlier sections, at higher MGCNC loadings, problems related to agglomeration occurs which prevents the perfect orientation of the nanofillers thereby leading to decrement in the electrical conductivity values. Similar anisotropic electrical conductivity behavior and subsequently the reduction in the electrical conductivity of the nanocomposites due to aggregations of the Fe_3O_4 nanoparticle coated on carbon nanotubes was also reported by Kim et al. [516] Therefore, it could be concluded that magnetically tuning the alignment of the MGCNCs in the PLA matrix leads to anisotropic electrical conductivity behavior, which has potential application in development of sensors or electronic-based packaging materials.



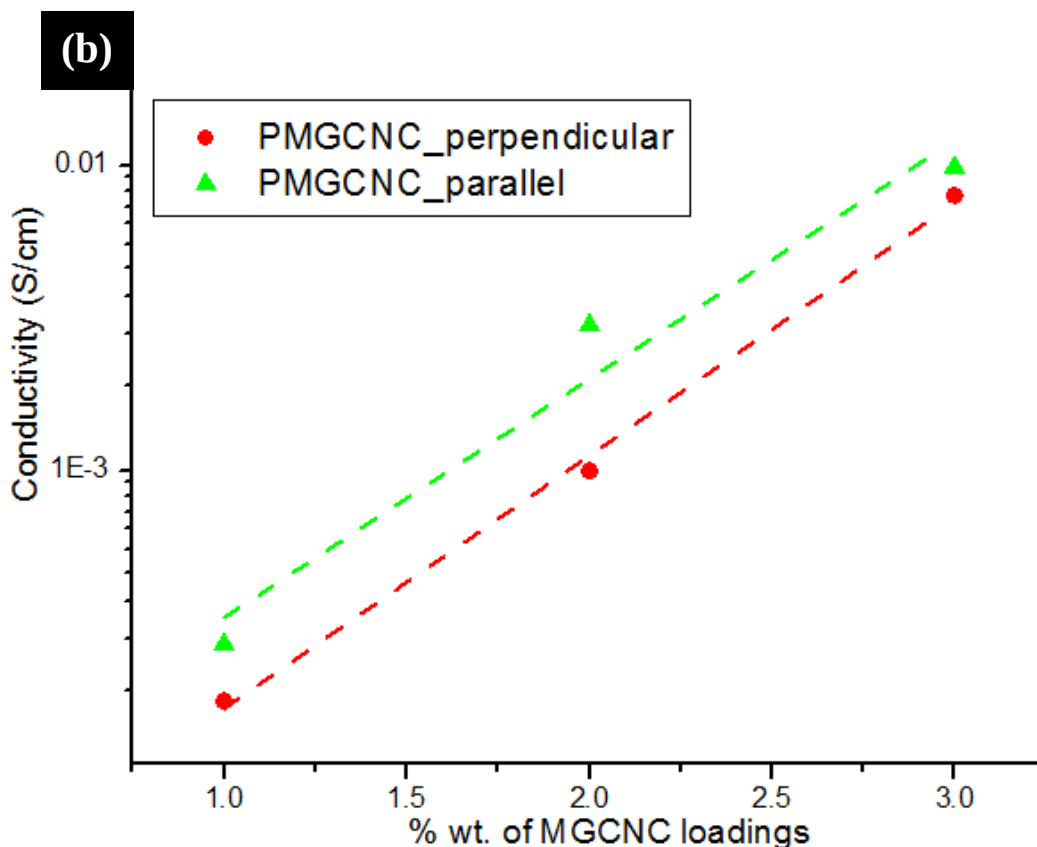


Figure 8.7: (a) The electrical conductivity of the PLA/MGCNC nanocomposites under parallel and perpendicular orientation of the nanofillers at constant loading of 1wt. % MGCNCs. (b) The electrical conductivity of the parallel and perpendicularly oriented nanocomposites as function of the 1-3 wt. % MGCNC loadings.

8.2.7 Magnetic Hyperthermia Behaviour of MGCNCs and their PLA-based Nanocomposite.

MGCNCs derived from renewable biomass (bamboo stems) with their surface decorated with ~50 wt% iron nanoparticles were evaluated for their application as hyperthermia agents, in the presence of alternating magnetic fields (AMF). Magneto-responsive CNCs, due to its non-toxicity, improved biocompatibility along with the hyperthermia behaviour can be potentially used as nano-vehicles in biological environments for theranostic applications,

especially inactivating the cancer or tumor cells at targeted sites. Such deactivation of cancer cells occurs due to generation of high temperature at specific localized regions, which damages the cells. The additional release of certain anticancer drugs at the target site can synergistically improve the efficacy of hyperthermia-based treatments. To evaluate the capability of the MGCNCs to generate local heat when placed under an oscillating magnetic field, two different concentrations 1 and 2.5 mg/ml of MGCNCs suspensions dispersed in water are studied (as shown in Figure 8.8(a)). At both concentrations, MGCNCs show drastic increase in temperature (by $\sim 16^{\circ}\text{C}$) from room temperature ($\sim 30^{\circ}\text{C}$) to $\sim 46^{\circ}\text{C}$, within a period of ~ 600 seconds. It has been reported that sudden increase in temperature of tumoral/cancerous cells above $\sim 42^{\circ}\text{C}$ is an ideal condition to destroy the natural enzymatic metabolism of cells which results in more effective and selective killing of the cancer cells [519]. Figure 8.8(a) shows a steep rise in the temperature of MGCNC suspension up to $\sim 42^{\circ}\text{C}$ within 300 seconds, probably due to their high magnetic saturation values, which makes them an ideal candidate for potential application as hyperthermia based anti-tumor/cancer agents. The heat generation by such MGCNC based bio-nano magnetic fluids is probably through Brownian and Néel relaxation phenomena which occurs in the presence of alternating magnetic fields [520]. In this phenomenon, MGCNCs, due to their high magnetic moment, tend to get aligned in the direction of the external magnetic field, which occurs in two different ways. Firstly, during the Brownian relaxation process, physical rotation of MGCNCs occurs in such a way that magnetic moment lines up in the direction of the applied external fields. However, its motion is hindered due to the viscous effect provided by the surrounding media. Secondly, during the Néel relaxation process, the nanoparticles remain static but the magnetic moment relative to crystal lattice undergoes rapid change in direction which is hindered by the anisotropy energy [521].

Presence of both these phenomena leads to heat generation, the degree of which depends on the magnetic saturation values, shape or dimensions of magnetic nanoparticles and amplitude/frequency of the applied field.

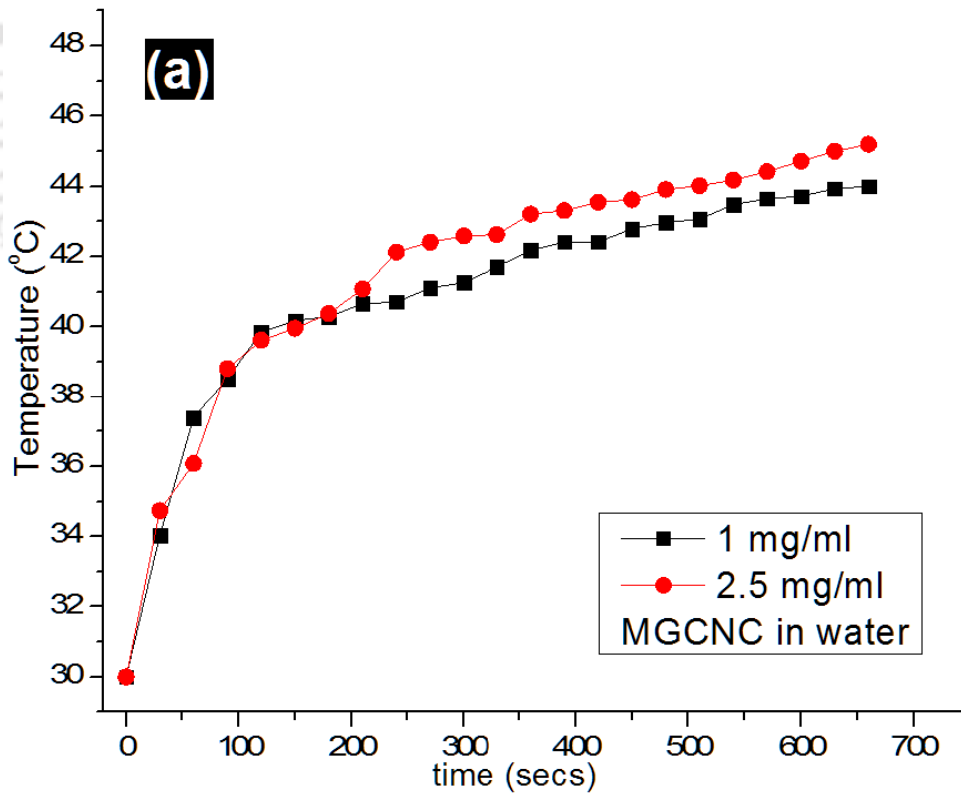
Specific absorption rate (SAR) is a parameter to measure the heating efficiency of magnetic nanoparticles, i.e., their ability to transform magnetic energy into heat when exposed to AMF. SAR is dependent on several factors such as magnetization values, fraction of magnetic elements and rate of rise in temperature, and is predicted using the following equation:

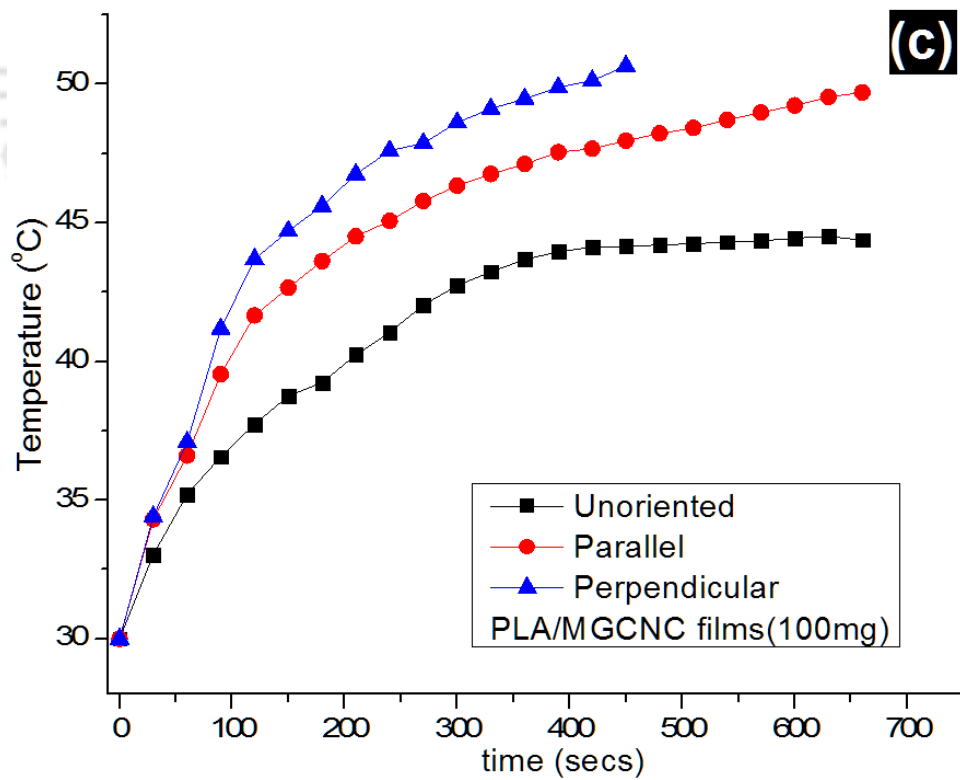
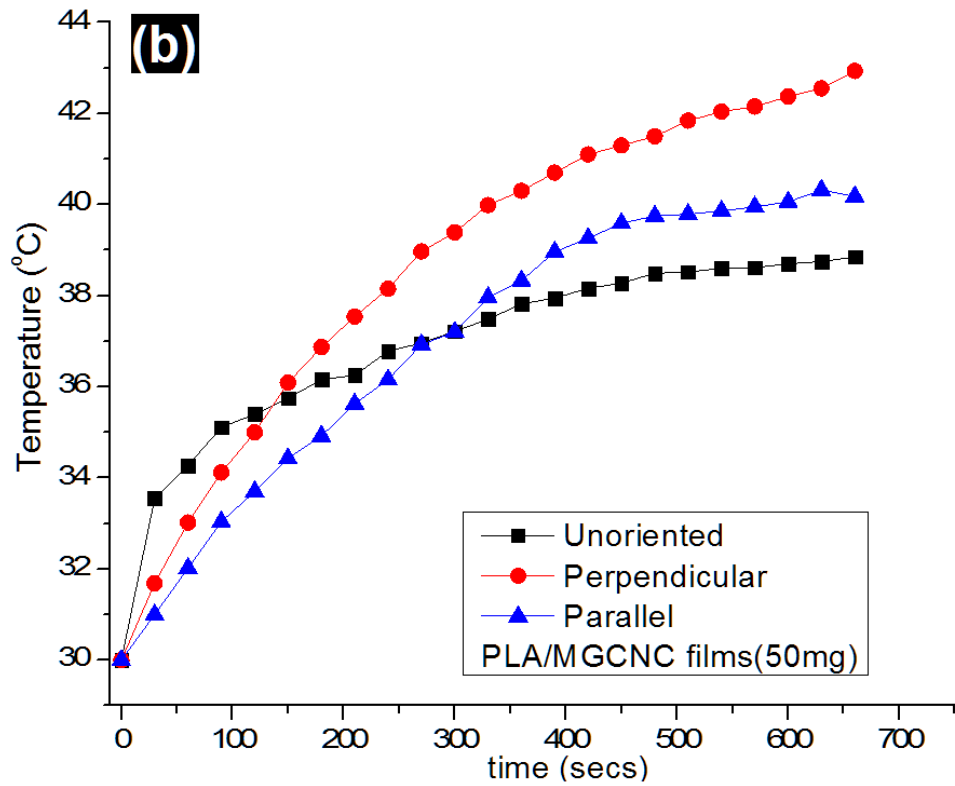
$$SAR = C \times \left(\frac{\Delta T}{\Delta t}\right) \times \frac{1}{(m_M)} \text{ or } C \times \left(\frac{\Delta T}{\Delta t}\right) \times \frac{m_s}{m_n} \quad (8.2)$$

where C refers to specific heat of the medium in which MGCNCs are dispersed, $\frac{\Delta T}{\Delta t}$ is the slope of the rise in temperature versus time curve, m_M is the weight fraction of magnetic element in the composite, m_s is the mass of the solvent and m_n is the mass of the nanoparticles. MGCNCs show a SAR value of ~ 270 W/g when dispersed in water at a concentration of 1mg/ml. It was observed that increasing the concentration of MGCNCs to 2.5 mg/ml did not have a significant effect on the temperature rise, and the SAR value decreased to ~ 117 W/g (Figure 8.8(d)). Similar behaviour was also reported by Carlos et al. [522], which is probably due to agglomeration of MGCNCs at higher loadings that limits its heat generation efficiency. Therefore, comparison of SAR values of MGCNCs with other nanomaterials [523] shows comparable results. Due to the non-toxic nature, biocompatibility and good SAR values of MGCNCs, they are promising materials for theranostic applications.

The magnetic hyperthermia behaviour of PLA/MGCNC films with ~ 3 wt. % MGCNC loading and the effect of MGCNC orientation in parallel or perpendicular directions are shown

in Figure 8.8(b) and (c) respectively. The 50 mg PLA/MGCNC films show a maximum rise in temperature up to $\sim 43^{\circ}\text{C}$ within a period of ~ 600 seconds, whereas for 100 mg PLA/MGCNC films, the temperature shoots above $\sim 50^{\circ}\text{C}$ (for perpendicularly oriented films) within a period of ~ 400 seconds. Moreover, significant variations in the rate of increase in temperature of the films are observed for the unoriented and the perpendicular/parallel aligned films. For both the cases (50 and 100 mg), perpendicularly oriented films show highest rate of increase of temperature followed by parallelly oriented and unoriented films. The SAR value for unoriented films is ~ 30 W/g, which increased to ~ 34 and 36 W/g for parallel and perpendicular alignment under constant loading of ~ 3 wt% MGCNCs. In comparison to the MGCNC ferrofluids, SAR values for PLA/MGCNC films reduced due to suppression of the Brownian relaxation [524].





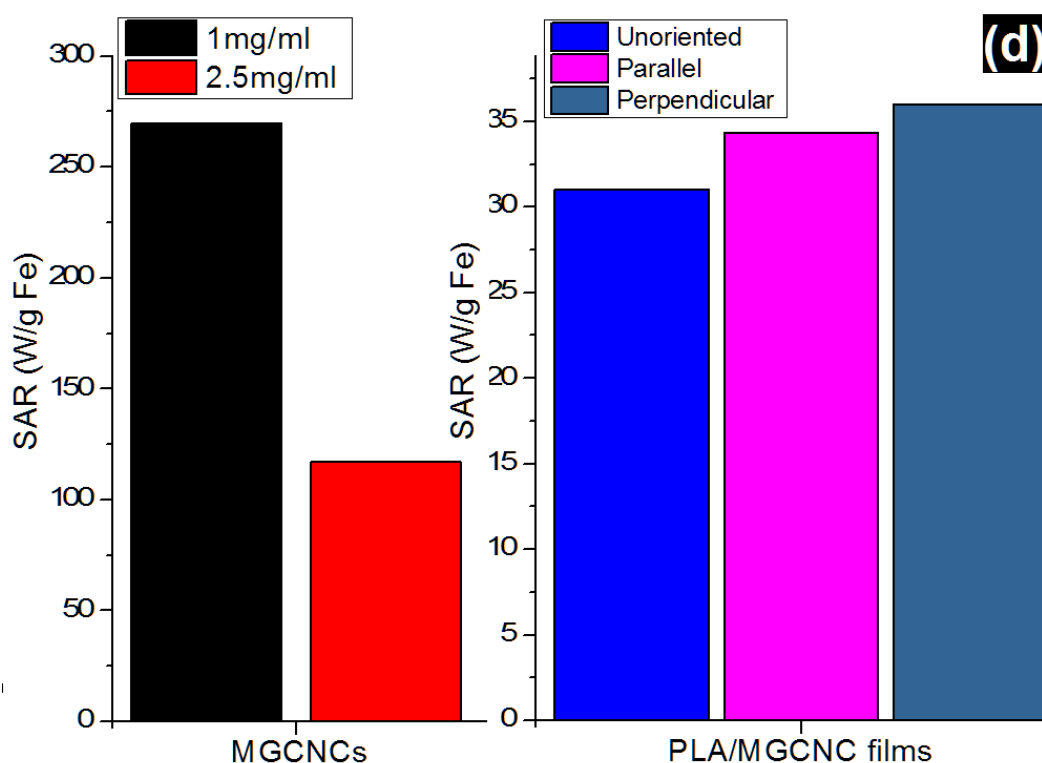


Figure 8.8: Heating curves showing the increase in temperature when exposed under alternating magnetic field of 300e at 2.8 kHz frequency applied for a fixed duration of ~660 seconds for (a) MGCNCs dispersed in water at 1 and 2.5mg/ml concentrations respectively. Hyperthermia behaviour of PLA/MGCNC (~3wt.% loadings) films at two different weights (b)~50mg and (c)~100mg respectively, without any orientation and with perpendicular or parallel alignment of MGCNC fillers in the polymeric matrix. (d)Comparison of specific absorption rate (SAR) for the MGCNCs dispersed in water and PLA/MGCNC films under different orientation of MGCNCs.

The enhanced hyperthermia properties for the oriented samples is due to the presence of interconnected columnar structures of anisotropically aligned MGCNCs (as shown in Figure 8.3), which improves the heat transfer rate from the magnetic materials to films surface.

Such biodegradable and biocompatible films, with self-heating ability in the presence of alternating magnetic fields, can have potential applications in areas related to medical diagnostics, in the development of sutures or bandages with improved anti-inflammatory properties, as membranes with reduced bio-film formation and in electronics as efficient heat exchangers. Therefore, it can be concluded that magneto-responsive CNCs can be used efficiently as hyperthermia agents, with enhanced heat generation both in dilute suspension as well as when embedded in polymeric matrix.

8.2.8 Functional Laminated Films Processed using Anisotropic PLA/MGCNC Nanocomposites

We demonstrate the potential applications of anisotropic PLA/MGCNC nanocomposites by laminating different layers of films oriented at two different conditions (as shown in Figure 8.9(a) & (b)) using a rotating hot press. Polymer lamination technology is an industrially viable approach in which stacked layers of polymeric films are hot pressed under a roller to form superior polymeric products for day-to-day applications. Therefore, in our study, we laminated PMGCNC || films in such a way that in one case the MGCNCs are aligned parallel to each other and in latter case films are laminated such that it formed a crisscrossed MGCNCs (one layer of MGCNC is perpendicular to other layer) forming a squared-network like structure. The presence of such aligned structures after the lamination was confirmed from the optical micrographs of the fabricated films as shown in Figure 8.9(a') and (b'). In the former case, the parallel red lines (as marked with black arrows) represents the parallely aligned MGCNCs confirms that orientation is retained in the final laminated composite (Figure 8.9(a')). In the latter case, optical micrographs shows the presence of crisscross structures (with the parallel MGCNCs represented by black arrows and perpendicular MGCNCs represented by blue

arrows), which is due to the presence of two different orientations of MGCNCs (one parallel and other perpendicular) forming an interlocked network structure in the laminated films. As a conceptual study, we used these aligned bilayer laminated composites and investigated their thermo-mechanical behavior over a wide temperature range. DMA analysis showed that the elastic modulus of the parallelly laminated PMGCNC||films are ~1.5 times higher than the unoriented PMGCNC laminated films. As discussed in previous section, the higher modulus of CNCs along the axial direction as well as the presence of the improved interaction between the two parts lead to the formation of mechanical locking thereby improving the mechanical properties significantly. Because of the presence of mechanical locking between the parallelly aligned MGCNCs, it leads to the formation of high stress bearing zones at their contact sites leading to improved reinforcing effect. Interestingly, we found the PMGCNC||films underwent drastic increase in elongation after it crossed the glass transition temperature compared to the unoriented sample. We find that by simply using two layers of the oriented PMGCNC||films we could tune in desirable mechanical properties into the final laminated nanocomposites. Therefore, the proposed concept could be exploited for laminating multiple layers of films with oriented nanofillers to fabricate nanocomposites with programmed mechanical properties in a specific direction to multi-directions. Figure 8.9(d), shows an anisotropic PMGCNC||laminated film containing the IIT Guwahati logo fixed in between. This polymer lamination technology using the anisotropic polymeric nanocomposites could be used for manufacturing packaging based products, especially for sensor based active food packaging, laminates or bio-inspired human body parts (knees, bone joints etc.) with accurately tuned reinforcing efficiency in three dimensions. The proposed concept could be further extended to introduce multifunctional anisotropic thermal, magnetic, optical and electrical

behaviors in the nanocomposites through accurately tuning the alignment of the anisotropic MGCNCs using magnetic field.

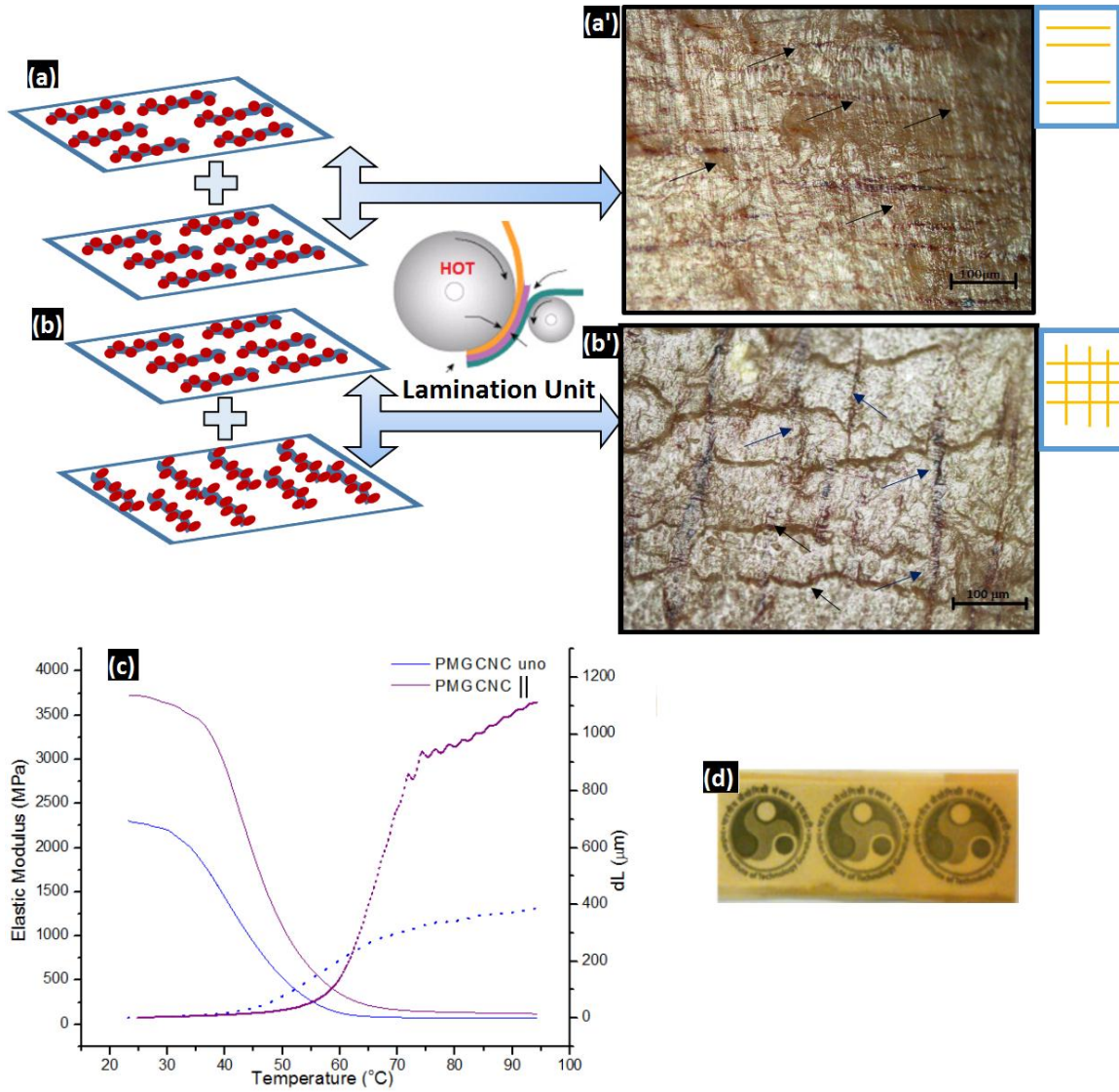


Figure 8.9: Schematic illustrations of (a) parallel alignment and (b) cross alignment of two PMGCNC|| films during lamination process. Optical micrographs showing (a') parallel aligned (marked in black arrows) and (b') cross-aligned MGCNCs (parallelly aligned marked in black arrows and perpendicular MGCNCs are marked in blue) (c) Dynamic mechanical analysis of the parallelly aligned laminated films as

shown in (a) and unoriented PMGCNC films (d) Laminated PMGCNC || films with a white piece of paper in between for visual display.

8.3 CONCLUSION

Magnetic cellulose nanocrystals (MGCNCs) were successfully fabricated by a simple one step co-precipitation technique, during which high content (~51 wt. %) of Fe_3O_4 nanoparticles of dimensions ~18–30 nm were adsorbed on CNCs. MGCNCs were further characterized through XRD, FTIR and Raman spectroscopy studies to understand the mechanism of precipitation of Fe_3O_4 nanoparticles and their interaction with the hydroxyl groups of CNCs. VSM studies showed that the fabricated MGCNCs were superparamagnetic in nature with very high magnetization values of ~50.5 emu/g, thus incorporating magneto-responsive behavior into CNCs. Surface modification of CNCs with the Fe_3O_4 nanoparticles enhances the dispersion of MGCNCs into PLA through simple sonication approach. The MGCNCs were further aligned (in parallel and perpendicular directions relative to the film surface) through directionally tunable magnetic field. The alignment of the MGCNCs in the PLA matrix was confirmed through FESEM morphological analysis followed by the EDX mapping. FTIR and XRD studies showed improved interaction of the MGCNCs with PLA matrix along with improvement in the overall crystallinity of nanocomposites under aligned conditions. Alignment of MGCNCs in PLA matrix (in parallel and perpendicular directions) resulted in incorporation of anisotropic behavior in the mechanical, thermal and electrical properties of the fabricated nanocomposites. Mechanical properties of the parallelly aligned samples (TS and % elongation improved by ~70 and 240%) were found to be highest followed by the perpendicularly aligned (TS and % elongation improved by ~58 and 172%) and unoriented PLA/MGCNC (1 wt. % loading) nanocomposites. Further, the Cox and Halpin-

Tsai models were used to determine the degree of orientation of MGCNCs in PLA matrix which confirmed the presence of complete alignment, although the MGCNCs self-assembled into high aspect ratio fibers in the presence of magnetic field. Thermo-mechanical studies showed improvement in the elastic modulus and the glass transition temperature (by $\sim 15^{\circ}\text{C}$) for the oriented samples. Orientation of MGCNCs in the parallel and perpendicular directions significantly altered the thermal properties as evident from DSC studies which show complete absence of the cold crystallization peaks. Alignment of MGCNCs in parallel and perpendicular direction led to improvement in the percentage crystallinity by ~ 11 and 4% respectively. Interestingly, the orientational effect of MGCNCs also led to incorporation of the anisotropic electrical and magnetic properties in the fabricated nanocomposites which can have potential applications in self-biased electronic products. . MGCNCs when dispersed in water or embedded in polymeric (PLA) matrix showed enhanced hyperthermia behaviour with considerable rise in temperature of $\sim 16^{\circ}\text{C}$ and $\sim 12^{\circ}\text{C}$ respectively, in less than 10 min. We also found that these anisotropic polymeric films could be laminated into novel products with accurately tuned three-dimensional reinforcing efficiency by application of magnetic field. Thus, this study provides a novel, low cost approach of orienting CNCs in the polymer matrix, thereby leading to incorporation of anisotropic behavior in the nanocomposites which is a critical requirement for fabrication of advanced materials for high performance applications.

Conclusions and Future Scope

This chapter summarizes the key findings and inferences drawn from the research work, and provides the perspectives for future studies on cellulose nanocrystals.

9.1 CONCLUSIONS

The main objective of the research work was to utilize the inherent characteristics of cellulose nanocrystals (CNCs), followed by their strategic modifications to develop high performance polymeric nanocomposites and advanced functional materials with CNCs as the building blocks. The major innovations and diverse applications of the surface-modified CNCs presented in this thesis include: (i) the fabrication of CNCs from wide range of renewable resources including newspaper with a strategy to convert ‘waste to wealth’, followed by its utilization as reinforcing agents (ii) to develop completely ‘green’ and biodegradable polymeric nanocomposites for food packaging applications, (iii) iron nanoparticle-modified CNCs with self-propagating behaviour for targeted drug delivery vehicles, (iv) as catalysts for bioremediation and conversion of chemical moieties, and (v) for fabrication of hydrogen storage devices. The thesis has addressed some fundamental issues related to the processing of CNCs and bio-based polymer/CNC nanocomposites, which has the potential to scale-up through industrially viable techniques. The key achievements reported in this regard are: (i) indigenously developed CNC production unit-*cum*-continuous dialysis system which reduces the CNC processing time from 7 days to 24–26 hours, and (ii) reactive extrusion of PLA-based CNC nanocomposite films with improved physicochemical and structural properties required for food packaging applications, which has been successfully tested on a pilot-plant scale

extruder. The research work presented in this thesis focusses on the fabrication and functionalization of CNCs through sustainable routes to tailor the surface properties for diverse applications. The major findings and conclusions drawn from the research work are summarized next.

- CNCs have been fabricated by using various renewable biomass resources such as bamboo stems, rice husks, commercial pulp, and waste newspaper. The CNCs thus obtained have distinct rod-like morphology with the dimensions depending on the initial cellulosic precursor. It has been observed that by simply varying the concentration of the hydrolysing agents, various varieties of nanocelluloses such as cellulose nanofibers (CNFs), CNCs and cellulose nanoparticles can be developed through simultaneous decoloration and acid hydrolysis of waste newspaper. CNCs have been fabricated with different cellulose polymorphs and through utilization of various acids as hydrolysing agents. The fabricated CNCs show significant variation in structural, physicochemical, thermal and surface characteristics. An indigenously developed CNC production unit equipped with continuous dialysis system has been developed, which successfully overcomes the techno-economic challenges reducing both the processing time as well as environmental hazards associated with CNC processing.
- The fabricated CNCs have been successfully dispersed into microbially derived polyhydroxybutyrate (PHB) to develop green and sustainable polymeric nanocomposites with improved intrinsic properties and biodegradability. CNCs formed percolating network-like structure in the PHB matrix through intra/intermolecular hydrogen bonding, which led to significant improvement in the thermal and mechanical properties, processing ability and rheological behaviour of PHB. In addition to the improved barrier properties

and non-toxic nature, the developed PHB/CNC films show overall migration values within the standard limits as per the set legislations, which makes them an ideal alternative to non-degradable petroleum-derived packages for potential food packaging applications.

- CNCs fabricated with different cellulose polymorphs and obtained from hydrolysis with different acids have been shown to significantly alter the structural, physicochemical, thermal and barrier properties of PLA when dispersed through either solution-casting or extrusion-based approach. CNCs with different cellulose polymorphs have significantly altered intermolecular hydrogen bonding which results in significant variation in their reinforcing capability. This results in improved structural and barrier properties for the PLA/CNC II nanocomposites, with CNC II (containing cellulose II polymorph) as the filler. CNCs obtained from hydrolysis of cellulose with different acids have different anionic moieties on their surface, resulting in tunable crystallization as well as dispersion in PLA matrix. Both polymorphic and acid-derived CNC types show significant variation in their percolating-network forming ability, and exhibit different thermal and structural properties. Therefore, the study presented in this chapter provides a sustainable approach of modifying the surface of CNCs, which leads to tailored properties of the resulting nanocomposites that can be finely tuned depending on the targeted applications.
- Processing of PLA/CNC nanocomposites through extrusion is a challenging task due to the thermal and shear-induced degradation as well as non-recyclability, which limits the application of these nanocomposites for development of value-added products through industrial routes. A single-step reactive extrusion-based strategy of processing PLA/CNC films has been developed which results in improved melt strength along with enhanced physicochemical and structural properties. In comparison to the traditional process, the

PLA/CNC films processed through this approach have considerably improved processability, dimensional stability along with reduced thermal or shear-induced degradation of both CNCs and PLA chains. During reactive extrusion, PLA chains grafted on the CNC surface through C-C chemical cross-linkages, leading to the formation of grafted gel-like structures with high relaxation time, which results in reduced necking behaviour and improved film properties. Interestingly, the reactively grafted PLA/CNC films show improved barrier properties and have overall migration values within the standard limits. The processed films show promising results for the storage of oil-based and dairy-based beverage products with a shelf life of ~5 months and ~2 weeks respectively. Therefore, this study provides a novel approach of processing the sustainable and biodegradable PLA/CNC films through an industrially scalable process, resulting in improved structural stability, processability and adequate physicochemical properties required for food packaging applications.

- The abundant hydroxyl functional groups on the surface of CNCs provide sites for modification with different iron nanoparticles such as zerovalent iron (Fe-CNC) and iron oxide nanoparticles (Fe_2O_3 -CNC) along with catalytically active species such as Pd and Pt nanoparticles (Pd/Pt- Fe_2O_3 -CNC). CNC-supported zerovalent iron nanoparticles show improved stability with potential applications as biocatalysts for bioremediation and chemical conversion of moieties, and as targeted drug delivery agents owing to the autonomous motion in the presence of chemo-magnetic field gradients. The fabricated Pd/Pt- Fe_2O_3 -CNCs and Fe_2O_3 -CNCs, where the nanoparticles are supported on the CNCs, can be employed as structure directing agents for rolling planar graphene sheets into nanoscrolls with tunable morphology and encapsulated nanoparticles in its core. Thus,

CNCs derived from renewable biomass resources such as bamboo stem have been used as catalysts in bioremediation, as potential drug delivery vehicles, and as structure directing agents for the fabrication of hydrogen storage devices.

- CNCs are inherently helically-shaped nanocrystals with anisotropic mechanical behaviour where $E_{\parallel} \sim 110\text{--}220$ GPa and $E_{\perp} \sim 2\text{--}50$ GPa. This anisotropic material has been used to develop PLA-based nanocomposites with directionally tunable properties. Surface modification of CNCs with Fe_3O_4 nanoparticles (MGCNCs) leads to incorporation of magneto-responsive behaviour in CNCs, which has been used to orient them in the polymeric matrix through application of low magnetic fields. Magnetic field-assisted alignment of MGCNCs in parallel and perpendicular directions results in anisotropic mechanical, thermal, electrical and magnetic properties of the nanocomposites. The anisotropic films show excellent magnetic hyperthermia behaviour with the capability to heat up in the presence of alternating magnetic fields. Such self-heating feature of the PLA-based biodegradable films with incorporated anisotropic characteristics has potential applications in the manufacturing of three-dimensional composites with microstructural features comparable to biological materials.

9.2 SCOPE FOR FUTURE WORK

The work presented in the thesis can be further advanced in several directions as listed below:

- The continuous dialysis system of CNCs can be integrated with other acid separation systems such as filtration in series which will further reduce the CNC processing time. Novel ways for recycling of the acids for further extraction of nanocellulose and utilization of the sugar derivatives produced during hydrolysis in energy generation such as in glucose derived fuel-cells will improve the LCA assessment of CNC production.
- Fabrication of PHB/CNC films through novel extrusion approaches followed by its biodegradability tests, shelf-life prediction for the storage of food products and biocompatibility needs to be studied.
- Self-propelling magnetic CNCs can be loaded with drugs/biomolecules and their ability to release the drugs at targeted sites needs to be studied alongwith their cytotoxicity in presence of various cell lines.
- Graphene Nanoscrolls can further be tested for their potential applications as charge storage devices as Li-ion batteries.
- CNCs modified with metallic Pt and Pd nanoparticles can be potentially used as catalyst in fuel cells or for hydrogen storage devices.
- Anisotropic polymeric nanocomposites with biodegradable, biocompatible and non-toxic characteristics can be used to develop complex biological structures using 3D printing techniques through magnetic orientation of nanostructures.

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Research Output

Patent Application

1. Vimal Katiyar, Amit Kumar and Prodyut Dhar, Poly (lactic acid) Nanocomposites formulation and method of making thereof. Indian Patent Application Number: 201631022079.

Journal Articles

1. **Dhar P**, Kumar A, Katiyar V. Magnetic Cellulose Nanocrystal Based Anisotropic Polylactic Acid Nanocomposite Films: Influence on Electrical, Magnetic, Thermal, and Mechanical Properties. *ACS Applied Materials & Interfaces* 6; 8(28):18393-409, 2016.
2. **Dhar P**, Bhasney SM, Kumar A, Katiyar V. Acid functionalized cellulose nanocrystals and its effect on mechanical, thermal, crystallization and surfaces properties of poly (lactic acid) bionanocomposites films: A comprehensive study. *Polymer* 101, 75–92, 2016.
3. **Dhar P**, Tarafder D, Kumar A, Katiyar V. Effect of cellulose nanocrystal polymorphs on mechanical, barrier and thermal properties of poly (lactic acid) based bionanocomposites. *RSC Advances* 5 (74):60426-40, 2015.
4. **Dhar P**, Tarafder D, Kumar A, Katiyar V. Thermally recyclable polylactic acid/cellulose nanocrystal films through reactive extrusion process. *Polymer* 22; 87:268-82, 2016.
5. **Dhar P**, Kumar A, Katiyar V. Fabrication of cellulose nanocrystal supported stable Fe (0) nanoparticles: a sustainable catalyst for dye reduction, organic conversion and chemo-magnetic propulsion. *Cellulose* 1; 22(6):3755-71.
6. **Dhar P**, Gaur, S S, Gupta A, Narendaren S, Bhasney S M and Kumar A, Katiyar V. “Reactive Extrusion of Polylactic Acid /Cellulose Nanocrystal Films for Food Packaging

Applications: Influence of Filler type on Thermo-mechanical, Rheological and Barrier Properties”, *Industrial & Engineering Chemistry Research* 2017, (Accepted).

7. **Dhar P**, Bhardwaj U, Kumar A, Katiyar V. Poly (3-hydroxybutyrate)/cellulose nanocrystal films for food packaging applications: Barrier and migration studies. *Polymer Engineering & Science*. 1; 55(10):2388-95, 2015.
8. **Dhar P**, Bhardwaj U, Kumar A, Katiyar V. Investigations on rheological and mechanical behavior of poly (3-Hydroxybutyrate)/ cellulose nanocrystal based nanobiocomposites. *Polymer Composites*. 2015.
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10. V Katiyar, **P Dhar** and A Kumar, “Cellulose nanocrystals: Potential nanofiller for food packaging and catalytic applications”, *J Material Sci Eng* 2015, 4:4 (doi: 10.4172/2169-0022.S1.023).
11. **Dhar P**, Kumar A and Katiyar V, “Improved structural properties of polyhydroxybutyrate composites”, *SPE Plastics Research Online*. (Invited feature article in category of Plastics Nanotechnology) 2016 Feb. (doi: 10.2417/spepro.006379).
12. Devi RR, **Dhar P**, Kalamdhad A, Katiyar V. Fabrication of Cellulose Nanocrystals from Agricultural Compost. *Compost Science & Utilization*. 2015 Apr 3; 23(2):104-16.
13. **Dhar P**, Gaur S., Chakroborty G., Kumar A., & Katiyar V. “Cellulose Nanocrystal based templates for fabrication of Graphene nanoscrolls with high hydrogen storage capacity: An experimental and molecular dynamics study.” (in process of submission).

14. **Dhar P**, Gupta A, and Kumar A, Katiyar V. “Nucleating, Anti-Nucleating and Synergistic Nucleation of the Reactively Extruded Polylactic Acid based Cellulose Nanocrystal Films”. (Manuscript under preparation).

Book Chapters

1. **Dhar P**, Bhardwaj U, Kumar A and Katiyar V, book chapter titled as 'Cellulose Nanocrystals: A Potential Nano-filler for Food Packaging Applications' Food Additives and Packaging, Chapter 17, pp 197–239, American Chemical Society Symposium Series, Vol. 1162, ISBN13: 978084123024.
2. Bhardwaj U, **Dhar P**, Kumar A and Katiyar V, book chapter titled as 'Polyhydroxyalkanoates (PHA)-Cellulose based Nanobiocomposites for Food Packaging Applications, Food Additives and Packaging, Chapter 19, pp 275–314, American Chemical Society Symposium Series, Vol. 1162, ISBN13: 978084123024.
3. **Dhar P**, Bhardwaj U and Katiyar V, book chapter titled as ‘Bio Based Nanocomposites: Prospects in Green Packaging Applications' in book 'Polymers for Packaging Applications'. (CRC Press, Taylor & Francis Group, ISBN: 9781926895772.
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Conference Presentations

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