

Bio-based nanocomposites from poultry feather keratin

by

Manpreet Kaur

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ABSTRACT

Chicken feathers are byproduct of poultry industries and except some minor low end application, they are generally dumped into landfills. An effort has been made to utilize this by-product by transforming them into nano-reinforced bioplastics which is not only a solution to feather waste disposal but also an attractive option to develop high performance nano-structured biomaterials from renewable and sustainable bioresource. To obtain bionanomaterials from these feathers, in-situ nanodispersions and modifications of feathers were carried out to obtain bionanocomposite (BNC) powders suitable for processing by compression molding technique. The obtained BNC powders were then simultaneously plasticized, cross-linked and processing conditions were optimized for film formation. Nano-fillers such as layered silicates and cellulose nanocrystals were used as nano-reinforcements for enhancing the material properties of the obtained films. The obtained bio-nanocomposite films were characterized by various analytical techniques. The thermal properties were analyzed by thermogravimetric analysis (TGA) and Differential Scanning Calorimetry (DSC). The thermomechanical properties of the samples were also investigated by Dynamic Mechanical Analysis (DMA) over a temperature range of -50 °C to 150 °C. The glass transition temperatures for CNC reinforcements were found to be higher (225 to 242 °C) than MMT (199 to 221 °C) reinforcement. Slight increase in glass transition temperatures has been observed with increase in MMT content from 1% to 5% while bio-nanocomposites with 1% and 10% CNC content showed improvements in glass transition temperatures. Structural analysis was carried out by wide angle X-ray diffraction (WAXD) and transmission electron microscopy (TEM). The arrangement of MMT in the bio-nanocomposite matrix ranged from intercalated to exfoliated at low content of the MMT (1% and 3%). However WAXD and TEM analysis displayed aggregations at higher concentration (10%) of both MMT and CNC nanoparticles. The mechanical properties of

biomaterials were investigated by Universal Testing Machine (autograph AGS-X Shimadzu, Canada) instrument. The two nanoparticles had different effect on tensile strength and elongation at break of the nanocomposites. MMT enhanced the tensile strength while CNCs incorporated samples showed higher percent elongation. The preliminary investigations on biodegradability of these biomaterials were also carried out using soil burial tests. The onset of degradation was found to be soil moisture dependent with ~ 25-30% weight loss within 10 days.

PREFACE

This thesis holds the original work done by Manpreet Kaur and has been written according to the guidelines for a paper format thesis of the Faculty of Graduate Studies and Research at the University of Alberta. The concept of this thesis originated from my supervisor Dr. Aman Ullah. The thesis consisted of three chapters: Chapter 1 provides a general introduction on the chicken feathers keratin and nanotechnology and the objectives of the thesis; Chapter 2 shows the comparative studies of two nanoparticles namely Montmorillonite and Cellulose nanocrystals on the material properties of chicken feather keratin based films; Chapter 3 is the biodegradation of the feather keratin based bio-nanocomposites reinforced with nanoparticles.

Dr. Aman Ullah greatly contributed to the experimental design, data interpretation, thesis preparation and edits. I was responsible for literature search relevant for the above studies, designing and performing laboratory experiments, data collection and analysis, and drafting the thesis.

DEDICATION

Dedicated to my beloved parents

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LIST OF ABBREVIATIONS

ATR-FTIR	Attenuated total reflectance-Fourier transform infrared spectroscopy
BE	Binding energy
CFK	Chicken feather keratin
CFF	Chicken feather fiber
CNC	Cellulose nanocrystals
DMA	Dynamic mechanical analysis
DSC	Differential scanning calorimetry
DTG	Derivative thermogravimetric
E	Elongation at break
MMT	Montmorillonite
PLA	Poly-lactic acid
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
T _g	Glass transition temperature
TG	Thermogravimetric
TGA	Thermogravimetric analysis
T _m	Melting temperature
TS	Tensile strength
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction

CHAPTER 1. INTRODUCTION AND OBJECTIVES

1.1 Introduction

Degradable and renewable nature of bio-polymers has led to renewed interest in replacing the plastic packaging derived from petro-based sources. The disposal problems and pollution created by plastic materials has been increased in last 50 years. It can be minimized to some extent by replacing petro-based packaging with bio-based packaging materials, which are renewable and environmentally friendly. Biopolymers are the polymers derived from natural resources such as proteins, cellulose, starch and other polysaccharides. These can be synthesized chemically or can be prepared from natural monomers such as lactic acid. Generally, the bio-polymer based packaging films are brittle in nature and are unable to meet the standards of plastics until their mechanical and barrier properties has not been improved. Therefore plasticizers are required to impart flexibility to bio-polymer based films. However addition of plasticizers decreases the tensile strength (TS) significantly (Wang, Sue & Jane, 1996).

Annually a million tons of feathers are produced as waste from poultry processing industries throughout the world. The percent of feathers are around 5- 7% of the body weight of chickens. A slaughterhouse with a capacity of 50,000 birds can produce 2-3 tons of dry feathers per day. Chicken feathers are approximately 91% protein (keratin), 1% lipids, and 8% moisture. The amino acid sequence of a chicken feather is very similar to that of other feathers and also has a great deal in common with reptilian keratins from claws (Fraser & Perry 1996). The sequence is largely composed of cysteine, glycine, proline, serine and contains almost no histidine, lysine or methionine (Schmidt, 1998). Despite its high protein concentration, feather keratin has limited applications

commercially. It is used for animal feed as dietary protein supplement, however it has poor digestibility with expensive meal production process.

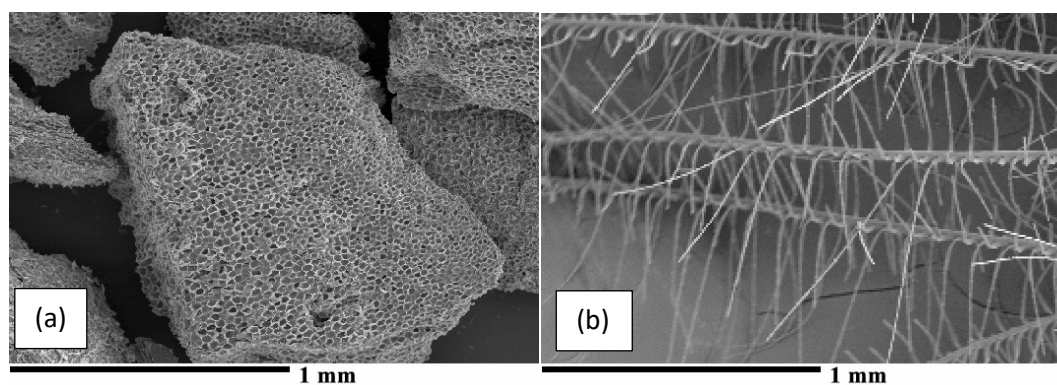
In order to successfully develop applications for chicken feathers in the realm of packaging industry, the material properties must be enhanced. Bio-nanocomposites (bio-polymer matrix reinforced with nanoparticles) have become promising solution to enhance the material properties of bio-polymers. This technique has been a success in polymer composites and elevated the polymer materials to a whole new level of materials properties. Nanoparticles are particles having at least one dimension in nanometer range (1-100nm) and high aspect ratio. Due to their high surface area bio-nanocomposites exhibit improved properties (Ray & Bousmina, 2005; Rhim & Ng, 2007; Zhao, Torley & Halley, 2008). The most commonly used nanoparticles are layered silicates such as montmorillonite (MMT), hectorite, saponite, and laponite. These clay minerals are famous for their unique structure and high elastic modulus (178 GPa). Nano-particles can improve mechanical properties of biopolymers by carrying a significant portion of the applied stress due to high elastic modulus (Zeng, Elder & Ragauska 2005; Fornes & Paul, 2003).

1.2 Chicken feather keratin overview

An enormous quantity of keratins in the form of hairs, feathers, horns and hoofs is available. Keratins are broadly classified as hard (5% sulfur) and soft (1% sulfur) keratins. Keratin is mechanically robust and chemically unreactive due to tight packing of protein chain in the form of α -helix or β -sheet into a supercoiled polypeptide chains crosslinked with di-sulfide bonds. Acid, alkali or enzymes can hydrolyze keratin and the obtained hydrolysates have number of applications.

The chicken feather keratin contains number of amino acids such as glycine, alanine, serine, cysteine and valine and lower content of lysine, methionine and tryptophan. The flexible nature of

keratin fibrous tissues provides toughness to the keratin. Also it is possible to dissolve chicken feather keratin into natural protein by using alkali or acid. The proteins are soluble in alkali or acid and then can be digested by trypsin and pepsin. In case of feathers the β -keratins have β -pleated sheets twisted together which are stabilized and hardened by disulfide bonds. To make the protein soluble, these disulfide bonds should be broken which in turn reduce the strength of keratin. The approximate molecular mass for β -keratin is about 10kDa and α -keratin is subdivided into two categories which are acidic keratins with 40-50kDa and neutral/basic keratins with 55-65kDa (Gillespie, 1990). There are five types of feathers such as contour, down, semiplume, filoplume and bristle. Schmidt and Line reported 30 macroscopically different poultry feathers (Schmidt & Line 1996). Winandy *et al.* reported that large contour feathers are half feather fibre and half quill by mass (Winandy, Muehl, Micales, Raina & Schmidt, 2003). This information was useful because the proprietary processing technologies yield two fractions, fiber and quill as shown in figure 1.1. Fiber keratin and quill keratin are two characteristic forms of microcrystalline keratin in feathers. Fibers are hollow and quill is hard (Hong & Wool, 2005). Figure 1.1 shows the keratin fiber diameter of 50 μm as reported by Misra (Misra, 2001). The scanning electron microscopic images of outer quill (c), inner quill (d), and fiber (e) are given in figure 1.1.



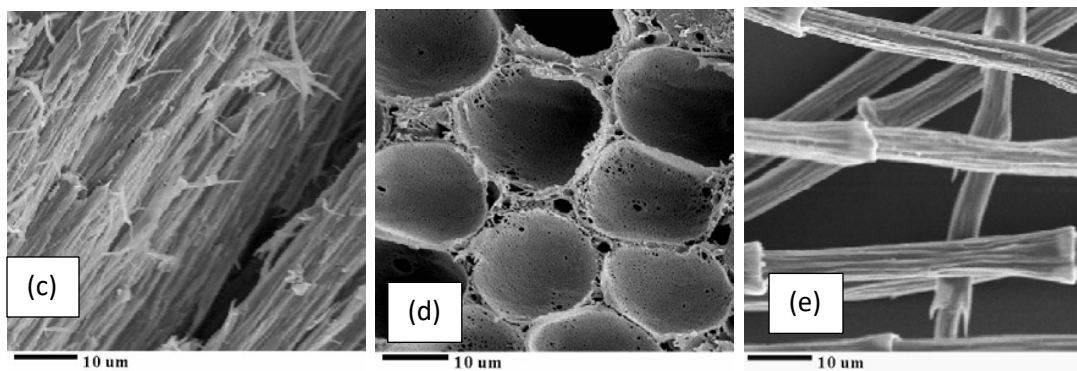


Figure 1.1. Scanning electron micrographs showing chicken feather : (a) inner quill; (b) fiber; (c) outer quill; (d) inner quill, and (e) fiber. (adapted from Misra, 2001)

The keratin can be extracted by different methods. The extraction of wool keratins was carried by Yamauchi *et al.* (Yamauchi, Yamauchi, Kusunoki, Khoda & Konishi, 1996). They have used aqueous solution of urea, 2-mercaptoethanol and sodium dodecyl sulfate (SDS). Increase in extraction yield and accelerated extraction was observed with SDS. It was found that the surfactant forms a complex with keratin and stabilizes the aqueous protein solution even after the urea was removed through dialysis with water containing 2-mercaptoethanol (0.08%). The amino acid composition of the feather keratin is provided in table 1.1.

Table 1.1. The amino acid composition of chicken feathers

Amino acid	$\mu\text{M}/\text{mg Protein}^*1$	% Amino acid
Aspartic acid	0.358	4.76
Threonine	0.345	4.11
Serine	1.292	13.57
Proline	0.875	1.01
Glutamic acid	0.624	9.18
Glycine	1.008	7.57
Alanine	0.411	3.66
Valine	0.618	7.24

Cystine	0.088	2.11
Methionine	0.017	0.025
Isoleucine	0.376	4.93
Leucine	0.570	7.48
Tryosine	0.102	1.85
Phenylalnine	0.267	4.11
Lysine	0.039	0.57
Histidine	0.001	0.016
Arginine	0.377	6.57

*based on sample as 100% protein

1 micro mole per milligram of protein (adapted from Gupta *et al.*, 2011)

1.2.1 Applications of chicken feathers

Various potential applications have emerged due to recent interest in transforming the chicken feathers into useful products. This led several researchers to investigate the fundamental properties of the feathers and to modify them for specific applications. The use of chicken feather as a contaminant adsorbent is one of the current area of research. Paper, erosion control fabrics and composite applications are also under investigation.

The native or processed fibers from feathers have also been tested for composite applications. Cheng *et al.* investigated the mechanical and thermal properties of chicken feather fiber/PLA green composites. Poly (lactic acid) (PLA) composites reinforced with chicken feather fiber (CFF) were processed by twin-screw extruder and an injection molder. They have prepared composites with different feather fiber content (2, 5, 8 and 10 wt%). The tensile strength of pure PLA was found to be lower than that of composites with chicken feather fiber. In case of 5% CFF highest strength of 4.2 GPa was obtained without any substantial change in weight. The morphology of composites investigated by scanning electron microscopy (SEM) showed uniform dispersion of CFF in composites. The thermal stability of PLA composites was enhanced with the addition of

CFF as indicated by thermogravimetric analysis (TGA). The results obtained from this study can help in further research for the formation of environmentally friendly composites from biodegradable polymers (Cheng, Lau, Liu, Zhao, Lam & Yim, 2009)

Keratin protein has fibrous matrix which act as nano-filtering sponge can be used for cleaning industrial effluents by the process of adsorption. The chicken feather fiber has a network of 0.05-0.10 μm nanopores arise from partial hollow structure resulting in high surface area of 12 m^2/g . Presence of various active functional groups in the feathers provides them unique capability to bind the heavy metals from waste water. Misra *et al.* investigated the metal uptake by the chicken feather fiber and found that pH, temperature and amount of keratin fiber are the factors which affect metal uptake (Misra, Kar, Priyadarshan & Licata, 2001). The metal cations which were effectively removed by feather fiber were chromium, copper, lead, mercury, nickel and zinc. Khosa and Arshad *et al.*, investigated modified feather keratin as sorbent for arsenic and other contaminates from waste and oil sands process affected water. The ground chicken feathers were chemically modified to examine the effect in arsenic removal from water. The four different dopants were used. The modified chicken feathers found to be effective biosorbents with high As(III) removal capacity of upto 11.5×10^{-2} and 11.0×10^{-2} mg/g from 100 ml arsenic polluted water solution respectively.

Al-Asheh *et al.* performed comparative studies of binding properties of natural feathers and chemically-activated feathers. They treated one group of feathers with alkaline solutions of NaOH and Na₂S and other group was treated with SDS, an anionic surfactant. At lower concentration of copper and zinc, non-activated feathers with SDS were effective than the activated feathers. However at higher concentration of zinc, activated ones displayed better results and found to be best adsorbers (Al-Asheh, Banat & Al-Rousan, 2003).

Another application which attracted attention is use in computer chips. Wool & Hong tried to replace the silicon with chicken feather keratin, a new generation microchip (Wool & Hong, 2004). Feathers have unique conducting and insulating properties which makes them suitable for this application. Circuit boards produced with chicken feathers found to be lighter in weight (50%) and feather fiber chip transmitting electrical signals fast as compared to silicon chips nearly as twice.(Barnes, 2002; Jacobson, 2002) However, higher flammability was concern and hampered such application. We have recently used nano-engineered keratin fiber to prepare green biocomposites with substantially enhanced flame resistance. Arshad *et al* studied the effect of polyhedral oligomeric silsesquioxanes (POSS) onto keratin biofibers by surface grafting. The modifications enhanced the thermal stability and reduction in moisture uptake.

Ye & Broughton developed a nonwoven insulation (batting) from chicken feather fiber and a small amount of binder fiber. Polyester fiber and goose down batting were compared with chicken feather fiber. The insulating properties including filling power which refers to volume a given weight of material can occupy under standard pressure was observed for all battings. Chicken feather batting was better than polyester fibers, but showed poor properties as compared to goose down batting (Ye & Broughton, 1999).

Durham has made decorative paper with 51% feather fiber and 49% wood pulp with an unusual texture and dyeing properties. This was fabricated to reduce the pressure on environment by replacing paper made from wood pulp (Durham, 2002).

George *et al.* developed geo-fabrics from latex-bonded non-woven turkey feathers for erosion control. Turkey feather fabrics showed similar light and water transmittance as conventional products made of jute and coconut. They did not affect pH, nitrogen, or phosphorous

content of soil and actually increased moisture content while decreasing compaction (George, Bockarie, McBride, Hoppy & Scutti, 2003)

Several studies were also carried out to investigate chicken feather fiber based composites. Winandy *et al.* investigated feather fiber board composite panels with 20% to 95% amounts with phenol formaldehyde as an adhesive. The feather fiber was used as replacement for aspen fiber. The chicken feather fiber panels with 47.5% showed 27% loss in modulus of elasticity and 18% loss in bending strength as compared to 95% aspen fiber (0% chicken feather fiber). However chicken feather fiber with 95% panels showed 51% loss in modulus of elasticity and 39% loss in bending strength. The panels with feather fiber demonstrated significant improvement in water absorption resistance linked with swelling and mold growth. The possible reason may be the hydrophobic elements in keratin's amino acid sequence. In addition 47.5% chicken feather fiber panels demonstrated 38% swelling in 2 hours and 48% water absorption. On the other hand 95% chicken feather fiber panels demonstrated 27% swelling in 2 hours and 36% water absorption (Winandy *et al.*, 2003).

1.3 Nanotechnology

Nanotechnology refers to the use and study of materials having at least one dimension in nanometer scale. At nanoscale, materials demonstrate better mechanical and barrier properties than those at the micro scale. Composite is a combination of dispersed filler in polymer matrix. While preparing nanocomposites, fillers are generally used in nanometer scale. Many researchers have reported improved mechanical and barrier properties by incorporation of nano-fillers as compared to conventional composite materials (Le Corre *et al.*, 2010; Tang *et al.*, 2008). Bio-nanocomposite refers to dispersion of inorganic or organic nanoparticles in a biopolymer matrix, which has a tendency to improve mechanical, barrier and insulation properties of natural biopolymer-based

packaging films (Arora & Padua, 2010; de Azeredo, 2009). Poor thermal, mechanical and barrier properties are a challenge for developing high performance bio-based packaging films. Clay-based nanoparticles improved the tensile strength and the barrier resistance against gas, moisture and volatiles as reported for bio-degradable materials. (Arora & Padua, 2010; Weiss *et al.*, 2006). Thus, biopolymer-based nanocomposites have potential for novel packaging materials to substitute the petroleum-derived plastics.

1.3.1 Layered silicates

Different nano-particles based on their physical shapes, including spheres, rods, plates, particles, etc. have been proposed by researchers. The composition, dimension, and shape of nanofillers with their spatial distribution in the matrix, are important factors which affect the properties of resulting nanocomposite products (Capek, 2006). The most commonly used layered silicates are smectites and montmorillonites (MMT). Due to their availability and low cost they have been used to enhance the material properties of protein-based materials. Increase in mechanical strength has been reported by incorporating these nanoparticles (De Azeredo, 2009). Montmorillonite (MMT) is naturally occurring clay and has been commonly used to develop polymer nanocomposites (Arora & Padua, 2010; Capek, 2006). MMT consists of a layer of octahedral sheet sandwiched with two silica tetrahedral parallel layers. The different layers are attracted with weak electrostatic forces due to negatively charged surfaces (Essington, 2003; Kumar, 2009; Tang, Alavi & Herald, 2008). MMT has high elastic modulus (178 GPa), high surface area (750 m²/g), and high specific aspect ratio (50-1000). These physical characteristics contribute to both enhanced mechanical and barrier properties of nanocomposite materials (de Azeredo, 2009; Essington, 2003; Gunister, Pestreli, Unlu, Atici & Gungor, 2007; Le Corre *et al.*, 2010). The layered silicates belong to phyllosilicate family. Also they display barrier resistance against carbon dioxide

, oxygen, moisture and volatile compounds and resistance to degradation by ultraviolet radiations. The major challenge faced by these nanofiller is dispersion within a polymer matrix due to their tendency to form aggregates (Angellier-Coussy, Chalier, Gastaldi, Guillard, Guillame, Gontard & Peyron, 2013).

1.3.2 Nanocrystalline Cellulose (NCC)

Nanocrystalline cellulose derived from acid hydrolysis of native cellulose possesses different morphologies depending on the origin and hydrolysis conditions. NCCs are rigid rod like crystals with diameter in the range of 10-20 nm and having lengths of few hundred nanometers; e.g. crystallites from tunicates and green algae have lengths in the range of a few micrometers and crystallites from wood and cotton have lengths of the order of a few hundred nanometers, while some spherical shape NCCs were also produced during the acid treatment (Zhang, Elder, Pu & Ragauskas, 2007; Wang, Ding, & Cheng, 2008), therefore the relative degree of crystallinity and the geometrical aspect ratio, i.e. the length to diameter (L/d) are very important parameters controlling the properties of NCC-based materials. An important characteristic of NCCs, when prepared in sulphuric acid is that they possess negative charges on their surface due to the formation of sulphate ester groups during acid treatment, which enhances their stability in aqueous solutions. In order to characterise the morphology of NCC, various types of analytical techniques can be used. The most conventional and common one is the transmission electron microscopy (Araki, Wada & Kuga, 2001) which directly provide a high resolution images. Moreover, scanning electron microscopy (SEM) (Miller & Donald, 2003), atomic force microscopy (AFM) etc are also helpful for their characterization. The use of NCC as reinforcement in polymeric matrix of nanocomposite materials could be a potential application. Favier *et al.* reported the use of NCC as reinforcing fillers in poly (styrene-co-butyl acrylate) (poly(S-co-BuA))- based nanocomposites for the first time

(Favier, Chanzy & Cavaille, 1995). After that several nanocomposites were made by dispersing NCC into wide range of polymeric matrices. The types and characteristics of NCC and polymeric matrices largely determined the properties of these cellulosic nanocomposites (Samir, Alloin & Dufresne, 2005). The dispersibility of NCC can be enhanced in organic solvents by chemical functionalization, which also expand its potential applications in various sectors.

1.4 Potential application of chicken feathers

Literature review and limited applications of the chicken feather provides the basis for investigating the feather keratin for composite formation. The renewable and large production of chicken feathers from poultry processing industries makes it attractable protein source. While some articles related to thermoplastic processing of chicken feathers are available. However, the data on chicken feathers processed thermos-plastically with the addition of nano-particles is not available. The nanoparticles can be used for enhancing the material properties of the bio-nanocomposite films. The mechanical and thermal properties of the developed bio-nanocomposites can be compared to existing films in market. This data can be used to target applications.

1.5 Hypothesis and research objectives

Chicken feathers are considered to be by-product of poultry processing industries and a source of protein which can be utilized for bioplastic application. Thermoplastic processing is a viable technique to form protein based bioplastics and simulate industrial processing of plastic films. However weak material properties of protein based films have been reported. Addition of nanoparticles may lead to improved mechanical and thermal properties due to their high elastic modulus and nanoscale dimensions. This study was undertaken with the idea that

- In-situ dispersion of nanoparticles will enhance homogeneity as compared to physical mixing approach and in turn may improve the material properties of chicken feathers keratin based bioplastics
- Homogenous dispersions may also affect the biodegradability

1.5.1 Research objectives

With the major objective of exploring chicken feather keratin potential for bio-nanocomposite formation, the specific objectives are outlined below.

1. Optimization of the processing parameters and processing aids.
2. To prepare chicken feather keratin based films with suitable plasticizer and cross-linking agent
3. To prepare bio-nanocomposite films using different nanoparticles e. Montmorillonite (bentonite) and cellulose nanocrystals.
4. To investigate the effect of nano-reinforcement i.e. Montmorillonite (bentonite) and cellulose nanocrystals on material properties.
5. To characterize the bio-nanocomposite films with different analytical techniques which include structural analysis (ATR-FTIR, XPS, (XRD), thermal analysis (TGA, DSC), mechanical analysis (DMA, Tensile testing).
6. To analyze the effect of nanoparticles on the biodegradation of the chicken feather keratin films.

The major aim of this project is to find resourceful alternative for the disposal of chicken feathers with the purpose to form feather keratin based packaging films for commercial applications. The success of this work will not only benefit poultry industries but also can provide renewable, sustainable and biodegradable material.

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CHAPTER 2. LITERATURE REVIEW

2.1 Biopolymer films

Due to various environmental concerns over petro-based polymers recent research efforts are inclined towards the use of bio-polymers as packaging materials which can be environmentally friendly and sustainable.

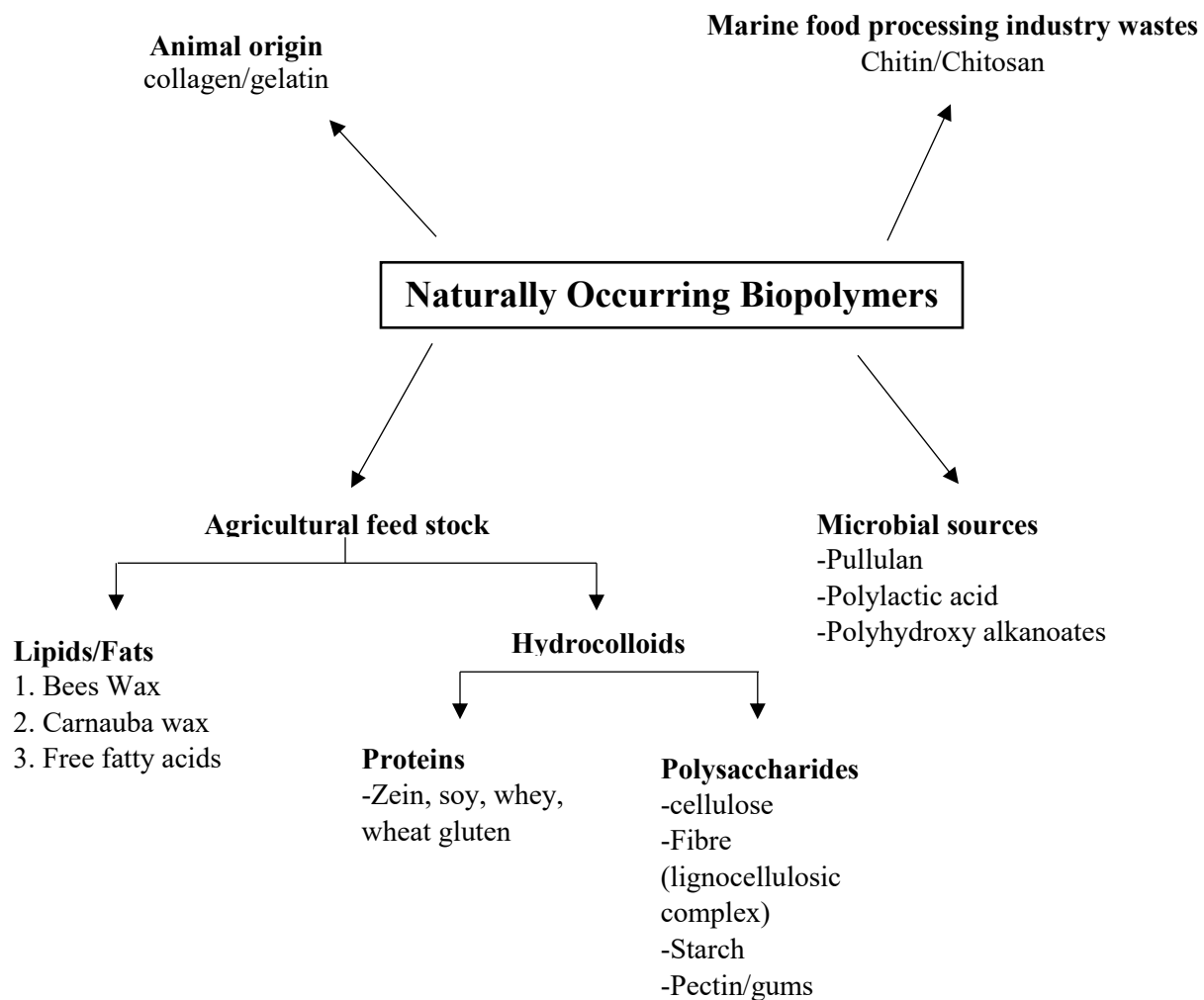


Figure 2.1. Classification of bio-polymers as packaging materials from different sources (Source: Tharanathan, 2003).

Biopolymers are classified into three categories: 1) extracted from natural raw materials (2) produced by microorganisms and (3) synthesized from bio-derived monomers. Cellulose, starch, polyhydroxyalkanoates (PHB) and polylactide acid (PLA) are among the acceptable biodegradable polymers representing these groups (Tharanathan, 2003). Classification of biopolymers is also represented in figure. 2.1.

Cellulose, starch and chitosan are among the popular polysaccharides that are investigated as packaging films. Cellulose is the most abundant polymer in nature with high crystallinity. Derivatization of cellulose is an expensive process due to its technique and difficult processing (Petersen, Nielsen, bartelsen, Lawther, Olsen, Nilsson & Mortensen, 1999). Carboxymethyl cellulose (CMC) is derived from cellulose and proved to have excellent film forming properties for packaging. It is water soluble and compatible with most of other biopolymers. High molecular weight CMC showed better barrier and mechanical properties (Tharanathan, 2003). Starch is another abundant and cheap biopolymer. It can be obtained from different sources and have thermoplastic properties. Film forming methods include extrusion, blow molding and injection molding. Starches are hydrophilic in nature which makes them moisture sensitive and have moderate gas barrier properties. Starch derived products are used in fermentation. Dextrin is used for fermenting poly(lactic) acid, which is new bio-based packaging material available in market (Petersen *et al.*, 1999). Another polysaccharide which can be used for making films is chitosan. It is also used as cross-linking agent. Packaging films from chitosan possess antifungal and anti-bacterial properties (Tharanathan, 2003).

Proteins are composed of different amino acids and classified into different levels depending upon their structure. Different levels of protein structures are primary, secondary, tertiary and quaternary. The different combinations of amino acids provide wide range of different proteins

having different functionalities. The responses of proteins to physical and chemical treatments like heat, mechanics, pressures, irradiation, liquid interfaces and metal ions differ from each other. Different types of proteins have been investigated to obtain protein based films but the most common one are soy protein, corn zein and whey proteins. However nowadays, the research has been extended to other proteins also. Protein network consists of intermolecular disulfide bonds, electrostatic bonds, hydrogen bonding and hydrophobic interactions between protein chains. Films obtained by only protein are brittle in nature, therefore plasticizers are necessary to reduce their brittleness, to impart flexibility and mobility to polymer chains. Glycerol, sorbitol, polyethylene glycol, lipids and fatty acids are the generally used plasticizer in the production of protein films.

2.2 Protein based Nanocomposites

2.2.1 Proteins

Various raw materials from agricultural resources have been used to produce agricultural substances for many years due to renewability and biodegradability. Edible packaging also being produced. Among bio-based products, proteins have been used as packaging material from long time (e.g. traditional lipoproteins skins in Asia and collagen sausage casings). Various vegetable proteins (corn, wheat gluten, soy protein etc.) have been used to produce bio-plastics (Pommet, Redl, Morel, Domenek & Guilbert, 2003; Cuq, Gontard & Guilbert, 1998). Proteins are made up of 20 different amino acids. Proteins are also known as polypeptides which are organic compounds made up of amino acids and are joined together by the peptide bonds between the carboxyl and amino groups. In stabilizing protein's three dimensional structure, non-covalent bond interactions play a major role. The unique structure of protein with wide range of functional properties provide advantage over other bio-polymers as non-food applications. (Angellier-Coussy *et al.*, 2013). To

prepare plastics from proteins requires the reconfiguration of the protein structure. There are four levels of protein structure. The primary structure is the linear chain of amino acids. The secondary structure is the folding of the primary structure by strong hydrogen bonds. This folding produces two most common patterns known as α -helix and β -sheet. The tertiary structure creates linkages in the secondary structures by bonds between side groups of the amino acid. This structure also involve hydrophobic interaction, disulfide and hydrogen bonds. The large folded molecules then get arranged to form quaternary structure (Angellier-Coussy *et al.* 2013)

2.3 Processing of protein-based nanocomposites

To process the proteins into different materials, a new three-dimensional network need to be formed which involves unfolding and rearranging of protein network and stabilized by inter and intra-molecular interactions. This can be achieved by the following steps: rupturing of low energy intermolecular bonds to unstable polymer chains in the native state, arrangment and orientation of these polymer chains and final step is the formation of a new network stabilized by new interactions and bonds.

2.3.1 Wet process

The wet process is based on the solubilisation of proteins in a solvent (water, ethanol and occasionally acetone), also called solution casting. The addition of reducing agents, pH variations with acids or bases, and/or ionic strength controlled by electrolyte leads to the denaturation of the proteins. Different type of proteins require different solubilisation techniques depending upon the amino acid sequence (Cuq *et al.*, 1998). In aqueous medium, proteins tends to arrange themselves with polar amino acids (hydrophilic part) at the interface with solvent and hydrophobic parts away from the solvent. To disperse the nanoparticles into the polymer chains is the major concern to form

nanocomposites (Dufresne, 2010). In this method solvent is used to disperse the clay nanoparticles initially and proteins should also be able to disperse in this solvent. The delaminated surfaces of clay nanoparticles enter the protein chains, after the solvent is removed or evaporated sheets formed by reassembling and sandwiching proteins. The pH of the film-forming solution can be modified to facilitate the mobility of proteins into clay galleries as the net positive charge on proteins favors the electrostatic interaction by attracting negatively charged clay particles.

2.3.2 Dry process

Dry processes involves thermoplastic processing in which protein matrix is mixed with the nano-particles in the molten state during heating and shearing. Therefore formation of a homogenous melt is required for this process and processing temperatures are usually above the protein denaturation point. Chemical additive and reducing agents such as sodium sulfite, sodium dodecyl sulfate, urea etc. are used to disrupt the covalent and non-covalent interactions with plasticizers as processing aids. Limited literature is found in processing of proteins by extrusion wheat gluten and SPI have been used to make composites by dry process.

2.3.3 Two-step process

The two-step process is the combination of both processes i.e. solution casting and dry processing via melt extrusion or melting. In this method the nanoparticle is mixed with distilled water and then added to the protein emulsion with constant mixing. In this mixture plasticizer is added and blended. After which this blend is subjected to melting. This method promises the dispersion of the nanoparticles into the protein matrix. The resultant material is stabilized by hydrogen bonding at the interfaces of protein and nanoparticles. It also promotes the intercalation

and delamination of the clay particles (Chang, Yang, Huang, Xia, Feng & Wu, 2009; Chen & Zhang, 2006).

2.4 Compression molding

The type and extent of physical modifications occurring during thermoplastic processing depends largely on the processing conditions. (Moraru & Kokini 2003). The transformation of protein-plasticizer mixtures into viscoelastic melts in compression molding results from the combination of high temperature, short times, high pressures and low moisture contents. Upon cooling, protein films with new interactions such as hydrogen, ionic, hydrophobic and covalent bonding will be obtained (Pol, Dawson, Acton & Ogale, 2002). The films processed with compression molding provide a wide range of mechanical and barrier properties depending on the formulation and processing conditions. This technology is suitable to inquire the properties of thermoplastic proteins. Similar to extrusion, compression molding can provide continuous and high speed processing for manufacturing films. A number of researchers have tried to make protein films with this technique.

Pommet *et al.* prepared wheat gluten blends with different plasticizers and compression molded at 100 °C for 5 min or 130 °C for 15 min. Five different plasticizers such as water, glycerol, 1,4-butanediol, octanoic acid, and lactic acid were used. The wheat gluten was mixed with plasticizers in a mixing chamber at a speed of 100 rpm and temperatures of 80 and 60 °C for hydrated and dry gluten respectively for 5 min after reaching maximum torque. Generally during mixing aggregation (due to temperatures > 60 °C) and de-aggregation of gluten molecules (due to shear) takes place, however it was observed that compression molding results only in aggregation as indicated by an increase in the sodium dodecyl sulfate (SDS)- insoluble gluten fraction (Pommet, Redl, Guilbert & Morel, 2005).

Corn gluten meal was blended with polar and amphiphilic plasticizers and compression molded by di Gioia & Guilbert. Corn gluten meal is rich in zein protein. Polar plasticizers namely water and glycerol and amphiphilic plasticizers namely octanoic acid and palmitic acids, dibutyl tartrate, phthalate and diacetyl tartaric acid ester of monoglycerides (DATEM) were used. The plasticizers and corn gluten meal were mixed at 25 rpm for 6 min in a controlled mixing chamber at 80 °C with circulating water. Bars (60×5× 2mm) were produced by compression molding at 110 to 130 °C, 1.4 MPa and dwell time of 10 min. Depending upon the type of plasticizer different degrees of plasticizer exudation were detected (di Gioia & Guilbert 1999).

Several researchers have also studied protein denaturation by compression molding. Cunningham *et al* have produced soy protein isolate-glycerol films at an optimum temperature of 150 °C, a pressure of 10 MPa and a dwell time of 2 min through compression molding. Ogale *et al.* observed that soy protein degraded at temperatures above 180 °C as indicated by thermogravimetric analysis (Ogale, Cunningham, Dawson & Acton, 2000). Similarly whey protein isolate-glycerol films were prepared by Sothornvit *et al.* (Sothornvit, Olsen, McHugh & Krotcha, 2003). They reported that dwell times above 2 min resulted in film degradation at temperatures above 140 °C, while film degradation was observed at both the minimum (0.81 MPa) and the maximum (2.25 MPa) pressures. At 104 °C with dwell times less than 2 min, no film formation occurred. They also found that films formed at 104 °C were flexible and partially soluble but the films obtained at 140 °C were nearly insoluble and inflexible. It concludes that higher compression molding temperatures promotes higher cross-linking with extensive protein denaturation and reduced solubility. Soluble and insoluble films can be used for different applications. A higher solubility and hydration is preferred in the edible film packaging of dry soup mixes or controlled drug delivery. Insoluble films could be desirable as wraps or casings for food with higher moisture content.

Laminated zein sheets were produced by compression molding by Rakotonirainy & Padua (Rakotonirainy & Padua, 2001). They have obtained the individual components by solution-casting and then pressed them with the Carver Press at 120 °C for 5 min to prepare 1-4-ply laminated zein sheets. The lamination process induced the flow of the oleic acid-zein films through melting. The decrease in voids and defects resulted in improved mechanical and oxygen permeability properties.

Pol *et al.* used thermoplastic properties and differences in molecular weight as an advantage to produce laminated films. Soy protein and corn zein protein was used to produce single and double coat laminates by compression molding. The higher molecular weight soy protein was plasticized with glycerol, thermally compacted at a temperature of 150 °C and used as the base film and laminated by zein at 125 °C without significant degradation of the soy protein film (Pol *et al.* 2002).

2.5 Process related factors

During the process of film making, various factors affect the functional properties of the films due to change in their molecular interactions. Several studies have been done on these process-related factors with a sense to achieve enhanced protein film characteristics. Every individual protein has different parameters, optimization differs for different combinations. This has been one of the major challenge faced in commercialization of the protein-based films.

2.5.1 Plasticizers

Plasticizers are added to the polymer matrix to enhance the processability and lead to the modification of final properties. Plasticizers can be used internally and externally. Internal plasticizers refers to plasticizers which are copolymerized or reacted with the polymer. On the other

hand external plasticizers are substances having low molecular weight and low volatility added to the system to produce swelling of the polymer chains (Sothornvit & Krotcha 2005).

Water is the most commonly used plasticizer in bio-polymer processing due to which it facilitates glass transition, deformation and processability of the polymer matrix. It also helps in delaying thermal degradation of the protein films (Tolstoguzov, 1993). An optimum amount of water should be used for processing, excessive water can lead to decrease in melt viscosity which results in low product temperature and interferes with protein transformation and interactions. Other common plasticizers include monosaccharides, oligosaccharides, polyols, lipids and derivatives used for edible films (sothornvit & Krotcha, 2005). Properties of Plasticizer such as composition, size, shape and ability to attract water affects film barrier properties as shown in solution-cast whey protein films (Sothornvit & Krotcha, 2000).

Glycerol ($C_3H_8O_3$) has been widely used in the thermoplastic processing of proteins. It has low molecular weight and hydrophilic in nature (Redl, Morel, Bonicel, Guilbert & Vergnes, 1999; Cunningham, Ogale, Dawson & Acton, 2000; Zhang, Mungara & Jane, 2001; Pommet *et al.*, 2003 ; Sothornvit *et al.*, 2003; Hernandez-Izquierdo, 2007). It has been an effective plasticizer due to its positioning and easy penetration into the 3-dimensional biopolymer network (di Gioia & Guilbert 1999). The plasticizing effect of sucrose ($C_{12}H_{22}O_{11}$) and sorbitol ($C_6H_{14}O_6$) have also been studied with fish myofibrillar proteins to produce bio-packaging materials by thermal compression-molding. (Cuq, Gontard & Guilbert, 1997). Pommet *et al.* also investigated several compounds as wheat gluten plasticizers with different chemical functions, number of functional groups and degree of hydrophobicity (Pommet *et al.*, 2005). The critical factors for a good plasticizer were determined by this study found to be low melting point, low volatility and protein compatibility. Also permanence in the film and amount of plasticizer should be taken into account while choosing

plasticizer (di Gioia & Guilbert 1999; Sothornvit & Krotcha, 2001). Quantification of the plasticizer efficiency in specific mechanical and barrier properties can also be done (Sothornvit & Krotcha, 2001).

2.5.2 Temperature

Processing temperature is another important parameter in film formation and lead to structural changes in protein. During heating protein molecules undergo transformation, chains become disordered and mobile. Heating treatment leads to protein network formation via crosslinking (Hernandez-Izquierdo, 2007; Sothornvit & Krotcha, 2005; Sabato, Ouattara, D'Aprano, Le Tien, Mateescu & Lacroix, 2001). Extreme or high temperature also causes protein degradation leading weaker protein network of films. Differential scanning calorimetry is a technique to characterize the thermal transitions in polymers. DSC can be used to detect thermal glass transition temperature (T_g), melting, crystallization, thermal degradation, protein denaturation and aggregation. In case of soy protein film single T_g was observed instead of two characteristic T_g values after thermal degradation (Zhang *et al.*, 2001). In addition in whey proteins an exothermic peak was observed which shows the slow formation of intermolecular bonds during thermal gelation (Fitzsimons, Mulyihill & Moris, 2007).

2.5.3 Other additives

Cross-linking agents and enzymes can also be used during film formation process. The enzymes such as transglutaminases and peroxides are incorporated to improve the moisture resistance, cohesion, mechanical strength and barrier properties. Egg protein treated with transglutaminases showed significant reduction in water vapor permeability (Motoki, Aso, Seguro & Nio, 1987; Lim, Mine & Tung, 1998). Various cross-linking agents can be used to improve

network formation. Formaldehyde is used as cross-linking agent in gluten based films. Improved mechanical properties were observed with increased covalent bonding between protein molecules (Micard, Belamri, Morel & Guilbert, 2000).

2.6 Film properties

2.6.1 Mechanical properties

Mechanical properties are important to assess packaging materials because of their usage in transportation, handling, and storage. Tensile strength (TS), elongation (E) and elastic modulus are the main parameters to assess mechanical properties of films. Tensile strength is the maximum stress tolerated before break point. It has units in MPa (Rhim & Lee, 2004). Elongation is referred to the extent to which film can stretch before break point and also it shows the flexibility of films. The mechanical properties are largely depends on inter and intra molecular interactions.

Table 2.1. Mechanical properties of proteins based films

Film	Tensile strength (MPa)	Elongation (%)	Thickness (mm)	Temp. (°C)	Rh (%)	Reference
Cellulose acetate	65.6	30	-	-		Briston, 1988
Low density polyethylene	12.9	500	-	-		Briston, 1988
Myofibrillar proteins	17.1	22.7	0.034	25	57	Cuq <i>et al.</i> , 1995
Whey protein isolate	13.9	30.8	-	23	50	McHugh & Kotcha 1994

Soy protein	1.9	35.6	0.088	25	50	Gennadios <i>et al.</i> , 1993
Wheat gluten proteins	0.9	260	0.088	25	50	Gennadios <i>et al.</i> ,1993

2.6.2 Water vapor permeability

Water vapor permeability is another important factor affecting the use of various packaging material for different applications. The packaging film should be able to provide barrier to moisture, CO₂ and O₂ for food safety issues and storage purpose. Packaging film is responsible for the transport of moisture which can lead to deterioration of the product in different ways (Grossman, Nuabunma, Dufresne, Thomas & Pothan, 2013). The major challenge faced during preparation of protein based films is the optimization of the processing conditions to achieve desired water vapor permeability.

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CHAPTER 3. NANO-REINFORCED BIOMATERIALS FROM NATURAL KERATIN AND MONTMORILLONITE/CELLULOSE NANOCRYSTALS

3.1 Introduction

Rise in environmental concerns have led the current research interest to replace traditional materials with greener alternatives (Cheng et al., 2009; Wu & Liao, 2012). Due to environmental disturbances and envisaged future shortfall of oil and oil derived products, the interest has been increased for the development of environment friendly materials from renewable resources such as lipids, polysaccharides, and proteins. Currently the main focus has been repositioned towards proteins being more robust than carbohydrates(Hardy & Scheibel, 2010; Liu, Jiang, Liu, & Zhang, 2010). Several works by numerous researchers have been reported to prepare plastics from proteins by solvent castings, extrusion or compressions molding techniques(Anderson & Ng, 2000; Mangavel, Barbot, Guéguen, & Popineau, 2003; Schrooyen, Dijkstra, Oberthür, Bantjes, & Feijen, 2001; Ullah, Vasanthan, Bressler, Elias, & Wu, 2011; W. Wei & Baianu, 1999).

Biopolymers have numerous advantages over synthetic polymers because of their biodegradability, renewability and sometimes low cost and eco-friendly nature(Mohanty, Misra, & Hinrichsen, 2000). Previously protein based films have been developed from soy protein, whey protein, casein, collagen, corn zein, gelatin and wheat gluten by many researchers(Cuq, Gontard, & Guilbert, 1998). However due to their limited availability as feedstock for bio-plastics, brittle nature and weak material properties act as hindrance to their commercial utilization. Chicken feathers are the natural source of keratin protein, which can be utilized to develop high performance biomaterials. Chicken feathers are a renewable cheap feedstock and poultry processing plants generate over 65 million of feathers worldwide every year(Zhao, Yang, Zhang, & Wu, 2012). Currently, the feathers are either processed into a low nutritional value animal feed(Schrooyen et

al., 2001) or land filled, with few application in composite preparation and other products(Bernhart & Fasina, 2009; Reddy & Yang, 2007; Saber, El-Metwally, & El-Hersh, 2010; Schrooyen et al., 2001). Both of the above mentioned disposal methods contribute to the environmental pollution. So by creating alternative ways to use chicken feathers on industrial scale will help reduce environmental impacts and health risk, which are directly linked to the disposal of these materials to landfills(Saber et al., 2010). Feathers consists of 90% keratin(Reddy & Yang, 2007) and 7% of cysteine, which results in α -helix formation in keratin protein through disulphide linkage(Arai, Takahashi, Yokote, & Akahane, 1983) in polypeptide chains. These linkages are responsible for the stiffness and hardness of the keratin protein making it difficult to dissolve in organic solvents(Onifade, Al-Sane, Al-Musallam, & Al-Zarban, 1998). Therefore alkaline and acid hydrolysis, oxidation and reduction methods have been employed and reported by many researchers for dissolution of keratin(Fan & Yu, 2012; Hill, Brantley, & Van Dyke, 2010; J. Zhang et al., 2013).

Previous studies have been done to modify poultry feather, either by surface grafting of synthetic polymers or by blending with plasticizer, to transform them into films using casting, compression molding or extrusion techniques. Apart from using keratin as a biosorbent(Arshad, Khosa, Siddique, & Ullah, 2016; Muhammad A. Khosa & Ullah, 2014; Muhammad Arshad Khosa, Wu, & Ullah, 2013), there are few reports on the formation of composite films using keratin fiber. Jin et al. modified native chicken feather fiber through graft polymerization with methyl acrylate, using $K_2S_2O_8/NaHSO_3$ as redox system, and prepared films by compression molding(Jin, Reddy, Zhu, & Yang, 2011). Results indicated higher tensile strength than soy protein isolate (SPI) and starch acetate (SA). Barone et al. prepared compression molded films from keratin feather fibers using high density polyethylene (HDPE) and observed increase in stiffness of HDPE with reduced tensile breaking stress(Barone, Schmidt, & Liebner, 2005). Ullah et al. studied the effect of different

plasticizer namely glycerol, propylene glycol, ethylene glycol and diethyl tartrate on bioplastics developed from feather quill processed by extrusion. They found ethylene glycol as a compatible plasticizer responsible in improvements of mechanical properties(Ullah et al., 2011).

However, despite abundant availability of feathers and some efforts to process them into bioplastics, their weakness such as hygroscopic nature (Saravanan & Dhurai, 2012) and low thermal stability(Zhan & Wool, 2013), need to be addressed to demonstrate a basis for their use in innovative technologies and high performance composite applications.

Addition of nanofiller/nanoparticles into synthetic(Arshad, Huang, & Ullah, 2016) or natural composite materials(Angellier-Coussy et al., 2013; Swain, 2014) is a common method to improve thermal mechanical, barrier and other properties. Recent reports on the improvements of different protein based composite films include addition of montmorillonite into soy protein(Echeverría, Eisenberg, & Mauri, 2014; Kumar, Sandeep, Alavi, Truong, & Gorga, 2010), zein nanoparticles and nano-SiO₂ in whey protein isolate(Oymaci & Altinkaya, 2016) and whey protein isolate/pululan(Hassannia-Kolae, Khodaiyan, Pourahmad, & Shahabi-Ghahfarrokhi, 2016) respectively, cellulose nanofibres in gelatin matrix(Mondragon, Peña-Rodriguez, González, Eceiza, & Arbelaz, 2015) and soy protein isolate(S. Zhang et al., 2016). But there are no reports on the preparation of chicken feather (keratin) based composite films reinforced with montmorillonite (MMT) and cellulose nano-crystals (CNCs). Unique structure and properties of clay minerals have been established as an effective materials to improve various properties of biopolymers including thermal, barrier and mechanical(Fornes & Paul, 2003; Zeng, Yu, Lu, & Paul, 2005). CNCs are new type of nano-particle with long crystalline rod shaped needles with size ranging from 1 to 20 nm in width and 1-100 nm in length. Due to its impressive material properties such as high elastic modulus, surface area, specific strength and young's modulus, low density and

coefficient of thermal expansion(de Souza Lima & Borsali, 2004; Matos Ruiz, Cavaillé, Dufresne, Gérard, & Graillat, 2000; Orts et al., 2005), it has been used as reinforcement in various polymers(Habibi, Lucia, & Rojas, 2010; Klemm et al., 2011; Moon, Martini, Nairn, Simonsen, & Youngblood, 2011). Recently, we have reported the dispersion of nanoclay as a reinforcement in a keratin matrix and its regeneration into hybrid fiber(Arshad, Kaur, & Ullah, 2016). Here in, we explore the in situ dispersion of MMT and CNCs in keratin solution and their comparison after preparing bio-nanocomposite films by compression molding, where 1, 2 butanediol and glycerol were used as plasticizers. The effect of MMT and CNCs concentration on keratin based nanocomposite films have also been investigated to evaluate their impact on thermal and mechanical properties.

3.2 EXPERIMENTAL SECTION

3.2.1 Materials and methods

White chicken feathers from broilers were provided by the Poultry Research Centre (University of Alberta) were processed. The first step performed was cleaning of chicken feathers which was done by washing with soap and water. Drying of the feathers was employed to evaporate water by spreading them under a closed fume hood for 4 days. To ensure the moisture is completely removed from feathers, the dried feathers were ventilated in oven at 50 °C for 8h. Scissors were used for processing after drying and feathers (fiber and quill portion) were ground using Fritsch cutting Mill with sieve of 0.25mm. (Pulverisette 15, Laval Lab. Inc., Laval Canada). To remove grease, ground feathers (8g) were washed with hexane solvent for 4 hours by soxhlet apparatus followed by drying and storing at room temperature for further modification.

Urea (99%), sodium sulphite (≥ 98), EDTA (99%), n-hexane ($\geq 95\%$), tris-base ($\geq 99.8\%$), glycerol (99.6%), chitosan, 1, 2-butanediol (≥ 98), HCL, hydrophilic nanoclay/Montmorillonite ($\geq 95\%$), were purchased from Sigma Aldrich and used as received. The cellulose nanocrystals (CNCs) were provided by the Alberta Innovates Technology Futures (AITF).

3.2.2 In-situ modification/ reinforcement of feather keratin with MMT and CNCs:

The weight ratio of 8 M urea solution to chicken feathers of 17:1 was used to completely immerse chicken feathers. In this immersion, EDTA (0.438g), tris-base (12.102g) and sodium sulfite (10g) were added and stirred at a temperature of 60 °C for a week. The pH of the immersion was adjusted to 9.0 to solubilize the protein monitored regularly during dissolution process. To study the effect of nanoparticles, MMT and CNCs concentrations of 1%, 3%, 5% and 10% based on the weight of chicken feathers were prepared. To enhance the dispersion of nano-particles, keratin-nanoparticle dispersions were stirred for 20 minutes and sonicated for 10 minutes. After which, the pH of the keratin-nanoparticle dispersion was adjusted to iso-electric point (4.0-4.2) with 1 M HCl and was centrifuged at 10,000 rpm for 10 min to obtain precipitates. The precipitates were washed three times with distilled water under centrifugation of 10,000 rpm for 10 min. This freshly collected nano-reinforced keratin material was then dried at 95 °C for 24 hours in oven, pulverized and sieved with mesh (180 μm).

3.2.3 Bio-nanocomposite film preparation by compression molding:

Keratin and keratin based nanocomposites reinforced with MMT and CNCs were prepared by compression molding. After screening of several plasticizers 1, 2-butanediol and glycerol were selected as suitable plasticizers for MMT and CNC reinforcements respectively. Bio-nanocomposite with glycerol and 1, 2 butanediol (20%) as plasticizers, chitosan (10%) as cross-

linking agent, sodium sulfite (3%) as reducing agent and moisture content (25%) were taken to prepare blends. The ingredients were mixed intensively for 5 min in a beaker and placed for 4 hr in a sealed plastic bag for hydration and plasticizer penetration in the blend. Sodium sulfite was added into the system in order to dissociate disulfide bonds between the cysteine residues of the keratin chains to achieve efficient mixing among keratin based nanocomposites and plasticizers. These blends were then thermally compacted using a carver laboratory press (Bench Top Manual Heated Press, Model CH (4386), Carver, Inc. USA). Approximately 3.5 g of each blend was placed within a 5-cm dia at the centre between two 12 × 12 in aluminium plates. The sample was compressed for 15 min within the aluminium plates between the two platens at 145 ± 2 °C with pressure of 10 MPa distributed uniformly over the platens. The plates were allowed to cool to 50 °C before removing from press and cooled to room temperature. Then the samples were cut to the required gage dimensions for further testing. Mechanistic diagram of processing of bio-nanocomposites is shown in figure 3.1.

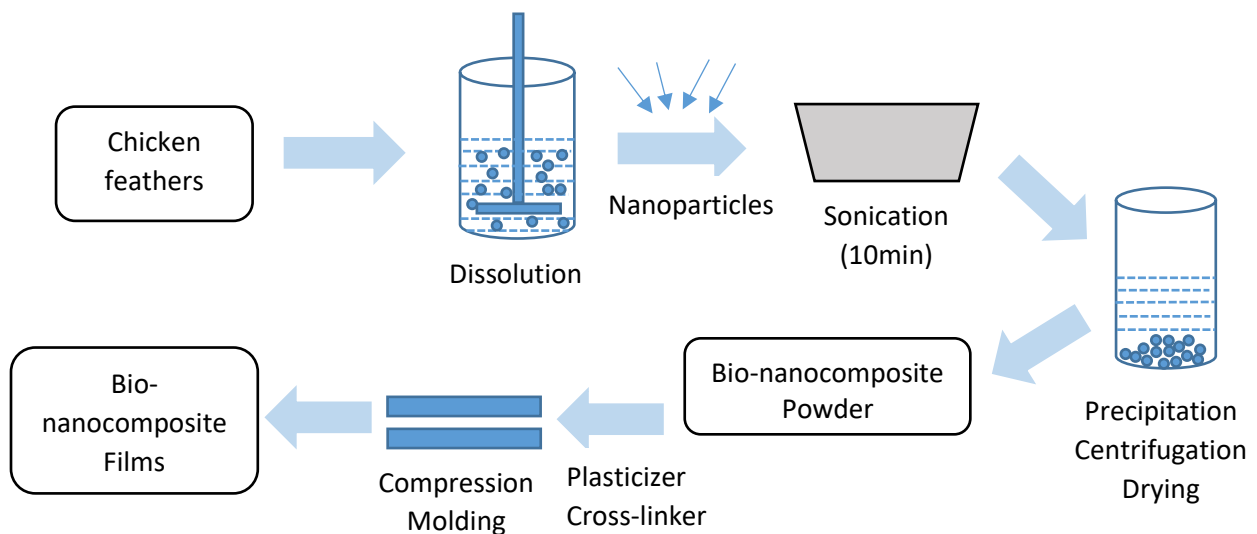


Figure 3.1. Mechanistic representation of processing of bio-nanocomposite films from chicken feather keratin

3.2.4 Screening and optimization of the parameters:

Different plasticizers and concentration were tried for preparation of the films viz. sorbitol, 1,4 butanediol, glycerol, polyethylene glycol and 1, 2 butanediol. Glycerol found to be suitable plasticizer for keratin-CNC composites, however 1, 2 butanediol found to be suitable for Keratin-MMT composite films. Cross-linking agent was used to enhance the processability and plasticity of keratin films. It also imparts flexibility to keratin-based nanocomposites as films made without cross-linking agent found to be brittle. Different concentrations of cross-linking agent 1%, 3%, 5% and 10% were investigated, out of which 10% chitosan showed most cohesive, homogenous and transparent films. Also the compression molding parameters viz. temperature, pressure and time were optimized before carrying out the actual experiments.

3.2.5 Film thickness:

Thickness of the film was measured at three different randomly selected locations using a digital Vernier calliper (digi-max caliper, sigma-aldrich, USA). The average value of film thickness was used in determining mechanical properties and dynamic mechanical properties. Three thickness measurements at different positions were taken on each specimen being in the range of 70-90 μm in all cases.

3.2.6 Differential scanning calorimetry (DSC):

The thermal properties of bio-nanocomposite films were demonstrated by differential scanning calorimeter (2920 Modulated DSC, TA instrument, USA) under the stream of nitrogen. To calibrate the heat flow and temperature of instrument, pure indium sample was used. All samples were analyzed in a temperature range of 25 to 300°C at a heating rate of 10°C/min. The samples weighing between 5 to 10 mg were encapsulated in aluminium pans.

3.2.7 Thermogravimetric analysis (TGA):

The thermal stability of CNC and MMT reinforced nanocomposite films were investigated by thermogravimetric analysis TGA Q50 (TA instrument, USA) under nitrogen flow. The temperature of the sample was increased from room temperature to 600 °C at a heating rate of 10 °C/min with sample size between 10-12mg. weight loss of the sample was measured as a function of temperature.

3.2.8 Mechanical testing:

Tensile strength (TS) and percent elongation (%E) at break of the bio-nanocomposite films were determined by tensile testing using Universal Testing machine (autograph AGS-X shimadzu, Canada) equipped with 5 kN static load cell according to the ASTM standard D882-02 (ASTM standards, 2002). Films were cut into rectangle pieces (50 x 5mm). The initial grid separation was set at 2.5 cm and the cross-head speed was 50 cm/min. Tensile strength was calculated by dividing peak load to initial specimen cross-sectional area. Percent elongation at break was calculated as the percentage change in length of the specimen between the grips. Three specimens of each sample were evaluated.

3.2.9 Dynamic mechanical analysis (DMA):

The mechanical properties of bio-nanocomposites films were analyzed by using a dynamic mechanical analyzer (Q800, TA Instrument) under the flow of nitrogen at a frequency of 1 Hz and amplitude of 15µm. The length and width of the film sample were 4 cm and 0.6 cm, respectively. The samples were heated from -50 °C to 150 °C at a heating rate of 3 °C/min. The storage modulus (E'), loss modulus (E'') and loss tangent ($\tan \delta = E''/E'$) were recorded as a function of temperature.

Glass transition temperature (T_g) was determined as the temperature at which $\tan \delta$ attained its peak value.

3.2.10 Wide angle X-ray diffraction (WAXD):

X-ray diffraction studies of all bio-nanocomposites films were performed with a diffraction unit Rigaku Ultima IV operating at 38kV and 38mA. The radiation was generated from a Cu-K α source with a wavelength (λ) of 0.154 nm. The diffraction data were collected from 2θ values of 5 to 45° with a step size of 0.02°, where θ is the angle incidence of the X-ray beam on the sample. Samples of bio-nanocomposite films were prepared by drying of the films at 70 °C in oven overnight followed by grinding. The ground sample of 5g was used for X-ray diffraction.

3.2.11 X-ray photoelectron spectroscopy (XPS):

The chemical composition of the keratin fiber surface was studied by XPS. The XPS measurements were conducted on Ultra spectrometer (Kratos Analytical). The base pressure in the analytical chamber was lower than 3×10^{-8} Pa. Monochromatic Al K α source ($h\nu = 1486.6$ eV) was used at a power of 140 W. The analysis spot was 400×700 μm . The survey scans were collected for binding energy spinning from 1000 eV to 0 with analyzer pass energy of 160 eV and a step of 0.4 eV. For the high resolution spectra the pass energy was 20 eV with a step of 0.1 eV.

3.2.12 Transmission electron microscopy (TEM):

The structure and morphology of all bio-nanocomposite films were visualized by a transmission electron microscope (CM20 FEG TEM/STEM Philips) operating at 200kV. An FEI Morgagni 268 instrument operated at 80 kV, equipped with Gatan Orius CCD camera was used to investigate the nano-reinforced samples. The samples were embedded in a polymer resin and thin

slices of thickness 80 nm were prepared by ultra-microtome. A slice of sample was put on a fine mesh of copper TEM support grid.

3.2.13 Statistical analysis:

All experiments were performed in triplicates. Mechanical properties data was subjected statistical analysis one-way analysis of variance (ANOVA) followed by Duncan test at a significance level of 0.05. The analysis was done to observe the significance difference among mechanical properties. It was performed by SPSS software (SPSS software, Version 23, Inc. USA).

3.3 RESULTS AND DISCUSSION

3.3.1 Differential scanning calorimetry (DSC):

The thermal transitions of neat chicken feather keratin (CFK) and its bio-nanocomposites reinforced with MMT and CNCs were studied by DSC and are presented in figure 3.2A and 3.2B respectively. Neat CFK exhibited two broad peaks, the first peak at 83 °C occurs usually due to evaporation of residual moisture of the protein and the second peak occurring at 242 °C is due to the crystalline melting of the keratin belongs to α -helix denaturation(Spei & Holzem, 1987). The thermograms of bio-nanocomposites reinforced with MMT shown in figure 3.2A contain endothermic peaks (crystalline melting) at lower temperature (195 to 221 °C) as compared to neat CFK. The presence of two peaks in this region might be attributed to plasticizer-rich domains and protein-rich domains. The decrease in melting could be due to addition of chitosan on the crystallinity of biopolymer matrix. Similar influence of chitosan/chitin was observed on the melting point of poly(vinyl alcohol) which shifted to a lower temperature accompanied by broadening due to inhomogeneous distribution (Kadokawa, Takegawa, Mine, & Prasad, 2011). It can be clearly seen in the figure 3.2A that the composite films with 3% and 5% MMT have 4 °C and 3 °C lower

melting temperature (T_m) respectively as compared to 1% MMT composite films, which could be attributed to good dispersion of MMT in case of 1% bio-nanocomposites. Also TEM images of 3% and 5% MMT composite films showed presence of some of the aggregated phases which may results in lower crystalline melting (figure 3.7.). However in case of 10% MMT composite, a rise of 22 °C has been observed. This rise in melting temperature relates to higher ratio of clay contents. The moisture loss peak has been disappeared in thermograms of all MMT composites, which shows the removal of all water molecules during the drying process. Several authors did not observe important changes in the glass transition temperature (T_g) of composites reinforced with CNC and MMT (Bodkhe, Rajesh, Kamle, & Verma, 2014; Diaconu, Asua, Paulis, & Leiza, 2007; Klopogge, Evans, Hickey, & Frost, 2002; Lönnberg, Fogelström, Berglund, Malmström, & Hult, 2008; Petersson & Oksman, 2006)

CNC reinforced bio-nanocomposites have two broad peaks as can be seen in figure 3.2B. The first broad peak belongs to evaporation of residual moisture of the protein and second broad peak corresponds to crystalline melting temperature of bio-nanocomposites. Out of 1%, 3%, 5% and 10% CNC reinforced composites, the composite material with 1% CNC displayed 12 °C (254 °C) higher stability with no residual moisture peak when compared with neat CFK (242 °C), which could be ascribed to well and homogenized dispersion of CNCs along with improved chitosan dispersability. While gradual increase in melting temperature (T_m) from 229 °C to 247 °C was observed in case of 3%, 5% and 10% CNC composites with the increase of CNCs ratio, which represents that increase in concentration of CNCs improves homogeneity of chitosan which results in higher stability of composites materials. Thermograms of both bio-nanocomposite materials have some additional peaks of very low intensity in the region of 160 °C to 200 °C might be ascribed to different types of interaction of 1, 2 butanediol and glycerol (plasticizers) with keratin biopolymer.

Chen and Zhang also observed similar plasticizer and protein-rich zones domains, while studying transitions and microstructures of glycerol plasticized soy protein (Chen & Zhang, 2005). Above results showed that both of the nano-particles have different effect on the thermal properties of chicken feather based composites. CNCs were found to be preferred nano-particle for chicken fiber keratin with chitosan and glycerol as compatible plasticizer for enhanced thermal stability.

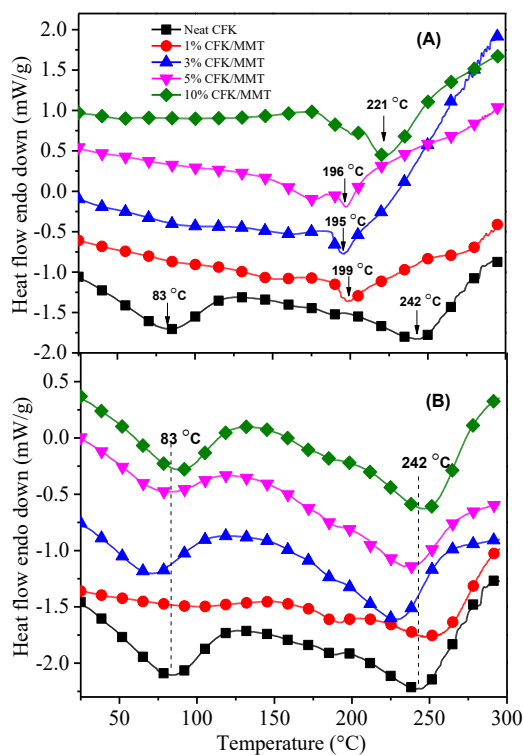


Figure 3.2. DSC thermo-grams of neat CFK and nano-composites reinforced with different ratio of MMT (a), and CNC's (b).

3.3.2 Thermogravimetric analysis (TGA):

To investigate the thermal stability and degradation behavior of the MMT and CNC reinforced composites, TG analysis were performed (figure 3.3). Three weight loss were noticed in the TG and DTG curves of MMT composites. The first weight loss of up to 9% in the temperature range of 50-125 °C belongs to the evaporation of residual moisture, while the second weight loss

form 10-30% in between 160-250 °C can be attributed to plasticizer evaporation and the final weight loss starting from 250 °C belongs to the decomposition of keratin and chitosan. It can be observed from the TGA data that the composite films with 1% and 10% MMT at 600 °C displayed weight loss of 74% and 73% respectively which is around 6% less than the neat fiber composite film. While 3% and 5% MMT composites showed total weight loss of 76% and 77% respectively showing around 3% less decomposition than the neat fiber film. The lesser weight loss in case of 1% MMT composite film may be attributed to homogenous dispersion of MMT, while in 10% MMT composite could be due to higher contents of nanoclay (Yousefian & Rodrigue, 2016) as compared to 3% and 5% MMT composites.

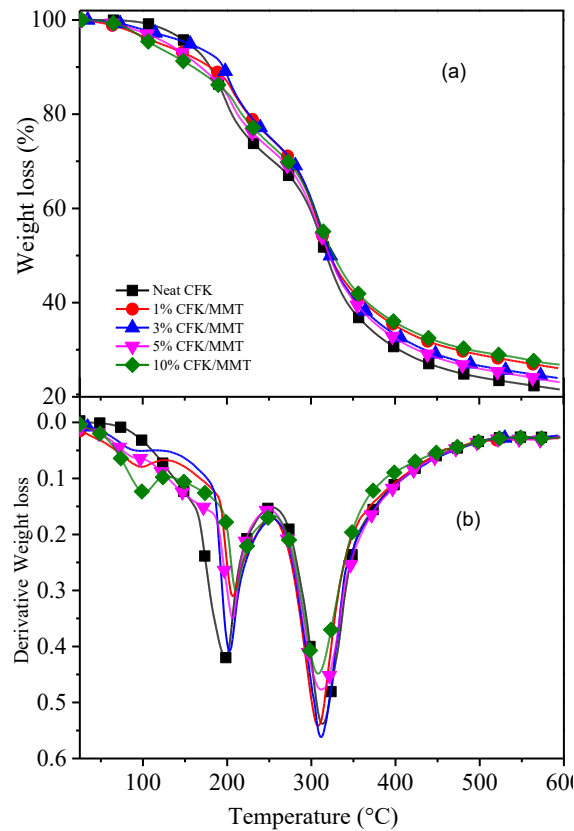


Figure 3.3. TGA (a) and DTG (b) curves of CFK-MMT films nanoreinforced with different MMT content under nitrogen flow.

On the other hand, thermal study of CNC reinforced composites was also investigated by TG analysis as shown in figure 3.4. TG and DTG curves of all CNC based composites also showed three stages of weight loss except biocomposite with 1% CNC, moisture loss peak is absent. In the DTG curve, the highest peak intensity represents the maximum weight loss at that specific temperature. The first weight loss peak for all other biocomposite films occurring below 110 °C is due to moisture, loosely bound to glycerol used as a plasticizer and protein molecules. The second stage of weight loss shows two peaks in DTG curve, which is due to either weak or strong interaction of CNC, chitosan and glycerol with protein.

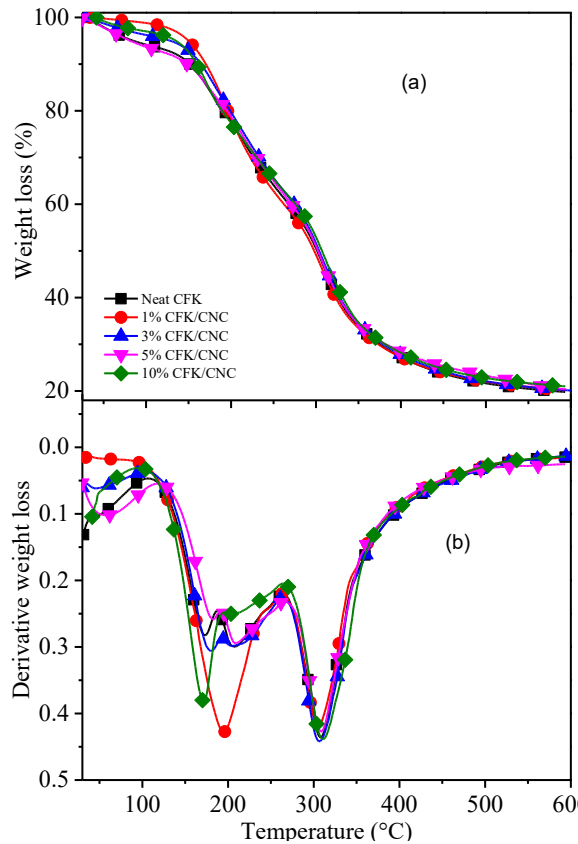


Figure 3.4. TGA (a) and DTG (b) curves of CFK-CNC films with different ratio of CNC under nitrogen flow.

The regions which contain less protein, CNC and Chitosan and more glycerol results in early weight loss due to weak interaction of glycerol with protein molecules, while those which are homogenous have strong interaction of glycerol and protein molecules resulting in weight loss at higher temperature. Same kind of degradation behavior has already been noticed by Grevellec *et al* (Grevellec, Marquié, Ferry, Crespy, & Vialettes, 2001) for cottonseed proteins . The third weight loss corresponds to the degradation of protein content, where DTG curve represents more stability for 5% and 10% CNC composites as their maximum weight loss was observed at bit higher temperature than neat keratin biomaterial. Nanocomposites with 1% CNC content showed better stability at initial stages and also showed crystalline melting at higher temperature as indicated in DSC. This might be attributed to the better dispersion of CNC's and shows higher hydrogen bonding within the matrix. The higher extent of CNC dispersion results in the high performance composite materials (Arias, Heuzey, Huneault, Ausias, & Bendahou, 2015). At 600 °C, all of the CNC reinforced composites demonstrated less weight loss and higher stability as compared to neat CFK. In DTG curves of both bio-nanocomposites, the maximum weight loss observed at slightly higher temperature for CNC based composites than MMT based composites when compared with neat CFK. While MMT containing composites provided less weight loss after complete decomposition as compared to CNC composite materials.

3.3.3 X-ray diffraction (XRD) spectroscopy:

X-ray diffractograms of pure MMT, neat feather keratin and nano-composites reinforced with different concentrations of MMT are given in figure 3.5. Pure MMT powder exhibited a diffraction peaks at 2θ angle of 6° , 28° , 35° , 54° , 61° and 73° respectively. It can be clearly seen from the XRD patterns of MMT biocomposites (figure 3.5.) that all of the crystallinity peaks of MMT have been completely disappeared in 1% and 3% MMT bio-nanocomposite films, while these

peaks have been greatly reduced in 5% and 10% MMT. Also 5% and 10% composites retain some of the pristine clay peaks which shows incomplete dispersion at higher concentration of layered silicates. It has been observed by many authors that physical blends showed tactoids and aggregates of MMT platelets during nanocomposite formation (Diaconu et al., 2007; Hoogsteen, Postema, Pennings, Ten Brinke, & Zugenmaier, 1990). However in this study nanocomposites showed good dispersion which possibly results from sonication of keratin-MMT suspension. These results illustrate that the inter layer spacing of MMT has been greatly increased, which represents a high extent of intercalation/exfoliation of nanosilicate layers in Keratin-nanoparticles suspension.

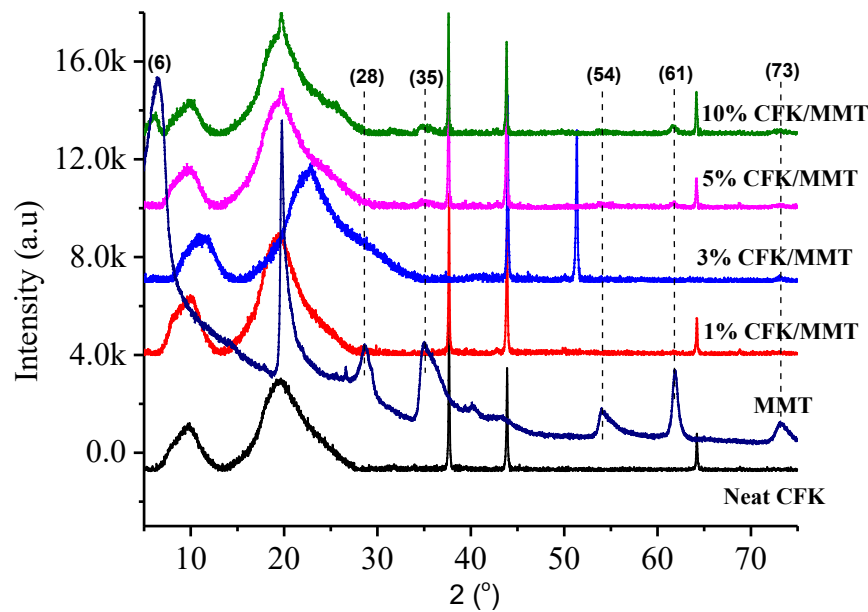


Figure 3.5. Wide angle X-ray diffractograms of neat keratin, MMT and biomaterials reinforced with different %ages of MMT

A similar phenomenon was observed in case of CNC reinforced keratin biomaterials as shown in Figure 3.5. XRD diffractograms of neat keratin, pure CNC and biocomposites reinforced with different ratio of CNC can be seen (figure 3.6). The crystallinity peaks at 2θ of 15° , 22° , 28° , 34° and 45° assigned to pure CNC dispersion have been completely vanished in 1% and 3% CNC

biocomposites. While in case of 5% and 10% biocomposites the crystallinity peaks are retained at 2θ of 22° and 34° , although significant decrease in peak intensities was observed. The absence of the crystalline peaks in 1% and 3% CNC content suggests well dispersion of CNC, which were further supported by their TEM analysis in figure 3.7. XRD spectrum of 1% CNC confirmed the results achieved in TGA and DSC which implies better dispersion lead to enhanced thermal stability. The peaks at near 22° observed in 5% and 10% CNC nanocomposites are due to the CNC crystallinity (figure 3.6). The 22° peak is explicit since CNC content was higher, this shows that at higher concentration CNC retains its crystallinity in polymer structure (Abdollahi, Alboofetileh, Rezaei, & Behrooz, 2013; Sain et al., 2013; Xu et al., 2013). The diffraction behavior of the composite films is dominated by the keratin phase which is the primary phase of the composite.

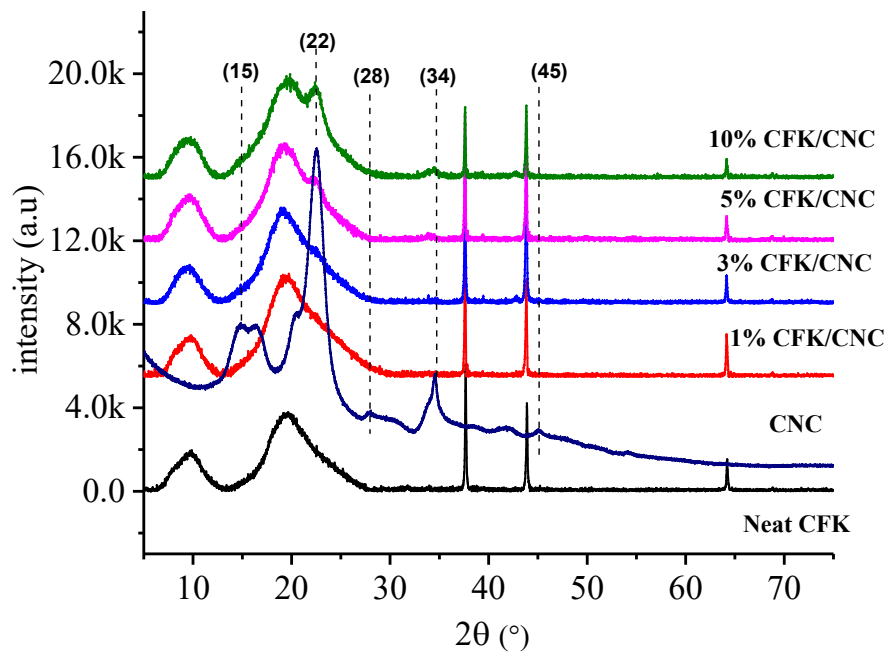


Figure 3.6. Wide angle X-ray diffractograms of neat keratin, CNC and biomaterials reinforced with different %ages of CNC

In order to ascertain the crystal structure of the reinforcing phase, the as-received CNC/water suspension was dried and the diffraction pattern of the resultant CNC mat is also shown in figure

3.6. Crystalline cellulose exists in different allomorphic forms, including Cellulose I with chains aligned in parallel and cellulose II having anti-parallel chain arrangement (Habibi *et al.*, 2010). The CNC display primary peaks at $2\theta = 15.1^\circ$, 17.5° , and 22.7° with a weak diffraction peak at 34.4° which corresponds to cellulose I (Fortunati *et al.*, 2012; Maiti *et al.*, 2013; Xu *et al.*, 2013).

3.3.4 Transmission electron microscopy (TEM):

TEM studies are necessary to verify the nanoparticles extent of dispersion (exfoliation/intercalation). TEM micrographs are consistent with those of the previous XRD pattern studies (figure 3.5 and 3.6). TEM images with different contents of MMT and CNC reinforced with neat CFK are displayed in figure 3.7 and 3.8 respectively. Figure 3.7 represents MMT composite films having well dispersed regions. It can be clearly seen from the images of 1% MMT composite films, where the layered structures of MMT are mostly exfoliated. However in case of 3% and 5% the layered silicates were mostly intercalated and exfoliated to some extent in the keratin matrix. While 10% MMT composite films contains tactoids and aggregated regions.

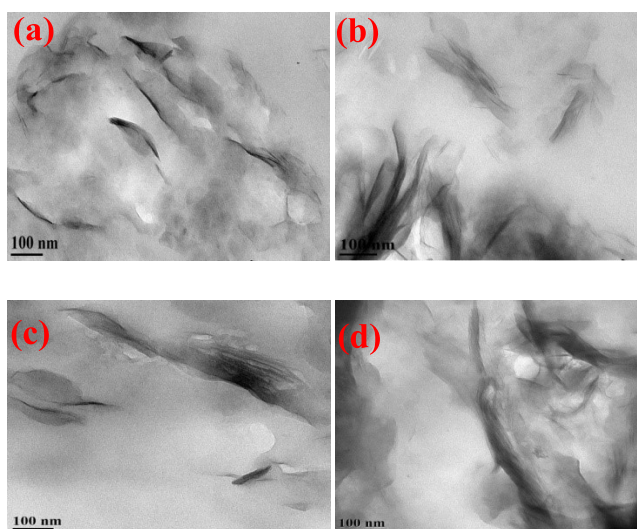


Figure 3.7. TEM images of bio-nanocomposites reinforced with 1% MMT (a), 3% MMT (b), 5% MMT (c) and 10% MMT (d).

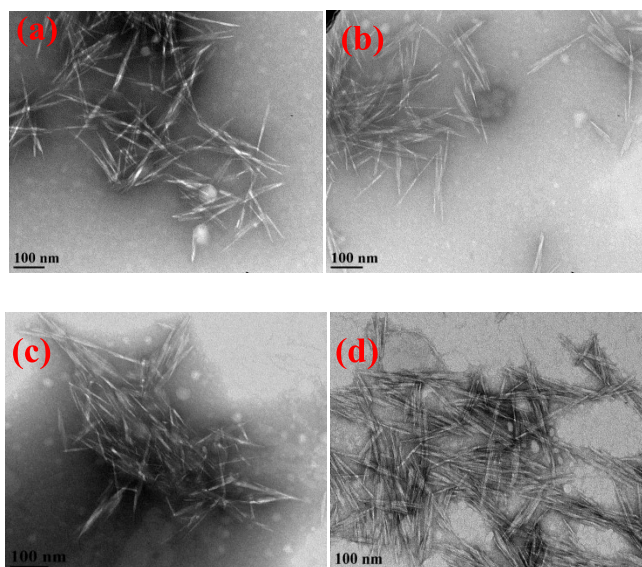


Figure 3.8. TEM images of bio-nanocomposites reinforced with 1% CNC (a), 3% CNC (b), 5% CNC (c) and 10% CNC (d).

TEM micrographs of CNC reinforced bio-nanocomposites also confirmed the well dispersion of cellulose nano-crystals which are present in the form of needles (figure 3.8). The extent of dispersion is more obvious in case of 1%, 3% and 5 % CNC bio-nanocomposites as compared to 10% CNC films. TEM results also support XRD data, where crystallinity peaks of MMT and CNC have been greatly reduced. The length of rod-like shape CNC was around 200-400nm.

3.3.5 Dynamic mechanical analysis (DMA):

Dynamic Mechanical Analysis (DMA) measures the changes in the viscoelastic properties of the polymers as a function of temperature. Thermal transitions occur because of chain mobility when temperature varies. The most important transition includes glass transition (T_g) which belongs to the beginning of chain motions. Normally DMA data for solids is displayed as storage modulus and damping or tan delta versus temperature. The size of tan delta peak reflects the volume fraction of the material undergoing transition. Effect of temperature on the tan delta of CFK-MMT films

with different MMT concentration has been shown in figure 3.9. T_g was determined from the peak of tan delta curve. Two transitions are obvious from all CFK-MMT composites, which could be attributed to two different zones present in the bio-nanocomposite films depending upon the strong/weak interaction between biopolymer, chitosan and plasticizer. The first could be attributed to plasticizer (1, 2-butanediol) and chitosan rich domains, while the second may be nanoparticle rich zone. The similar behavior among plasticizer and biopolymer has already been reported (Chen & Zhang, 2005; Ullah *et al.*, 2011). Figure 3.9 illustrates that the T_g for neat CFK film is $45 \pm 2^\circ\text{C}$, while it has been improved in all MMT bio-nanocomposites where first peak is occurring near $68 \pm 5^\circ\text{C}$ and second peak exhibiting a T_g of $116 \pm 5^\circ\text{C}$. The effect of temperature on storage modulus (E') of CFK-MMT composites with 0%, 1%, 3%, 5% and 10% MMT are also shown in figure 3.9a. At -50°C , neat CFK has the highest storage modulus as compared to all CFK-MMT bio-nanocomposites. With the increase in temperature, a rapid decline in storage modulus was observed for neat CFK, while CFK reinforced with MMT displayed a gradual decrease. This gradual decrease in storage modulus of all bio-nanocomposites ranges from -45°C to 30°C , represent the transition of bio-nanocomposite films from glassy state to rubbery state. The bio-nanocomposites containing 1%, 3% and 10% MMT demonstrated higher value of storage modulus as compared to 5% MMT composites. At -11°C and above, all MMT composites exhibited higher and sustained storage modulus as compared to neat CFK, which could be attributed to strong interaction of reinforced keratin with plasticizer and nanofillers in this temperature range. As protein interaction with polymer matrix plays an important role in storage modulus. Generally stronger interactions results in a higher storage modulus (L. Wei, Stark, & McDonald, 2015).

Tan delta of CFK-CNC films containing different contents of CNC are displayed in figure 3.10. The glass transition temperature for 0%, 1%, 3%, 5% and 10% CNC composite films are 46

°C, 52 °C, 62 °C, 31°C and 39 °C respectively. There was increase in glass transition temperature with increase in CNC content from 0% to 3%, while decrease in T_g was observed in case of 5% and 10% CNC content. The higher value of T_g in 1% and 3% CNC might be attributed to better dispersion of CNC causing hindrance in chain motions and increased cross linking of keratin with 1, 2-butanediol.

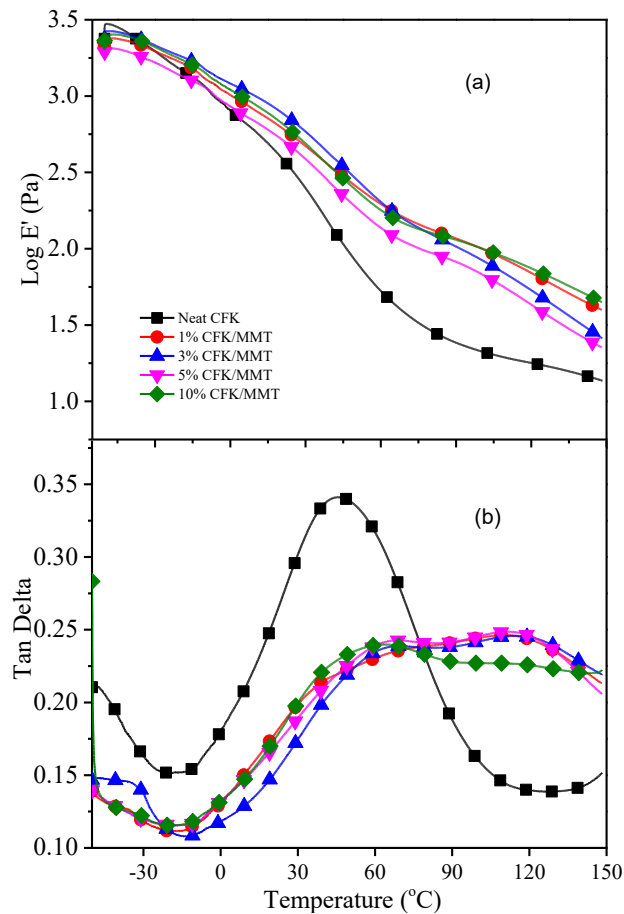


Figure 3.9. Effect of temperature on Log E' (a) and tan δ (b) of CFK-MMT films with different MMT contents.

However, lower T_g in 5% and 10% CNC films represents poor CNC dispersion and less capability of keratin polymer chains to cross link with the plasticizer results in weak bonding. In case of CFK-CNC films, at -46 °C 1% CNC composite demonstrated improved storage modulus as compared to

neat CFK, 3% CNC composite have storage modulus similar to neat CFK, while composite films with 5% and 10% CNC content exhibited low storage modulus (figure 3.10a). With increase of temperature from -46 to 55 °C, the storage modulus remains higher than neat CFK for 1% and 3% CNC composite films, while it drops for 5% and 10% CNC composites. So the higher value of E' for 1% and 3% CNC bio-nanocomposites is due to better dispersion of CNC leading to improved interactions with keratin and reduced mobility of biopolymer chains reinforced with CNC.

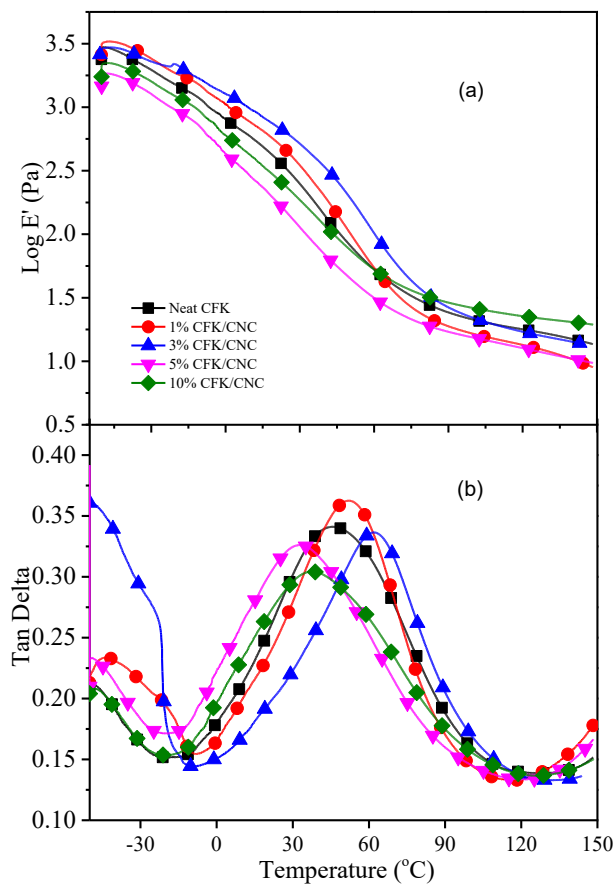


Figure 3.10. Effect of temperature on Log E' (a) and $\tan \delta$ (b) of CFK-CNC films with different CNC contents.

The addition of layered silicates and cellulose nano-crystals in biopolymers has significant influence on tensile properties (tensile strength and % elongation) as reported by many researchers. Tensile strength and percent elongation obtained for biomaterials reinforced with both nano-

particles can be seen in table 3.1 & 3.2. The effect of MMT on the feather keratin based composites is shown in table 3.1. By addition of MMT, the improvement in tensile strength and % elongation was observed compared to neat keratin based films. Particularly, film with 5% MMT demonstrated highest tensile strength. A gradual increase in % elongation was observed, when the MMT concentration was increased from 1% to 10%. Generally, the improvement in mechanical properties of the bio-nanocomposites films can be attributed to the high aspect ratio and rigidity that results from the strong affinity between the biopolymer and MMT.

On the other hand, effect of CNC on tensile properties of keratin based nano-composites is given in table 3.2. Tensile strength was not considerably affected but percent elongation was substantially increased. At low CNC content, the tensile strength of the CFK-CNC composite films was comparable to that of the neat CFK films. Highest tensile strength (5.3 MPa) was reported in 1% CNC based composite which is in accordance with other characterization. But it has decreased at higher CNC contents. Also higher percent elongation was observed in CNC based composites as compared to MMT's which may be attributed to the greater extent of hydrogen bonding. This might be due to incomplete dispersion or presence of aggregates of CNC's in the keratin bio-polymer matrix. In case of 3% MMT based film significant difference was reported as compared to neat and other nanocomposites based on MMT. However there was no significant difference on percent elongation was reported except in 1% MMT. In CNC based composites 3% and 5% CNC films showed significant difference as compared to neat along with percent elongation.

Table 3.1. Effect of type and content of MMTs on the tensile strength (TS) and percent elongation at break (%E) of CFK-MMT films.

Sample	Tensile strength (MPa)	Elongation at break (%)
Reference	6.505 ± 0.06b	6.834 ± 0.10a
1% CFK-MMT	5.892 ± 0.80b	7.444 ± 1.89ab
3% CFK-MMT	4.485 ± 0.99a	4.496 ± 2.37b
5% CFK-MMT	6.748 ± 0.14b	8.734 ± 0.98b
10% CFK-MMT	5.985 ± 0.07b	9.380 ± 0.49b

Table 3.2. Effect of type and content of CNCs on the tensile strength (TS) and percent elongation at break (%E) of CFK-CNC films.

Sample	Tensile Strength (MPa)	Elongation at break (%)
Reference	4.503 ± 0.20a	6.587 ± 0.22a
1% CFK-CNC	5.314 ± 0.19c	24.760 ± 3.41c
3% CFK-CNC	4.634 ± 0.04a	24.493 ± 0.41cd
5% CFK-CNC	4.969 ± 0.05b	27.627 ± 0.75b
10% CFK-CNC	4.678 ± 0.07a	21.053 ± 0.44d

*Values of mean of three replicates ± standard deviation. Means in the same column followed by the same letter are not significantly different (P > 0.05)

3.3.6 Scanning electron microscopy (SEM):

SEM images of the fractured surface (cross-sectional surface) of CFK-MMT bio-nanocomposite films with 1%, 3%, 5% and 10% contents of MMT are shown in figure 3.11. The white strands in the SEM images correspond to MMT platelets. At MMT content of 5%, MMT platelets were well dispersed in the bio-nanocomposite films, suggesting exfoliation of MMT in the

films. The fracture surface of the films with both nano-particles become rougher as the MMT content increased to 10%. This can be attributed to the formation of intercalated and disordered intercalated structures arrangements at higher MMT content. In agreement with the TEM results of intercalated structures, larger aggregates of MMT were found in bio-nanocomposite films with MMT content of 10%. Based on the XRD, TEM and SEM results, it can be concluded that compression molding of CFK and MMTs resulted in intercalated and exfoliated morphologies. At higher MMT content (10%), the structure of bio-nanocomposites ranged from intercalated to disordered intercalated. SEM images of fractured surfaces of CFK-CNC films also shown in figure 3.12.

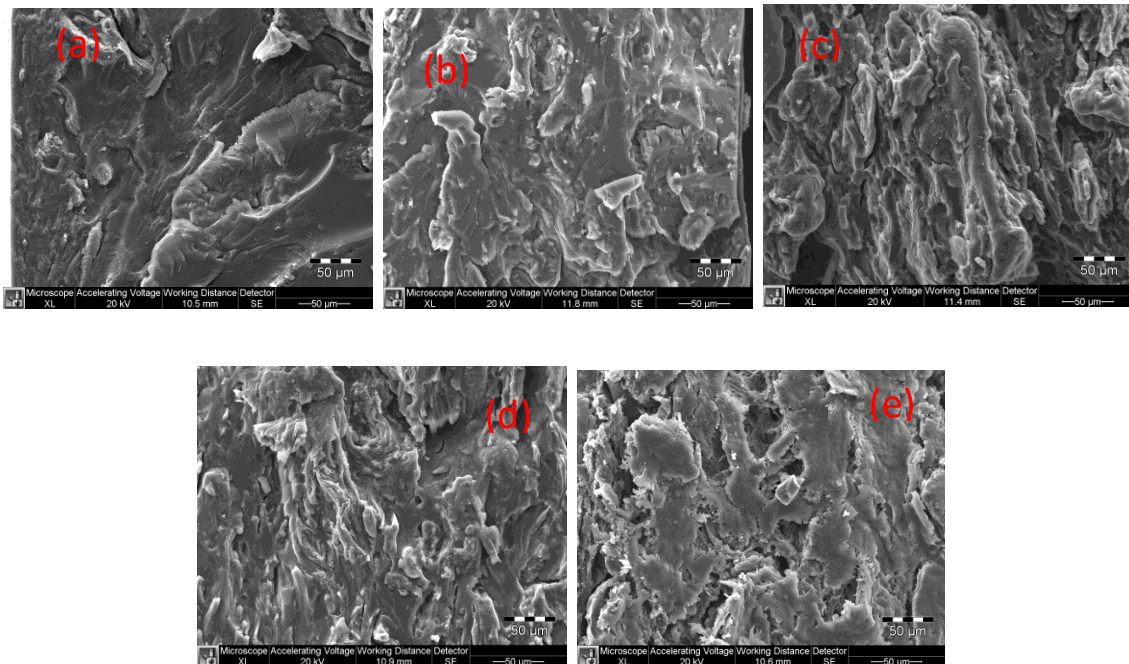


Figure 3.11. SEM images of (a) 0% CFK (b) 1% CFK/MMT (c) 3% CFK/MMT (d) 5% CFK/MMT (e) 10% CFK/MMT

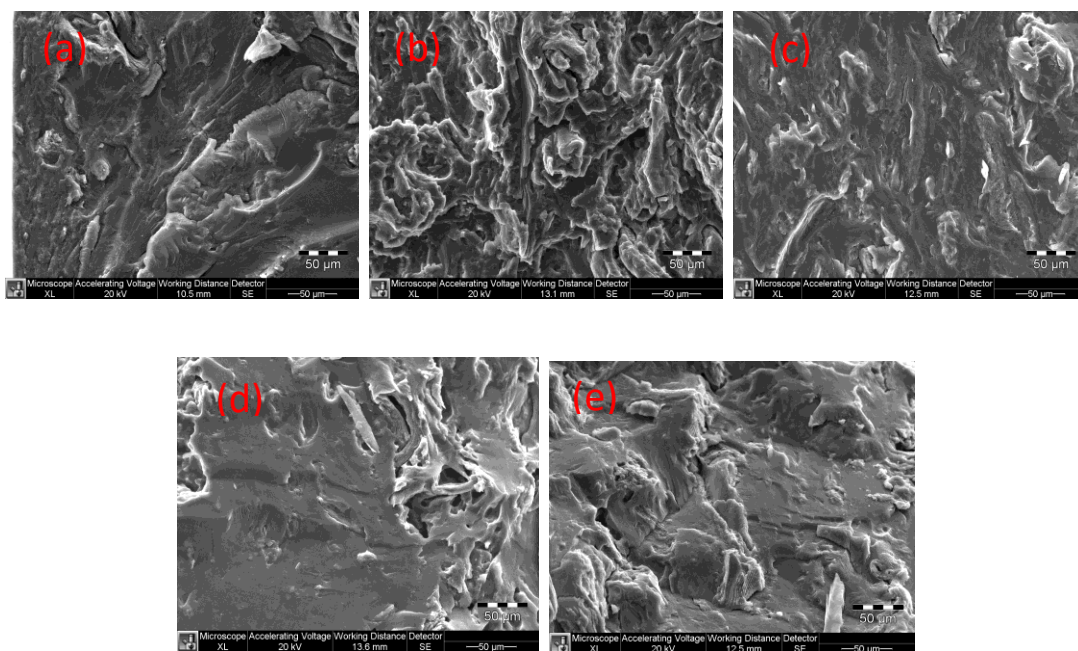


Figure 3.12. SEM images of (a) 0% CFK (b) 1% CFK/CNC (c) 3% CFK/CNC (d) 5% CFK/CNC (e) 10% CFK/CNC

3.3.7 ATR-FTIR spectroscopy:

ATR-FTIR investigation was done to analyze the structural changes in protein. figure 3.13 contains FTIR results of neat CFK, MMT and the composites with different concentration of MMT. Keratin being protein in nature, typically it has three amide regions. The amide I region lies in the range between 1600 and 1700 cm^{-1} , while amide II and amide III absorption bands comes at around 1530 and 1220 cm^{-1} . Amide I refers to the secondary structure of protein and arises mainly from C=O stretching, with a minor contribution from C-N stretching while the amide II band originates from N-H bending and C-H stretching vibrations. In the interval from 3600 to 3100 cm^{-1} neat CFK shows stretching vibration related to N-H of keratin. Overall, there are no significant changes in the spectra of neat and modified keratin fibers, but a slight difference in the peak intensities of neat and modified keratin fibers can be observed (figure 3.13).

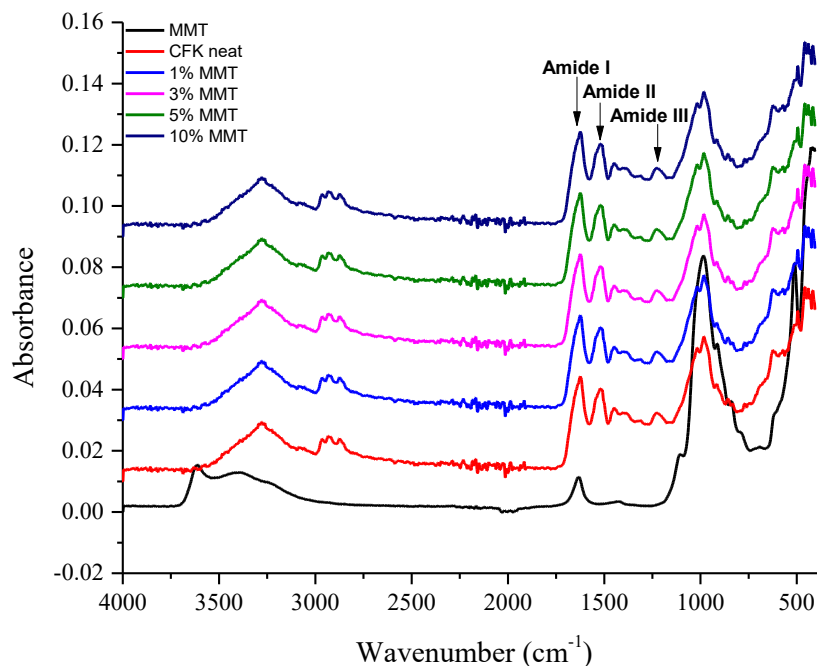


Figure 3.13 FT-IR spectra of MMT, chicken feather keratin and keratin reinforced with different MMT content

ATR-FTIR of CFK reinforced with CNC and CNC alone are shown in figure 3.14. FT-IR shows the spectra of nanocomposites and neat components which shows the structure and hydrogen bonding interactions between CNC and CFK. A broad band in neat CFK starting from 3600 to 3100 cm^{-1} belongs to stretching vibration of N-H groups of keratin, while the broad band in CNC corresponds to -OH stretching vibrations. A very slight variation in this broad region (3280 cm^{-1}) of composites could be attributed to difference in hydrogen bonding of N-H with other functional groups (figure 3.14).

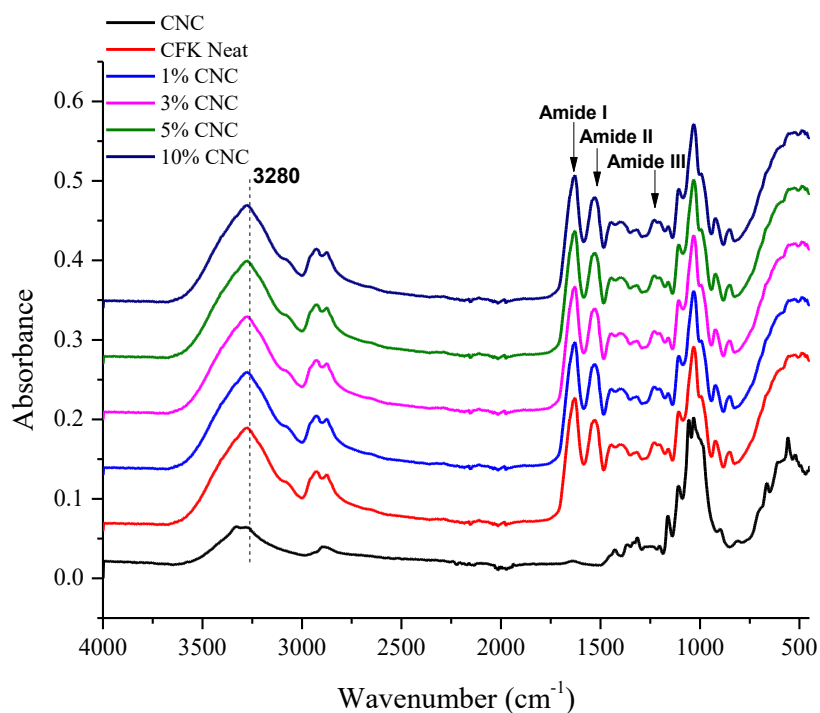


Figure 3.14 FT-IR spectra CNC, chicken feather keratin and keratin reinforced with different CNC content

3.3.8 X-ray photo electron spectroscopy:

X-ray photoelectron spectroscopy (XPS) was used to identify and estimate the surface elemental composition and corresponding chemical bonding status of neat MMT, CNC, CFK and modified MMT-CFK, and CNC-CFK composites. XPS survey spectra of neat MMT, CFK, and keratin reinforced with MMT (MMT-CFK) are given in figure 3.15. Carbon, nitrogen and oxygen are major elements in neat keratin (CFK) as well as in keratin reinforced with MMT. However pure MMT spectrum contains additional elemental peaks related to silicon and aluminium. These additional elemental peaks were not observed in bio-composites reinforced with lower concentration of MMT (1%, 3% and 5%), but at higher MMT contents (10%) the peaks corresponding to silicon and aluminium are obvious. In addition to that a gradual increase in the atomic concentration of oxygen, sodium and silicon has also been observed with the increase of

nanoclay ratio in the modified bio-composites. The high resolution C1s spectra (figure 3. 16) revealed the change in chemical bonding occurred during regeneration of keratin fiber in the presence of nanoclay. Modification changed the relative amount of C1s components. The neat CFK has three distinctive peaks with binding energies (B.E) 284.4 eV (C-C, C-H), 285.6 eV (C-O, C-N) and 287.6 eV (C=O, C-O-C), while MMT has four peaks at 284.2 eV (C-Si), 284.7 eV (C-C, C-H), 286.5 eV (C-O, C-N) and 289.2 eV (-COOR) which are consistent with the already reported data (Arshad, Kaur & Ullah, 2016; Hansson, Trouillet, Tischer, Goldmann, Carlmark, Barner-Kowollik & Malmstorm, 2013; Senoz & Wool, 2010; Wang, Hu, Gao, Zhao, Zhang & Wang, 2015) However, the C1s spectrum of CFK reinforced with 10% MMT has an additional peak at 284.2 eV corresponding to C-Si, which was not present in the neat CFK. Instead of that, in 10% MMT-CFK the area and peak intensities of some peaks have been increased, while a slight shift in their position towards higher B.E can also be seen. The peaks at 284.4 eV (C-C, C-H) and at 285.6 eV (C-O, C-N) of untreated CFK have been shifted to 284.5 eV and 285.9 eV respectively in MMT-CFK biocompoiste, which can be attributed to the nature of substituent on the carbon or the change in its environment after modification. The increase in the area of C-C/C-H, C-O-C/C=O and C-Si peaks also illustrate that bonding of C1s has been increased affirming the diverse interaction of nanoclay with CFK.

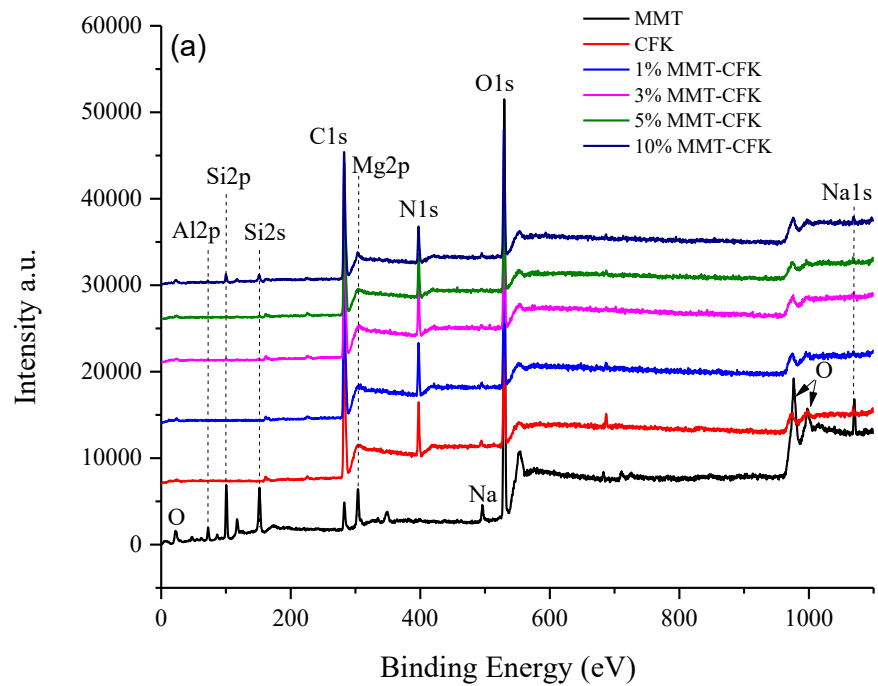


Figure 3.15. XPS survey spectra of MMT, neat chicken feather keratin and its nanocomposites with MMT

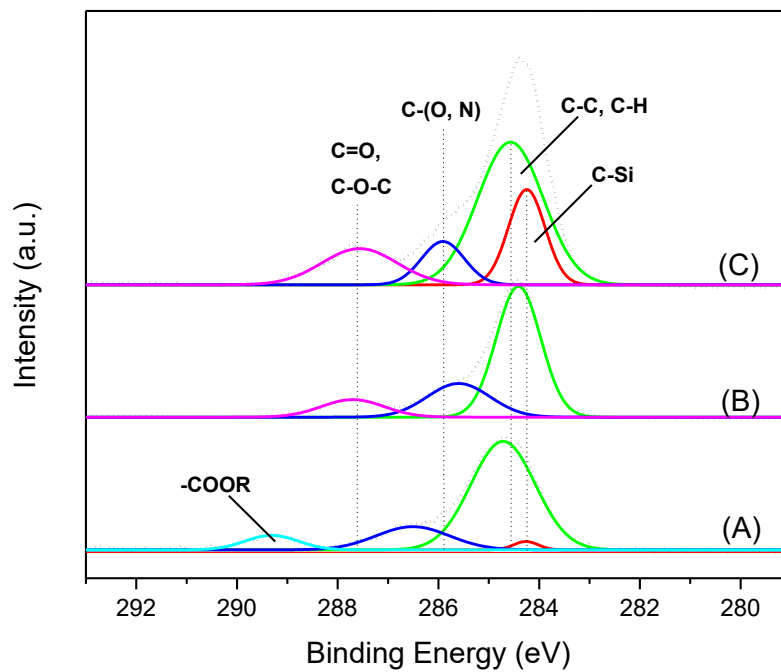


Figure 3.16. High resolution C1s spectra of MMT (A), Neat biopolymer (B) and keratin Biopolymer reinforced with 10% MMT (C)

XPS survey and C1s high resolution spectra of CNC, CFK and modified CNC-CFK composites are given in figure 3.17 and 3.18 respectively. The survey spectrum of CNC, CFK and CNC-CFK contains mainly five elements S, C, N, O and Na with binding energies at 168.3, 283, 395.7, 527.3 and 1068.5 eV respectively. The only distinction which can be seen from figure 3.17 is the extent of difference in their intensities. It has been noticed that, on increasing the ratio of CNC in modified composites, an increase in the atomic concentration of oxygen, nitrogen and sulphur was observed in case of 1%, 3% and 10% CNC-CFK composites when compared to neat CFK. Whereas 5% CNC-CFK displayed inconsistent behaviour, which could be attributed to inhomogeneous dispersion of CNC. On the other hand, the high resolution C1s spectra of 10% CNC-CFK has three additional peaks compared to neat CFK. Moreover, CNC also has three peaks with B.E at 284.6, 286.3 and 287.6 eV corresponding to C-C/C-H, C-O-C/C-OH and C=O/O-C-O respectively (Kaboarani & Riedl, 2015). The presence of extra peaks in 10% CNC-CFK spectra suggests the interaction of CNC with CFK resulting in the formation of new chemical bonding (figure 3.18). The additional peak at 287.8 eV can be attributed to ester (Arshad, Kaur & Ullah, 2016; Hansson *et al.*, 2013), which might be produced from esterification of -COOH from CFK and -OH from CNC. While the second additional peak at 286.3 eV belongs to C-O-C/C-OH bonding and is due to the presence of CNC contents as this bonding exists in neat CNC as well. The third additional peak at 286.0 eV could be attributed to either C-N (quaternary ammonium groups) or C=N bonding created during dispersion of CNC. In addition to the presence of extra peaks in 10% CNC-CFK composite, the peak intensities and area of other peaks related to C-C/C-H, C-O/C-N and C=O/O-C-O have been increased in comparison to neat CFK, which represent the greater ratio of carbon bonding in the modified composite.

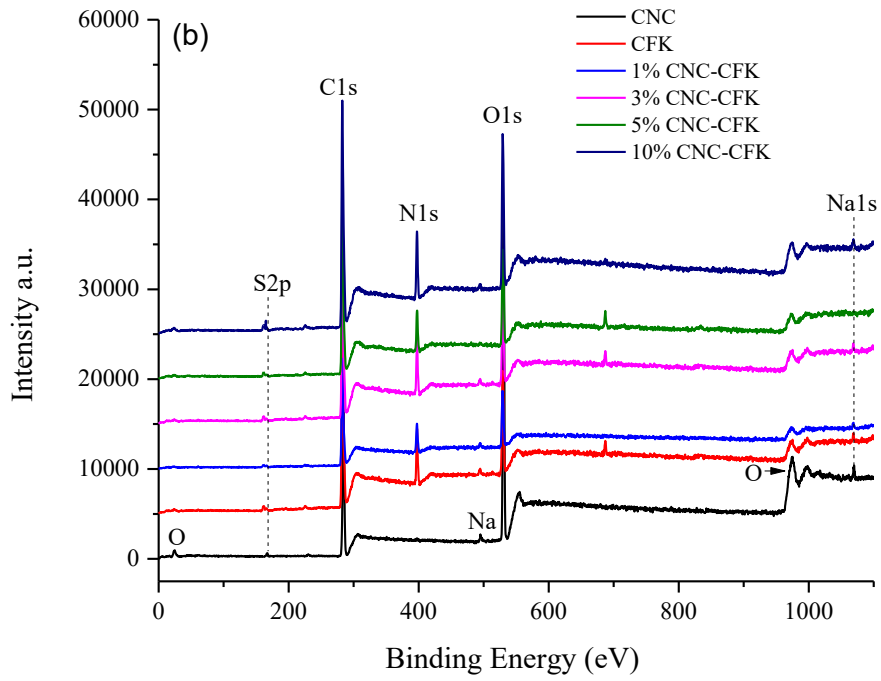


Figure 3.17. XPS survey spectra of neat CFK, CNC and CNC-CFK composites.

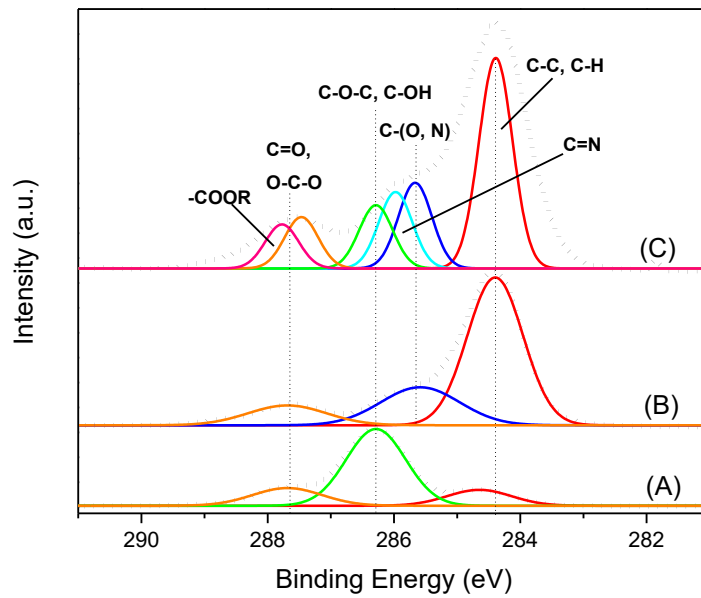


Figure 3.18. High resolution C1s spectra of CNC (A), Neat biopolymer (B) and keratin Biopolymer reinforced with 10% CNC.

Table 3.3. Elemental composition (%) of neat CFK, MMT and MMT-CFK composites.

Samples	C1s	O1s	N1s	Na1s	Si2p	S2p	Al2p	Mg2p
CFK	76.01	11.93	9.66	0.39	0	0.93	0	0
MMT	12.02	58.82	0.05	2.02	17.12	0	6.82	2.59
1% MMT-CFK	75.18	13.38	9.50	0.19	0.19	0.98	0.13	0
3% MMT-CFK	75.58	13.38	9.23	0.21	0.43	0.92	0.11	0
5% MMT-CFK	74.15	14.07	9.78	0.30	0.32	0.97	0.20	0
10% MMT-CFK	65.53	19.98	9.07	0.43	2.85	0.88	0.97	0

Table 3.4. Elemental composition (%) of neat CFK, CNC and CNC-CFK composites.

Samples	C1s	O1s	N1s	Na1s	S2p
CFK	76.01	11.93	9.66	0.39	0.93
CNC	59.10	39.76	0.34	0.53	0.27
1% CNC-CFK	75.99	12.51	9.67	0.49	0.96
3% CNC-CFK	71.83	17.51	9.47	0	1.06
5% CNC-CFK	76.49	12.70	8.30	0.04	1.04
10% CNC-CFK	72.51	16.04	9.85	0.37	1.20

3.4 Conclusions:

In this study feather keratin/MMT films were prepared by compression molding method. The focus of the study was to compare reinforcement effect of montmorillonite (MMT) and cellulose nanocrystals (CNC) on the chicken feather keratin matrix. Better dispersion of nano-particles is desirable for improving the properties of bio-nanocomposite films. The Glass transition temperatures for CNC reinforcements (225 to 242 °C) found to be higher than MMT (199 to 221 °C) reinforcement. Slight increase in glass transition temperatures has been observed with increase in MMT content from 1% to 5% while bio-nanocomposites with 1% and 10% CNC content showed improvements in glass transition temperatures. Similar improvement in thermal degradation behaviour was observed for both nano-particles reinforced biomaterials. The arrangement of MMT in the bio-nanocomposite matrix ranged from exfoliated to inter-calated at low content of the MMT (1% and 3%). However XRD and TEM analysis displayed aggregations at higher concentration (10%) of both MMT and CNC nanoparticles. Well dispersed rod-like shape cellulose nanoparticles were clearly observed in TEM images. Significant increase in T_g observed by DMA has been reported with the addition of nanofillers. MMT reinforced keratin composites showed higher T_g as compared to CNCs. The two nanoparticles had different effect on tensile strength and elongation at break of the nanocomposites. MMT enhanced the tensile strength while CNCs incorporated samples showed higher percent elongation.

3.5 References

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CHAPTER 4. BIODEGRADATION OF CHICKEN FEATHER KERATIN REINFORCED WITH MONTMORILLONITE/CELLULOSE NANOCRYSTALS

4.1 Introduction

In recent years bio-based polymers have attracted lot of attention due to their advantageous nature over petro-based polymers including renewability, sustainability and biodegradability. To solve the problem of waste disposal and environment pollution, various researchers are trying to find the replacements for plastics and undoubtedly biodegradation and recycling are preferred solutions (Sun Yang, Sun Yoon, & Nam Kim, 2005). Therefore agricultural or natural derived polymers are gaining much attention as source for packaging material. Two processes employed for the preparation of the polymers are thermoplastic method and solution casting. Every year billion pounds of feather are wasted from poultry industry. Studies on converting these chicken feathers into useful products have been done. These were also processed thermoplastically by extrusion, compression molding. Horticultural application of processed feather keratin can be one of the attractive application, such as mulching films and nursery containers. This industry also produces plastic waste which should be disposed. There is lack of information on biodegradability and compostability of various natural polymers.

Polymers like polylactic acid (PLA) and polyhydroxybutyrate (PHB) are bio-based polymers and also are biodegradable. PHB found to be highly degradable than PLA (Rosa, Filho, Chui, Calil & Guedes, 2003) . Various other natural polymers were also thermally processed and evaluated for its biodegradability. Biodegradation of starch based polymers were studied by Gasper et al. in the presence of amylase and amloglucosidase enzymes without simulating composting conditions. The polymers were prepared thermally and hydrolysis was observed (Gasper, Benko, Dogossy, Reczey & Czigany, 2005). Compostability of unprocessed corn starch and corn zein

protein has been studied in simulated compost conditions. In high moisture environments, these were fully degradable in 2 weeks with neutral pH (Imam & Gordan, 2002). Lodhi and Netravali studied the soy protein polymers processed thermoplastically. Weight loss measurements and FT-IR analysis showed that there was significant degradation occurred after 20 days which infers slow degradation with the addition of additives. They have used hydrophobic stearic acid by replacing glycerol as plasticizer (Lodha & Netravali, 2005)

The effect of nanoparticles on the biodegradation of the biopolymers has been studied. Some authors reported that nano-particles accelerate the polymer degradation (Ray, Yamada, Okamoto, Ogami & Ueda, 2003). However other studies observed that nanoclays retarded the degradation of aliphatic polyesters during biodegradation, attributing to enhanced barrier properties of the layered silicate nanocomposites. PLA is naturally degradable polymer in soil or compost (Tsuji, Mizuno, & Ikada, 2005; Ho, Pometto, Gadea, Briceno & Rojas, 1999). Although the degradation in natural environment is slower as compared to aliphatic biodegradable polymers (poly (ϵ -caprolactone) PCL) (Pranamuda, Tokiwa & Tanaka, 1997). It has been reported that products from hydrolytic degradation are completely assimilated by micro-organisms such as fungi or bacteria (Hoshino, Tsuji, Ito, Momochi, Mizutani & Takakuwa, 2003; Tsuji *et al.* 1998) The major limitations of PLA are its poor mechanical and gas barrier properties which hinder its industrial applications. It has observed that addition of nanoparticles can not only enhance the material properties of PLA but also accelerates the biodegradation in compost medium (Lee, Park, Lim, Kang, Cho & Ha, 2002). The possible reason for this was high relative hydrophilicity of the nano-clays which allows water to permeate easily resulting in hydrolytic degradation.

Many authors have listed work on various proteins such as soy protein, whey proteins, corn zein etc, but little work has been done on converting chicken feather keratin to bioplastics and on

the evaluation of its biodegradability. Barone & Arikan investigated the composting and biodegradation of thermally processed feather keratin polymers. They composted the polymers in self-heating laboratory composters with three-month old compost inoculum. The biodegradation was observed for 30 days and temperature and carbon dioxide development was monitored regularly. FT-IR showed degradation of feather keratin within 10 days as indicated by reduced molecular weight. DSC analysis showed the change in crystallinity and visual inspection also showed deterioration of the films (Barone & Arikan, 2007).

The aim of this study was to observe the biodegradation of chicken feather keratin and its bio-nanocomposites reinforced with layered silicates and cellulose nanocrystals prepared by compression molding in soil as biodegradation medium.

4.2 EXPERIMENTAL SECTION

4.2.1 Materials and methods

White chicken feathers from broilers were provided by the Poultry Research Centre (University of Alberta) and were processed. The first step performed was cleaning of chicken feathers which was done by washing with soap and water. Drying of the feathers was employed to evaporate water by spreading them under a closed fume hood for 4 days. To ensure the moisture is completely removed from feathers, the dried feathers were ventilated in oven at 50 °C for 8h. Scissors were used for processing after drying and feathers (fiber and quill portion) were ground using Fritsch cutting Mill with sieve of 0.25mm. (Pulverisette 15, Laval Lab. Inc., Laval Canada). To remove grease, ground feathers (8g) were washed with hexane solvent for 4 hours by Soxhlet apparatus followed by drying and storing at room temperature for further modification.

Urea (99%), sodium sulphite (≥ 98), EDTA (99%), n-hexane ($\geq 95\%$), tris-base ($\geq 99.8\%$), glycerol (99.6%), chitosan, 1, 2-butanediol (≥ 98), HCL, hydrophilic nanoclay/Montmorillonite ($\geq 95\%$), were purchased from Sigma Aldrich and used as received. The cellulose nanocrystals (CNCs) were provided by the Alberta Innovates Technology Futures (AITF).

4.2.2 In-situ modification of feather keratin with MMT and CNCs:

The weight ratio of 8 M urea solution to chicken feathers of 17:1 was used to completely immerse chicken feathers. In this immersion, EDTA (0.438g), tris-base (12.102g) and sodium sulfite (10g) were added and stirred at a temperature of 60 °C for a week. The pH of the immersion was adjusted to 9.0 to solubilize the protein monitored regularly during dissolution process. To study the effect of nanoparticles, MMT and CNCs concentrations of 1% and 10% based on the weight of chicken feathers were prepared. To enhance the dispersion of nano-particles, keratin-nanoparticle dispersions were stirred for 20 minutes and sonicated for 10 minutes. After which, the pH of the keratin-nanoparticle dispersion was adjusted to iso-electric point (4.0-4.2) with 1 M HCl and was centrifuged at 10,000 rpm for 10 min to obtain precipitates. The precipitates were washed three times with distilled water under centrifugation of 10,000 rpm for 10 min. This freshly collected nano-reinforced keratin material was then dried at 95 °C for 24 hours in oven, pulverized and sieved with mesh (180 μm).

4.2.3 Bio-nanocomposite film preparation by compression molding:

Keratin and keratin based nanocomposites reinforced with MMT and CNCs were prepared by compression molding. After screening of several plasticizers 1, 2-butanediol and glycerol were selected as suitable plasticizers for MMT and CNC reinforcements respectively. Bio-nanocomposite with glycerol and 1, 2 butanediol (20%) as plasticizers, chitosan (10%) as cross-

linking agent, sodium sulfite (3%) as reducing agent and moisture content (25%) were taken to prepare blends. The ingredients were mixed intensively for 5 min in a beaker and placed for 4 hr in a sealed plastic bag for hydration and plasticizer penetration in the blend. These blends were then thermally compacted using a carver laboratory press (Bench Top Manual Heated Press, Model CH (4386), Carver, Inc. USA). Approximately 3.5 g of each blend was placed within a 5-cm dia at the centre between two 12 × 12 in aluminium plates. The sample was compressed for 15 min within the aluminium plates between the two platens at 145 ± 2 °C with pressure of 10 MPa distributed uniformly over the platens. The plates were allowed to cool to 50 °C before removing from press and cooled to room temperature.

4.2.4 Screening and optimization of the parameters:

Different plasticizers and concentration were tried for preparation of the films. Glycerol found to be suitable plasticizer for keratin-CNC composites, however 1, 2 butanediol found to be suitable for Keratin-MMT composite films. Cross-linking agent was used to enhance the processability and plasticity of keratin films. It also imparts flexibility to keratin-based nanocomposites as films made without cross-linking agent found to be brittle. Different concentrations of cross-linking agent 1%, 3%, 5% and 10% were investigated, out of which 10% chitosan showed most cohesive, homogenous and transparent films. Also the compression molding parameters viz. temperature, pressure and time were optimized before carrying out the actual experiments.

4.2.5 Biodegradation studies:

Biodegradability of the samples was studied at room temperature in at constant humidity of 30-60%. Soil was used as the biodegradation medium. Test specimens were prepared from

compression-molded samples. Around 8 samples were vertically buried at 4-6cm depth to guarantee aerobic degradation conditions at a horizontal distance of 5-6cm between the samples. At selected times (every five days) samples were collected, washed with water and dried at room temperature until constant weight was obtained. Based on the sample weight before and after composting, the average percentage of residual mass for each material was calculated.

Indoor soil degradation

Experiments were carried out in a series of plastic box containing 4000g (dry basis) of agricultural soil. Samples were cut into rectangular shape and weighed. Specimens were put in direct contact of the soil. The specimens were buried at 5-6 cm depth from soil surface to ensure aerobic conditions of degradation. The relative humidity was kept around 40% by adding water periodically and kept at room temperature (24 ± 2 °C). Samples were removed from soil at specific intervals (t), carefully cleansed with distilled water, superficially dried with a tissue paper and weighed. Water loss (%WL) was quantified by the following equation:

$$\%WL = \frac{m_i - m_f}{m_i} \times 100$$

Where m_i and m_f are the initial and the residual mass at time = t, respectively. The reported values are the average of two measurements. After water sorption determination, samples were dried in oven to constant weight. The specimens were weighed on an analytical balance in order to determine the average weight loss (%WL).

4.2.6 Differential scanning calorimetry (DSC):

The thermal properties of bio-nanocomposite films were demonstrated by differential scanning calorimeter (2920 Modulated DSC, TA instrument, USA) under the stream of nitrogen.

To calibrate the heat flow and temperature of instrument, pure indium sample was used. All samples were analyzed in a temperature range of 25 to 300°C at a heating rate of 10°C/min. The samples weighing between 5 to 10 mg were encapsulated in aluminium pans.

4.2.7 Scanning Electron Microscopy (SEM):

The morphology of the degraded samples of bio-nanocomposites was observed using a scanning electron microscope (SEM, FEI XL30, USA) operating at 20 kV. The samples were coated with Au/Pd with the help of hummer 6.2 sputter coater by Anatech Ltd.

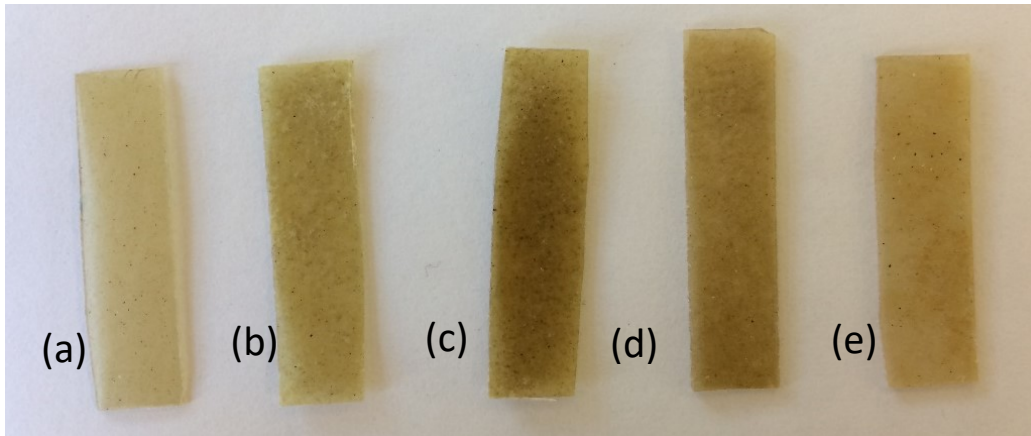
4.2.8 ATR-FTIR spectroscopy:

ATR-FTIR analysis of all films were done on Nicolet 8700 spectrometer (Madison, WI, USA). The samples were observed as films. Spectra were recorded using 64 scans in the wavelength range of 4000–500 cm⁻¹, with 4 cm⁻¹ resolution.

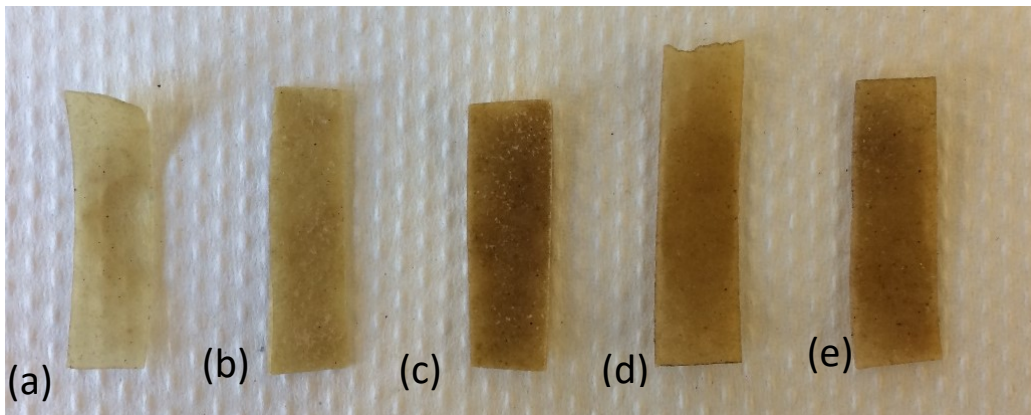
4.3 Results and discussion

4.3.1 Visual inspection:

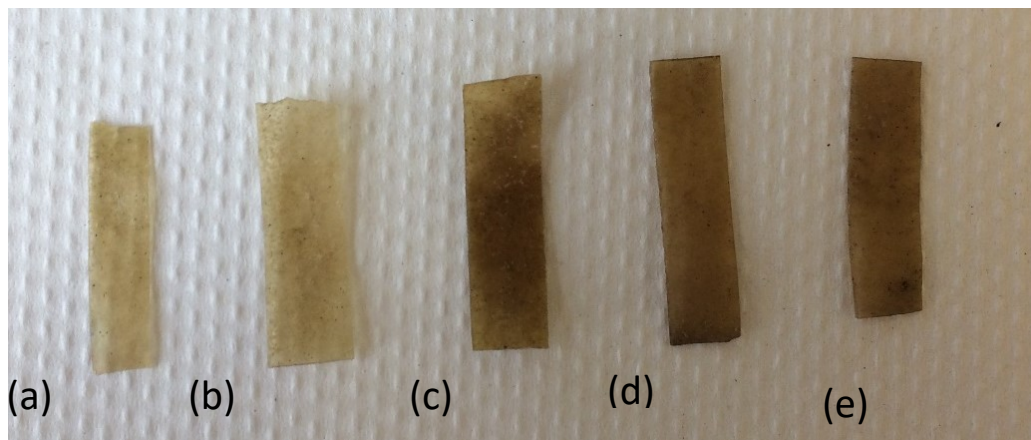
The samples undergoing degradation were visually analyzed. Degraded samples after 0, 5 and 10 days are shown respectively (figure 4.1). After 10 days there was clear degradation of the CFK films with severe discoloration and shortening of the original films. This continued over the entire 30-day period. The darkening of the samples could be the result of enzymatic degradation. The films incorporated with nanoclay were more stable as compared to films with cellulose nanocrystals. However moisture loss was high in case of MMT based films with less discoloration.



Films with a) 0% b) 1% MMT c) 10% MMT d) 1% CNC e) 10% CNC



Films after 5 days of interval



Films after 10 days of interval

Figure 4.1. Pictures of the recovered samples of the control, nanocomposites after 5 and 10 days.

Pictures of the recovered samples of the control, nanocomposites after 5 and 10 days of soil burial are shown in fig. It has been reported that the surface whitening also occurs in some cases due to the process of hydrolytic degradation of the polymer matrix, thus inducing a change in the refraction index of the sample as a consequence of water absorption and/or presence of products formed by the hydrolytic process (Latizia, Scandola, Dobrzynski & Kowalczyk, 2002; Zhang, Xiong & Deng, 1996)

4.3.2 Weight loss of films buried in soil:

Soil has microflora which is a mixture of microbial population including bacteria, fungi, protozoa and actinomycetes. This microflora act together while degradation. Also different types of enzymes produced by micro-organisms are capable of reacting with exposed protein surfaces (Lodha & Netravali, 2005). Beyond the 10th day, samples became brittle. The weight loss for pure feather keratin found to be 23.38% after 5 days. In case of 1% MMT and 10% MMT, it was 26.23% and 21.43% respectively (figure 4.2). Composite films with CNCs showed lower weight loss as compared to MMT based films with 18.88% and 19.32% for 1% and 10% CNC content. However after 10 days, CNC based films showed higher weight loss with 27.37% and 32.42% for 1% and 10 % CNC content respectively. It can be seen from weight loss that there was not significant effect of concentration of nanoparticles. The weight loss was not more than 5% for higher nanoparticle content. In case of CNCs, the weight loss was approximately same for films with 1% and 10% CNC content.

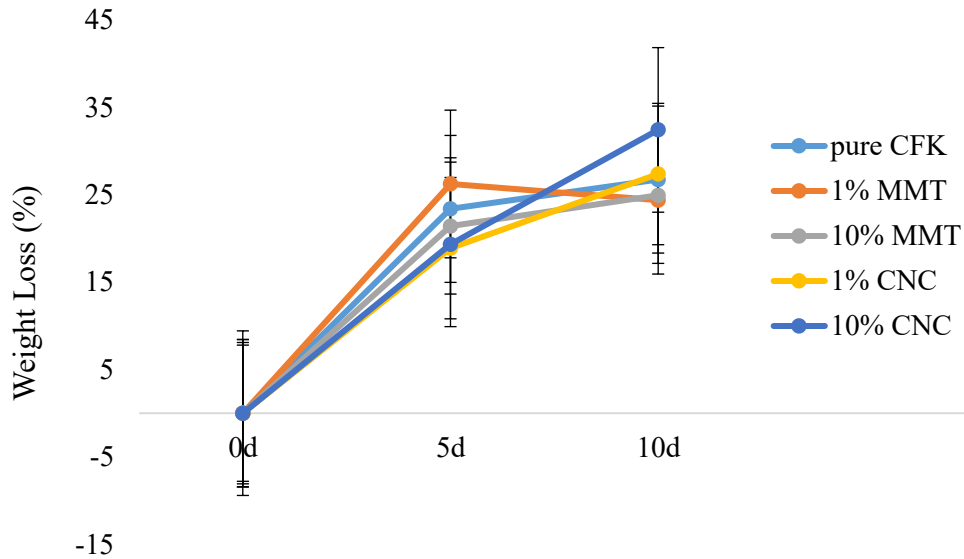


Figure 4.2. Percentage weight loss during biodegradation in the soil at room temperature

4.3.3 Differential scanning calorimetry (DSC):

DSC scans of the degraded samples of pure keratin and composites have been shown in figure 4.3. large peak at 100 °C indicative of moisture loss. Peak at 195 °C shows the keratin crystallinity in the presence of plasticizer. After 5 days of degradation, water loss peak has decreased to lower temperature. The denaturation temperature were observed at 200 °C. The original crystallinity for feather keratin was observed near 250 °C. Similar increase in crystallinity were observed during composting of soy protein isolate. This may be attributed to the fact that micro-organisms attack amorphous regions first. Therefore there is increase in crystallinity (Lodha and Netravali, 2005). Also multiple peaks were observed in case of PLA during degradation which could be related to different crystal structures.

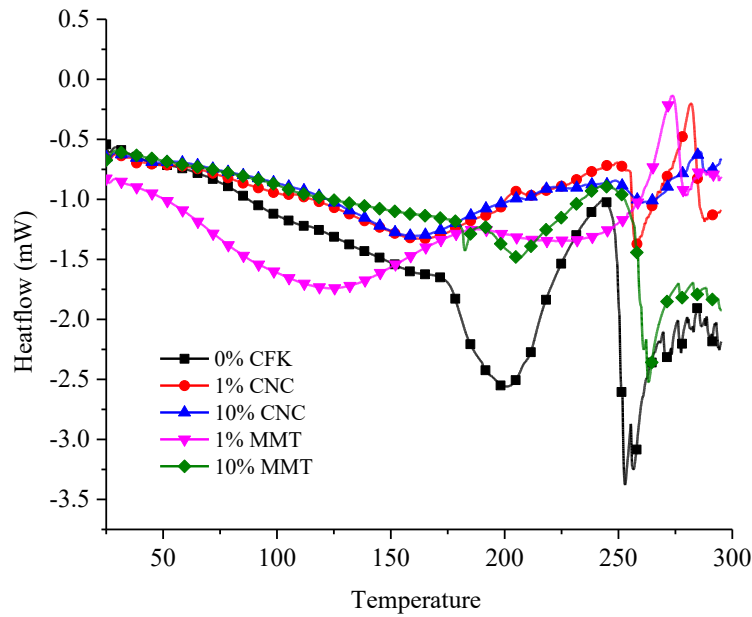


Figure 4.3. DSC thermograms of chicken feather keratin and its composites after 5 days of biodegradation

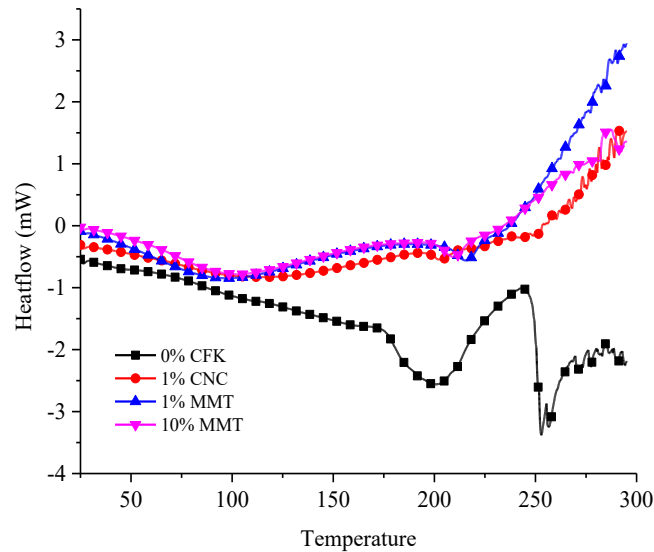


Figure 4.4. DSC thermograms of chicken feather keratin and its composites after 10 days of biodegradation

4.3.4 ATR-FTIR spectroscopy:

ATR-FTIR investigation was done to analyze the structural changes in protein. Figure 4.5. contains FTIR results of neat CFK, MMT and the composites with CNC with 1% and 10% concentration of MMT and CNC after 5 days of interval. Keratin being protein in nature, typically it has three amide regions. The amide I region lies in the range between 1600 and 1700 cm^{-1} , while amide II and amide III absorption bands comes at around 1530 and 1220 cm^{-1} . Figure 4.6 represents the samples after 10 days of biodegradation.

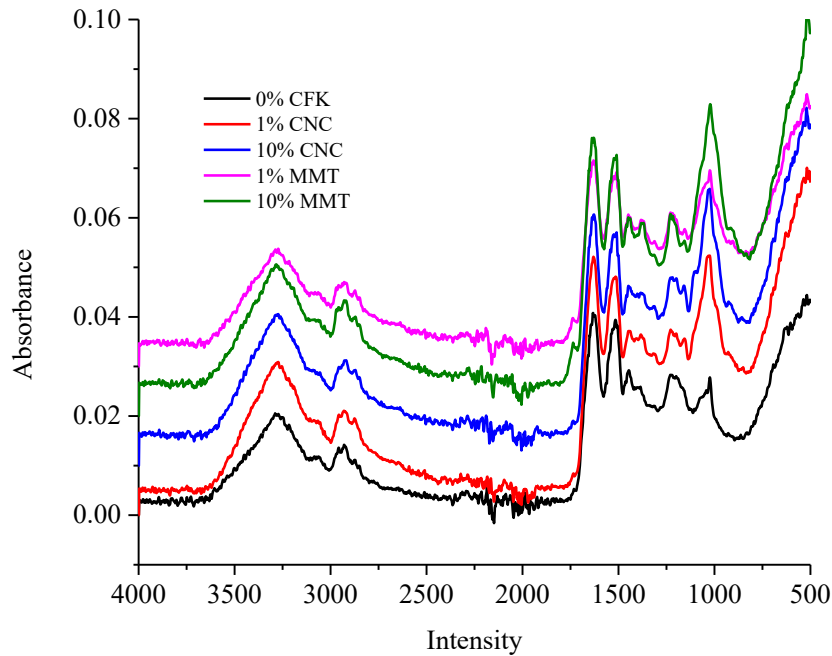


Figure 4.5. FT-IR analysis of chicken feather keratin and its composites after 5 days of biodegradation

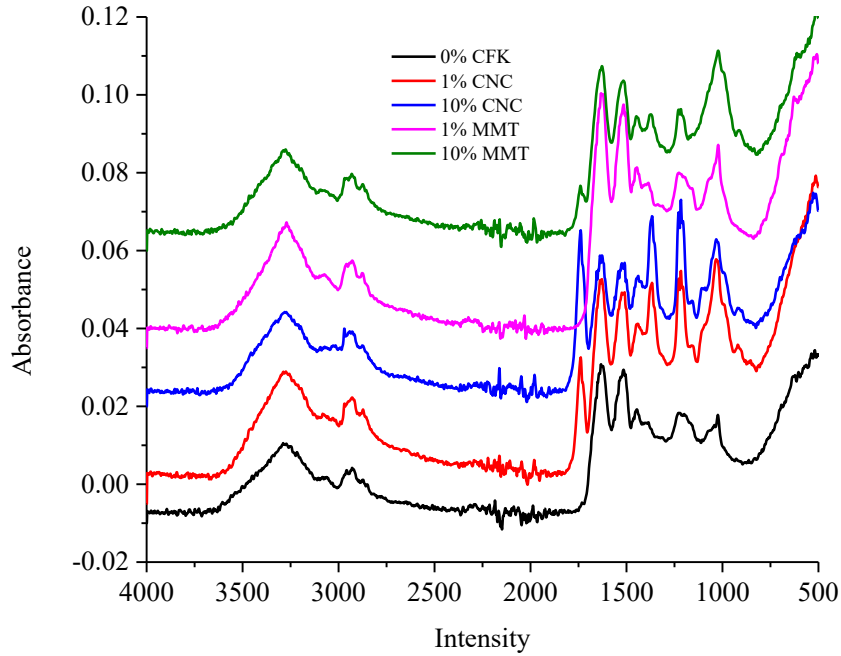
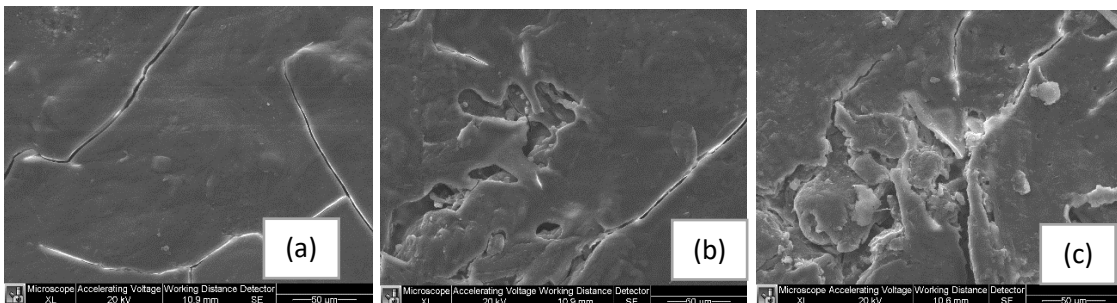


Figure 4.6. FT-IR analysis of chicken feather keratin and its composites after 10 days of biodegradation

4.3.5 Scanning Electron Microscopy (SEM):

The SEM images of the surface has been shown. The images were taken after 5 and 10 days of biodegradation.



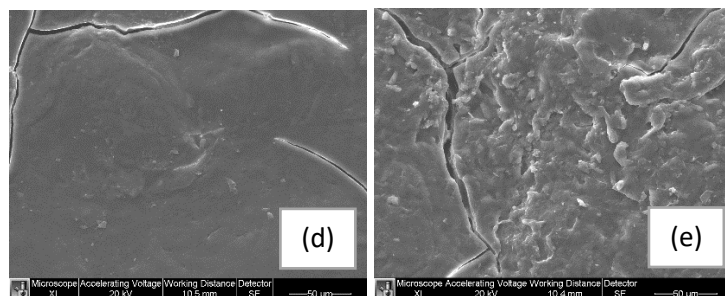


Figure 4.7. SEM images after 5 days of biodegradation (a) neat chicken feather (b) 1% MMT (c) 10% MMT (d) 1% CNC (e) 10% CNC.

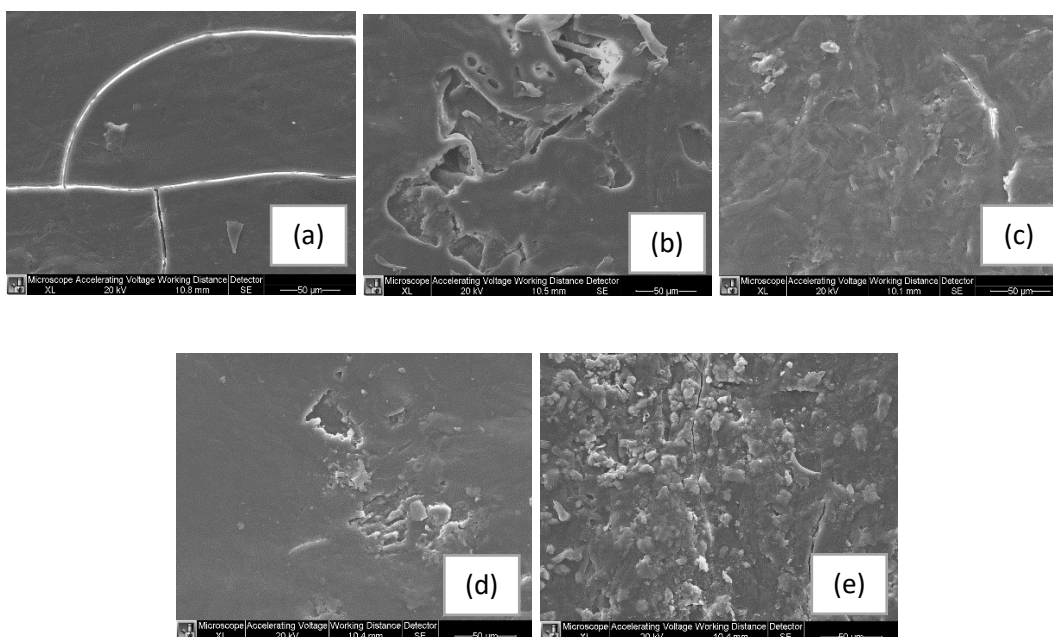


Figure 4.8. SEM images of samples after 10 days of biodegradation (a) neat chicken feather (b) 1% MMT (c) 10% MMT (d) 1% CNC (e) 10% CNC

4.4 Conclusions:

The observed biodegradation was lower than for other proteins such as soy protein. The limited biodegradation of the chicken feather keratin may be due to absence of keratinase producing microbes in the medium. DSC analysis shows change in the crystallinity of the feather keratin due to the loss of plasticizer over time.

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CHAPTER 5. GENERAL CONCLUSION AND FUTURE WORK

5.1 Major findings of the project

As described in Introduction, the major objective of this project was in-situ dispersion of the nanoparticles into the protein matrix and to explore the value added potential of these bio-nanocomposites for packaging industrial applications. The first study was focused on optimization, in-situ dispersion and comparative study of two different nanoparticles dispersed in chicken feather keratin matrix processed by compression molding. Second study represents the biodegradation of the obtained bio-nanocomposites.

It can be challenging to prepare a homogenous biopolymer nanocomposites. Improved thermal stability was observed in nanocomposites prepared with MMT's and CNC's. Crystalline melting temperature shifted to higher temperature in both the cases as indicated by DSC.

In case of mechanical properties, MMT based composites showed higher tensile strength as compared to CNC based composites. However percent elongation was higher in CNC based nanocomposites. Glass transition showed the viscoelastic behaviour, single transitions occurred in CNC based composites as compared to MMT's where two broad transitions were observed.

Structural analysis showed the dispersion of the nanoparticles in the protein matrix. The dispersion was better in low content of nanoparticles. Aggregation was reported at higher content of nano-filler lead to poor homogeneity of the matrix. MMT and proteins involved electrostatic as well as hydrogen bonding between MMT and protein molecules. Crosslinking was demonstrated to be an effective method to improve the interaction the protein-based nanocomposite system and helps in the network formation in the continuous phase during processing.

During biodegradation, weight loss was observed higher in case of CNC's and continued till 10th day. In case of MMT based nanocomposites and neat chicken feather keratin film weight loss found to less after 10 days. Changes in the crystallinity were observed after biodegradation on 5th and 10th day investigated by DSC.

In conclusion, the in-situ dispersion of nanoparticles can yield better interactions between nano-fillers and proteins as compared to physical mixing to carry out thermoplastic processing which in turn can improve the mechanical and thermal properties.

5.2 Future work

Although the bioplastic application of chicken feather keratin has been discussed in this study, the material should ideally display the typical advantages as higher stability, better mechanical properties, biodegradability, lower price, ease of handling, abundance and renewable character. Therefore, there are still further studies that needed to be considered for future on chicken feather protein.

Apart from the weak mechanical properties, high moisture sensitivity is also observed in protein based films. Moisture uptake tests and water vapor permeability tests should be carried out to investigate the effect of nanoparticles on the moisture sensitivity of the films. In proteins moisture is absorbed by the polar groups presence on the surface. Hence undesirable for packaging and other bioplastic applications and adversely affect the durability of the bioplastic films (Ullah & Wu, 2013).

The mechanical properties viz. tensile strength and percent elongation were not substantially enhanced using the above processing method and due to other factors. However study reported by Ullah *et al.* showed higher tensile strength in chicken feather keratin based bioplastic films

processed by extrusion without the addition of any nano-filler. They have studied the effect of different plasticizers on the material properties of chicken feather keratin films. They have reported tensile strength above 15 MPa in all different plasticizers. There is a possibility that extruded and in-situ dispersed nano-filler combined together provides higher strength to chicken feather keratin based nanocomposites (Ullah & Wu, 2013).

Biodegradability studies showed that at low moisture (30%), weight loss was observed within 5 days of experiment which is not desirable. The biodegradation studies were conducted for 10 days period. However the data is not enough to predict the behaviour of these nanocomposite films. Therefore the biodegradation experiment should be conducted for longer period to observe the behavior of these films. In addition the biodegradation can be carried in several ways. For instance compost can be used for conducting biodegradation using municipal solid waste with standard method. Composting of feather keratin films has been reported in which three-month old compost was used to biodegrade thermally processed feather keratin for 30 days. The results showed 24% of biodegradation in 30 days. FT-IR analysis showed reduction in amide peaks attributed to chain scissioning. Also there was change in crystallinity as indicated by DSC analysis (Barone *et al.*, 2007).

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