APPLICATIONS OF CELLULOSE AND CHITIN BASED MATERIALS AS SUSTAINABLE PLASTICS

A Dissertation
Presented to
The Academic Faculty

by

Chinmay C. Satam

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy in the
School of Chemical & Biomolecular Engineering

Georgia Institute of Technology
May 2020

COPYRIGHT © 2020 BY CHINMAY CHARUHAS SATAM
APPLICATIONS OF CELLULOSE AND CHITIN BASED MATERIALS AS SUSTAINABLE PLASTICS

Approved by:

Dr. J. Carson Meredith, Advisor
School of Chemical & Biomolecular Engineering
Georgia Institute of Technology

Dr. Robert J. Moon
School of Materials Science & Engineering
Georgia Institute of Technology

Dr. Meisha L. Shofner
School of Materials Science & Engineering
Georgia Institute of Technology

Dr. Christopher O. Luettgen
School of Chemical & Biomolecular Engineering
Georgia Institute of Technology

Dr. Yulin Deng
School of Chemical & Biomolecular Engineering
Georgia Institute of Technology

Date Approved: March 06, 2020
To my parents who have always been by my side
ACKNOWLEDGEMENTS

This thesis would not be possible without the contribution and guidance of several people. I would like to thank my advisor, Dr. Carson Meredith for his valuable technical guidance as well as providing materials and making available human resources to achieve our research objectives. I greatly appreciate the patience he showed during the time I have been his student and the support he has provided in my academic and career pursuits. I thank all the undergraduate students who have helped execute and shape this research – Jerel Jallorina, Rozhin Parvaresh, Akshay Chiddarwar, Riley Geran, Cameron Coffey, Rogelio Ibarra-Rivera and Eunhyang Lim. I thank all the graduate student collaborators who have helped me execute my vision for this project – Cameron Irvin, Augustus Lang, Yue Ji and Wei Liu. I would like to also thank my graduate student friends who have contributed to my research - Michael McBride, Nils Persson, Jianshan Liao, Vincent Li, Jacob Deneff, and Juan Hazbon.

I would like to thank my thesis committee members – Dr. Meisha Shofner, Dr. Yulin Deng, Dr. Robert Moon and Dr. Christopher Luettgen who provided valuable comments that have shaped this thesis research. I would also like to thank Dr. John Reynolds, Dr. Victor Breedveld and Dr. Matthew Realff who provided valuable expertise, guidance and inputs for my research.

I am greatly indebted to past and present group members - Haisheng Lin, Natalie Girouard, Zihao Qu, Zifu Li, Timi Fadiran, Yi Zhang, Omotola Okesanjo, Songcheng Wang, Aaron Liu, Zeyang Yu, Ezgi Dogan-Guner, Tanner Hickman, Rahul Venkatesh, Madelyn Smart, and Samantha Waters.
Last but not least, I would like to thank my family – my mother, father and my who have always been with me – providing love and support as I complete this journey.
TABLE OF CONTENTS

ACKNOWLEDGEMENTS iv
LIST OF TABLES ix
LIST OF FIGURES xi
LIST OF SYMBOLS AND ABBREVIATIONS xvi
SUMMARY xix

CHAPTER 1. Introduction 1
1.1 Structure & Composition of Natural Cellulose and Chitin 2
1.2 Properties of Cellulose and Chitin 4
  1.2.1 Chemical Structure 4
  1.2.2 Polymorphism & Structural Arrangement 8
1.3 Nanomaterials from Cellulose and Chitin 9
  1.3.1 Mechanical Properties of Cellulose and Chitin Nanomaterials 12
  1.3.2 Gas Permeability 13
1.4 Thesis Overview 17
1.5 REFERENCES 19

CHAPTER 2. Spray-Coated Multilayer Cellulose Nanocrystal – Chitin Nanofiber Films for Barrier Applications 22
2.1 Introduction 22
2.2 Materials & Methods 25
  2.2.1 Materials 25
  2.2.2 Chitin Purification 26
  2.2.3 Extraction of ChNFs 26
  2.2.4 ChNF Characterization 27
  2.2.5 PLA Characterization 28
  2.2.6 Spray Coating 28
  2.2.7 Sprayed Film Characterization 29
  2.2.8 ChNF, CNC Blend Characterization 30
2.3 Results & Discussions 31
2.4 Conclusions 51
2.5 REFERENCES 53

CHAPTER 3. Controlling Barrier and Mechanical Properties of Cellulose Nanocrystals by Blending with Chitin Nanofibers 57
3.1 Introduction 57
3.2 Materials & Methods 60
  3.2.1 Materials 60
  3.2.2 Chitin Purification 60
  3.2.3 Extraction of ChNFs 61
3.2.4 CNC Surface Charge Characterization 62
3.2.5 ChNF Surface Charge Characterization 62
3.2.6 Particle Size and Distribution of ChNFs and CNCs Suspensions 63
3.2.7 Characterization of ChNFs, CNCs and Blends of ChNFs-CNCs 63
3.2.8 Preparation of Neat and Blended Films 64
3.2.9 Characterization of Neat and Blended Films 64

3.3 Results & Discussions 66
3.3.1 Physicochemical Properties 66
3.3.2 Oxygen Permeability 77
3.3.3 Mechanical Properties 79
3.3.4 Optical Properties 83

3.4 Conclusions 91
3.5 REFERENCES 94

CHAPTER 4. Optimization of High-Pressure Homogenization to Produce Chitin Nanofibers for Barrier Films 99
4.1 Introduction 99
4.2 Materials & Methods 102
4.2.1 Materials 102
4.2.2 Deacetylation of Commercial Chitin 103
4.2.3 Crab shell Chitin purification and Deacetylation 103
4.2.4 Extraction of ChNFs 104
4.2.5 ChNF and Suspension Characterization 105
4.2.6 Preparation of Solution Cast Commercial and Crab Shell ChNF films 106
4.2.7 Characterization of Solution Cast ChNF films 106

4.3 Results & Discussions 108
4.3.1 Effect of Deacetylation 108
4.3.2 Effect of Pressure 123
4.3.3 Optimized Homogenization of Crab Shell Chitin 132

4.4 Conclusions 136
4.5 REFERENCES 138

CHAPTER 5. Future directions in high oxygen and water vapor barrier materials – Thermally treated Poly(glucuronic acid)-Chitosan films 143
5.1 Introduction 143
5.2 Materials & Methods 146
5.2.1 Materials 146
5.2.2 Preparation of TEMPO derived PG 147
5.2.3 Preparation of Ch and PEG-Ch-PG Membranes 147
5.2.4 Preparation of Ch-PG Membranes 147
5.2.5 Heat treament of Membranes 148
5.2.6 Characterization of Heat Treated and Untreated Films 148

5.3 Results & Discussions 149
5.4 Conclusions 158
5.5 REFERENCES 160

CHAPTER 6. Conclusions and Recommendations 164
6.1 **Summary and Conclusions** 164
  - 6.1.1 Develop Coatings Based on ChNFs and CNCs 164
  - 6.1.2 Optimize the Utilization of ChNFs in ChNF-CNC Based Composites 165
  - 6.1.3 Increase Production Efficiency ChNFs 166
  - 6.1.4 Maximize Synergistic Interactions between Chitin and Cellulose based Materials 167

6.2 **Recommendations for future work** 168
  - 6.2.1 Simulations for Fiber Assembly and Hydrogen Bonding 169
  - 6.2.2 Modified Cellulose and Chitin as Platform for Plastic-like Materials 169

**APPENDIX A. particle size and diameter distribution for deacetylation studies** 171
  - A.1 89 % DA ChNFs 171
  - A.2 79 % DA ChNFs 173
  - A.3 72 % DA ChNFs 175
  - A.4 LP Processed ChNFs 177
  - A.5 HP Processed ChNFs 178
LIST OF TABLES

| Table 1.1  | Degree of Polymerization of various forms of cellulose. | 7 |
| Table 1.2  | Permeability of different gases in CNF & CNC films (Sisal) at 25 °C & 1 bar pressure, Permeability of different gases in ChNF films & coagulated ChNF [C-ChNF] films. | 14 |
| Table 1.3  | Water Vapor Permeability rates of different polymers. | 15 |
| Table 2.1  | Properties of neat PLA. | 33 |
| Table 2.2  | Thickness of equal volumes of 0.5 wt. % loading solution cast films. | 43 |
| Table 3.1  | Composition and surface charge of suspensions of CNCs, ChNFs and blends. | 69 |
| Table 3.2  | Two tailed P-values for mean ultimate tensile strength (UTS). Values highlighted in red indicate differences in means between the two samples being compared is significant and the level of 0.05. | 80 |
| Table 3.3  | Two tailed P-values for mean strain at break. Values highlighted in red indicate differences in means between the two samples being compared is significant and the level of 0.05. | 80 |
| Table 4.1  | Properties of ChNF samples produced from commercial chitin. | 108 |
| Table 4.2  | Two sample KS values for particle length distributions. Values highlighted in red indicate differences in distributions between the two samples being compared is significant at the significance level of 0.01. | 111 |
| Table 4.3  | Two sample KS values for particle width distributions. Values highlighted in red indicate differences in distributions between the two samples being compared is significant at the significance level of 0.01. | 111 |
| Table 4.4  | Two tailed P-values for mean ultimate tensile strength (UTS). Values highlighted in red indicate differences in means between the two samples being compared is significant and the level of 0.01. | 119 |
| Table 4.5  | Two tailed P-values for mean strain at break. Values highlighted in red indicate differences in means between the two samples being compared is significant and the level of 0.01. | 119 |
Table 4.6  Two sample KS values for particle length distributions. Values highlighted in red indicate differences in distributions between the two samples being compared is significant at the significance level of 0.01.

Table 4.7  Two sample KS values for particle width distributions. Values highlighted in red indicate differences in distributions between the two samples being compared is significant at the significance level of 0.01.

Table 4.8  Two tailed P-values for mean ultimate tensile strength (UTS). Values highlighted in red indicate differences in means between the two samples being compared is significant and the level of 0.01.

Table 4.9  Two tailed P-values for mean strain at break. Values highlighted in red indicate differences in means between the two samples being compared is significant and the level of 0.01.

Table 4.10 Properties of solution cast films of homogenized crab shell with different values of DA.

Table 5.1  Thickness and Oxygen Transmission of films.

Table 5.2  Two tailed P-values for mean Ultimate Tensile Strength. Values highlighted in red indicate differences in means between the two samples being compared is significant at the level of 0.05.

Table 5.3  Two tailed P-values for mean strain at break. Values highlighted in red indicate differences in means between the two samples being compared is significant at the level of 0.05.
LIST OF FIGURES

Figure 1.1  Hierarchical structure of woody material. 3
Figure 1.2  Hierarchical arrangement of chitin micro fibrils. 4
Figure 1.3  Structure of cellulose & chitin. 5
Figure 1.4  Structure of crab shell chitin. 6
Figure 1.5  a) TEM image of Sisal CNCs (b) Scanning Electron Microscope (SEM) image of SISAL CNFs. 11
Figure 1.6  Oxygen transmission rate of CNF films as a function of relative humidity (RH) for different film grammages. 16
Figure 2.1  Spray coating apparatus. 28
Figure 2.2  FTIR spectrum of pure chitin from Alfa Aesar and processed crab shell chitin. 31
Figure 2.3  Haze of pristine and aged 0.5 wt. % suspensions of (a) ChNF (b) CNC (d) sprayed ChNF (d) sprayed CNC. 32
Figure 2.4  DSC curve of neat PLA showing heat flow vs temperature. Inset shows section of DSC curve between 40 and 100 °C used to determine the T_g of neat PLA. 34
Figure 2.5  DSC curve of neat PLA film showing heat flow vs time. 35
Figure 2.6  TGA curves for (a) neat ChNF film (b) neat CNC film (c) neat PLA film. 36
Figure 2.7  Film thicknesses of PLA films with and without single and multilayer coatings. Error ranges are 95 % confidence intervals. 37
Figure 2.8  a) Photos of neat PLA and (b) PLA-(CNC-ChNF)_1 films covering CMYK and RGB colored swatches. (c) Light transmission versus number of layers of films measured at 550 nm (d) Optical haze calculated over the visible spectrum (380-780 nm). 38
Figure 2.9  Representative AFM images of coated PLA films. (a) Height image and (b) phase image of top layer coating of ChNFs on a (PLA-(ChNF-CNC))_1-ChNF) multilayer (c) height image and (d) phase image of top layer coating of CNCs on a (PLA-(ChNF-CNC)_2) multilayer. 40
Figure 2.10  SEM cross-sectional images of fractured films: (a) PLA-(ChNF)$_1$, (b) PLA-(ChNF-CNC)$_1$, (c) PLA-(ChNF-CNC)$_1$-ChNF, (d) PLA-(ChNF-CNC)$_2$, (e) PLA-(ChNF-CNC)$_2$-ChNF, (f) PLA-(ChNF)$_5$, (g) PLA-(CNC)$_5$, (h) PLA-(CNC)$_1$.

Figure 2.11  (a) O$_2$ permeability of PLA films with and without single and multilayer coatings of ChNFs and CNCs at 23 ºC and 50 % RH. (b) O$_2$ permeability versus RH of permeant gas at 23 ºC.

Figure 2.12  (a) WVTR of PLA films with and without single and multilayer coatings of ChNFs and CNCs at 23 ºC and 50 % RH. Measurement errors at 50 % RH smaller than 7 g-water/m$^2$/day. (b) WVTR versus RH of permeant gas at 23 ºC.

Figure 2.13  Selected individual stress vs strain curves determined from HTMECH biaxial indentation.

Figure 2.14  (a) UTS, (b) breaking strength and (c) strain at break of coated and uncoated PLA films deformed biaxially with an instrumented probe.

Figure 3.1  Number distributions of (a) length (b) of LChNF & HChNF fibers. Number distributions of (c) length & (d) width of CNCs.

Figure 3.2  Figure 3.2 (a) Zeta potential and (b) Zetasizer particle size of cast CNC, LChNF, HChNF, CNC-LChNF & CNC-HChNF composite films.

Figure 3.3  Images of representative CNC, LChNF, HChNF & blended films. The scale bar is 50 mm.

Figure 3.4  Light Transmission at 550 nm of cast CNC, LChNF & CNC-LChNF composite films. Inset compares HChNF & LChNF neat and composite films.

Figure 3.5  Observed thickness of CNC, LChNF & CNC-LChNF composite films. Inset compares HChNF & LChNF neat and composite films.

Figure 3.6  Oxygen permeability of cast CNC, LChNF & CNC-LChNF composite films. Inset shows oxygen permeability of HChNF & LChNF neat and composite films.

Figure 3.7  Roughness of solution cast films measured from an AFM image of 5 μm x 5 μm size.

Figure 3.8  Box plots showing the mechanical properties of films. (a) UTS, (b) strain at break.

Figure 3.9  Optical microscope images of (a) neat CNC, (b) 3:1 CNC-LChNF, (c) 1:1 CNC-LChNF, (d) 1:3 CNC-LChNF, (e) neat LChNF, (f) neat
HChNF, and (g) 1:1 CNC-HChNF. Images are exposure-corrected. All scale bars are 200 μm in length.

Figure 3.10 AFM images of (a) neat CNC, (b) 3:1 CNC-LChNF, (c) 1:1 CNC-LChNF, (d) 1:3 CNC-ChNF, (e) neat LChNF, (f) neat HChNF, and (g) 1:1 CNC-HChNF.

Figure 3.11 SEM images of fractured films (a) neat CNC, (b) 3:1 CNC-LChNF, (c) 1:1 CNC-LChNF, (d) 1:3 CNC-ChNF, (e) neat LChNF, (f) neat HChNF, (g) 1:1 CNC-HChNF, and (h) magnified image of 1:1 CNC-HChNF. Scale bars are 2 μm.

Figure 4.1 Number distributions of (a) length (b) width of ChNF fibers with different DA values in suspension determined from AFM measurement.

Figure 4.2 Variation of (a) suspension pH (b) suspension light transmission as a function of number of homogenization passes for commercial chitin samples with different values of DA.

Figure 4.3 Variation of (a) light transmission and (b) oxygen permeability for dried cast films prepared from commercial ChNF samples with different values of DA. Permeability is measured at 50 % RH and 23 °C.

Figure 4.4 (a) Ultimate tensile strength and (b) strain at break of dried films cast from homogenized commercial ChNFs with different values of DA.

Figure 4.5 AFM images of the top surface of dried cast films of (a) 89% DA, (b) 79 % DA, and (c) 72 % DA ChNFs. Height images are on the left and phase images are on the right hand side.

Figure 4.6 SEM images of cross-sections of (a) 89% DA, (b) 79 % DA, and (c) 72 % DA ChNF films. Scale bars are 500 nm.

Figure 4.7 Number distributions of (a) length (b) width of ChNF fibers with different DA values in suspension.

Figure 4.8 Variation of (a) Suspension pH and (b) Suspension light transmission for different homogenization passes for commercial chitin samples with different values of homogenization pressures.

Figure 4.9 Variation of solution cast film (a) light transmission (b) oxygen permeability for commercial chitin samples with different values of homogenization pressures.
Figure 4.10  (a) Ultimate tensile strength, (b) strain at break of solution cast films of homogenized commercial chitin with different values of DA.

Figure 4.11  AFM images of (a) LP (b) HP processed ChNFs. Each image set from left to right. Height image and phase image.

Figure 4.12  SEM images of cross-sections of (a) LP (b) HP ChNF films. Scale bars are 1 μm.

Figure 4.13  Variation of (a) Suspension pH and (b) Suspension light transmission for different homogenization passes for crab shell chitin samples with different values of DA.

Figure 4.14  (a) Ultimate tensile strength, (b) strain at break of cast films of homogenized crab shell ChNF with different values of DA.

Figure 5.1  Water Vapor Transmission Rate of films.

Figure 5.2  (a) Ultimate Tensile Strength and (b) Strain at Break of untreated and treated films

Figure 5.3  FTIR spectrum of films, PG.

Figure A.1  AFM height image of 89 % DA ChNF.

Figure A.2  AFM height image of 89 % DA ChNF.

Figure A.3  AFM height image of 89 % DA ChNF.

Figure A.4  AFM height image of 79 % DA ChNF.

Figure A.5  AFM height image of 79 % DA ChNF.

Figure A.6  AFM height image of 79 % DA ChNF.

Figure A.7  AFM height image of 79 % DA ChNF.

Figure A.8  AFM height image of 79 % DA ChNF.

Figure A.9  AFM height image of 72 % DA ChNF.

Figure A.10  AFM height image of 72 % DA ChNF.

Figure A.11  AFM height image of 72 % DA ChNF.

Figure A.12  AFM height image of LP processed ChNF.

Figure A.13  AFM height image of LP processed ChNF.
Figure A.14  AFM height image of LP processed ChNF. 178
Figure A.15  AFM height image of HP processed ChNF. 178
Figure A.16  AFM height image of HP processed ChNF. 179
Figure A.17  AFM height image of HP processed ChNF. 179
LIST OF SYMBOLS AND ABBREVIATIONS

AFM  Atomic Force Microscopy
Ch   Chitosan
ChNC Chitin Nanocrystals
ChNF Chitin Nanofibers
ChNW Chitin Nanowhiskers
CNC  Cellulose Nanocrystals
CNF  Cellulose Nanofibrils/Nanofibers
DA   Degree of Acetylation
DDA  Degree of Deacetylation
DI   Deionized
DP   Degree of Polymerization
DSC  Differential Scanning Calorimetry
EVOH Ethylene vinyl alcohol
FTIR Fourier Transform Infra-Red
HChNF ChNF with DA >90%
HP   High Pressure
HPH  High Pressure Homogenization
HPLC High Performance Liquid Chromatography
HTMECH High-Throughput Mechanical Characterization
KS   Kolmogorov-Smirnov
LChNF ChNF with DA in the 50-90 % range
LDPE Low Density Poly(ethylene)
LP  Low Pressure
m  Equivalents of NH2 per gram of chitin

MTM  Montmorillonite
NIR  Near Infrared
OP  Oxygen Permeability
PE  Poly(ethylene)
PEG  Poly(ethylene glycol)
PEL  Polyelectrolyte
PET  Poly(ethylene terephthalate)
PG  Poly(glucuronic acid)
PLA  Poly(lactic acid)
PVA  Poly(vinyl alcohol)
PVDC  Poly(vinylidene chloride)
RH  Relative Humidity

TEMPO  2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl

T  Light transmission (%)
T1  Transmittance values for geometries without sample
T2  total sample transmittance (diffuse and specular)
T3  Transmittance values for geometries without sample
T4  only diffuse transmittance
Tg  Glass Transition Temperature

TGA  Thermogravimetric Analysis
SEM  Scanning Electron Microscopy
UTS  Ultimate Tensile Strength

UV-Vis  Ultraviolet-Visible
WVTR  Water Vapor Transmission Rate

$\phi$  Void fraction

$\rho_{\text{air}}$  density of air

$\rho_{\text{bulk}}$  bulk density of films

$\rho_{\text{mix}}$  weight averaged theoretical density of mixtures
SUMMARY

Plastics waste is a land management and logistical problem, with difficulties in recycling packaging being of particular concern. A composite barrier material was developed by spray coating chitin nanofibers (ChNFs) and cellulose nanocrystals (CNCs) onto poly(lactic acid) (PLA). The resulting renewable flexible film had similar barrier properties to poly(ethylene terephthalate), with oxygen permeability of 19.6 cm³-μm/m²/day/kPa, resulting from structures driven by synergistic interactions of ChNFs and CNCs. Films formed from blended ChNF and CNC aqueous suspensions were investigated in order to determine whether synergy between the components leads to optimal mechanical and barrier properties. Solution cast films from CNCs were found to have higher oxygen permeability (OP) than deacetylated ChNF films. Addition of 25 wt. % ChNFs to CNCs resulted in an OP reduction by 87 % to a value of 1.7 cm³-μm/m²/day/kPa similar to that of pure deacetylated ChNF films. These developments allow lowering the amount of ChNFs used in the ChNF-CNC formulations without significant impact to barrier properties if deacetylated ChNFs were used. Additionally, the homogenization process for the ChNF manufacture was optimized through controlled deacetylation. The resulting surface cationization allowed ChNFs to be homogenized in 8 passes as opposed to 30 passes at a lower pressure of 551 bar. Deacetylated material had similar barrier properties, higher light transmission (up to 85 %T) and showed 164 % and 162 % improvement in tensile strength and strain at break, respectively. Finally, a hybrid film was produced using chitosan, cellulose derived poly(glucuronic) acid and polyethylene glycol, which exhibited high oxygen barrier (0.2 cm³-μm/m²/day/kPa) and polymer-like water
vapor permeation (8.2 g-mm/m²/day). This material takes advantage of reactions between chitosan and carboxylic acid groups on poly(glucuronic acid) that could form the basis for a new class of biodegradable materials. In summary, these developments contribute to optimization of renewable bio-based nanomaterial blends to produce alternative barrier packaging that could be produced in a circular manner at a lowered cost.
CHAPTER 1. INTRODUCTION

Natural materials are omnipresent in our everyday lives. One specific material that is a subject of extensive engineering research is cellulose, which has been used by mankind for centuries. Cellulose is most widely used in the form of wood as a high-strength durable building material.[1] It has been used as clothing in the form of cotton. Derivatives of cellulose have been used for fibers and plastics and as special chemicals for building and construction as well as specialized applications in paint, oil exploration, food and pharmaceutical products, etc. Cellulose nitrate and cellulose acetate were historically important in film preparation for photographic and packaging applications. It is the major constituent of paper. Synthetic composites of native cellulose or regenerated cellulose are widely known and used. Vast amounts of cellulose are produced naturally by plants and form an important food source for many animals.[2]

Chitin is said to be the second most abundant polysaccharide and like cellulose it is synthesized by living organisms.[3, 4] Unlike plant-based cellulose, chitin is primarily synthesized by crustaceans, fungi and insects. It is structurally very similar to cellulose and finds application in metal chelation, food additives, seed and fertilizer coatings, surface treatment in the pulp and paper industry, moisturizers, and cosmetics. It has especially found use in biomedical applications including tissue engineering, drug delivery, wound dressing, scaffolds, cancer diagnosis, etc., due to its antibacterial properties. The annual natural chitin production is estimated to be around $10^{10} - 10^{12}$ tons per year[3] and is about the same order of magnitude as cellulose, whose annual bio-production by photosynthesis is estimated to be of the order of $10^{11}-10^{12}$ tons per year.[4]
Despite a fair number of applications this discarded chitinous material has very little commercial value[5] as of yet, possibly due to supply outweighing demand or due to purity constraints. It can however potentially be used in a manner similar to cellulose, and the significant opportunities in expanding chitin utilization justify further research and development. Specifically, it is the intent of this dissertation to engineer raw chitinous material into a flexible barrier material that can compete with commercially existing food packaging barrier materials. The potential for sustainable sourcing and production may thereby reduce the dependence of the packaging industry on petroleum based raw materials.

1.1 Structure & Composition of Natural Cellulose and Chitin

Cellulose occurs primarily as wood in the form of lignocellulosic materials with the cellulose content of wood being 40-50 % (without differentiating between hardwood and softwoods). Other materials such as water plants, grasses and plant matter also contain cellulose to different extents with the cellulose content of cotton being as high as 95 %. [4] Cellulose occurs naturally with lignin and hemicelluloses along with small amounts of extractives & inorganic salts.[4, 6]

Cellulose is present as 3-35 nm (depending on source) diameter elementary fibrils, which in turn are bundled into 10-20 μm diameter[4, 6] micro fibrils arranged in a complex hierarchical structure with hemicelluloses and lignin as seen in Figure 1.1. Chitin is naturally found alongside proteins and minerals that constitute the extracellular matrices of animals. For example, typical crustacean shells containing about 20-30% chitin along
with 30-40% proteins & the rest minerals (mainly calcium carbonate). Some species of lobster contain as much as 70% chitin.[7]

Figure 1.1 Hierarchical structure of woody material. Figure shows the middle lamella (ML), the primary wall (P), the outer, middle and inner layers (S1,S2,S3) layers of secondary wall, the warty layer (W), cellulose (C), hemicellulose (H), lignin (L), microfibril (MF), elementary fibril (EF), crystalline domain (Cr) and amorphous domain (Am). Adapted from Nechyporchuk et al.[6]

Chitin from low cost commercial sources like crustaceans also exists as microfibrils packaged in a complex hierarchical fashion similar to cellulose as can be seen in Figure 1.2, albeit along with different materials. Approximately 18 to 25 chitin crystal molecules are arranged as long narrow crystalline units which are further encased in proteins. This unit is about 2-5 nm in diameter and about 300 nm in length and is called the nanofibril. The nanofibrils are clustered into long fibrils of about 50-300 nm in diameter which are further woven into a planar periodically branched chitin–protein network. The
spaces are filled with proteins and minerals (mostly CaCO₃) which can be microscopic in size. The sheets are arranged in a helicoidal stacking sequence referred to as the Bouligand (or twisted plywood) pattern.[8]

Figure 1.2 Hierarchical arrangement of chitin micro fibrils showing the complex arrangement in crustacean shells. Figure adapted from Raabe et al.[8]

1.2 Properties of Cellulose and Chitin

1.2.1 Chemical Structure

Chitin and cellulose are both structurally (chemically) similar (Figure 1.3). Cellulose is a linear homopolymer of glucose while chitin is a linear homopolymer of N-acetyl glucosamide. [7]
One of the most well recognized facts about cellulose is the presence of strong inter and intramolecular hydrogen bonding which is one of the primary factors that renders it insoluble in water. Some solvents like ionic liquids and exotic blends like lithium chloride/N, N-dimethylacetamide can dissolve cellulose but their toxicity, volatility or cost prevents their large scale commercial use.[9] The structural similarity of chitin also gives it strong intermolecular hydrogen bonding and thus chitin also does not dissolve in water. In addition to that it has hydrophobic -COCH₃ groups which are not found in cellulose. It can be however be solubilized in a few solvents such as hexafluoroisopropanol, hexafluoroacetone, and chloroalcohols in conjunction with aqueous solutions of mineral acids, and dimethylacetamide containing 5% lithium chloride. [10]
Structurally the chitin of interest to us is not pure chitin but is partially deacetylated with a few acetamide groups replaced by amine groups as seen in Figure 1.4. This is the natural form of crustacean chitin. The percentage of acetamide groups amongst all amino and acetamide groups in the chitin structure is called the degree of acetylation (DA). When the degree of acetylation is 0% there are amino groups on the carbon chain and the material is a homopolymer of glucosamine called chitosan, which occurs naturally only in the walls of certain fungi.

Chitosan unlike chitin can be dissolved quite easily in dilute acids. This unique property makes it extremely useful in many applications in place of chitin. However, the main source of chitosan is still the chemical deacetylation of chitin. Generally, when referring to chitosan, we refer to material whose degree of acetylation is less than 50%. If DA is more than 50 % it is generally referred to as chitin.[7] Generally chitin extracted from crustacean shells has a DA of about 90% with DA varying due to the nature of the extraction process.[12] The reported DA of crab shell chitin is 92%. [13]
Naturally occurring cellulose has a varied degree of polymerization (DP) depending on the source with DP of various wood of various species falling in the 6,000-25,000 range and bacterial cellulose being in the 4,000-6,000 range. Pulping, bleaching and other refining processes usually reduce the DP. Table 1.1 illustrates the variety of celluloses available with different degree of polymerizations.[2]

Table 1.1 Degree of Polymerization of various forms of cellulose. Data from Zugemaier.[2]

<table>
<thead>
<tr>
<th>Source</th>
<th>Degree of Polymerization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulp</td>
<td>500-2000</td>
</tr>
<tr>
<td>Sulfate pulp</td>
<td>950-1300</td>
</tr>
<tr>
<td>Chemical pulp bleached</td>
<td>700</td>
</tr>
<tr>
<td>Cotton linters bleached</td>
<td>1000-5000</td>
</tr>
<tr>
<td>Textile flax</td>
<td>9000</td>
</tr>
<tr>
<td>Rayon</td>
<td>300-500</td>
</tr>
<tr>
<td>Cellophane</td>
<td>300</td>
</tr>
<tr>
<td>Cellulose acetate</td>
<td>200-250</td>
</tr>
</tbody>
</table>

Chitin has a weight-average molecular weight of about 1,030,000 to 2,500,000 Da [14] which roughly corresponds to a DP of about 5200-12600 assuming a 90% DA (Mol. wt. =199 x DP). N-Deacetylation and processes which lead to N-Deacetylation generally reduce the molecular weight to 100,000-500,000 Da[14] which corresponds to a DP of about 500-2500.

Cellulosic materials have degradation onset temperatures of about 200 °C [15] and melt at around 400 °C.[16] Since the material degrades before it melts, this prevents us from using industry standard techniques like melt processing in cellulose handling. Chitin also degrades at similar temperatures (above 200 °C) with degradation accelerated by presence of neighboring acetamide groups. It has been found that higher degree of
Acetylation causes earlier onset of degradation with 60% acetylated chitin losing up to 16% of its mass at 200 °C. Thus other methods are required to process chitin and cellulose, the favored amongst them being processing from aqueous suspensions, where charging of individual fibers can be taken advantage of to facilitate suspension stability.

1.2.2 Polymorphism & Structural Arrangement

Chitin is found in three polymorphic forms: α-chitin; β-chitin; and γ-chitin. The three different forms of chitin vary in packing and polarities of adjacent chains in successive sheets. α-chitin is the most stable form with chains arranged in antiparallel configuration while in case of β-chitin the chains are arranged in parallel. The least common is the γ-chitin form which is thought to be a combination of α & β forms. The intermolecular hydrogen bonding structure of α chitin gives it a very tight packing. The inter-chain distances in α chitin are reported to be about 0.47 nm. This makes α chitin particularly insoluble and intractable. The hydrogen bonded network of the β form is dominated by intra-plane hydrogen bonding rather than intermolecular hydrogen bonds thus it lacks the particular tight packing and is more susceptible to intra-crystalline swelling. It is no wonder that the α-chitin form serves as the resistance structure in insect cuticles, shells of crabs, lobsters and shrimp, fungal and yeast cell walls, marine sponges and is the most abundant form found in nature. β-chitin has been found in the extracellular fibers of diatoms, squid pens, spines and chaetae of annelids. γ-chitin has been found in the stomachs of squid and in beetle cocoons.

Cellulose exists in various crystalline polymorphic forms with the most common form (native) being Cellulose-I. Cellulose-II on the other hand has a more complex
structure than Cellulose-I which is attributed to its antiparallel structure leading to interplane hydrogen bonding as compared to Cellulose-I which is thought to have cellulose chains running in parallel.[4] Cellulose-II is rarely found in nature and is mostly prepared by regeneration of cellulose derivatives or by mercerization by soaking cellulose in aqueous NaOH (17% to 20%, w/v) followed by decomposition of the intermediate by neutralization or washing out the NaOH.[2, 4] Two other modifications of cellulose-Cellulose III and Cellulose IV also exist. Cellulose III is produced by treatment of native cellulose with liquid ammonia while treatment of modified cellulose at high temperature and under tension leads to Cellulose IV.[4] For our case we will specifically focus on Cellulose I and α-chitin as purified native versions of the respective materials which will simply be referred to as cellulose and chitin. The structural differences between these two native forms is the first major difference encountered when referring to cellulose and chitin. It has been shown in certain cases that microfibrillar β-chitin may actually be more crystalline than cellulose.[2]. Fan, et al.[18] have shown that α-chitin from crab shells is more crystalline than β-chitin from squid pens. Thus, there is reason to suspect that α-chitin is more crystalline than some celluloses.

1.3 Nanomaterials from Cellulose and Chitin

The unit structure of both naturally occurring chitin and cellulose are fibers of nanometer scale diameter. Removal of the surrounding matrix material naturally frees these fibers. Chitin and cellulose both have amorphous and crystalline domains and removal of amorphous regions in certain conditions can lead to highly crystalline product with special properties.[19] These nanomaterials can have properties that are significantly different from the parent material and a lot of research has been done on making efficient use of
these nanomaterials. For example, wood fibers have a tensile strength of less than 1000 MPa; however, cellulose crystals are found to have tensile strengths that are higher than 7000 MPa. A significant improvement in tensile strength is thus achieved by extraction of the native nanocrystals.[20]

Cellulose particles with at least one dimension in the nanoscale are referred to as nanocellulose. Nanocellulose in various forms contains unique structures and self-assembly features which can be exploited to give different types of green materials. For example it can lead to lightweight high performance composites that could eventually replace carbon fiber and lead to better automotive parts.[20] Nanocellulose is further subdivided into cellulose nanocrystals or whiskers and cellulose nanofibers and the classification is rather loose and based on the production technique, which can heavily influence the dimensions, composition and properties of these nanomaterials. Generally, the cellulose nanocrystals (CNCs) are rigid rod like particles of 3-35 nm diameter and length of about 200-500 nm. These are produced by acid hydrolysis of cellulose which degrades amorphous cellulose regions and leaves crystalline regions untouched. Cellulose nanofibrils (CNFs) are generally produced by applying high shear to cellulose pulps and have a diameter of about 5-50 nm with length of several microns.[6] Figure 1.5 shows both CNCs and CNFs from sisal.

We shall similarly define nanochitin as chitin with one dimension in the nanoscale. Similar to cellulose, chitin can also occur as chitin nanofibers (ChNFs) and chitin nanocrystals (ChNCs) depending on the processing conditions. Acid hydrolysis of chitin produces ChNCs or Chitin whiskers with diameter of about 10-50 nm with lengths varying from 150-2200 nm depending on the source of chitin.[21] Similar to cellulose, chitin can
also be subjected to high shear treatment to give nanofibers of 10-20 nm diameter and several microns in length.[13, 22] There are several methods of high shear treatment like sonication, micro fluidization, homogenization, etc. of which homogenization is particularly attractive due to its preexistence in the industry (especially dairy industry where it is used to homogenize and stabilize raw milk).

Homogenization is a process whereby the suspension under high pressure is passed through a very small orifice resulting in external and internal fibrillation of fibers. It is expected that due to degradation of amorphous regions both cellulose and chitin nanocrystals are more crystalline than their nanofiber counterparts.

Figure 1.5 (a) TEM image of Sisal CNCs (b) Scanning Electron Microscope (SEM) image of SISAL CNFs. Figures (a) and (b) from Siqueira et al.[21] (c) Chitin whiskers from Zeng et al.[19] (d) Chitin Nanofibers from crab shells (this work).
1.3.1 Mechanical Properties of Cellulose and Chitin Nanomaterials

The mechanical properties of cellulose nanofibers are expected to be at least more homogeneous than their cellulose fiber counterparts due to the homogeneous nature of the fibers. The degree of polymerization of CNFs is strongly correlated to the length and aspect ratio of the nanofibers. By viscosity measurements, researchers determine the length and hence degree of polymerization of CNFs. It has been proven that disintegration processes reduce the DP of the CNFs. In addition, it is noted that a reduction in DP also reduces strength of films formed from CNFs.[23]

The modulus of elasticity of perfectly crystalline native cellulose has been estimated to be around 130-250 GPa while the tensile strength is around 800-10000 MPa.[24] The modulus of elasticity of CNFs is a mixing rule type average between the moduli of the amorphous and crystalline domains. The average value of CNF moduli are expected to be around 40-70 GPa while tensile strengths of around 700 MPa[24] have been reported. The average value of CNCs is expected to be higher due to their more crystalline nature and average values of 130 GPa have been reported. It has been estimated that the tensile strength of CNCs would be of the order of 750 MPa.[25] If we consider the fact that this material is lightweight with densities of about 1.5 g/cm³, this makes it similar to Kevlar with density of about 1.45 g/cm³. The specific young’s modulus (modulus per unit density) is actually higher than steel.[26]

Chitin is expected to be a comparable material to cellulose and stronger than most other materials. Chitin nanofiber membranes were reported to similar strain to failure of about 10 %[27] compared to membranes composed of cellulose nanofibers (10 %).[28]
Modulus of elasticity of chitin nanofiber membranes from crustaceans was measured to be around 3 GPa with tensile strengths of around 40 MPa.[22] Mushi et al.[29] reported that the Chitin nanocrystals from crab shells were also measured to have an elastic modulus of around 5.3 GPa and tensile strength of around 110 MPa.[18] These two values are lower than those for corresponding cellulosic materials. However, Mushi et al.[29] theorized that pretreatment methods may contribute significantly to the lowering of chitin properties. They devised a milder pretreatment method and reported improved chitin membrane elastic moduli of 8.3 GPa and tensile strength of about 150 MPa with improved strain to failure of around 10 % (almost the same as cellulose as stated by Mushi et al.). The improvement in properties correlated well to protein removal despite the low elastic moduli values compared to cellulose.

1.3.2 Gas Permeability

Packaging is an especially stable industry and currently petroleum based polymers dominate the packaging industry. This is primarily because of their excellent barrier properties & cost effectiveness. Nanocellulosic or nanocellulose-reinforced materials show incredible promise and are of great interest to this industry. The primary reasoning behind this is due to the capability of nanocellulosic materials to produce films having high optical transparency and good mechanical and barrier properties.[30]

The mechanism of barrier to penetrating molecules is twofold: difficulty of molecules to penetrate the highly crystalline cellulose domains (solubility) and formation of dense percolating networks held together by strong inter-particle bonding (diffusion
The gas permeability in barrer units (1 barrer = $10^{-10}$ cm$^3$ (STP) cm cm$^{-2}$ s$^{-1}$ cmHg$^{-1}$) of nanocellulose and nanochitin is presented in Table 1.2. Lower barrer is better.

Table 1.2 Permeability of different gases in CNF & CNC films (Sisal) at 25 °C & 1 bar pressure

<table>
<thead>
<tr>
<th>Test Gas</th>
<th>CNF</th>
<th>CNC</th>
<th>ChNF</th>
<th>C-ChNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon Dioxide</td>
<td>0.10</td>
<td>118.8</td>
<td>0.0180</td>
<td>0.0080</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.15</td>
<td>161.7</td>
<td>0.0034</td>
<td>0.0030</td>
</tr>
<tr>
<td>Oxygen</td>
<td>0.09</td>
<td>140.7</td>
<td>0.0060</td>
<td>0.0030</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>-</td>
<td>-</td>
<td>0.0240</td>
<td>0.1000</td>
</tr>
<tr>
<td>Methane</td>
<td>-</td>
<td>-</td>
<td>0.0027</td>
<td>--</td>
</tr>
</tbody>
</table>

Belbekhouche[30] concluded that the CNF films outperform CNC films due to having a more tortuous diffusion path. The smaller CNCs are more crystalline yet their packing properties are poor and they end up having more porosity. Selectivity of one molecule over the other has generally not been observed.[30] As can be seen in Table 1.2, ChNFs produced by high pressure homogenization have been found to have barrier properties comparable to cellulose. Interestingly one can see that the barrier properties are not the same for all gases and there is some separation especially between carbon dioxide and oxygen. This difference is even more pronounced for hydrogen and methane and it has been found that the permeability is correlated to the kinetic diameter of the diffusing molecule.[13, 30]

Duan et al.[31] produced plasticized chitin films by coagulation where the chitin was dissolved in Urea-NaOH mixture followed by freezing and thawing and the resulting solution was spread on a glass plate and treated with ethanol (coagulant) The coagulated chitin films gave slightly better barrier properties for all gases except hydrogen resulting
in an extremely high selectivity with respect to hydrogen probably due because the organic solvent coagulant led to restructuring and orderly alignment of chitin chains to form a homogeneous close chain packing.

It is well known that polysaccharides like cellulose are susceptible to water which can strongly affect properties of the same. The hydrophilicity of cellulose nanofibers or nanocrystals is strongly dependent on the treatment and processing methods used to prepare them. Pretreatment methods, structure & morphology of films and chemical modifications all have significant impact on water vapor transmission rates as well as permeability for CNF films.

Table 1.3 Water Vapor Permeability rates of different polymers. Values for CNF from Nair et al. and others from Lange & Wyser.

<table>
<thead>
<tr>
<th>Polymer/Nanofiber composite</th>
<th>Water Vapor Permeability (g-mm/m²/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNF</td>
<td>9.83 @ 50% RH</td>
</tr>
<tr>
<td>Poly(ethylene) (PE)</td>
<td>0.5-2 @ 85 % RH</td>
</tr>
<tr>
<td>Poly(ethylene terephthalate) (PET)</td>
<td>0.5-2 @ 85 % RH</td>
</tr>
<tr>
<td>Poly(vinylidene chloride) (PVDC)</td>
<td>0.1 @ 85 % RH</td>
</tr>
<tr>
<td>Ethylene vinyl alcohol (EVOH)</td>
<td>1-3 @ 85% RH</td>
</tr>
</tbody>
</table>

Water vapor transmission rates of CNFs are generally seen to be more than those of low-density polyethylene (LDPE) and other frequently used polymers as seen in Table 1.3. It can also significantly impact the oxygen permeability of films. As seen in Figure 1.6, the oxygen permeability of CNF films increases with increasing relative humidity, possibly due to the replacement of CNF-CN F hydrogen bonds with CNF-water hydrogen bonds. The high water vapor transmission rates and degradation of barrier properties due to water are important effects that may be key areas that need to be improved for
cellulose films. Similar water sorption behavior is seen in chitin & chitosan[35]. Nanoclays as parts of composites have been successfully utilized to reduce both the water vapor and oxygen transmission rates. Bartel et al.[36] reduced the oxygen permeability (at 50% RH) of poly(lactic acid) (PLA) from 160 cm³-µm/m²/day/kPa to 92 cm³-µm/m²/day/kPa by adding 6 wt % Cloisite 30B to PLA followed by extrusion. Similar, 33 % reduction in water vapor transmission can also be seen for the composites. Wu et al.[37] were able to produce tempo oxidized CNF-montmorillonite (MTM) composites.

![Figure 1.6 Oxygen transmission rate of CNF films as a function of relative humidity (RH) for different film grammages. Figure adapted from Aulin, et al.[32]](image)

They observed a reduction in oxygen permeability (50% RH) from 0.2 to less than 0.05 cm³-µm/m²/day/kPa at loading of 50 % MTM opening up the possibility of utilizing clays with bio-based materials like cellulose and chitin in their nanomaterial forms. However such composites with inorganic content is not the current topic of this thesis and the reduction of water vapor transmission rates and improved resilience of oxygen
permeability of chitin to water vapor is one of the key areas of research as a part of this thesis.

1.4 Thesis Overview

Biodegradable plastics have gained a lot of attention in recent times due to the growing concerns over ocean pollutions and accumulation of trash in landfills. At the same time there is increasing concern over the renewability and sustainability of plastics due to depletion of resources. Bio-based polymers offer a promising alternative to plastics due to their inherent abundance, renewability, promise of sustainability and biodegradability.

Chitin and cellulose based nanomaterials are especially attractive due to their unique mechanical and barrier properties. ChNFs have a positive charge due to the presence of surface amines, while CNCs have a negative charge due to presence of sulfate half ester groups. In addition, these specific materials have tremendous potential to be utilized together either as coatings or blends to manufacture materials which have enhanced barrier properties. At the same time the production of chitin is orders of magnitude less than that of cellulose which is made by the pulp and paper industry.[7, 38, 39] Thus efficient production and utilization of chitin- especially in its nanofiber form is one of the core aims of this thesis.

The specific aims of the thesis are to:

1. Develop coatings based on ChNFs and CNCs

2. Optimize the utilization of ChNFs in ChNF-CNC based composites
3. Increase production efficiency of ChNFs

4. Maximize synergistic interactions between chitin and cellulose based materials

In Chapter 2 we describe the manufacture of spray coated ChNF and CNC based films with enhanced barrier properties. Alongside, we also investigated the optical and mechanical properties of the films. In chapter 3, we investigate blended films of ChNFs and CNCs with varying degrees of acetylation and ChNF/CNC composition. The barrier, mechanical and optical properties of these films were studied. In, chapter 4 we investigated the influence of deacetylation on the homogenization efficiency of chitin nanofibers. The particle size distributions and optical properties of the suspensions were studied. Alongside we also studied the mechanical, barrier and optical properties of solution cast films from these suspensions. Chapter 5 discusses a novel film made from crosslinked poly (glucuronic acid) and chitosan with enhanced oxygen and water vapor barrier properties. Chapter 6 of this thesis provides concluding remarks and recommends future directions.
1.5 REFERENCES


CHAPTER 2. SPRAY-COATED MULTILAYER CELLULOSE
NANOCRystal – CHITIN NANOFIBER FILMS FOR BARRIER
APPLICATIONS

2.1 Introduction

Cellulose and chitin are the first- and second-most abundant naturally-occurring
biopolymers, respectively, with annual natural production estimated to be $10^{11}$-$10^{12}$ tons
per year (cellulose)[1] and $10^{10}$ - $10^{12}$ tons per year (chitin).[2] Cellulose is sourced most
abundantly from plants, but is also available from bacteria and tunicates. Chitin is produced
by shellfish, insects and fungi. Chemically, cellulose is a linear homopolymer of glucose
and chitin is a linear homopolymer of N-acetyl glucosamine.[3] In nature, chitin occurs as
a copolymer in which some fraction of the acetamide groups on the 2 carbon are replaced
by amine (-NH$_2$) groups. These amine groups can be protonated in acidic media to create
charged sites that aid in the colloidal stabilization of chitin fibers, or complete
solubilization with high degrees of deacetylation (as in the case of chitosan).

Both cellulose and chitin are found in hierarchical structures (in plant cell walls as
well as crustacean exoskeletons, respectively), from which nanocrystals or nanofibers can
be extracted. Because of their high crystallinity, cellulose nanocrystals (CNCs), cellulose
nanofibers (CNFs),[4, 5] chitin nanowhiskers, and chitin nanofibers (ChNFs)[6] exhibit a
high modulus and tensile strength and are excellent candidates for gas barrier films.[7, 8]
The nanocrystalline or nanofibrous forms of these biopolymers represent promising
renewable, compostable sources of materials for replacing traditional plastics in packaging
applications. Typically, CNCs are produced by acid hydrolysis of a wide variety of fibrillar cellulose sources,[4] while CNFs are produced by mechanical defibrillation processes, which includes refining, homogenization and grinding.[9] In a manner similar to CNFs, when acidified chitin suspensions are subjected to high shear treatment, ChNFs of 10-20 nm diameter and several microns in length are obtained.[6, 8] Previously, Wu et al.[8] produced chitin nanofibers in suspension from purified crab α-chitin. Suspension cast films were found to have O\(_2\) and CO\(_2\) gas permeabilities of 0.006 and 0.018 barrer, respectively. In comparison, poly (ethylene terephthalate) (PET) has an O\(_2\) permeability of 0.015-0.076 barrer[10] and CO\(_2\) permeability of 0.08-0.15 barrer.[11] This makes these chitin films excellent candidates in sustainable barrier packaging applications.

The inability to melt-process cellulose and chitin as neat materials is a principal obstacle to their use in packaging. However, the ease of suspending cellulose and chitin nanomaterials in water suggests their application as coatings to produce barrier films. Coatings allow fabrication of multilayered materials in an efficient manner, by combining multiple thin functional layers. A challenge of water processing of these materials is the low solids content at which high viscosity and gelation occur, which leads to the need to remove large quantities of water through drying. Rod coating[12] as well as a slot-die coating[13] have been used for coating CNF suspensions onto paperboard for barrier applications, taking advantage of the suspension’s shear thinning behavior. Multilayered CNF films also have been prepared on silica substrates by using dip coating.[14] Spin coating was also applied to coat CNC films on polypropylene[15] and gas barrier properties of spin-coated multilayered CNC films have been reported.[16]
Spray coating is a versatile method for film deposition that offers enhanced drying rates due to the large surface area of droplets.[17] One of the main features of spray coating is contactless delivery of material to the surface, allowing uneven or delicate surfaces to be coated.[18] Beneventi et al.[19] spray-coated slurries of microfibrillated cellulose onto wet, highly-porous papers allowing complete retention of the microfibrillated cellulose on the surface of the substrate and improved barrier properties of the final product. Under sufficiently acidic conditions, ChNFs are cationic, and sulfate-modified CNCs are anionic. The opposite charges of chitin and cellulosic nanomaterials may facilitate their fabrication into multilayered barrier materials by promoting adhesion and rapid densification of their interfaces. For example, previous work by Qi et al.[20] successfully demonstrated layer-by-layer deposition of chitin nanofibers and TEMPO oxidized CNFs onto PET by using dip coating; however, the resulting films did not have improved barrier properties relative to uncoated PET. Park et al.[21] successfully spray-coated aqueous chitosan solutions containing suspended clay to add barrier properties to poly (lactic acid) (PLA) films.

While PLA is a compostable polymer, especially in film form, PLA formulations typically suffer from the lack of cost effective compostable additives. Thus, current efforts are being focused on producing partially renewable PLA based materials.[22] CNCs[23] and ChNFs[24] are renewable alternatives for barrier property modification of PLA. The goal of the present study is to demonstrate that a fully biologically-sourced multilayer barrier film can be fabricated through a process compatible with roll-to-roll coating, by using oppositely-charged ChNFs and CNCs applied to a suitable model compostable substrate (PLA). Single and multiple layers of ChNFs and CNCs were spray-coated from their aqueous suspensions onto PLA. Film structure was analyzed by scanning electron
microscopy (SEM), atomic force microscopy (AFM), and by UV-Vis spectroscopy. The resulting barrier and mechanical properties were determined, revealing an important synergy between CNCs and ChNFs that yielded enhanced O$_2$ barrier properties in their multi-layered coatings compared to films composed solely of ChNFs or CNCs.

2.2 Materials & Methods

2.2.1 Materials

Deionized water (DI) (18.2 MΩ cm) was obtained from a Millipore MilliQ Advantage A10 water purification system. Commercial purified chitin was obtained from Alfa Aesar (Ward Hill, MA), to be used as a standard for comparison to chitin purified from crab shells in this work. Hydrochloric acid and sodium hydroxide were purchased from EMD Chemical Inc (Burlington, MA). Acetic acid was purchased from Sigma Aldrich (St. Louis, MO). Natureworks LLC (Minetonka, MN, USA) 4032D PLA was used for this study, from which films of 25.4 μm thickness were extruded by Frito Lay, Inc (Plano, TX).

Crab shells were purchased from Neptune’s Harvest (Gloucester, MA). CNCs as a 5.5 wt. % aqueous suspension were provided by the USDA Forest Products Laboratory (Madison, WI). The aqueous CNC suspension was prepared from mixed southern yellow pine dissolving pulp via 64% sulfuric acid digestion as described previously (Beck-Candanedo et al., 2005). The CNCs had 0.86 wt. % sulfur content via sulfate functionality with Na$^+$ counterions. The CNC suspension was diluted to 0.5 wt. % by addition of deionized (DI) water. Research-grade oxygen and ultra-high purity nitrogen were purchased from Airgas (Atlanta, GA). HPLC-grade water was purchased from J.T. Baker.
(Center Valley, PA). CNCs as a 5.5 wt. % suspension were provided by the USDA Forest Products Laboratory (Madison, WI).

### 2.2.2 Chitin Purification

Purification was done as described in Wu et al.[8] The crab shells were washed several times and were then ground into powder using a commercial grinder (Sunbeam Products Inc.). The powder was refluxed with 5 wt. % NaOH for 6 h. The resulting solids were filtered and washed with DI water until the wash water had a pH of 7. The filtered solids were then treated with a 7 wt.% HCl bath for 6 h. The solids were again filtered and washed until the wash water pH was 7. The acid-treated solids were then refluxed with 5 % NaOH for 48 h and the resultant solids were filtered and washed with DI water until the wash water pH was 7. This resulted in a bright white purified chitin powder, which was dried in an oven at 60 °C for 24 hours.

### 2.2.3 Extraction of ChNFs

A 0.5 wt. % purified chitin suspension was prepared in DI water. The pH of the suspension was adjusted to 3.0 using glacial acetic acid and the acidified suspension was homogenized in a Mini DeBEE Homogenizer (BEE International, South Easton, MA). The first sequence of homogenization was carried out at a pressure of 1034 bar by using a 0.2 mm nozzle for 20 passes followed by a second sequence at 1516 bar and utilizing a 0.13 mm nozzle for an additional 10 passes. The resulting suspension was then used for spray coating. Because of the fluid expansion through the nozzle resulting in shear heating, a product cooler was used throughout the processing to keep the ChNF suspension at a maximum temperature of 35 °C.
2.2.4 *ChNF Characterization*

The degree of acetylation (DA) of the chitin was determined by potentiometric titration. A volume of 25 ml of the homogenized suspension was ion exchanged using 300 ml of anion exchange resin (Amberlite IRN-78, Alfa Aesar, Ward Hill, MA). The ion exchange resin was washed with about 200 ml of DI water to remove entrapped chitin. 20 ml of 0.25 N HCl was added to the resulting suspension which was then titrated against 0.5 N NaOH using a Mettler Toledo Seven Excellence S400 pH meter. The inflection points as determined by the pH vs volume of titrant curve were then used to determine the amount of NaOH consumed. The NaOH equivalents of HCl present in the suspension was subtracted from the total consumed NaOH to yield the amount of NaOH consumed by the protonated chitin. The number equivalent of NaOH consumed divided by the mass of the starting material gives m [mol NH$_2$/g chitin (dry basis)] which was used to determine the degree of acetylation by

$$\text{DA} \, [\%] = 100 \times \frac{203.21}{42.04 + m} \quad (2.1)$$

where 203.21 [g/mol] is the molecular mass of a chitin monomer and 42.04 [g/mol] is the difference in molecular mass between a chitin and a chitosan (deacetylated chitin) monomer. The titrations were repeated thrice for each sample.

The O$_2$ permeability values of these neat films were obtained by using a MOCON OXTRAN 1/50 instrument at values of relative humidity (RH) ranging from 0 to 80 %. The water vapor transmission rate (WVTR) of these films were obtained by using a MOCON PERMATRAN-W 1/50 instrument at values of RH ranging from 30 to 90 %.
2.2.5 **PLA Characterization**

Water content of neat PLA films was found by thermogravimetric analysis (TGA) using a TA Instruments TGA Q50. The glass transition temperature ($T_g$) and percent crystallinity of the neat PLA films was obtained by differential scanning calorimetry (DSC) using a DSC Q200 from TA Instruments.

2.2.6 **Spray Coating**

![Spray coating apparatus](image)

**Figure 2.1. Spray coating apparatus.**

The spray coating apparatus is shown in Figure 2.1 and consists of a 1.52 mm spray nozzle (Central Pneumatic, GA) connected to a liquid reservoir containing a 0.5 wt. % ChNF or 0.5 wt. % CNC suspension. The nozzle was supplied with a carrier gas by connection to a nitrogen cylinder at 4 bar. The PLA film was heated to 60 °C while being held on a heated surface normal to the spray. Each coating layer was deposited by spraying the PLA with a 30 ml volume of the ChNF or CNC suspension, with 2 min drying time allowed between subsequent coatings. As a control the procedure was repeated by spraying
only DI water (no CNC or ChNF) onto the PLA film, henceforth referred to as uncoated PLA films.

2.2.7 Sprayed Film Characterization

Water content of coated PLA films was found in a similar manner to neat PLA films using TGA. Light transmission measurements were made by using a Cary 5000 UV-Vis-NIR equipped with an integrating sphere attachment (DRA 2500). PLA and multilayer-coated films were measured using a diffuse / normal geometry (di:0°) with the sample in the transmission port. Optical haze was calculated according to the following equation per ASTM D1003:

\[
\text{Haze} (\%) = \left( \frac{T4}{T2} \cdot \frac{T3}{T1} \right) \times 100 \tag{2.2}
\]

where \( T1, T2, T3, \) and \( T4 \) are transmittance values integrated from 380 – 780 nm for four different sample configurations. \( T1 \) and \( T3 \) correspond to geometries without the sample in the transmission port as an instrument correction. \( T2 \) corresponds to the total sample transmittance (diffuse and specular) whereas \( T4 \) captures only the diffuse transmittance.

In order to determine the effect of the spray process on suspension haze, ChNF and CNC suspensions were sprayed into a glass bottle. The haze of the collected ChNF and CNC sprayed suspensions was studied 1 week apart in a similar way to that for ChNF and CNC characterization.

Films were cut with blades and SEM (LEO 1530) was used to image the cross section. The thicknesses of the coatings were determined by analysis of the SEM images. For each coating thickness at least 10 measurements were done at different points on SEM
images. AFM (Bruker Icon) was utilized to image the top surface of coated films. The size of structures on the coated films were measured by visual analysis of the AFM phase images. Film roughness was determined from the AFM images by using Bruker Nanoscope Analysis software.

The O2 permeability values of the coated and uncoated PLA films were obtained as described for neat ChNF but at RH values from 10 to 90 % RH. The WVTR of films was obtained similar to neat films but at 30 to 90 % RH. The measurement errors for multilayered composites were estimated to be proportional to the magnitude of the reading and extrapolated from the measurement error for O2 permeability of neat PLA at 50 % RH. Mechanical properties of uncoated and coated films were determined for comparison by using a high-throughput mechanical characterization (HTMECH) instrument.[25] This instrument utilizes a hemispherical indenter to deform films biaxially normal to the film plane until failure. The deformation rate was 10 mm/s and 3 measurements were taken on each film sample by using a 1.2 mm diameter indenter with a length of 34 mm. Stress vs. strain curves obtained from one sample of each type of coated or uncoated film. The maximum of the stress strain curve is determined to be the ultimate tensile strength while the stress at break is determined to be the breaking strength of the material.

2.2.8 ChNF, CNC Blend Characterization

To determine the stability of ChNF and CNC suspensions, the haze of 0.5 wt. % suspensions was studied in a similar manner to sprayed films using a Cary 5000 UV-Vis-NIR equipped with an integrating sphere attachment (DRA 2500). Haze was measured for pristine suspensions and for suspensions that had been stored for one week. The suspensions were found to be stable with there being negligible difference in haze between
the pristine and 1 week aged suspensions indicating that the suspensions have a stability over a timescale of at least 1 week. This is less than the time between suspension manufacture and spray coating.

For film thicknesses, 50 ml of 0.5 wt. % ChNF and CNC solutions were air-dried for 14 days at room temperature and humidity (45 % relative humidity (RH)) in a poly(tetrafluoroethylene) dish to yield free-standing films. In addition, equal volumes of 0.5 wt. % ChNF and 0.5 wt. % CNC suspensions (25 ml each) were blended together in a beaker at 500 rpm for 4 hours to produce a mixture. The resultant mixture was sonicated for 30 s at a power of 80 W. The sonicated mixture was air-dried like the ChNF and CNC films to produce a 1:1 ChNF-CNC film. The thickness of these films was measured with a micrometer at more than 5 different points on the films.

2.3 Results & Discussions

![FTIR spectrum](image)

Figure 2.2: FTIR spectrum of pure chitin from Alfa Aesar (dashed line) and processed crab shell chitin (solid line).
Figure 2.2 compares the spectrum of pure chitin obtained from Alfa Aesar and the FTIR of the crab shell-derived chitin processed by our method outlined above. The FTIR spectra are identical showing that the treatment procedure produces purified chitin similar to commercially purified chitin.

Figure 2.3: Haze of pristine and aged 0.5 wt. % suspensions of (a) ChNF (b) CNC (d) sprayed ChNF (d) sprayed CNC.

Haze was measured for pristine suspensions and for suspensions that had been stored for one week as shown in Figure 2.3(a) and (b). The suspensions were found to be stable with there being negligible difference in haze between the pristine and 1 week aged
suspensions indicating that the suspensions have a stability over a timescale of at least 1 week. This is less than the time between suspension manufacture and spray coating.

To fabricate multilayer coatings, ChNFs were first spray coated directly onto the PLA, followed by alternating spray-coated layers of CNCs and ChNFs. In describing the multi-layer films, we use the terminology PLA-(X)_{n}, where (X)_{n} describes either single layers or bilayers of material. Thus, a PLA film coated with a single layer of CNCs is described as PLA-(CNC)_{1}, while PLA coated with 5 alternating layers of ChNFs and CNCs, starting with ChNF first is written as PLA-(ChNF-CNC)_{2}-ChNF.

**Table 2.1. Properties of neat PLA.**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystallinity</td>
<td>Semicrystalline (&lt; 45 %)</td>
<td>[26]</td>
</tr>
<tr>
<td>L-Lactide content</td>
<td>98.5 %</td>
<td></td>
</tr>
<tr>
<td>Molecular Weight (GPC)</td>
<td>200 kDa</td>
<td></td>
</tr>
<tr>
<td>Polydispersity Index (GPC)</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Glass Transition Temperature</td>
<td>61 °C</td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>1.24 g/cm³</td>
<td>[27]</td>
</tr>
<tr>
<td>Melting Point</td>
<td>155-170 °C</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.1 shows the typical properties of Natureworks 4032D PLA used in this study. The PLA is semicrystalline and has a L-Lactide content of 98.5 % with a corresponding glass transition temperature of 61 °C. This is similar to the Tg of 63 °C obtained from the DSC curves in Figure 2.5.
Figure 2.4. DSC curve of neat PLA showing heat flow vs temperature. Inset shows section of DSC curve between 40 and 100 ºC used to determine the $T_g$ of neat PLA.

The $T_g$ was determined from the slope of the first minimum observed for the first increasing temperature ramp and the melting curve is the second minimum observed as seen inset of Figure 2.4. Figure 2.5 shows the DSC curve (heat flow vs time) of neat PLA. The cold crystallization peak is the first maximum observed after the glass transition. The crystallinity of PLA films is determined to be the percentage value of the area under the melting curve less the area under the cold crystallization curve divided by the melting enthalpy value of 93.1 J/g for 100 % crystalline PLA.[28]
Figure 2.5. DSC curve of neat PLA film showing heat flow vs time.

Figure 2.5 shows the cold crystallization enthalpy and melting enthalpy used for calculation of neat PLA crystallinity. The crystallinity of the PLA from DSC measurements in Figure 2.5 was obtained to be 9.5\%.
Figure 2.6: TGA curves for (a) neat ChNF film (b) neat CNC film (c) neat PLA film.
Figure 2.6 shows the TGA curves for the neat ChNF, CNC and PLA films. The water content calculated from the TGA measurements for PLA-(CNC)$_5$, was 1.5 wt. %, 0.9 wt. % for and PLA-(ChNF)$_5$ films and 0.3 wt. % for PLA. The DA of chitin as determined by potentiometric titration was found to be 92.6 ± 2.8 %. Cross sectional SEM images were taken in order to visualize the change in film thickness with each coating layer. The evolution of film thickness is shown in Figure 2.7. To within experimental uncertainty, the coating thickness increases with the increase in number of coated layers. The coating thickness for a multilayer PLA-(ChNF-CNC)$_2$-ChNF film increases to 3.7 μm while the coating thicknesses increases to 7.7 μm and 6.4 μm for PLA-(ChNF)$_5$ and PLA-(CNC)$_5$ films respectively.

![Figure 2.7](image.png)

**Figure 2.7.** Film thicknesses of PLA films with and without single and multilayer coatings. Error ranges are 95 % confidence intervals.
The optical transmittance of neat and coated PLA films is summarized by the photographs, transmittance, and haze values shown in Figure 2.8. As shown in the photographs, the visible appearance of neat PLA (Figure 2.8(a)) and PLA-(CNC-ChNF)$_1$ (Figure 2.8(b)) films are similar and the underlying colored patterns are clearly visible. The coated PLA-(ChNF-CNC)$_1$ has a matte finish due to its diffuse light reflection compared to the neat PLA. The optical transmittance for each film at 550 nm is shown in Figure 2.8(c) where a slight decrease in %T is seen going from neat PLA (92.7 %T) to the multilayer coated-PLA (91 %T).

Figure 2.8. (a) Photos of neat PLA and (b) PLA-(CNC-ChNF)$_1$ films covering CMYK and RGB colored swatches. (c) Light transmission versus number of layers of films measured at 550 nm (d) Optical haze calculated over the visible spectrum (380-780 nm). Filled squares denote PLA (layer 0) coated with ChNF (layer 1), then CNC (layer 2), then (ChNF), and so on. The same convention is used in the remaining Figures.

The PLA-(CNC)$_5$ sample shows a notably decreased transmittance (88.3%) due to a large amount of scattering in the sample. The haze of each film was calculated using
Equation 1 and is shown in Figure 2.8(d). The addition of a single spray-cast layer onto PLA increases the haze from 0.8 % to 2.7 % (ChNF) or 6.9 % (CNC). Interestingly, the film haze does not increase with additional layers. The 5-layer sample (PLA-(ChNF-CNC)2-ChNF) has a haze of 6.3 % which is far lower than the haze of equivalent five layered films composed solely of ChNF (23 %) and CNC (46 %). The lower haze for multilayered films suggests that alternating the oppositely charged ChNFs and CNCs produces denser films with fewer voids to scatter light. Wagberg et al.[29] made smooth, well organized multilayer films with CNF and polyelectrolytes (PELs). They concluded that during adsorption of the CNF to the PE surface, electrostatic repulsion between the fibrils prevents their aggregation and allows better organization into thin layers. The PEL charge characteristics and solution ionic strength had a large influence on the thickness of CNF-PEL multilayers. Increasing the PEL solution ionic strength by salt addition caused an increase in layer thickness, indicating that packing was inefficient due to screening of long-range electrostatic interactions. In addition, PELs formed from weaker, less-charged bases produced thicker layers than stronger basic PELs. While the PELs are molecularly dissolved and the ChNFs are suspended particles, the similarities in charge characteristics between the two systems (CNF-PEL & CNC-ChNF) are significant enough to warrant a comparison as long range electrostatic forces are expected to be the most dominant forces in both systems. We expect that the attractive electrostatics between ChNFs and CNCs promotes strong adsorption of thin alternating layers, and that self-repulsion between ChNFs or CNCs in any layer allows each layer to pack more efficiently. It is known that the pKa of sulfate ester group on CNCs is 2.46[30] as compared to that of acetic acid (used for homogenization of chitin), which is 4.76.[31] Thus, the deprotonated, negatively-
charged sulfonate groups on CNC would promote the adsorption of positively-charged ChNFs on the CNC surface, and repulsive interactions between similarly charged ChNFs (or CNCs) would promote their rearrangement into more efficiently packed layers. This effect could produce thinner films with reduced voids in the light transmission path resulting in less light scattering.

**Figure 2.9.** Representative AFM images of coated PLA films. (a) Height image and (b) phase image of top layer coating of ChNFs on a (PLA-(ChNF-CNC)_1-ChNF) multilayer; (c) height image and (d) phase image of top layer coating of CNCs on a (PLA-(ChNF-CNC)_2) multilayer.

AFM measurements were next used to visualize the surface density of particles in the multilayer films. In Figure 2.9(a) we see the height image of the top ChNF layer on (PLA-(ChNF-CNC)_1-ChNF). Figure 2.9(b) shows the corresponding phase image. Individual fibers can be seen on the surface, some of which are several microns in length. The fibers vary in diameter from 20 nm to 100 nm. It must be pointed out that fibers smaller than 20 nm will not be resolved in these images. Figure 2.9(c) shows the height image of
the top CNC layer on (PLA-(ChNF-CNC)₂) with the phase image shown in Figure 2.9(d). In these images, individual CNC whiskers are dried into a compressed layer with ordered regions suggestive of formation of a nematic phase during drying. This is consistent with the known structural ordering of CNCs at semi-dilute concentrations.[32] The CNCs appear to be 150 ± 30 nm in length and have varying diameters of 20-60 nm. The typical Forest Products Lab CNCs from dissolving pulp are 150-200 nm in length and approximately 5 nm in diameter[33] suggesting clusters of longitudinally oriented CNCs.

The ChNF films have an average surface roughness of 11.8 ± 2.0 nm while the CNC films have a roughness of 7.2 ± 1.2 nm based on averages of 3 samples. The roughness of CNC films is consistent with the diameter of CNCs while the roughness of ChNFs is consistent with single ChNF fiber diameters as mentioned before. The ChNF fibers show much larger variation in fiber diameters which might lead to a higher surface roughness than CNCs, which are more uniform in size.

Film cross-sections were imaged by SEM and analyzed to understand the evolution of film thickness with the number of layers in the coating. Figure 2.10 shows cross-sectional SEM images of PLA films coated with ChNFs, CNCs or both in multilayers. The films with ChNF coating on the top (outermost) layer appear rougher than those with CNC as the top layer, which is consistent with the AFM images shown in Figure 2.9. In all cases the films completely cover the underlying surface, except for the PLA-(ChNF)₅ and PLA-(CNC)₅ films where cracks can be seen in the layers. The transverse directional cracks suggest that these thick neat layers are brittle, and this may be related to the apparently lower density and drying pathway followed in the thick neat films. However, SEM-induced cracking artifacts cannot be excluded.
The reduction in coating thickness observed in going from PLA-(ChNF)$_1$ to PLA-(ChNF-CNC)$_1$ is unexpected.
Table 2.2 Thickness of equal volumes of 0.5 wt. % loading solution cast films.

<table>
<thead>
<tr>
<th>Material</th>
<th>Neat film thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 wt. % ChNF solution casted films</td>
<td>127±61</td>
</tr>
<tr>
<td>0.5 wt. % CNC solution casted films</td>
<td>133±15</td>
</tr>
<tr>
<td>0.5 wt. % ChNF-CNC mix- 1:1 volume ratio</td>
<td>48±6</td>
</tr>
</tbody>
</table>

Table 2.2 shows the thicknesses of blends of ChNF and CNC films. It has been observed that a blended mixture of 0.5 wt. % ChNF and CNC suspensions produces thinner films than equivalent volumes of ChNFs and CNCs alone. In the multilayered films, rewetting of underlying ChNF layers by the next deposited CNC suspension may allow for some interfacial mixing of ChNFs and CNCs resulting in thinner mixed ChNF-CNC films. While this explanation is supported by the observations on blended films, we cannot rule out inaccuracy in measurement or in the deposited volume of ChNF or CNC as a possible cause for the small PLA-(ChNF-CNC)$_1$ film thickness. However, the thickness-corrected O$_2$ permeability of this sample is markedly lower than the single ChNF or CNC samples, and is almost identical to that of the thicker multilayer films, indicating that the density and intrinsic transport properties of PLA-(ChNF-CNC)$_1$ are similar to subsequently-deposited layers (discussed below with Figure 2.11). The five-layered PLA-(ChNF-CNC)$_2$-ChNF films are thinner than the PLA-(ChNF)$_5$ and PLA-(CNC)$_5$ films, which suggests denser packing when the films are fabricated from thin, alternating multilayers compared to the equivalent sprayed volume of neat material.

The impact of this structure on the multilayer film’s barrier properties was determined by measuring the oxygen permeability and the WVTR. Figure 2.11(a) shows the O$_2$ permeability of PLA films coated with ChNFs, CNCs, and alternating layers of
ChNF and CNC measured at 50% RH. Upon coating with 1 ChNF layer, the film shows a slight increase in O₂ permeability from 70 to 80 cm³-μm/m²/day/kPa indicating that the first ChNF layer with a thickness of 1.4 μm does not improve barrier performance. The added ChNF instead increases the thickness of the composite material causing an increase in the effective permeability of the composite. This effect is also observed more dramatically in the thicker PLA-(ChNF)₅ films that are coated with 5 times the volume of ChNF suspension as the PLA-(ChNF)₁ film. For PLA-(ChNF-CNC)₁ films, the permeability drops to around 20 cm³μm/m²/day/kPa, and adding additional alternating layers of ChNF and CNC, up to PLA-(ChNF-CNC)₅, results in essentially the same permeability. In comparison, a single 5–layer-equivalent of neat CNCs does not decrease the permeability significantly indicating that CNC coatings delivered as a single neat layer have roughly the same permeability as the neat PLA.

Figure 2.11(b) shows the O₂ transport characteristics of a PLA-(ChNF-CNC)₂-ChNF film as a function of RH. As RH increases, the oxygen transmission rate of coated PLA films increases in contrast to uncoated PLA films where the O₂ permeability remains in the range of 45-70 cm³μm/m²/day/kPa without an obvious trend. The O₂ permeability of semicrystalline PLA has been reported by others to be between 95-115 cm³μm/m²/day/kPa,[34] while the permeability of amorphous PLA lies in between 130-190 cm³μm/m²/day/kPa,[34] which can change based on molecular mass and processing
history. These values are 2 to 4 times higher than the neat PLA utilized in this study, indicating that reductions in permeability reported here are conservative values.

Figure 2.11. (a) O₂ permeability of PLA films with and without single and multilayer coatings of ChNFs and CNCs at 23 °C and 50 % RH. (b) O₂ permeability versus RH of permeant gas at 23 °C.

The O₂ permeability of 10 µm thick neat solution-cast ChNF film also increases with an increase in RH as can be seen in Figure 2.11(b). In addition, Aulin et al.[35] showed
that the O$_2$ permeability of cellulose nanofiber films increases with increasing RH. Belbekhouche et al.[7] also reported higher permeability values for CNCs than CNFs and reported similar water vapor sorption isotherms for both materials. Therefore, it is reasonable to state that the O$_2$ transmission rates of CNC films in this work increase with RH and are at least as high as those reported for CNF films.[34]

At lower RH (< 80%), the combined ChNF-CNC multilayers have a significantly lower O$_2$ permeability than the PLA alone and appear to control the O$_2$ permeability of the composite film. At higher RH, the O$_2$ permeability of the ChNF-CNC multilayer film approaches the range of permeability displayed by the neat PLA. This result indicates that the O$_2$ transmission is controlled by the PLA layer at RH exceeding 80%.

The WVTR of various barrier films is shown in Figure 2.12(a). Multilayer films exhibit a WVTR in the range of 64 - 150 g/m$^2$/day, similar to neat PLA, even after coating with up to 5 alternating layers of ChNF and CNC. The WVTR of PLA-(CNC)$_1$ films also lies in the same WVTR range as neat PLA films. The WVTR of PLA-(ChNF)$_5$ films is similar to the multilayered films, but WVTR of PLA-(CNC)$_5$ is slightly higher than for PLA-(ChNF)$_5$. This is decidedly not due to off-gassing of water from the film as even PLA-(CNC)$_5$ and PLA-(ChNF)$_5$ films with the most amount of water sprayed on them due to higher spray coat volumes had less than 2 wt. % water. Figure 6(b) shows the WVTR characteristics as a function of RH for PLA, PLA-(ChNF-CNC)$_2$-ChNF, and a solution cast ChNF film. The multi-layer film shows a slightly lower WVTR compared to neat PLA which increases in a similar fashion with RH. The WVTR of neat solution-cast ChNF film increases much more rapidly with RH compared to neat PLA. These two results indicate that the PLA controls the RH-dependence of WVTR for the PLA-(ChNF-CNC)$_2$-ChNF
WVTR of neat CNF films has been reported to be around 230 g/m²/day for a 42 µm film at 50 % RH and 23 °C,[36] which after normalizing for thickness is 9,800 g µm/m²/day and is similar to the 11,400 g µm/m²/day found for ChNFs here. Similar adjusted WVTR values were obtained for CNF films as reported by Bedane et al.[37] as well as by Nair et al.[38]

Figure 2.12. (a) WVTR of PLA films with and without single and multilayer coatings of ChNFs and CNCs at 23 °C and 50 % RH. Measurement errors at 50 % RH smaller than 7 g-water/m²/day. (b) WVTR versus RH of permeant gas at 23 °C.
These results suggest that the ChNF-CNC multilayers have a much higher water vapor permeability than PLA and thus the PLA layer controls the transport of water in the composite as can be seen in Figure 2.12(a). In summary, the composites have an O₂ permeability that is controlled by the ChNF-CNC coating and a WVTR that is controlled by the underlying PLA layer.

Figure 2.13. Selected individual stress vs strain curves determined from HTMECH biaxial indentation.

Figure 2.13 shows the typical stress-strain curves evaluated using the HTMECH biaxial indentation measurement system. Ultimate tensile strength (UTS) was evaluated as the maximum in the stress-strain curve. The breaking strength was evaluated as the value of stress at the break point of the film. The strain at break was evaluated as the strain value at the break point. The results summarized in Figure 2.14 show that upon adding layers of ChNFs or CNCs, both the UTS and breaking strength decrease with a dramatic loss of
strength for the 5 layer coated films (PLA-(ChNF)$_5$, PLA-(CNC)$_5$, and PLA-(ChNF-CNC)$_2$-ChNF)). Figure 7(c) shows that the strain at break initially decreases after adding one layer of ChNF in PLA-(ChNF)$_1$ but increases again with subsequent layering of CNCs, until 5 layers are reached where it decreases sharply. For each of the coated materials, the UTS and breaking strengths are similar indicating that the materials break close to their maximum tensile stress (UTS). The PLA-(ChNF)$_1$ films behave similarly to the PLA-(CNC)$_1$ films as evidenced by similar breaking strength and UTS, but the PLA-(CNC)$_1$ films have a higher strain at break.

To understand the reduction in mechanical strength and strain at break of multilayered films, it is useful to consider that the mechanical attachment of two different materials may lead to stress concentrations that can initiate cracks at the interface.[39] There is a significant mismatch in thermal and mechanical properties at the interface of PLA and ChNF/CNCs. For example, the neat PLA films have a high strain at break of about 200 %. Solution cast 0.5 wt. % ChNF and CNC films were found to have a much lower strain at break ranging from 10 - 50 % and 0.5-3 % respectively, indicating that ChNF and CNC films are more brittle than neat PLA. Thermal contraction during cooling and volumetric contraction during drying of ChNFs and CNCs is expected to result in large residual stresses at the interface of ChNF and PLA. Such stresses are known to adversely affect mechanical integrity.[40] Another reason multilayered materials may fail at a lower strain and tensile strength than their parent materials is due to “mutual interlayer destruction.” In this type of failure, a brittle layer cracks first and this crack then acts as a notch to localize the stresses in an adjacent ductile layer, causing premature failure of the ductile material, leading to failure of the whole composite.[41, 42] Youngblood et al.[43]
reported reduced UTS, lower Young’s modulus and higher strain at break in (compared to neat CNF films) CNF-polymer laminates, while they reported reduction in all of these properties in CNC-polymer laminates.

![Graph showing UTS, breaking strength, and strain at break of coated and uncoated PLA films](image)

**Figure 2.14.** (a) UTS, (b) breaking strength and (c) strain at break of coated and uncoated PLA films deformed biaxially with an instrumented probe.

All the five-layered films in Figure 2.14 are similar in UTS and breaking strength, suggesting similarity in the effects of interfacial mismatch or brittleness above a threshold thickness. This is similar to embrittlement induced by high loads of neat polystyrene
coextruded over high-impact (rubber-roughened) polystyrene, which fail due to mutual interlayer destruction.\textsuperscript{40} Thus, a trade-off exists between barrier versus mechanical effects, suggesting that the number of brittle ChNF/CNC layers should be kept to a minimum required to achieve desired transport properties.

2.4 Conclusions

This study provides an initial perspective of the potential for utilizing alternating layers of chitin and cellulose-based nanofibrous materials to tune barrier properties. Multilayered coatings consisting of alternating layers of ChNFs and CNCs can be applied to PLA films through spray coating of the charge-stabilized aqueous suspensions of each material. This process can be considered a type of layer-by-layer coating process, where the CNCs are negatively charged and the ChNFs are positively charged. We found that spraying onto a heated substrate (60 °C, just under the glass transition for PLA) facilitated drying of the sprayed coatings before significant dewetting of the aqueous suspension occurred on the PLA film. We found that films with at least two alternating coated layers, consisting of PLA-(CNC-ChNF)$_n$, showed reductions in O$_2$ permeability relative to neat PLA, even at elevated RH (70%). Interestingly, these improvements in O$_2$ barrier properties were not observed for films with single layers of neat ChNF or CNC sprayed from an equivalent volume. The improvement in O$_2$ permeability was correlated with SEM and AFM evidence of thin, densely-packed layers of ChNF and CNC. The composites were more brittle than neat PLA possibly due to mutual interlayer destruction, which could be addressed by minimizing the thickness of coated layers or the use of plasticizers to decreasing the brittleness of the coated ChNF-CNCs. In terms of engineering applications, these films could form the basis for a platform technology of 100% biorenewable barrier
packaging. A challenge to this goal that will form the basis of future work is the need to make improvements in WVTR, which was not intrinsically improved through the addition of ChNF or CNC layers (but continued to be controlled by the PLA film substrate). This technology can be applied to a variety of applications where oxygen permeability is a key problem, including packaging of foods, pharmaceuticals and electronics.
2.5 REFERENCES


CHAPTER 3. CONTROLLING BARRIER AND MECHANICAL PROPERTIES OF CELLULOSE NANOCRYSTALS BY BLENDING WITH CHITIN NANOFIBERS

3.1 Introduction

Cellulose and chitin are two of the most abundant polymers found in nature, and are available from bacteria, tunicates, wood, or other plants (cellulose) and crustacean food waste, insects, or fungi (chitin).[1-3] Cellulose is a linear homopolymer of D-glucose[1] and chitin is structurally similar with an acetamide group (-NHCOCH₃) instead of a hydroxyl at the C2 position. Cellulose and chitin are found in hierarchical structures from which nanocrystals or nanofibers can be extracted. Cellulose in the form of cellulose nanocrystals (CNCs) is produced by sulfuric acid hydrolysis that lends negatively charged sulfate groups to these particles of ~5 nm in width and 150-200 nm in length.[4] Chitin nanofibers (ChNFs) are often extracted from crustacean shells by a process of base and acid treatments to remove proteins and minerals, respectively, and followed by homogenization to produce fibers that are ~10-20 nm in diameter and several microns in length.[5] In native chitin, a certain fraction of acetamide groups are deacetylated to an amine that is easily protonated, attaining a positive charge in sufficiently acidic solutions. Therefore, the degree of acetylation, which can be adjusted through the intensity of chitin base hydrolysis, determines the extent of charging possible, i.e., more acetylation equates to lower charge density. The nanocrystal and nanofiber forms of neat cellulose and chitin have been reported to have excellent gas barrier properties.[5-7] This study explores the additional opportunities to tune barrier and mechanical properties simultaneously through
the blending of insoluble ChNF and CNC particle suspensions, by exploiting their chemical and charge complementarity. In addition to the ability to tune physical properties by blending, understanding trade-offs in mechanical or barrier properties with blend composition is quite important for practical reasons. For example, the current industrial capacity to produce cellulose-derived nanofibers and nanocrystals greatly exceeds that of chitin-derived materials,[8-10] making it advantageous to be able to blend cellulose nanomaterials with chitin nanomaterials to optimize based on the cost and supply of the feedstocks.

Both chitin and cellulose nanomaterials have been studied for biomedical applications,[11] water treatment,[12, 13] and fillers in composites.[14-16] Blends of both soluble and insoluble forms of chitin and cellulose have been explored. Chitosan, a water-soluble derivative of chitin with deacetylation greater than 50%,[8] has been reinforced by blending with CNCs, resulting in enhanced mechanical and water vapor barrier properties.[17] Chitosan and cellulose blended spun fibers,[18] cast films[19, 20] and electrospin nanomats[21] have been prepared from solution and certain blend compositions had improved mechanical or hardness properties, tensile strength and improved elongation at break than unblended membranes. Recently, Duan et al.[20] reported the preparation of chitin-cellulose composite films by dissolving chitin and cellulose in an ionic liquid and γ-valerolactone. The blends had improved tensile strength up to 80 wt% chitin. A relatively smaller amount of work has considered blends of insoluble chitin and cellulose in nanofibers or nanocrystals. Robles et al.[22] prepared blended films from microfibrillated/nanofibrillated cellulose along with chitin nanocrystals (ChNC). They studied compositions of up to 10 wt % ChNC and found that addition of ChNCs decreased
the water vapor permeability to 80-100 g-mm/m²/day. Qi et al.[23] prepared composite films by coating TEMPO oxidized cellulose nanofibers (CNF) and ChNFs onto plastic films in a layer-by-layer deposition process. But, the resultant films did not exhibit enhanced barrier properties. Poly(lactic acid) (PLA) films reinforced with ChNF and CNFs resulted in a rigid cellulose nanofiber percolated network, with chitin acting as a compatibilizer between relatively hydrophobic PLA and relatively hydrophilic cellulose.[24, 25]

Previously, we synthesized oxygen barrier composites formed by spraying multilayer coatings of CNC and ChNF on PLA.[26] The resulting composites film showed a 73% reduction (at 50% relative humidity (RH)) in oxygen permeability (OP) compared to neat PLA and single-layer CNC- or ChNF-coated PLA However, the mechanical properties were compromised relative to uncoated PLA, and as the number of alternating layers increased beyond four. Kim et al.[27] reported a multilayer spray coating of TEMPO-oxidized CNFs and ChNCs that had enhanced barrier properties. Tricomponent composites consisting of poly(vinyl alcohol) (PVA), ChNF and CNC had enhanced tensile strength and modulus at certain ratios of ChNF and CNC compared to ChNF or CNC used alone as the filler. These studies suggest that in blends, the ratio of chitin and cellulose can be adjusted to tune barrier and mechanical properties in unique manner compared to neat materials or multilayers. Relative to multilayer coatings, blending also provides a simpler one-step process for producing biomass-derived chitin- and cellulose-based materials.

In this study, we examined suspension-cast ChNF/CNC blends and explore the effect of composition and degree of acetylation (DA) of ChNF on oxygen permeability and mechanical properties, not been previously reported in literature for CNC/ChNF blends.
We analyzed the relationship between neat particle and blended particle size and charge with the resulting blended film density, morphology, optical transmission, barrier and mechanical properties. An important finding was that by adjusting the ChNF/CNC blend ratio, the extent of ChNF/CNC aggregation was adjusted rationally based on knowledge of their surface charges, and this led to an ability to optimize the mechanical properties while minimizing the oxygen permeability.

3.2 Materials & Methods

3.2.1 Materials

ACS grade Sodium hydroxide pellets were obtained from VWR (Radnor, PA). Hydrochloric acid was purchased from EMD Chemical Inc (Burlington, MA). Glacial acetic acid was purchased from Sigma Aldrich (St. Louis, MO). Crab shells were purchased from Neptune’s Harvest (Gloucester, MA). Deionized (D.I.) water was obtained from a Thermo scientific GenPure UV/UF xCAD plus water purification system. Research-grade oxygen and ultra-high purity nitrogen were purchased from Airgas (Atlanta, GA). The aqueous CNC suspension was obtained from USDA Forest Products Lab (Madison, WI) and was sourced from mixed southern yellow pine dissolving pulp via 64% sulfuric acid digestion as described previously.[4] The resulting CNCs had 0.86 wt % sulfur content via sulfate functionality with Na\(^+\) counterions.

3.2.2 Chitin Purification

Two different batches of crab shells were used to extract chitin, which led to ChNF suspensions with different DA. The procedure for chitin treatment was per previously
published studies.[5, 26] Each batch was washed several times in water and the chitin was then ground into powder using a commercial grinder. The powder was refluxed at a setpoint temperature of 110 °C with 5 wt % NaOH for 6 h. The resulting solids were filtered and washed with DI water until the wash water had a pH of 7. The filtered solids were then treated with a 7 wt % HCl bath for 6 h at room temperature. The solids were again filtered and washed until the wash water pH was 7. The acid-treated solids were then refluxed with 5% NaOH for 48 h at a setpoint temperature of 110 °C and the resultant solids were filtered and washed with DI water until the wash water pH was 7. This resulted in a white purified chitin powder, which was dried in an oven at 60 °C for 24 hours. The two batches of chitin were then homogenized as described below to produce ChNF suspensions.

3.2.3 Extraction of ChNFs

A 0.5 wt % purified chitin (of unknown DA) suspension was prepared in DI water. The pH of the suspension was adjusted to 3.0 using glacial acetic acid and the acidified suspension was homogenized in a Mini DeBEE Homogenizer (BEE International, South Easton, MA). The first sequence of homogenization was carried out at a pressure of 1034 bar by using a 0.2 mm nozzle for 20 passes followed by a second sequence at 1516 bar and utilizing a 0.13 mm nozzle for an additional 10 passes. Because of the fluid expansion through the nozzle resulting in shear heating, a water cooled heat exchanger was used throughout the processing to cool the product and keep the ChNF suspension at a maximum temperature of 35 °C.
3.2.4 CNC Surface Charge Characterization

Potentiometric titration was carried out to obtain the surface charge concentration of CNCs. For the titration testing, 100 ml of 5.5 wt % aqueous suspension of CNCs was diluted with 400 ml of DI water to approximately 1 wt %. The resulting suspension was ion exchanged with 200 ml of Merck Ion Exchanger I, a strongly acidic cation exchange resin, to remove any cations. The resin was then washed with DI water to wash out entrapped CNCs and the resulting suspension was titrated against a 1.5 N NaOH solution by potentiometric titration with a Mettler Toledo Seven Excellence S400 pH meter.

3.2.5 ChNF Surface Charge Characterization

The DA of the chitin was determined by potentiometric titration. A volume of 25 ml of the homogenized suspension was ion exchanged using 300 ml of anion exchange resin. The ion exchange resin was washed with about 200 ml of DI water to remove entrapped chitin. 20 ml of 0.25 N HCl was added to the resulting suspension which was then was titrated against 0.5 N NaOH using a Mettler Toledo Seven Excellence S400 pH meter. The inflection points as determined by the pH vs volume of titrant curve were then used to determine the amount of NaOH consumed. The NaOH equivalent of HCl present in the suspension was subtracted from the total consumed NaOH to yield the amount of NaOH consumed by the protonated chitin. The number equivalent of NaOH consumed divided by the mass of the starting material gives \( m \) [mol NH\(_2\)/g chitin (dry basis)] which was used to determine the DA by

\[
DA \, [\%] = 100 \times \frac{203.21}{42.04 + m} \quad (3.1)
\]
where 203.21 [g/mol] is the molecular mass of a chitin monomer and 42.04 [g/mol] is the difference in molecular mass between a chitin and a chitosan (deacetylated chitin) monomer. The titrations were repeated thrice for each sample.

After processing different batches of chitin and characterizing their DA, chitin nanofibers (ChNFs) with lower DA (50-90% DA) are referred to as LChNF and those from chitin with higher DA (>90%) are chitin referred to as HChNF.

3.2.6 Particle Size and Distribution of ChNFs and CNCs Suspensions

Atomic Force Microscopy (AFM) (Bruker Icon) was utilized to image the ChNFs and CNCs. To produce these samples, 0.5 wt % HChNFs, LChNFs were diluted with pH 3 acetic acid solution with Triton X-100 surfactant to 0.001 wt %. Similarly, to study CNCs, 0.5 wt % CNC suspensions were diluted to 0.001 wt % using DI water and Triton X-100. 20 µl of the diluted suspensions were drop cast onto a clean, ozone treated silicon wafers. The drops were allowed to dry at 60 °C for 24 hours. The length and number of particles were determined by visual analysis of the AFM height images with the help of ImageJ to mark, label and measure the nanoparticles. The diameter of the nanoparticles were estimated using the height sensor value from the AFM height image of the nanoparticles.

3.2.7 Characterization of ChNFs, CNCs and Blends of ChNFs-CNCs

40 µl volumes of 0.5 wt % suspensions of neat LChNFs, HChNFs, CNCs and blends of these materials were diluted with 20 ml of DI water. A Malvern Zetasizer Nano was used to measure the zeta potential and the size of dilute ChNF, CNCs and blends of ChNFs and CNCs.
3.2.8 Preparation of Neat and Blended Films

The as-received CNC suspension was diluted to 0.5 wt% by addition of deionized (DI) water. 50 ml of 0.5 wt% HChNF, LChNF and CNC suspensions were air-dried for 14 days at room temperature and humidity (45% RH) in a poly(tetrafluoroethylene) dish to yield free-standing, single-component films. In addition, different volumes of 0.5 wt% LChNF and 0.5 wt% CNC suspensions were blended together in a beaker at 500 rpm for 4 hours to produce mixtures of varying ratios of CNCs to LChNFs. In addition to neat LChNFs and CNCs, blends with three different CNC:LChNF ratios (by mass) were studied: 3:1, 1:1, and 1:3. The total volume of all blends was maintained to be 50 ml, so that the total mass of solids was equivalent in all castings. The resultant mixtures were sonicated for 30 s at a power of 80 W. The sonicated mixture was air-dried like the single-component films described above to produce a films with 25%, 50%, 75% LChNFs. Similarly, a mixture of 50% HChNF and 50% CNC was also produced by blending 0.5 wt% HChNF suspension and 0.5 wt% CNC suspension (total volume 50 ml) and casting and drying per the described procedure.

3.2.9 Characterization of Neat and Blended Films

The thickness of these films was measured with a micrometer at more than 10 different points on the films. Light transmission of these films was measured at 550 nm using a Shimadzu UV-1800 UV/Vis spectrophotometer. Mechanical properties of suspension-cast films were measured, specifically ultimate tensile strength (UTS) and strain at break, by using a high-throughput mechanical characterization (HTMECH) instrument, that measures force and deformation of film regions through transverse biaxial
loading by an instrumented thin contact tip.[28] The UTS of the films was taken at the maximum of the stress vs strain curve. The OP values of films were obtained by using a MOCON OXTRAN 1/50 instrument at 50% RH. The films were humidified to soften them before permeability testing using an Electrotech ultrasonic humidification system (Glenside, PA) operating at 100% RH. In general, humidification makes the films easier to handle and soft enough to cut without fracturing. The films were humidified until they could be bent without fracture and were cut into approximately 9 cm$^2$ squares and covered with a mask that allowed measurement of 5 cm$^2$ of this area. The interval between measurements was 1 h with the final measurement value taken at steady state. The criteria for steady state was that the contiguous readings must differ by less than 1% or 0.05 cc/m$^2$/day (convergence by cycles mode). Generally, convergence time was of the order of 24 h.

AFM was utilized to image the top surface of coated silicon wafers. To produce these samples, drops of 20 µl of mixtures of HChNFs, LChNFs and CNCs were drop cast onto a clean silicon wafer. The drops were allowed to dry at room temperature and 45% RH for 48 hours. The size of structures on the coated films were measured by visual analysis of the AFM phase images. Film roughness was determined from the AFM images by using Bruker Nanoscope Analysis software. The drop casted wafers were then fractured by breaking with a sharp blade and the resulting cross sections were imaged with Zeiss Ultra 60 Scanning Electron Microscope (SEM). The optical properties of films were further investigated using an optical microscope (Olympus BX51) equipped with two polarizers, referred to as the polarizer and the analyzer (Olympus U-AN360P). The density of suspension-cast films was measured by pycnometry. 99 % n-Hexadecane was purchased
from Alfa Aesar (Ward Hill, MA) and was used as a reference liquid for the pycnometry. Void fraction ($\emptyset$) of the films is calculated as below:

$$\emptyset = \frac{\rho_{\text{bulk}} - \rho_{\text{mix}}}{\rho_{\text{air}} - \rho_{\text{mix}}}$$ (3.2)

where, $\rho_{\text{bulk}}$ is bulk density of films as measured by pycnometry, $\rho_{\text{mix}}$ is the weight averaged theoretical density of mixtures assuming pure cellulose density of 1.5 g/cm$^3$[29] and pure chitin density of 1.462 g/cm$^3$.[30] $\rho_{\text{air}}$ is the density of air, assumed to be 1.225 g/cm$^3$.

### 3.3 Results & Discussions

#### 3.3.1 Physicochemical Properties

The number distribution of length and width of HChNFs, LChNFs and CNCs determined from AFM images of deposits from dilute suspension and are shown in Figures 3.1(a-d). HChNFs and LChNFs have a wide distribution in fiber length: 42 % of HChNF fibers are above 1 $\mu$m in length while only 21 % of LChNF fibers are above 1 $\mu$m. In addition, 21 % of HChNF fibers are 10 $\mu$m or larger in length, which is true of only 12 % of LChNF fibers. About 15 % of HChNF fibers have widths of about 3 nm while this figure increases to 32 % for LChNFs. This indicates that the suspensions of LChNFs are more fibrillated than HChNF suspensions, and HChNF suspensions have much wider, more heterogeneous length and width distributions than LChNFs. In comparison, CNCs are significantly more uniformly distributed in size with particle length of 154 ± 59 nm and width of 6.0 ± 2.6 nm.

Table 1 presents mass ratios of CNC, LChNF and HChNF used in each sample, as well as results of the titration measurements on the neat materials. Titration of the CNC
suspension resulted in surface charge of $-0.49 \pm 0.09$ meq/g. Similarly, titration of HChNFs yielded a charge of $0.35 \pm 0.12$ meq/g corresponding to $92.9 \pm 2.4\%$ DA (degree of acetylation), while LChNFs were found to have a charge of $1.37 \pm 0.08$ meq/g corresponding to $73.6 \pm 1.5\%$ DA.

Figure 3.1 Number distributions of (a) length (b) of LChNF & HChNF fibers. Number distributions of (c) length & (d) width of CNCs.
These two batches were used to compare the effect of acetylation on the properties of blended films. The samples, although treated with a similar process, can vary in their acetylation%[31] due to differences in the biomass (crab shell) source,[32-34] variations in the size of solids, and variation in the actual temperature-time behavior during the reflux with NaOH.[35-37] For example, even using the same master source, it is possible that the small batch size per process samples different distributions of shell content and size. The two prepared LChNF and HChNF samples are well-characterized and serve as high- and low-charge (or low and high acetylation) materials for comparison of this effect on film properties.

Based on the available surface charge per unit weight, and recognizing that CNCs are negatively charged while ChNFs are positively charged, we calculated the stoichiometric amounts required for surface charge neutralization in a blended suspension. The mass ratios for charge neutralization are 2.8 CNC:1 LChNF and 1.4 CNC:1 HChNF. In addition to the measured neat values, Table 3.1 lists the calculated surface charge content of particles in blends of LChNF, HChNF and CNCs assuming that positive and negatively charged surfaces attract each other and the the number of available surface charges is reduced by such interactions. The error values given in the Table are standard deviation errors for LChNF, HChNF and CNC charge content. To assign a standard deviation for blend charge content, the largest standard deviation value of the charge content of the blend components was used.
Table 3.1 Composition and surface charge of suspensions of CNCs, ChNFs and blends.

<table>
<thead>
<tr>
<th>Label</th>
<th>CNC content (wt %)</th>
<th>Net Charge (meq/g)</th>
<th>Zeta Potential (mV)</th>
<th>Fiber Length (nm)</th>
<th>Film Density (g/cm³)</th>
<th>Void fraction of films (ø)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNC</td>
<td>100</td>
<td>-0.49 ± 0.09</td>
<td>-35.9 ± 8.1</td>
<td>300 ± 80</td>
<td>1.12</td>
<td>0.25</td>
</tr>
<tr>
<td>3:1 CNC-LChNF</td>
<td>75</td>
<td>-0.02 ± 0.09*</td>
<td>-23.2 ± 10.7</td>
<td>1960 ± 370</td>
<td>1.20</td>
<td>0.20</td>
</tr>
<tr>
<td>1:1 CNC-LChNF</td>
<td>50</td>
<td>0.44 ± 0.09*</td>
<td>44.6 ± 3.6</td>
<td>1130 ± 180</td>
<td>1.19</td>
<td>0.20</td>
</tr>
<tr>
<td>1:3 CNC-LChNF</td>
<td>25</td>
<td>0.91 ± 0.09*</td>
<td>50.0 ± 5.3</td>
<td>1160 ± 180</td>
<td>1.24</td>
<td>0.16</td>
</tr>
<tr>
<td>LChNF</td>
<td>0</td>
<td>1.37 ± 0.08</td>
<td>50.4 ± 4.9</td>
<td>610 ± 100</td>
<td>1.28</td>
<td>0.13</td>
</tr>
<tr>
<td>HChNF</td>
<td>0</td>
<td>0.35 ± 0.12</td>
<td>31.7 ± 5.7</td>
<td>1270 ± 210</td>
<td>1.03</td>
<td>0.30</td>
</tr>
<tr>
<td>1:1 CNC-HChNF</td>
<td>50</td>
<td>-0.07 ± 0.12*</td>
<td>-9.08 ± 7.4</td>
<td>1730 ± 330</td>
<td>1.16</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*Calculated based on neat particle charge content.

The zeta potential of HChNFs and LChNFs was measured to be +31.7 ± 5.7 mV and +50.4 ± 4.9 mV while the zeta potential of CNC suspensions was measured to be -35.9 ± 8.1 mV. Neat LChNF suspensions have high stability while HChNF suspensions have incipient instability. The trends in zeta potential correspond well to the charges determined from titration.

Table 3.1 (and Figure 3.2a) reports the zeta potential of neat suspensions and blends. The zeta potential of the blends of CNCs and HChNFs crosses 0 mV closest to 3:1 CNC-LChNF mixtures, while it is 0 mV closest to 1:1 CNC-HChNF mixtures. This corresponds well to the calculated expected composition for neutral charge. Considering that suspensions with absolute values of zeta potentials greater than 30 mV are considered
to be electrostatically stabilized,[38] the blend suspensions with > 25 wt % LChNFs can be considered to be stable.

Figure 3.2 (a) Zeta potential and (b) Zetasizer particle size of cast CNC, LChNF, HChNF, CNC-LChNF & CNC-HChNF composite films.
The average suspension particle size per the Malvern Zetasizer are shown in Figure 3.2b and averages are reported in Table 3.1. The maximum size is observed for 3:1 CNC-LChNF mixtures as compared to LChNFs and CNCs. Similarly, 1:1 CNC-HChNF mixtures have the maximum size compared to HChNFs and CNCs respectively. This corresponds well to the stoichometric ratios at which maximum aggregation is to be expected. The average particle size of all blend suspensions of CNCs, LChNFs and HChNFs is greater than the average size of the starting CNCs, LChNFs or HChNFs. This observation corresponds to expected aggregation driven by electrostatic attraction of chitin and cellulose nanomaterials.

Figure 3.3 Images of representative CNC, LChNF, HChNF & blended films. The scale bar is 50 mm.

Figure 3.3 shows the cast, air-dried films made from mixtures of LChNFs, HChNFs and CNCs. The neat CNC films were relatively flat and took on the shape of the plastic boat that was used to dry them. As LChNF content of the films increased, the films were more distorted, with 1:3 CNC-LChNF and neat LChNF films being most distorted. Additionally, the neat LChNF films were highly transparent (63\% average light transmission (T)), and the presence of CNCs led to haziness in the mixed CNC-LChNF films (37-41\% T). The neat CNC films were transparent but also at the same time showed
iridescence leading to lower light transmission (27% T) that is not seen in any of the other films. Both HChNF and 1:1 CNC-HChNF films were distorted. Like neat LChNF films, neat HChNF films were also transparent (51% T). 1:1 CNC-HChNF films were visibly hazier (38% T) than neat HChNF films.

Iridescence in neat CNC films has been reported previously and was attributed to chiral nematic ordering of CNCs in the films after drying.[39, 40] Loss of the iridescence can be attributed to disruption of the chiral nematic ordering due to formation of aggregates of CNCs and LChNFs as evidenced by the dramatically increased haziness of 3:1 CNC-LChNF films. This ratio was near the stoichiometric charge-neutralized ratio (1.4 CNC:1 LChNF). Visibly increased haziness could also be seen for the near stoichiometric 1:1

![Figure 3.4 Light Transmission at 550 nm of cast CNC, LChNF & CNC-LChNF composite films. Inset compares HChNF & LChNF neat and composite films.](image)

CNC-HChNF films, presumably due to aggregation.
Figure 3.4 shows light transmission at 550 nm for films shown in Figures 3.3. The light transmission through neat CNC films was the lowest at 27 % with a high standard deviation, while the LChNF films had the highest light transmission of 63 %. Addition of 25 % LChNFs to CNCs increased the average light transmission of the films to around 37% for 3:1 CNC-LChNF films. The corresponding standard deviation in light transmission was also reduced indicating that the blended film may possess a higher-density, more homogenous microstructure with fewer voids. Further addition of LChNFs beyond this mass loading did not change light transmission significantly, and 1:3 CNC-LChNF films had average light transmission of 41%. Neat HChNF films had a light transmission of 51 %, which was lower than neat LChNF films. Addition of 50 wt. % HChNFs to neat CNCs lowered the light transmission to around 38 % for 1:1 CNC-HChNF films, which was similar to the blended 1:1 CNC-LChNF films.

Neat LChNF films are transparent and clear. Addition of LChNFs to CNCs in stoichiometric amounts should contribute to aggregate formation due to attraction between positively charged LChNFs and negatively charged CNCs, which should lower the light transmission due to light scattering from aggregates. Increasing LChNF amounts over the stoichiometric 3:1 CNC-LChNF mixtures should increase the LChNF content and thus the resultant films would have higher light transmission as the effect is to dilute the presence of aggregated particles. Neat CNCs have a lower light transmission than the blends due to film iridescence. The minimum light transmittance is reported to occur around 470 nm where up to 60% of light to be reflected. (Parit et al. 2018) Neat HChNF films are expected to be less transparent than LChNF films as it is the lower surface charge that would lead to a higher number of aggregates that would, in effect, lower the light transmission through
scattering. CNCs are iridescent and disruption of chiral nematic ordering should increase light transmission through addition of ChNFs. At the same time the HChNF-CNC aggregates that form, scatter light and the net result of the two opposing trends is that the light transmission remains almost unchanged for 1:1 CNC-HChNFs films, where neat HChNF films show lowered light transmission, likely due to aggregation of highly acetylated HChNFs.

Figure 3.5 shows the measured thickness of suspension-cast films of neat and mixed CNCs and LChNFs, as well as neat HChNFs. Even though each casting contained an equivalent mass of solids, the final observed thickness of dried films was strongly dependent on composition.

![Graph showing thickness of CNC, LChNF & CNC-LChNF composite films. Inset compares HChNF & LChNF neat and composite films.](image)

Figure 3.5 Observed thickness of CNC, LChNF & CNC-LChNF composite films. Inset compares HChNF & LChNF neat and composite films.

Neat CNC films had the highest observed thickness of about 133 μm and neat LChNFs had the lowest observed film thickness at 55 μm. Addition of up to 50 wt %
LChNFs to CNCs (1:1 CNC-LChNF) was found to decrease film thickness to 63 μm. Addition of ChNFs beyond this weight loading did not significantly reduce the observed film thickness. The HChNF films were thicker than LChNF films (127 μm for HChNF versus 55 μm for LChNF) and were in the same size range as neat CNC films. Addition of 50 wt % HChNFs to CNCs caused the average observed thickness of the resultant 1:1 CNC-HChNF film to reduce to 48 μm, lower than 1:1 CNC-LChNF films.

Miettinen et al.[41] reported that apparent thickness of mixtures of CNFs and TEMPO CNFs decreased with increasing TEMPO CNF content. Similarly, Rodionova et al.[42] also saw a similar decrease in thickness of TEMPO CNF films made from eucalyptus with increasing carboxylate content. Robles et al.[22] also reported a decrease in thickness of CNF-ChNC films with increasing ChNC content. In all cases, decrease in thickness was correlated with the presence of higher amounts of surface charges in the suspension. Robles et al.[22] reasoned that both the inclusion of NH₂ based surface charges on the ChNCs as well as smaller aspect ratio of ChNCs (relative to the CNF matrix) leads to better packing of the nanocrystals within the films, as evidenced by reduced porosity and permeability at higher ChNC content. Table 3.1 shows that LChNFs have a higher surface charge per mass than HChNFs and that CNCs have a higher surface charge per mass than HChNFs and have an opposite charge (-) relative to HChNFs (+). Based on the idea that highly charged fibrils or crystals promote repulsion that supports entropically-driven alignment and packing, then increasing LChNF content is expected to increase the packing efficiency and result in thickness reduction of resultant CNC-LChNF films. This agrees with our observation that LChNF films with the highest surface charge per unit mass have the lowest thickness. Similarly, HChNF and CNC films have the lowest surface
charges and as a consequence have much higher thickness as compared to neat LChNF films. However, addition of 50% CNCs to neat HChNF films decreases the thickness of the resultant 1:1 CNC-HChNF films despite a decrease in the net charge of the mixture as evident in Table 3.1, possibly due to the effect of inclusion of smaller aspect ratio and length CNCs in the larger fibrillar HChNFs. The decrease in thickness of ChNF films on addition of CNCs is indeed similar to what was reported by our group for spray coated multi-layer chitin nanofiber cellulose nanocrystal films.[26] It is important to note that our case is different from Robles et al.[22] In their case, the smaller sized particles and particles with higher surface charges were the same particles, the ChNCs, while in our case CNCs are the smaller sized particles while ChNFs have either similar or substantially higher charges per unit weight.

Table 3.1 presents the bulk density and void fraction for neat and blended films. In the series of blends of CNCs and LChNFs, the neat CNC films have the lowest density and the LChNF the highest, with blends falling between these limits. The densities follow the same trends as observed for the thickness measurements. The HChNF density is lowest of all solid films, at 1.03 g/cm³, which correlates to the high film thickness in Figure 3.5. The low HChNF density is likely influenced by the large and heterogeneous distribution in fiber lengths and diameters, and as will be discussed below, the morphology of HChNFs is quite heterogeneous compared to LChNFs and CNCs with large micron-sized voids intermixed with dense film interlayers.
3.3.2 Oxygen Permeability

Figure 3.6 shows the OP of blended LChNF, HChNF and CNC films. Neat CNC films had the highest OP of 18.5 cm\(^3\)-\(\mu\)m/m\(^2\)/d/kPa. Belbekhouche et al.\([6]\) reported OP values 650x lower for CNCs than reported in this study. The stark difference in measurement can be attributed to different conditions (0% RH) and a different method of measurement (‘time lag’ method) for gas permeation used by Belbekhouche et al.\([6]\) The OP of neat LChNF films was the lowest at 1.0 cm\(^3\)-\(\mu\)m/m\(^2\)/d/kPa. Addition of even 25 wt % LChNF to CNCs resulted in a decrease of the OP from 18 to 1.7 cm\(^3\)-\(\mu\)m/m\(^2\)/d/kPa for the resultant 3:1 CNC-

![Graph showing oxygen permeability of different films](image)

**Figure 3.6 Oxygen permeability of cast CNC, LChNF & CNC-LChNF composite films. Inset shows oxygen permeability of HChNF & LChNF neat and composite films.**

LChNF films. Further addition of LChNF to CNCs beyond this weight loading did not significantly change the OP. The OP of neat HChNF films was 4.4 cm\(^3\)-\(\mu\)m/m\(^2\)/d/kPa, about
4 times higher than neat LChNF films, but the 1:1 CNC-HChNF blends had OP of 1.9 cm$^3$-μm/m$^2$/d/kPa. The OP of all films containing LChNFs or HChNFs is considerably lower than that of CNC films and was also found to be lower than the OP of poly(ethylene terephthalate) (PET) which has a OP range of 9.8 to 49.3 cm$^3$-μm/m$^2$/d/kPa when measured at the same conditions as our films.[43] We note that the OP is calculated by using an apparent thickness measured by a micrometer, which includes the effects of surface roughness (shown in Figure 3.7) and microporosity that can result in an erroneously high thickness, which would lead to a high value of OP. So, these OP results can be considered conservative values.[41]

![Figure 3.7 Roughness of solution cast films measured from an AFM image of 5 μm x 5 μm size.](image-url)
3.3.3 *Mechanical Properties*

Figure 3.8 Box plots showing the mechanical properties of films. (a) UTS, (b) strain at break.

Figure 3.8 shows the mechanical properties of HChNF, LChNF, CNC and blended films. Tables 3.2 and 3.3 show two-tailed P values for the null hypothesis that difference between means of the compared samples is insignificant. P values less than the significance level (0.05) indicate that the difference between the means is significant.
Table 3.2 Two tailed P-values for mean ultimate tensile strength (UTS). Values highlighted in red indicate differences in means between the two samples being compared is significant and the level of 0.05.

<table>
<thead>
<tr>
<th></th>
<th>Neat CNC</th>
<th>3:1 CNC-LChNF</th>
<th>1:1 CNC-LChNF</th>
<th>1:3 CNC-LChNF</th>
<th>Neat LChNF</th>
<th>Neat HChNF</th>
<th>1:1 CNC-HChNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat CNC</td>
<td>1.00</td>
<td>0.0008</td>
<td>0.01</td>
<td>0.09</td>
<td>0.10</td>
<td>0.07</td>
<td>0.002</td>
</tr>
<tr>
<td>3:1 CNC-LChNF</td>
<td>0.0008</td>
<td>1.00</td>
<td>1.00</td>
<td>0.44</td>
<td>0.39</td>
<td>0.004</td>
<td>0.13</td>
</tr>
<tr>
<td>1:1 CNC-LChNF</td>
<td>0.01</td>
<td>1.00</td>
<td>1.00</td>
<td>0.43</td>
<td>0.40</td>
<td>0.046</td>
<td>0.17</td>
</tr>
<tr>
<td>1:3 CNC-LChNF</td>
<td>0.09</td>
<td>0.44</td>
<td>0.43</td>
<td>1.00</td>
<td>0.86</td>
<td>0.16</td>
<td>0.92</td>
</tr>
<tr>
<td>Neat LChNF</td>
<td>0.10</td>
<td>0.39</td>
<td>0.40</td>
<td>0.86</td>
<td>1.00</td>
<td>0.17</td>
<td>0.76</td>
</tr>
<tr>
<td>Neat HChNF</td>
<td>0.07</td>
<td>0.004</td>
<td>0.05</td>
<td>0.16</td>
<td>0.17</td>
<td>1.00</td>
<td>0.007</td>
</tr>
<tr>
<td>1:1 CNC-HChNF</td>
<td>0.002</td>
<td>0.13</td>
<td>0.17</td>
<td>0.92</td>
<td>0.76</td>
<td>0.007</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 3.3 Two tailed P-values for mean strain at break. Values highlighted in red indicate differences in means between the two samples being compared is significant and the level of 0.05.

<table>
<thead>
<tr>
<th></th>
<th>Neat CNC</th>
<th>3:1 CNC-LChNF</th>
<th>1:1 CNC-LChNF</th>
<th>1:3 CNC-LChNF</th>
<th>Neat LChNF</th>
<th>Neat HChNF</th>
<th>1:1 CNC-HChNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat CNC</td>
<td>1.00</td>
<td>0.14</td>
<td>0.0002</td>
<td>0.09</td>
<td>0.14</td>
<td>0.0004</td>
<td>0.00</td>
</tr>
<tr>
<td>3:1 CNC-LChNF</td>
<td>0.14</td>
<td>1.00</td>
<td>0.0007</td>
<td>0.10</td>
<td>0.19</td>
<td>0.0001</td>
<td>0.00</td>
</tr>
<tr>
<td>1:1 CNC-LChNF</td>
<td>0.0002</td>
<td>0.0007</td>
<td>1.00</td>
<td>0.32</td>
<td>0.73</td>
<td>0.53</td>
<td>0.05</td>
</tr>
<tr>
<td>1:3 CNC-LChNF</td>
<td>0.09</td>
<td>0.10</td>
<td>0.32</td>
<td>1.00</td>
<td>0.27</td>
<td>0.28</td>
<td>0.76</td>
</tr>
<tr>
<td>Neat LChNF</td>
<td>0.14</td>
<td>0.19</td>
<td>0.73</td>
<td>0.27</td>
<td>1.00</td>
<td>0.91</td>
<td>0.15</td>
</tr>
<tr>
<td>Neat HChNF</td>
<td>0.00004</td>
<td>0.0001</td>
<td>0.53</td>
<td>0.28</td>
<td>0.91</td>
<td>1.00</td>
<td>0.03</td>
</tr>
<tr>
<td>1:1 CNC-HChNF</td>
<td>0.0001</td>
<td>0.003</td>
<td>0.05</td>
<td>0.76</td>
<td>0.15</td>
<td>0.03</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Figure 3.8a shows the UTS values obtained. From these results, the neat CNC films had the lowest UTS with the mean value being 4 MPa while LChNF films had the highest mean UTS with a mean value of 16 MPa. The addition of LChNFs to CNCs was found to lead to an increase in UTS of the blended films with 1:3 CNC-LChNF films having at mean UTS of 15 MPa having the highest value amongst the blended samples studied here. In general, once LChNF is added to CNCs, the difference in UTS is significant (p < 0.05), but the difference between the various blend ratios is insignificant (1:3, 1:1 and 3:1). This dependence of UTS on LChNF content mirrors the OP values, where the values of blends are significantly different than neat CNC, but not different from blends at other concentrations. The neat LChNF films had a higher mean UTS than the neat CNC, but this difference was not statistically significant due to variability in measurements. Neat HChNF films had a mean UTS of about 7 MPa, lower than LChNF films, but this was not statistically significant. Despite this, adding up to 50% HChNFs to CNCs lead to an improvement in UTS of the resultant films, where the UTS of 1:1 CNC-HChNF films was increased to 11 MPa in the same range as that of 1:1 CNC-LChNF films, (mean value of 14 MPa). The breaking strength of the films was also found to be similar to the UTS and follow the same trends as the UTS. The LChNF- and HChNF-containing blends at a 1:1 CNC:ChNF ratio gave statistically distinct results from one another.

Figure 3.8b shows the strain at break of films. Neat CNC films as well as 3:1 CNC-LChNF films had the lowest strain at break with mean values of 2% and 3% respectively. The strain at break of films increased with increase in mass loading of LChNF up to 75 wt % LChNFs, where the strain at break reached a maximum with a mean value of 20%. Neat LChNF films had a lower strain at break with a mean value of 10%. Neat HChNF films
had similar strain at break compared to LChNF films with a mean value of about 10%. Addition of 50% HChNF to CNCs caused the strain at break to increase with the mean value being 18% for the resultant 1:1 CNC-HChNF films, values that were higher than 1:1 CNC-LChNF films (11% mean strain at break).

In general, it can be seen that neat CNC UTS and strain at break are significantly different than those of the 3:1 and the 1:1 CNC:HChNF. In addition, neat HChNF properties are always insignificantly different than LChNF properties. Blends containing HChNF are generally significantly different than blends containing LChNF. Interestingly, the neat LChNF and the 1:3 CNC:LChNF properties are never significantly different than any other sample. In addition, while there are significant differences between neat CNCs and blends with either LChNFs or HChNFs, the blends containing CNC:LChNF or CNC:HChNF at various ratios are not significantly different in most cases. These significance values indicate that adding LChNF in any quantity usually produces a significant difference compared to neat CNCs, and a significant difference compared to blends containing HChNF, but blends containing LChNFs at various ratios do not differ significantly from one another.

One study showed that CNFs were more effective than CNCs at reinforcing composites with poly(caprolactone), and the higher modulus and tensile strength in CNF-containing composites was attributed to the entanglements possible with the higher aspect ratio CNFs versus CNCs.[44] We suggest that the reinforcement of CNCs by ChNFs shown in Figure 3.8 is also related to the high aspect ratio and entanglement enabled by ChNFs. Blends of CNFs with ChNCs and showed that increasing strain at break as CNF content increased. Since CNCs are similar to ChNCs with regard to crystallinity and aspect ratio,
and ChNFs are similar to CNFs in aspect ratio, these trends correlate with our observations for ChNF-CNC blends.

### 3.3.4 Optical Properties

Figure 3.9 shows optical microscopy images of cast CNC, LChNF, HChNF and blended films under crossed polarization. It can be seen that neat CNC films exhibited large birefringent regions of the order of 200 μm in size (Figure 3.9a). Neat LChNF and HChNF films (Figure 3.9e and 3.9f) did not have such large regions of birefringence and were more homogenous at this length scale when compared to CNC films, although some bright spots were seen with ~100 μm sizes.

**Figure 3.9** Optical microscope images of (a) neat CNC, (b) 3:1 CNC-LChNF, (c) 1:1 CNC-LChNF, (d) 1:3 CNC-LChNF, (e) neat LChNF, (f) neat HChNF, and (g) 1:1 CNC-HChNF. Images are exposure-corrected. All scale bars are 200 μm in length.

Addition of 25 wt % LChNFs to neat CNCs reduced the birefringent domain sizes to an order of 100 μm as seen in Figure 3.9b. Increasing the LChNF content to 50% caused the films to appear more homogenous with decreasing size of birefringent domains to 100 μm in Figure 3.9c. 1:1 CNC-HChNF films (Figure 3.9g) were more homogenous under crossed polarizers than 1:1 CNC-LChNF films (Figure 3.9c) with almost no discernible
bright regions, indicating that the CNCs may have been better dispersed in the 1:1 CNC-HChNF films. Addition of 75 wt % LChNFs to neat CNCs reduced the number of birefringent domains even further than the 50 wt % LChNF films and these films had a fine granular appearance with structures of the size of 50 μm (Figure 3.9d).

In general, polarized light microscopy is used to detect nematic ordering, aggregation, and the state of dispersion at the optical scale in CNC-, CNF-, ChNC- and ChNF-containing suspensions and solids.[44-46] In the case of CNCs, the negative surface charges cause the excluded volume of the rods to be larger than the rod volume. The excluded volume coupled with the high aspect ratio allows CNCs to form nematic mesophase domains at sufficiently high concentrations [45] as can be seen in Figure 3.9a. ChNFs on the other hand have lower crystallinity than CNCs. They consist of rigid crystalline blocks (similar to CNCs, termed ChNCs) separated by flexible amorphous regions, so that the overall crystallinity is lower than CNCs and ChNCs. Strong interfibrillar hydrogen bonding is known to lower the long range orientational order leading to suppression of chiral nematic domains,[47] which is consistent with our observations in Figure 3.9e and 3.9f. From the images in Figure 3.9, addition of ChNFs to CNCs causes reduction in the birefringent mesophases. Addition of LChNFs to CNCs (3:1 CNC-LChNF) near the stoichiometric ratio would be expected to lead to suspensions with large numbers of aggregated particles with CNCs attached to LChNFs, driven by electrostatic attraction and hydrogen bonding. This expectation is consistent with the reduction in the chiral nematic domain size of the CNCs following addition of LChNFs in Figure 3.9. Due to the electrostatic attraction of ChNF protonated amine groups and CNC sulfate ester groups, we may expect aggregation of CNC at cationic sites on ChNF to increase localized
CNC concentrations and enable birefringent domains in those regions. (AFM data below supports the formation of CNC-ChNF aggregates on the outer surface of ChNFs.) The concentration of CNCs in the blended films is lower as compared to neat CNC films, thus the size and number of CNC-driven birefringent regions is expected to be lower. The HChNFs have a lower surface charge than LChNFs. At stoichiometric ratios, the HChNF aggregates have $1/3$rd the amount of CNCs as LChNF aggregates. The significantly lowered number of CNCs on the HChNF aggregates as compared to LChNF aggregates is expected to reduce the size and number of birefringent domains as compared to CNC-LChNF composites, and this is seen in Figure 3.9g. These effects are consistent with previous work [25] studying polarized light images of tricomponent composites of ChNFs, CNCs and PVA. The ChNFs utilized in that study had charges consistent with LChNFs. Composites with only CNCs and PVA showed large white features similar to Figure 3.10a. PVA-ChNF composites in Irvin et al.,[25] showed a lesser number of these regions than PVA-CNC and PVA-ChNF-CNC composites.
Figure 3.10 AFM images of (a) neat CNC, (b) 3:1 CNC-LChNF, (c) 1:1 CNC-LChNF, (d) 1:3 CNC-ChNF, (e) neat LChNF, (f) neat HChNF, and (g) 1:1 CNC-HChNF.

Figure 3.10 shows AFM images of cast CNC, LChNF, HChNF and blended films. The neat CNC film (Figure 3.10a) shows oriented CNCs in the height and phase images, with a length of $\sim 150$ nm, consistent with wood-based CNCs[4] and consistent with our length measurements for CNCs as shown in Figure 3.1c. The 3:1 CNC-LChNF shows LChNFs appear to be coated with CNCs that have a size consistent with neat CNCs in
Figure 3.10a. The phase image of 3:1 CNC-LChNF films shows that the visible fiber like particles of ChNFs and CNCs are oriented like CNCs. Films of 1:1 CNC-LChNF, 3:1 CNC-LChNF, neat LChNFs and neat HChNFs show little apparent ordering compared to 3:1 CNC-LChNF and neat CNC. For blends containing more than 50% CNC content, LChNFs appear to be in excess on the surface with few CNCs visible. The height image of 1:1 CNC-HChNF films shows HChNFs coated with CNCs like the 3:1 CNC-LChNF films.

From the optical microscopy of Figure 3.9a and AFM of Figure 3.10a, CNCs have a qualitatively higher ordering or alignment compared to LChNFs and HChNFs, associated with the CNC’s higher crystallinity and shorter length.[45] While not immediately apparent from Figures 3.9(e-f) and 3.10(d-f), LChNFs are expected to be more ordered than HChNFs due to their higher surface charge.[48, 49] Disruption of CNC ordering upon addition of ChNFs is also expected due to the formation of agglomerates that neutralize the negative surface charge responsible for repulsion. Indeed, the qualitative disappearance of birefringence in the blended films seen in Figures 3.3 and 3.9 on addition of LChNFs and HChNFs points to this.

Figure 3.11 shows SEM images of fractured surfaces of drop casted films on silicon wafers. The cross sections of ChNF-containing films show the presence of lamella arranged as parallel layers.
Figure 3.11 SEM images of fractured films (a) neat CNC, (b) 3:1 CNC-LChNF, (c) 1:1 CNC-LChNF, (d) 1:3 CNC-ChNF, (e) neat LChNF, (f) neat HChNF, (g) 1:1 CNC-HChNF, and (h) magnified image of 1:1 CNC-HChNF. Scale bars are 2 µm.

In contrast neat CNC films in Figure 3.11a lack lamella and appear friable. Figure 3.11b shows the presence of lamellar structures even with only 25% addition of LChNF to CNCs. With increasing LChNF content the lamellar structures are more distinct with neat LChNFs showing clearly lamellar multilayers in Figure 3.11e. Similarly, HChNF films
seen in Figure 3.11f also show lamellar morphology. Addition of 50% HChNFs to CNCs produces lamellar morphology as seen in Figure 3.11g and more clearly in the magnified image of the same 1:1 CNC-HChNF film seen in Figure 3.11h.

The ability of chitin to form lamellar structures is well known. In nature these fibers occur as chitin-protein composites that form planes stacked at angles. Our studies show planar fibrillar networks occurring at concentrations as low as 25% LChNFs in blends with CNCs. From the AFM, titration and zeta potential data, we know that CNCs aggregate with both HChNF and LChNF surfaces and it is possible that lamellar structure formation is aided by these attractive interactions. The primary difference between LChNFs and HChNFs seen in Figures 3.11e and 3.11f is that the HChNF network is more heterogeneous, with the presence of large voids in between denser lamellae. This correlates well with the lower measured density (and higher void fraction) of HChNFs as compared to LChNFs seen in Table 3.1. Raabe et al. described the chitin-protein planes in lobster cuticles interconnected by chitin-protein fibrils to form honeycomb-like structures between layers. Such “honeycomb” arrays are also seen in our studies in Figures 3.11f and 3.11h for neat HChNFs and 1:1 CNC-HChNF blended films. The formation of lamella and the accompanying honeycomb structure in nature arises due to the existence of strong ChNF-protein fiber texture and associated branching. Even though the blends reported here lack the protein and mineral components found in natural crustacean cuticle, the SEMs in Figure 3.11 clearly exhibit branching of ChNFs and honeycomb structures.

The structural and performance data presented above allow a structure-property relationship to be proposed for the dependence of OP and blend composition, especially the roles of density, alignment, and tortuosity that arise from fiber-fiber interactions and
fiber size and distribution. The densest film having the lowest void fraction was neat LChNF, which also has the best lowest OP value of 1.0 cm$^3$ μm/m$^2$ day kPa (best barrier). Blends of LChNFs with CNCs had OP values that increased up to 1.9 cm$^3$ μm/m$^2$ day kPa as CNC content was raised and as void fraction increased. This correspondence between blend film void fraction and OP points to an important role of dense structure formation in low OP values in LChNFs and their blends with CNCs. The neat HCNFs had the highest bulk void fraction, but SEM images indicated these voids were quite heterogeneous in size, with large voids distributed in honeycomb-like structures between denser lamellar layers. In addition, HChNF size distributions were considerably more disperse than LChNFs. The neat HChNF OP value was also the highest among chitin-containing samples, at 4.4 cm$^3$ μm/m$^2$ day kPa, but the 1:1 CNC:HChNF blends had low OP near the values observed for LChNF-containing blends, 1.3 cm$^3$ μm/m$^2$ day kPa. This suggests that HChNF possesses particles that are still capable of forming relatively dense structures (SEM) that are effective barriers, even though voids are also distributed between the lamellar layers. While CNCs have an optical and AFM appearance that is highly aligned, they have a relatively high void fraction intermediate between HChNFs:CNCs and neat HChNFs. Coupled with their very brittle mechanical behavior, and appearance of voids in SEM images, we suggest that defects present in the CNC film lead to the high OP values and void fraction. Defects could, for example, be related to ‘grain boundaries’ between highly-aligned regions or stresses that arise during drying that lead to crack formation.

In addition to void fraction (density) as a structural feature related to OP, the tortuous structures associated with CNC-chitin aggregation and chitin fiber branching appear to also support barrier functions. SEMs (Figure 3.11) indicate that ChNFs and ChNF-containing
blends have lamella with long, entangled structures formed through CNC-ChNF attraction that offer tortuosity to O\textsubscript{2}. This is consistent with significant improvement in O\textsubscript{2} permeability in the blended films relative to neat CNCs. The fundamental mechanistic difference in diffusion of gas molecules between neat CNC films and neat CNF films as described by Belbekhouche et al.[6] is that CNF films are highly entangled, which creates tortuosity and hence more barrier to the diffusion of gases through the thickness of the films. The mechanism of gas diffusion through ChNF films and blends of CNC-ChNF films could be attributed to similar tortuosity added by the layered chitin structures present in the blends and ChNF films. Structural observations from AFM, SEM, as well as zetasizer measurements (Figure 3.2(b)) indicate formation of aggregates of CNC and ChNF, driven by their opposite charges. The size of these aggregates is much larger CNCs or ChNFs used to form them. The effective fiber length of aggregates that bridge together multiple ChNFs can present a diffusion path that is longer than the individual ChNF fibers present, which may explain the especially large lengthscale associated with the 1:1 CNC-HChNF blends.

### 3.4 Conclusions

This study shows that when blended with CNCs, ChNFs improve the mechanical and barrier properties of CNC-ChNF films. The oxygen permeability of neat LChNF films (at 23 °C and 50% RH) was the lowest of all films, at 1.0 cm\textsuperscript{3}·μm/m\textsuperscript{2}/d/kPa. Blended CNC-ChNF films had a permeability that was an order of magnitude lower (1.4-1.9 cm\textsuperscript{3}·μm/m\textsuperscript{2}/d/kPa) than neat CNC films (18.5 cm\textsuperscript{3}·μm/m\textsuperscript{2}/d/kPa). One of the most interesting aspects is that the addition of either HChNFs (93% DA) or LChNFs (73% DA) to CNCs is able to lower the OP of the blend films significantly and nearly equivalently at concentrations as low as 25% ChNF by weight. A 25% addition of LChNFs lowered the
OP of blended 3:1 CNC-LChNF films by 91% compared to neat CNCs. The decrease in OP at relatively low concentration of chitin was attributed to aggregation of CNCs with LChNFs or HChNFs, driven by electrostatic attraction (in addition to hydrogen bonding) due to their opposite charge. Several structural effects attributed to this aggregation can explain the decreased OP in chitin-cellulose blends relative to CNCs. The blend systems are able to form dense layered lamellar structures in which the diffusion timescale oxygen is expected to be increased relative to neat CNCs. In addition, CNC-ChNF aggregates appear to form long diffusion length scales that likely add tortuosity within lamellae, even when ChNF is the minority component. The long length scales may be caused by bridging of chitin fibers by CNCs. LChNFs also had better mechanical properties of UTS and strain at break than HChNF films, which in turn had better UTS and strain at break than CNC films. Addition of LChNF and HChNFs to CNC films increased UTS and strain at break of the resultant blended films. Both 1:1 CNC-HChNF and 1:1 CNC-LChNF showed a 160% improvement in UTS. LChNFs were better at improving the strain at break of blended CNC-ChNF films with 1:1 CNC-LChNF films showing a 450% improvement while 1:1 CNC-HChNF films showed a 100% improvement over neat CNC films. All blended films showed reduced light transmission rates over LChNF films which had the best light transmission. The light transmission of neat CNC films was poor as a consequence of their iridescent properties. While cellulose is currently produced all over the world as part of the pulp and paper industry, the industrial processing of chitin lags by orders of magnitude.[8-10] The cost of cellulose-derived materials is expected to be lower than chitin derived materials like ChNFs due to economy of scale being in favor of CNCs. Thus ChNFs are always going to be more expensive than CNCs despite their superior barrier and
mechanical properties. These results show that there may be a way to impart superior mechanical and barrier properties of ChNFs to CNC based materials by blending ChNFs (as additives) with CNCs. Thus ChNFs are bio-derived additives that improve the mechanical and barrier properties of CNCs to support renewable materials in food, electronic and pharmaceutical packaging.
3.5 REFERENCES


[38] G.W. Lu, P. Gao, Emulsions and Microemulsions for Topical and Transdermal Drug Delivery, Handbook of Non-Invasive Drug Delivery Systems: Non-Invasive and


CHAPTER 4. OPTIMIZATION OF HIGH-PRESSURE HOMOGENIZATION TO PRODUCE CHITIN NANOFIBERS FOR BARRIER FILMS

4.1 Introduction

High pressure homogenization (HPH) is an extremely popular method for size-reduction of natural fibers, especially for production of nanofibers from polysaccharides like cellulose and chitin that occur naturally as nanofibers embedded in a matrix.[1-3] Homogenizers as devices are well known in the food processing and cosmetic industries.[4] Chitin is an abundant biomaterial that occurs in fungi, crustaceans and insects.[3] Within the exoskeleton of crustaceans, for example, chitin is found as microfibrils of 100-200 nm in diameter[5] and several microns in length.[6] Chitin is a polymer of 2-acetamido-2-deoxy-β-d-glucose[7], and is structurally very similar to cellulose, the only difference being that at the C2 position, it has an acetamide group (-NCOCH3). In nature some of these acetamide groups are deacetylated leading to amine groups that can be protonated under acidic conditions, making chitin a cationic polyelectrolyte. Both chitin and cellulose in their nanofiber form are of immense interest because of their excellent barrier properties that have been reported in the literature.[6, 8, 9] In addition, both biopolymers in their nanomaterial form are known to have high excellent physical properties including high tensile strength, Young’s modulus [10-12] and can be fabricated into highly transparent films[6, 13-15].
Homogenization refers to a broad class of processes where shear forces are used to create colloidal suspensions or emulsions with increased product stability, usually through shear forces driven by high pressure or by using a rotor-stator arrangement.[16] HPH particularly refers to a very common subclass of homogenization processes where a suspension at high pressure is forced through a homogenization valve where high turbulence and cavitation are thought to help provide the necessary energy for size reduction of particles (suspensions) or droplets (emulsions).[16] HPH has the capability to delaminate larger fibers (10-50 µm) to smaller diameters (less than 100 nm) while retaining the high aspect ratios of the nanofibers.[4] It is a relatively straightforward process but has been associated with elevated energy usage that cannot be lowered with mechanical pretreatment methods implying poor fluid-particle energy transfer efficiencies,[17] thus Chemical pretreatment methods have been explored to reduce energy intensity required to fibrillate materials.[1, 17] Homogenization of cellulose pulps has been widely reviewed.[1, 4, 18, 19] Cellulose slurries are usually passed through a small gap across which the pressure drops, causing the fibers to experience intense shear and impact forces.[4, 18] The suspensions are usually cycled through the homogenizer multiple times.[18] The number of homogenization passes and pass pressure affects the properties of the final suspension and nanofibers[18, 20, 21]. For cellulose nanofibers (CNF) it has been shown that homogenization (as compared to other defibrillation methods such as grinding) resulted in fibers with the highest specific surface area. Upon solvent casting, such fibers form films with the lowest water vapor transmission rate.[22] HPH of Chitin nanofiber (ChNF) suspensions is similar to cellulose, although using an acidic medium. The resulting suspensions show a colloidal behavior which can be attributed to protonation of surface
amino groups.[6, 23-25] These charged groups are exploited to lend stability to suspensions of chitin in acidic conditions.[12] In case of cellulose, chemical pretreatment methods like prehydrolysis[26], enzyme based pretreatment [27], and 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) mediated chemical oxidation have been shown to be effective in aiding homogenization.[28] TEMPO mediated oxidation introduces carboxylate groups that aid in suspension stability via electrostatic repulsion. Due to structural and chemical similarities of chitin and cellulose, similar methods can be applied to aid in homogenization of ChNF suspensions. Chemical oxidation of chitin has been utilized to introduce carboxylate groups on the surfaces that boost electrostatic repulsion and allow for successful preparation of chitin nanocrystals (ChNCs) just by gentle mechanical disintegration techniques. These nanocrystals are more crystalline than ChNFs and are have much smaller aspect ratios, with a width of 8 nm and lengths of 340 nm for ChNCs [29] compared to 20 nm and length of several hundred to thousands of nanometers of ChNFs.[6] Another method of increasing surface charges is N-deacetylation of chitin to convert the acetamide groups to amino groups. Complete N-deacetylation of chitin leads to formation of chitosan, which is soluble in water under acidic conditions.[3, 30] N-deacetylation of chitin can also be used to produce ChNCs.[31] Conversely, acetylation of ChNFs has been performed to improve compatibility with nonpolar solvents.[12]. As mentioned before, pretreatment procedures can have a significant impact on the energy usage[18] but it may also affect several key properties of the final films like optical transmission, tensile strength, strength to failure [32] and possibly even gas permeability.[33] Qi et al.[34] studied the influence of the type of acid required to cationize the surface of ChNFs and were able to optimize the suspension pH of ChNF to improve
nanofibrillation efficiency indicating the strong effect that suspension pH plays on final product properties.

The objective of this chapter is to demonstrate deacetylation as a method of increasing homogenization efficiency for HPH of chitin. The basis of this is that suspension pH is an important parameter for controlling the properties of nanofiber based products, including ChNFs[35, 36] and proteinaceous materials.[37, 38] Prior studies noted that pH varies during the homogenization to produce ChNFs,[6] and it tracks the number of protonated surface amines, which is a direct result of the new ChNF surface area formed during HPH. Previously, Fan et al.[35] prepared chitin nano-whiskers by partial deacetylation of α-chitin fibrils resulting in increased surface cationization. We utilize similar methods to Fan et al.[31] to demonstrate that N-deacetylation of chitin and increased cationization of the resulting ChNFs significantly reduces the required number of homogenization cycles required to produce fiber suspensions with equivalent or better properties (charge, light transmission and mechanical properties of formed films). Free standing solution cast films made from homogenized and partially N-deacetylated samples of commercially produced chitin were studied for their mechanical, light transmission and barrier properties.

4.2 Materials & Methods

4.2.1 Materials

Commercial grade chitin was purchased from Alfa Aesar (Ward Hill, MA). ACS grade NaOH pellets were obtained from VWR (Radnor, PA). Crab shells were purchased from Neptune’s Harvest (Gloucester, MA). Hydrochloric acid and sodium hydroxide were
purchased from EMD Chemical Inc (Burlington, MA). (Burlington, MA). Glacial acetic acid was purchased from Sigma Aldrich (St. Louis, MO). Deionized (D.I.) water was obtained from a Thermo scientific GenPure UV/UF xCAD plus water purification system. Research-grade oxygen and ultra-high purity nitrogen were purchased from Airgas (Atlanta, GA).

4.2.2 Deacetylation of Commercial Chitin

Commercial chitin was deacetylated per the procedure of Chang et al.[39] The degree of deacetylation (DDA) was kept below 50 % to prevent conversion of chitin to chitosan. Deacetylation was carried out with 35 wt.% NaOH solution obtained by mixing NaOH with D.I. water and 25 ml/g chitin loading of solution. The mixture was then refluxed at 110 °C for the specified amount of time. Experiments were carried out with three target deacetylation extents of 30 %, 20 % and 0 %. For a 30 % deacetylation target, a deacetylation time of 150 mins was used, and for a 20 % target, a time of 100 mins was used. For the 0 % DDA target, no deacetylation step was utilized.

4.2.3 Crab shell Chitin purification and Deacetylation

Crab shell chitin was purified per previous studies.[6, 9, 40] First, crab shells were washed several times and were then ground into powder using a commercial grinder. The powder was refluxed with 5 wt. % NaOH for 6 h. The resulting solids were filtered and washed with DI water until the wash water had a pH of 7. The filtered solids were then treated with a 7 wt. % HCl bath for 6 h. The solids were again filtered and washed until the wash water pH was 7. The acid-treated solids were then refluxed with 5 % NaOH for 48 h and the resultant solids were filtered and washed with DI water until the wash water pH
was 7. This resulted in a white purified chitin powder, which was dried in an oven at 60 °C for 24 hours. For experiments with crab shell chitin, two target deacetylation degrees were used: 0 % and 30 %. The purified crab shell powder was considered to be mostly acetylated (0-10 % DDA) and the same conditions for 30 % deacetylation target were used for preparation of 30 % DDA commercial chitin were used for crab shell chitin. The chitin will henceforth be referred to by its degree of acetylation (DA) which is 100 less its DDA.

4.2.4 Extraction of ChNFs

Suspensions containing 0.5 wt. % of purified chitin from each of the various preparations above were prepared in DI water. The pH of the suspension was adjusted to 3.0 using glacial acetic acid and the acidified suspension was homogenized in a Mini DeBEE Homogenizer (BEE International, South Easton, MA). Because of the fluid expansion through the nozzle resulting in shear heating, a product cooler was used through the processing to keep the ChNF suspension at a maximum temperature of 35 °C. Chitin suspensions with different DA values were homogenized using the procedure from Wu et al.[6] The first sequence of homogenization was carried out at a pressure of 1034 bar by using a 0.2 mm nozzle for 20 passes followed by a second sequence at 1516 bar and utilizing a 0.13 mm nozzle for an additional 10 passes.

For experiments involving optimization of pressure, a 30 % DDA target commercial chitin chitin was homogenized as above except the pressure was kept constant throughout the homogenization. Two different pressures were used: a low pressure (LP) of 551 bar and a high pressure (HP) of 1723 bar.
Unmodified crab shell chitin was homogenized as before utilizing the procedure of Wu et al.[6] as a control. Deacetylated crab shell chitin with an optimum DA was homogenized at a reduced number of homogenization passes suggested by experimentation with commercial chitin. A pressure of 1034 bar was utilized.

4.2.5 ChNF and Suspension Characterization

The DA of each of the produced chitin batches was determined by potentiometric titration. A volume of 25 ml of the homogenized suspension of commercial chitin of a particular target DA was ion exchanged using 300 ml of anion exchange resin. The ion exchange resin was washed with about 200 ml of DI water to remove entrapped chitin. 20 ml of 0.25 N HCl was added to the resulting suspension which was then was titrated against 0.5 N NaOH using a Mettler Toledo Seven Excellence S400 pH meter. The inflection points determined from the pH vs volume of titrant curve were then used to determine the amount of NaOH consumed. The NaOH equivalents of HCl present in the suspension was subtracted from the total consumed NaOH to yield the amount of NaOH consumed by the protonated chitin. The number equivalent of NaOH consumed divided by the mass of the starting material gives \( m \) [mol NH2/g chitin (dry basis)] which was used to determine the DA by

\[
DA \% = 100 \times \frac{203.21}{42.04 + \frac{1}{m}} \tag{4.1}
\]

where 203.21 [g/mol] is the molecular mass of a chitin monomer and 42.04 [g/mol] is the difference in molecular mass between a chitin and a chitosan (deacetylated chitin) monomer. The titrations were repeated thrice for each sample.
Particle size distribution of ChNFs upon homogenization was characterized by Atomic Force Microscopy (AFM) imaging using a Bruker Icon AFM. To produce these samples, the final ChNF suspensions (30 passes), were diluted with pH 3 acetic acid solution along with Triton X-100 surfactant to 0.001 wt %. 20 µl of the diluted suspensions were drop cast onto a clean, ozone treated silicon wafers. The drops were allowed to dry at 60 °C for 1 d. The length and number of particles were determined by visual analysis of the AFM height images with the help of ImageJ to mark, label and measure the nanoparticles. The width of the nanoparticles were estimated using the height sensor value from the AFM height image of the nanoparticles.

In addition, the stability of the final homogenized ChNF suspensions was characterized by Zeta potential measurements. 40 µl volumes of 0.5 wt % suspensions of ChNF suspensions were diluted with 20 ml of DI water. A Malvern Zetasizer Nano was used to measure the zeta potential and the size of dilute final ChNF suspensions.

4.2.6 Preparation of Solution Cast Commercial and Crab Shell ChNF films

A volume of 50 ml of 0.5 wt.% of each of the final homogenized chitin suspensions from pass 30 prepared above were cast in a in a poly (tetrafluoroethylene) dish (8.5 cm x 13 cm) and air-dried for 14 days at room temperature and humidity (45 % relative humidity (RH)) to yield free-standing films.

4.2.7 Characterization of Solution Cast ChNF films

The thickness of cast films was measured with a micrometer at more than 10 different points on each film. Light transmission of the films was measured at 550 nm using
a Shimadzu UV-1800 UV/Vis Spectrophotometer. Tensile mechanical properties of uncoated and coated films were determined by using a high-throughput mechanical characterization (HTMECH) instrument described previously.[41]

The O₂ permeability values of the neat films were obtained by using a MOCON OXTRAN 1/50 instrument at 50 % RH. The films were humidified to soften them using an Electrotech ultrasonic humidification system (Glenside, PA). The humidification facilitated cutting, handling and loading of the films into the MOCON without fracturing. The films were cut into squares of approximately 9 cm² size and covered with a mask that allowed measurement of 5 cm² of this area. The interval between measurements was 1 hour with the final measurement value taken at a steady state. The criteria for steady state was that the contiguous readings must differ by less than 1% or 0.05 cm³/m²/d (convergence by cycles mode offered by MOCON). Generally, convergence time was of the order of 24 h.

Atomic Force Microscopy (AFM) (Bruker Icon) was utilized to characterize the ChNFs. For AFM imaging, films of ChNF were drop casted onto a silicon wafer and dried for 1 d, followed by utilizing a Bruker Icon AFM to image the top surface of the films. The size of structures on the films were measured by visual analysis of the AFM phase images. Film root mean square roughness was determined from the AFM images by using Bruker Nanoscope Analysis software. The same films were fractured and the cross section was viewed using a Zeiss Ultra 60 FE-SEM.
4.3 Results & Discussions

4.3.1 Effect of Deacetylation

Table 4.1 shows the DA and the zetasizer measurements for chitin with different DA values homogenized per the procedure described in Wu et al.[6] The untreated commercial chitin has a DA of about 89%. For 80 and 70% target DA values we obtained DA values of 79% and 72% respectively indicating a good agreement with experiments done by Chang et al.[39] The zeta potential of the suspensions shows an increasing trend with decreasing DA with 89% DA suspensions having a zeta potential value of 32 mV. The zeta potential increases to 86 mV for chitin with 70% DA.

Table 4.1 Properties of ChNF samples produced from commercial chitin.

<table>
<thead>
<tr>
<th>Target DDA (%)</th>
<th>Actual DDA (%)</th>
<th>Actual DA (%)</th>
<th>Zeta Potential (mV)</th>
<th>Zetasizer Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>10.8±3.2</td>
<td>89.2±3.2</td>
<td>31.7±3.9</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>21.0±3.6</td>
<td>79.0±3.6</td>
<td>54.5±4.1</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>28.4±3.4</td>
<td>71.6±3.4</td>
<td>85.7±6.0</td>
</tr>
</tbody>
</table>

In general, as per Deryaguin–Landau–Verwey–Overbeek theory, increase in surface charge of the ChNFs is expected to increase the zeta potential and thus suspension stability. An operational definition of stability can be considered to be a suspension zeta potential value above 30 mV [42, 43]. The suspensions produced by homogenizing 89% DA chitin are on the border of incipient instability (10-30 mV) [43] while suspensions produced by homogenizing 79% DA chitin have good stability (40-60 mV).[43] The suspensions from 72% DA chitin have excellent stability (>60 mV).[43]
Table 4.1 also shows the zetasizer size measurements for different ChNF suspensions. These sizes reported by these zetasizer measurements is the hydrodynamic radius of the particles as reported by the Stoke-Einstein equation.[44] Decreasing value of DA is seen to decrease particle size with 89 % DA ChNF suspensions having average ChNF particle size of 1680 nm while deacetylation to 72% is seen to decrease the size to 930 nm. Fan et al.[35] utilized surface deacetylation to prepare chitin nanowhiskers (ChNWs) which have much lower aspect ratios (250 nm average length and 6.2 nm in width determined by Transmission Electron Microscopy[31]) than chitin nanofibers that are several microns in length and have an average width of 20 nm.[6] Surface deacetylation in our case certainly decreases the size of the ChNFs and towards the ChNW range.
Figure 4.1 Number distributions of (a) length (b) width of ChNF fibers with different DA values in suspension determined from AFM measurement.
Table 4.2 Two sample KS values for particle length distributions. Values highlighted in red indicate differences in distributions between the two samples being compared is significant at the significance level of 0.01.

<table>
<thead>
<tr>
<th>KS-values (KS Critical=0.23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>89 % DA</td>
</tr>
<tr>
<td>89 % DA</td>
</tr>
<tr>
<td>79 % DA</td>
</tr>
<tr>
<td>72 % DA</td>
</tr>
</tbody>
</table>

Table 4.3 Two sample KS values for particle width distributions. Values highlighted in red indicate differences in distributions between the two samples being compared is significant at the significance level of 0.01.

<table>
<thead>
<tr>
<th>KS-values (KS Critical=0.23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>89 % DA</td>
</tr>
<tr>
<td>89 % DA</td>
</tr>
<tr>
<td>79 % DA</td>
</tr>
<tr>
<td>72 % DA</td>
</tr>
</tbody>
</table>

Figure 4.1(a) shows the length distribution of ChNF fibers in suspensions for different DA values. The 89 % DA and 79 % DA suspensions have 49 % and 47 % of their fibers in the >950 nm range. The deacetylation process increases the number of 600-700 nm sized particles from 13 % (89 % DA) to 19 % (79 % DA).

A two-sample Kolmogorov-Smirnov (KS) Test was performed on the distribution lengths and widths in Table 4.2 and Table 4.3. The tables also describe the critical values for a significance value of 0.01 that were used to determine if the distributions were significantly different. It is seen from Table 4.2 that 89 % DA and 79 % DA length distributions are not significantly different (KS value = 0.08). Decrease in acetylation to 72 % DA leads to a drop in number of >950 nm particles to 10 %. Similarly, the number
of particles in the 300-500 nm range is increased to 62%. The 72 % DA particle length distribution is significantly different from the 89 % DA distribution (KS value=0.50) and 79 % DA distribution (KS value=0.58).

Figure 4.1(b) shows the width distribution for the same ChNF samples. For 89 % DA chitin, about 30 % of the particles are of 5-6 nm width while for the 79 % DA chitin, the peak width shifts to 3-4 nm with 25 % of the particles being in this range. For 72 % DA chitin the peak widths are similar to 79 % DA (3-4 nm) with 43 % of the particles of this width being observed in imaging and no particles with width greater than 6 nm observed. The widths for different degrees of acetylation are significantly different from each other as indicated by the KS test in Table 4.3.

The distributions show that the deacetylation of chitin from 89 % to 79 % causes the length distribution to remain same, however on further deacetylation to 72 % the length is drastically reduced to the 300-500 nm range as indicated by the KS test in Table 4.2. This corresponds well to the zetasizer sizes in Table 4.1 where the sizes for the 89 % and 79 % DA chitin are similar while the size of the 86 % DA chitin is the lowest. As indicated from the KS test in Table 4.3, increasing deacetylation leads to lower width particles across the board indicating increased fibrillation of fibers for deacetylated (72 & 79 % DA) samples as compared to the unmodified chitin (89 % DA).

Figure 4.2(a) shows the change in pH with number of homogenizer passes for commercial chitin samples with different degrees of acetylation. The biggest difference is seen with chitin having 89 % DA and that with 79 % DA. The final pH for 89 % DA chitin is around 3.10 while that for 79 % DA and 72 % DA is 3.19 and 3.22 respectively. The pH
change with homogenization mostly occurs between 0-15 passes with the change in pH being very gradual after 15 passes.

![Figure 4.2](image)

**Figure 4.2** Variation of (a) suspension pH (b) suspension light transmission as a function of number of homogenization passes for commercial chitin samples with different values of DA.

Figure 4.2(b) shows the light transmission at 550 nm for suspensions of chitin nanofibers sampled during the homogenization. The light transmission at pass (cycle) 0 is that of the chitin slurry after the deacetylation step. As the HPH process proceeds in cycles the light transmission is seen to decrease for the 100 % DA sample while it gradually increases for the 72 % and 79 % DA samples. The light transmission eventually increases
after 10 passes for the 100 % DA sample but after 30 passes it reaches only 36 %, whereas the 79 % and 72 % DA samples reach light transmission values of 74 % and 81 %, respectively.

As homogenization proceeds the pH is expected to increase as new surface area is created. The newly created surfaces have amino groups that on contact with the acidic suspension medium get protonated from NH₂ to NH₃⁺. Each NH₂ thus takes up one H⁺ from the volume of suspension increasing the pH. The magnitude of pH change depends on the (i) surface area of the fibers in suspension and (ii) the average number of amino groups per gram of chitin, which is represented by its DA. Higher DA values correspond to lower number of amino groups and consequently the maximum pH increase is less than the chitin having lower DA. However, it must also be noted that such suspensions are charge stabilized. Having lower DA values is advantageous as higher cationization of the fiber surface increases the repulsive electrostatic barrier to fiber aggregation. Consequently, in HPH, as fibrillation proceeds, the fibers tend to remain in suspension longer for chitin with lower DA thus increasing the efficiency of homogenization. The higher light transmission of lower DA chitin samples (89 % and 79 % DA) in Figure 4.2(b) points towards more stabilized ChNF fibers which are also more fibrillated as supported by smaller fiber widths, seen in Figure 4.1(b). These observations are consistent with higher zeta potential driven by higher surface charge (Table 4.1) on lower DA chitin samples.

The thickness of 89 % DA film was analyzed to be 83.5 ± 27.3µm, while that for 79 % DA was found to be 65.6 ± 12.5 µm. The thickness of 72 % DA film was found to be 55.0 ± 15.4 µm. The reduction in measured thickness of films formed from fibers with
increasing number of surface charges (lower DA) has been reported for TEMPO oxidized cellulose nanofibers as well as mixtures of chitin nanocrystals and cellulose nanofibers. [45, 46] Previous studies on blended ChNF-cellulose nanocrystal films have also reported this phenomenon.[40] The decrease in thicknesses with increasing

Figure 4.3 Variation of (a) light transmission and (b) oxygen permeability for dried cast films prepared from commercial ChNF samples with different values of DA. Permeability is measured at 50 % RH and 23 °C.
cationization of the fibers can be attributed to electrostatic repulsion that promotes entropically driven packing, leading to lower film thicknesses for lower DA ChNFs.

Figure 4.3(a) shows the light transmission at 550 nm of films cast from suspensions produced by homogenizing chitin with different DA values for 30 passes. It is seen that the average light transmission value of 89% DA chitin films is the lowest at 19% and that light transmission increases with decrease in DA values with 79% and 72% DA films having transmission values of 55% and 61%, respectively.

Figure 4.3(b) shows the oxygen permeability (OP) of films cast from suspensions produced by homogenizing chitin with different DA values for 30 passes. It is seen that the average OP values of the 89% DA samples are higher at 2.4±0.8 cm$^3$·μm/m$^2$/day/kPa than 79% and 72% DA samples which have average OP values of 1.9±0.4 and 2.0±0.5 cm$^3$·μm/m$^2$/day/kPa respectively. When error bars are considered the values are not considered to be significantly different to each other.

The intensity of transmitted light is at least inversely correlated to the radius of the particle (for spherical particles) with the exact dependence on radius being a function of several factors like particle shape and beam geometry.[47] This still holds for particles of other shapes. Instead of radius alone, it is also a function of length and diameter for ChNFs. The increase in the transmission of homogenized suspensions with decrease in DA, as seen in Figure 4.2(b), points to particles of lower diameter, length or both. This is supported both by Figure 4.1, Table 4.1 where particle diameters reduce with increasing DA and lengths decrease significantly between 79% DA and 72% DA. The increase in light
transmission of films from suspensions is also related to the increased zeta potential with decreasing DA which enables the particles to pack better through the drying process. The oxygen permeability (OP) of the solution casted films are similar to each other. The 89 % DA samples are expected to have large aspect ratio particles that would lead to lower OP values, however their alignment is expected to be less than particles with lower DA since the fiber surfaces have lower charge repulsions that would drive higher alignment. The resultant poor alignment increases OP.[48] Decreasing DA decreases particle size as seen in Table 4.1 and Figure 4.2 (increasing OP) but also the increased cationization increases alignment, which decreases OP. These two effects, those of particle size and alignment, are in opposition and the trade-off determined the OP value that results from the final dried structure. In fact, per this argument there could be a minimum value for an intermediate DA sample and on closer inspection the 82 % DA samples seem to have lower average OP than either of the other samples. Despite the minor improvement for the 82 % DA sample, the aspect ratio seems to be the most dominant driver of OP for the ChNF films as the presence of long fibers creates tortuosity in the thickness of the film, which is known to improve barrier properties. While cellulose nanocrystals (CNCs) of 100 nm in length and 5 nm width have been aligned with the desired effect of lowering OP,[15, 49] the improvement in alignment of lower DA fibers is not expected to be of the same magnitude due to the difficulty in aligning fibers that have much higher aspect ratios (300-500 length, 4 nm width) than CNCs, by charge stabilization alone. The average OP values in Figure 4.3(b) are low for both chitin with high and low DA and the resulting low OP is similar to the low OP values seen for blended ChNF-CNC filmsthat have been previously reported.[40]
Figure 4.4(a) shows the ultimate tensile strength (UTS) of films cast from ChNF suspensions produced by homogenizing commercial chitin with different DA values for 30 passes. UTS were determined by taking the maximum value in the stress versus strain data. The box-plot shows the median value at the intersection of the light and shaded areas, where the top of the light area is the 75\textsuperscript{th} percentile and the bottom of the dark area is the 25\textsuperscript{th} percentile. Error bars represent the total range of the data set. Tables 4.4 and 4.5 indicate two-tailed P values for the null hypothesis that difference between means of the compared samples is insignificant. P values less than the significance level (0.01) indicate that the difference between the means is significant. It is seen that the 89 \% DA and 79\%
DA films have statistically similar (p value=0.94) average UTS values of 18 MPa and 17 MPa respectively while the 72 % DA chitin had a higher average UTS value of 29 MPa.

Table 4.4 Two tailed P-values for mean ultimate tensile strength (UTS). Values highlighted in red indicate differences in means between the two samples being compared is significant and the level of 0.01.

<table>
<thead>
<tr>
<th></th>
<th>89 % DA</th>
<th>79 % DA</th>
<th>72 % DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>89 % DA</td>
<td>1.00</td>
<td>0.94</td>
<td>0.0017</td>
</tr>
<tr>
<td>79 % DA</td>
<td>0.94</td>
<td>1.00</td>
<td>0.0016</td>
</tr>
<tr>
<td>72 % DA</td>
<td>0.0017</td>
<td>0.0016</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 4.5 Two tailed P-values for mean strain at break. Values highlighted in red indicate differences in means between the two samples being compared is significant and the level of 0.01.

<table>
<thead>
<tr>
<th></th>
<th>89 % DA</th>
<th>79 % DA</th>
<th>72 % DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>89 % DA</td>
<td>1.00</td>
<td>0.010</td>
<td>0.0015</td>
</tr>
<tr>
<td>79 % DA</td>
<td>0.010</td>
<td>1.00</td>
<td>0.15</td>
</tr>
<tr>
<td>72 % DA</td>
<td>0.0015</td>
<td>0.15</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Figure 4.4(b) shows the strain at break values of films cast from suspensions produced by homogenizing chitin with different DA values for 30 passes. Similar to the UTS, it is seen that strain at break of suspensions increases with decreasing DA values. 89 % DA samples had average strain at break value of 18 %. The average value increased to 28 % for 79 % DA chitin samples. The strain at break is highest for 72 % DA samples with an average value of 34 %. The strain at break of the 79 % DA sample is significantly higher than 89 % DA sample. Similarly the 72 % DA sample shows significantly higher strain at
break than the 89 % DA sample while the values for the 72 % DA and 79 % DA samples are similar.

The UTS of the fibers corresponds well to their particle length distributions (Table 4.1) with the UTS of the 79% and 89 % DA samples being similar for since both have similar particle sizes. The dependence of tensile strengths on fiber lengths have been reported before for unidirectional plys where increase in UTS with decrease in fiber lengths is attributed to defects along fiber length.[50] This is mainly for cases where the single fibers are stronger than the composite, and as fiber length reduces, the number of defects decrease leading to increased UTS. Fan et al.[51] reported that surface deacetylated α-chitin nanofibers/nanowhiskers had significantly higher tensile strengths compared to regenerated chitin (amorphous) due to their higher crystallinity. The ChNFs in general are known to have crystalline regions with very high tensile strength separated by amorphous domains.[52] In addition it is known that the N-deacetylation of chitin preferentially occurs in the amorphous domains[53] leading to higher N-deacetylation of the amorphous domains (and conversion to chitosan) making them susceptible to dissolution in acidic HPH medium. The dissolution of these “weaker” amorphous regions would explain the particle length reduction leaving behind more crystalline regions that would be relatively defect free explaining the increased UTS. As seen in Table 4.3, and Figure 4.1(b) the HPH using deacetylated ChNFs creates more fibrillation leading to lower fiber widths. The lower fiber widths along with higher surface charge (Table 4.1) may lead to better packing and lower number of large randomly aggregated fibers leading to more effective stress distribution to surrounding load bearing fibers in case of random fiber breaks and consequently higher overall strain at break of deacetylated ChNF samples.
Figure 4.5 shows the AFM height and phase images of films cast from suspensions produced by homogenizing chitin with different DA values for 30 passes. The images show mats of chitin fibers. 89 % DA samples show large fibers both height and phase image. The number of larger fibers is less in the 79 % DA height and phase images. The 72 % DA images show a few large fibers, which are seen to have a smaller width than the larger fibers seen in in the 89 % DA images. The roughness of the 89 % DA films analyzed from AFM imaging is the highest at 8.4 nm and decreases with decrease in DA. The 72 % DA

![AFM images](image)

Figure 4.5 AFM images of the top surface of dried cast films of (a) 89% DA, (b) 79 % DA, and (c) 72 % DA ChNFs. Height images are on the left and phase images are on the right-hand side.
films have the smallest roughness at 4.8 nm while the roughness of the 79 % DA films is 5.8 nm. These changes in roughness values correspond well to peak fiber widths reported in Figure 4.1(b).

Figure 4.6 SEM images of cross-sections of (a) 89 % DA, (b) 79 % DA, and (c) 72 % DA ChNF films. Scale bars are 500 nm.

Figure 4.6 shows the SEM cross sectional images of films produced from ChNFs with different DA. It is seen that all films show lamellar structures of ChNFs throughout the film thickness. Films with 100 % DA show lamella that are loosely bound to each other and the structure of the stacked lamella appears to be loose. The interlamellar distance is
seen to reduce with decrease in DA with 79 % DA films showing lamella with lesser apparent delamination and the 72 % DA films showing almost no delamination.

Decrease in interlamellar spacing (or tighter interlamellar stacking) can be explained by the decreasing fiber widths in Figure 4.1(b) coupled with increase in cationization. The decrease in spacing with increased cationization of the fibers seems to counterintuitive at first glance, however it can be explained as follows. In absence of the charges, the alignment of fibers in suspension is largely driven by hydrogen bonding. Fiber-fiber collisions in the suspensions lead to formation of loose aggregates largely driven by hydrogen bonding between fibers.

The charge driven repulsions help overcome the weaker hydrogen bonding in a suspension of charged fibers effectively driving the alignment. Increased cationization leads to better alignment of charged fibers in suspensions as fibers are forced into optimal configurations that minimize potential energy in a field of highly charged fibers. As the suspension water content is reduced during drying, the fibers are forced into close vicinity of each other. In the case of highly charged fibers, they are still more aligned than less charged fibers due to the strength of the repulsive interactions. This leads to better alignment in the dried films for highly charged fibers as compared to less charged ones.

4.3.2 Effect of Pressure

Chitin with a 72 % DA was homogenized at two different pressures referred to as LP, and HP. The zeta potential of the suspensions was measured to be 60.0±5.4 for LP, and 86.1±6.3 for HP suspensions, indicating good metastability as per the criterion in Kumar
et al. [43]. The Zetasizer-determined size of the suspensions was measured to be 512±76 nm for LP, and 506±90 nm for HP suspensions.

Figure 4.7 Number distributions of (a) length (b) width of ChNF fibers with different DA values in suspension.
Table 4.6 Two sample KS values for particle length distributions. Values highlighted in red indicate differences in distributions between the two samples being compared is significant at the significance level of 0.01.

<table>
<thead>
<tr>
<th></th>
<th>LP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP</td>
<td>0.00</td>
<td>0.22</td>
</tr>
<tr>
<td>HP</td>
<td>0.22</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 4.7 Two sample KS values for particle width distributions. Values highlighted in red indicate differences in distributions between the two samples being compared is significant at the significance level of 0.01.

<table>
<thead>
<tr>
<th></th>
<th>LP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP</td>
<td>0.00</td>
<td>0.10</td>
</tr>
<tr>
<td>HP</td>
<td>0.10</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Figure 4.7 (a) shows the particle length distribution and 4.7(b) shows the particle width distributions obtained with different homogenization pressures. Both LP and HP suspensions have 4 % of their particle lengths greater than 950 nm. LP suspensions have particle length peak in 700-800 nm range and HP suspensions have particle length peaks in the 400-500 nm range. The particles subjected to LP have a particle width peak in the 4-5 nm range with about 30 % of the particles in this range. Particles processed with HP have a peak particle width of 3-4 nm with 26 % particles falling in this range. Tables 4.6 and 4.7 show the KS tests for particle lengths and widths of ChNF suspensions obtained by using different HPH pressures. Table 4.6 and 4.7 show that the LP and HP sample length and width distributions are statistically similar. Homogenization pressure does not create significantly shorter particles for HP samples but fibrillates equally as evidenced by the similar distributions in particle width when a 72 % DA chitin is used.
Figure 4.8. Variation of (a) Suspension pH and (b) Suspension light transmission for different homogenization passes for commercial chitin samples with different values of homogenization pressures.

The effective stabilization of particles due to the low DA is expected to create a high homogenization efficiencies even at LP conditions such that it leads to similar widths when LP and HP are used. Increase in pressure to HP conditions is however expected to reduce particle lengths.
Figure 4.8(a) shows the change in pH with number of passes for chitin samples processed at different homogenization pressures. Chitin processed at LP has a final pH of 3.33 which is lower than chitin processed with HP which has final pH of 3.42. With increasing pressure, the final pH is seen to increase, albeit to a lower extent when compared to pH changes with DA seen in Figure 4.2(a-b). This is to be expected as the final distribution of particle sizes and widths (Figure 4.7) are similar to each other than of the samples in Figure 4.1. Since the starting DA of the samples is the same, the surface NH$_2$ groups per unit area are expected to be similar and the uptake of H$^+$ from the surrounding solution will be similar leading to similar end pH values.

Figure 4.8(b) shows the light transmission at 550 nm for suspensions of chitin nanofibers sampled during the homogenization. The light transmission at pass 0 is that of a chitin slurry. As the process proceeds the light transmission is seen to increase for all samples. The light transmission of LP and HP samples are 87 % and 84 % respectively and are similar. Compared to the light transmission values seen in Table 4.2(b) the light transmission values of the LP and HP samples are similar. This is expected due to similar charge stabilization of the particles in suspensions due to same starting DA of the chitin and similar particle size distributions of the final suspensions as seen in Tables 4.6 and 4.7.

Figure 4.9(a) shows the light transmission at 550 nm of films cast from suspensions produced by homogenizing chitin at different pressures. It is seen that the average light transmission value of LP chitin is 73±4 %. HP chitin samples have a similar light transmission value at 65±4 % when error bars are considered due to similar reasons as mentioned for their suspensions.
Figure 4.9 Variation of solution cast film (a) light transmission (b) oxygen permeability for commercial chitin samples with different values of homogenization pressures.

Figure 4.9(b) shows the oxygen permeability (OP) of films cast from suspensions produced by homogenizing chitin at different pressures. It is seen that the average OP values of the HP samples are similar (1.6±0.4 cm³-µm/m²/day/kPa) to the LP chitin samples with OP values of 1.4±0.3 cm³-µm/m²/day/kPa. The particle length distributions encountered for different pressures per the KS test (Table 4.7) indicate that the LP and HP
Figure 4.10 (a) Ultimate tensile strength, (b) strain at break of solution cast films of homogenized commercial chitin with different values of DA.

Table 4.8 Two tailed P-values for mean ultimate tensile strength (UTS). Values highlighted in red indicate differences in means between the two samples being compared is significant and the level of 0.01.

<table>
<thead>
<tr>
<th></th>
<th>LP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP</td>
<td>1.00</td>
<td>0.15</td>
</tr>
<tr>
<td>HP</td>
<td>0.15</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 4.9 Two tailed P-values for mean strain at break. Values highlighted in red indicate differences in means between the two samples being compared is significant and the level of 0.01.

<table>
<thead>
<tr>
<th></th>
<th>LP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP</td>
<td>1.00</td>
<td>0.014</td>
</tr>
<tr>
<td>HP</td>
<td>0.014</td>
<td>1.00</td>
</tr>
</tbody>
</table>
length and width distributions are similar and thus the aspect ratios of particles are largely unchanged with processing conditions. The OP values are thus expected to be similar due to similar charge distributions which is expected to produce similar alignment.

Figure 4.10 (a) shows the UTS of films cast from suspensions produced by homogenizing chitin at different pressures. It is seen that the LP chitin samples has an average UTS value of 32 MPa and is similar to HP chitin samples with average UTS value of 36 MPa. Table 4.8 indicates the two-tailed P values for the null hypothesis that difference between means of the compared samples is insignificant. P values less than the significance level (0.01) indicate that the difference between the means is not significant. The table shows that UTS of HP and LP samples are not significantly different (p value=0.15). The similarity in UTS between the LP and the HP samples is expected due to similar overall particle lengths and widths.

Figure 4.10(b) shows the strain at break values of films cast from suspensions produced by homogenizing chitin at different pressures. LP samples have a mean strain at break values of 30 %. HP chitin samples have lower strain at break with a mean value of 20 %. However, as Table 4.9 shows, the HP samples are not significantly different than the LP samples (p value=0.014). The similar strain at breaks are expected, again due to similar length and width distributions and also due to similar alignment produced since the DA of the chitin is similar.
Figure 4.11 AFM images of (a) LP (b) HP processed ChNFs. Each image set from left to right. Height image and phase image.

Figure 4.11 (a-b) shows the AFM height and phase images of films cast from suspensions produced by homogenizing chitin at different pressures. The roughness of the chitin films is similar with LP ChNFs having roughness of 2.9 nm and HP ChNFs 3.2 nm. The LP sample shows a large fiber in the image. The samples themselves appear largely similar to each other and the similarity is supported by the similar size range of fiber lengths and widths (Figure 4.7) and similar roughness values as compared to AFM images of samples seen for different DA values (Figure 4.5) which are visually different.

Figure 4.12 shows the SEM cross sectional images of films produced from ChNFs under different homogenization pressures. LP and HP chitin films are similar to the 72 % DA chitin films seen in Figure 4.6(c) due to the DA being the same. Thus, cationization of the fibers is the same which drives similar alignment.
Figure 4.12 SEM images of cross-sections of (a) LP (b) HP ChNF films. Scale bars are 1 μm.

4.3.3 Optimized Homogenization of Crab Shell Chitin

The experiments performed above with commercial chitin were repeated with crab shell chitin processed and extracted in our lab. The DA of the unmodified acetylated chitin was 91.0±9.3. The deacetylated chitin was found to have a DA value of 64±3.2 corresponding to a target 30 % DDA (or 70 % DA.)

Figure 4.13(a) shows the change in pH with number of passes for crab shell chitin samples with different DA. The 91 % DA chitin reaches pH of 3.27 in 30 passes. In comparison the 64 % DA chitin reaches pH of 3.24 in 8 passes. This is similar to the experiments done on commercial chitin (Figure 4.2 (a) and (b)). The experiment was stopped at 8 passes since the suspension had similar light transmission to the water at 8 passes indicating that further homogenization passes may not necessarily lead to increased fibrillation.
Figure 4.13. Variation of (a) Suspension pH and (b) Suspension light transmission for different homogenization passes for crab shell chitin samples with different values of DA.

Figure 4.13(b) shows the light transmission at 550 nm for suspensions of chitin nanofibers sampled during the homogenization. The light transmission at pass 0 is that of a chitin slurry. As the process proceeds the light transmission is seen to decrease for both 64 % DA and 91 % DA samples and increases after 5 passes. 91 % DA chitin reaches 40 % light transmission after 30 passes while the 64 % DA chitin reaches 85 % light transmission.
transmission in 8 passes mirroring the trends observed in Figure 4.2(b) for commercial chitin.

Table 4.10 summarizes the properties of dried films cast from of 91 % DA and 64 % DA crab shell ChNFs. 64 % DA crab shell chitin films have a light transmission value of 40 % while those from 91 % DA crab shells have a light transmission value of 7 %. The 91 % DA films have a thickness of 131 µm and are thicker than 64 % DA chitin films with thickness of 56 µm. The OP of the 64 % DA chitin films is 1.92 cm³-µm/m²/day/KPa and is slightly smaller than the 91 % DA chitin with an OP value of 2.56 cm³-µm/m²/day/KPa within the errors considered. The trends in these values are similar to those observed for experiments with commercial chitin (Figure 4.3(a-b)).

Table 4.10 Properties of solution cast films of homogenized crab shell with different values of DA.

<table>
<thead>
<tr>
<th>Property</th>
<th>91 % DA</th>
<th>64 % DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness (µm)</td>
<td>131 ± 41</td>
<td>57 ± 22</td>
</tr>
<tr>
<td>Light Transmission@ 550 nm (%)</td>
<td>6.6 ± 1.8</td>
<td>40.2 ± 14.1</td>
</tr>
<tr>
<td>Oxygen Permeability (cm³-µm/m²/day/KPa)</td>
<td>2.5 ± 0.8</td>
<td>1.9 ± 0.7</td>
</tr>
</tbody>
</table>

Figure 4.14(a) shows the UTS of films cast from suspensions produced by homogenizing crab shell chitin having different degrees of acetylation. It is seen that the 91 % DA chitin samples have UTS values in 5-10 MPa range while deacetylated chitin samples have higher UTS values in the 20-30 MPa range.

Similarly, Figure 4.14(b) shows the strain at break values of films cast from suspensions produced by homogenizing crab shell chitin having different degrees of acetylation. It is seen that the 91 % DA chitin samples have strain at break values in 5-15 % range while 64 % DA chitin samples have higher strain at break values in the 20-30 %
range. The clear improvement in mechanical properties is similar to that observed for commercial chitin (Figure 4.4(a-b)).

![Graph showing ultimate tensile strength and strain at break for homogenized crab shell ChNF with different values of DA.]

**Figure 4.14 (a) Ultimate tensile strength, (b) strain at break of cast films of homogenized crab shell ChNF with different values of DA.**

It is important to reiterate that based on studies with commercial chitin, the homogenization with crab shell chitin was stopped earlier at 8 passes for 64 % DA chitin as compared to the regular 30 passes required for 91 % DA chitin to arrive at the same value of light transmission as the initial suspension (near to 100 % T). The mechanical properties of the 64 % DA chitin show clear improvements compared to 91 % DA chitin, while the thicknesses are lower. Along with this, the OP values of both 64 % DA crab shell chitin and 91 % DA crab shell chitin are similar. The trends are similar to the studies done
with commercial chitin, while the deacetylated chitin was obtained using a lower number of homogenizer passes, leading to lower equipment residence times and lower expected energy consumption.

4.4 Conclusions

This study shows that the efficiency of HPH can be improved when we use deacetylation to increase cationization of ChNF fibers. The ChNFs from commercially available chitin, when deacetylated, produce suspensions with higher light transmission (74 % and 81 % T) at 30 passes as compared to their untreated counterpart (36 % T). The increased cationization aids in the stabilization of suspensions and increases the efficiency of the homogenization process. This produces films which are more transparent than the 89 % DA ChNFs. The light transmission was highest at 61 % T for the 72 % DA films as compared to the 19 % T for 89 % DA films. Furthermore, the deacetylation process is also found to improve the mechanical properties of the films. The UTS of the 72 % DA chitin improved to a value of 29 MPa compared to the 89 % DA ChNF sample with value of 18 MPa. The strain at break improved with a decrease in DA with 79 % and 72% DA films having breaking strain values of 28 % and 34 % respectively compared to the untreated ChNF films having a value of 18 %. Interestingly, we did not see a change in the barrier properties with the OP of all films being in the 1.9-2.4 cm³ μm/m²/day/kPa range without any obvious trend.

At high deacetylation values (72 % DA) the use of high pressures did not improve the properties of the films. LP and HP processed ChNF suspensions had similar light transmission values in the 84-87 % T range and similar light transmission values of films
(65-73 % T). The UTS of LP and HP (32 and 36 MPa respectively) samples was similar with the breaking strain of the to the samples being similar in the 20-30 % range. The OP values of the LP and HP chitin samples were similar in the range 1.4-1.6 cm$^3$-$\mu$m/m$^2$/day/kPa. This indicates that at 72 % DA, the ChNFs can be homogenized at lower pressures than previously reported by us [6, 9, 40] and still produce films with similar light transmission, UTS, strain at break and OP values due to the particle width and lengths being similar.

The results were replicated using crab shell chitin, where instead of homogenizing for 30 passes, the process was halted at 8 passes. The resultant films were stronger than the non-deacetylated samples, similar to the results with commercial chitin. These results show that efficiency of HPH can be increased for chitin by deacetylation. Increase in efficiency reduces machine residence time and energy consumption decreasing both the capital and operating costs for HPH of chitin. The skip in the higher-pressure step at 1479 bar further reduces energy consumption. These improvements bring the process of ChNF production closer to cost-competitiveness. In the future, using wider diameter orifices, lowered pressure homogenization at 551 bar with high DA chitin could be attempted at low number of passes to increase cost efficiency and higher energy efficiency.
4.5 REFERENCES


CHAPTER 5. FUTURE DIRECTIONS IN HIGH OXYGEN AND WATER VAPOR BARRIER MATERIALS – THERMALLY TREATED POLY(GLUCURONIC ACID)-CHITOSAN FILMS

5.1 Introduction

Cellulose is one of the most abundant biopolymers.[1] However it is insoluble in almost all solvents and often substitution reactions of the hydroxyl groups with carboxymethyl ether or sulfate ester groups are used to make the end product soluble in solvents.[2] Poly(glucuronic acids) are homopolymers of glucuronic acid.[2] They can be found naturally or can be produced synthetically by regioselective oxidation of natural glucans such as cellulose.[2] Isogai & Kato[3] produced water soluble β-(1,4)-D-poly(glucuronic acid) (called cellouronic acid) acid by regioselective oxidation of C₆ primary hydroxyls of regenerated cellulose using 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO). It must however be pointed out that several sources of natural water soluble β-(1,4)-D-poly(glucuronic acids) have also been identified, one among them being an exopolysaccharide excreted by the Sinorhizobium meliloti.[2] Chitin is another abundantly available biopolymer, with natural production being of the same order of magnitude as cellulose.[4] An important derivative of chitin is chitosan, which is a linear polysaccharide and a homopolymer of D-glucosamine and produced by deacetylation of chitin.[5] Due to the presence of amino groups chitosan is soluble in dilute acids like acetic, formic, succinic etc.[5] Crosslinking of chitosan with itself[6] or other reagents like o-phthalaldehyde,[7] glutaraldehyde,[8] vanillin,[9] and many others, has been studied. Similarly, cellulose and chitosan has been used to produce antibacterial composites[10]
and polymer blends of these two have been studied by Isogai.[11] Alam and Christopher produced crosslinked cellulose-chitosan crosslinked superabsorbent hydrogels by crosslinking partially-oxidized cellulose aldehyde with carboxymethylated chitosan.[12] Tang et. al were able to couple chitosan and TEMPO oxidized cellulose nanofibrillated cellulose by exploiting their electrostatic attraction at room temperature, followed by heating to produce covalent amide linkages.[13] Soni et al. produced transparent gas barrier films using TEMPO oxidized cellulose nanofibers and chitosan. They found that the films had better oxygen gas barrier, thermal and mechanical stability properties than chitosan films.[14] Choo et al. produced polyvinyl alcohol-chitosan composite films and reinforced them with TEMPO oxidized cellulose nanofibers. [15] Yan et al. prepared a polyelectrolyte complex by utilizing a negatively charged carboxylic curdlan bearing a β-1,3-poly(glucuronic acid) structure and positively charged chitosan.[16]

Sannino et al.[17] describe a method for preparation of a polymer hydrogel, using a polycarboxylic acid (including poly(glucuronic acid)) as the cross-linking agent. Valentova [18] describe a chitosan-glucan complex for wound healing applications. Buchanan et al.[19] have described methods of oxidation of cellulose ester interpolymers using TEMPO which can also work with chitosan. They also describe complexation between oppositely charged macromolecules using anionic cellulose ester interpolymers and chitosan for solubility modifiers of therapeutic agents.

Polyethylene glycols (PEGs) are polymers made from ethylene and due to their minimal skin irritation, high stability and excellent physical characteristics they finds wide use in detergents, cosmetics, drug tablets, as well as food applications.[20] PEGs with molecular weight of up to 20,000 Da[20] are known to be biodegradable, and in aqueous
waste do not lead to serious environmental pollution. In addition, PEGs <9500 Da are generally recognized to be safe as food additives by the US FDA.[21] In 2008, Yan and Gao[22] described dissolving cellulose in 1 wt.% PEG/9 wt.% NaOH solution. They could produce cellulose solutions of 1-11.5 wt.% cellulose by mixing cellulose in the PEG/NaOH solution, freezing the mixture at -15 °C for 12 hours and then thawing the mixture under vigorous stirring to produce a homogenous cellulose solution. Tang et al. [23] described producing water soluble TEMPO oxidized cellulose by dissolving cellulose in a Urea/NaOH solution. The Urea/NaOH treated cellulose was then oxidized utilizing TEMPO-Sodium Bromide-Sodium hypochlorite to yield water-soluble poly (glucuronic acid). In this case urea is a hydrogen bond acceptor and stabilizes the cellulose solution. The solvation of cellulose allows for the TEMPO to oxidize the whole substance rather than just cellulose surface allowing the resulting TEMPO oxidized cellulose to be self-stabilized even after removal of urea (hence producing dissolving cellulose).[23]

Synergistic interactions of chitin and cellulose based nanomaterials have been documented,[24, 25] where blending ChNFs with CNCs produced films that had superior oxygen barrier and mechanical properties than ChNFs and CNCs alone. However, maximizing these interactions on a molecular level between the two materials is difficult given their insolubility and the requirement of harsh or exotic solvent systems for solvation. However, chemical modification of both of these materials allows for the modified components to be water soluble. The described PEG/NaOH system by Yan and Gao is a relatively simple system with non-toxic components where PEG functions as a hydrogen bond acceptor and could serve as a greener solvent for the TEMPO oxidation[22] that allows for a soluble cellulose to be produced. In addition, PEG is a cheap petrochemical
derived non-toxic material. It is a hydrogen bond acceptor that could provide an opportunity to further optimize the interactions between chitin and cellulose and may be key to development of degradable functional barrier materials. This study describes crosslinked cellulose derived poly (glucuronic acid) (PG) and chitin derived chitosan (Ch) membranes with and without the presence of PEG for advanced barrier applications. The method for fabrication of these membranes utilizes the TEMPO oxidation procedure as described by Tang et al.[23] with modifications, the primary modification being the utilization of PEG/NaOH solution as described by Yan and Gao[22] instead of Urea/NaOH for dissolution of cellulose. Free standing solution cast films were analyzed for their mechanical and barrier properties.

5.2 Materials & Methods

5.2.1 Materials

ACS grade Sodium hydroxide pellets and ACS grade crystalline sodium bromide was obtained from VWR (Radnor, PA). Solid Polyethylene glycol 2000 was obtained from Fluka Biochemika (Steinheim, Germany). TEMPO free radical was obtained from Alfa Aesar (Ward Hill, MA). Deionized (D.I.) water was obtained from a Thermo scientific GenPure UV/UF xCAD plus water purification system. Freeze dried cellulose nanocrystals (CNCs) were obtained from Forest Products lab (Madison, WI). Glacial acetic acid was purchased from Sigma Aldrich (St. Louis, MO). Ch powder (min. 90% deacetylation) was obtained from Spectrum Chemicals (Gardena, CA). Sodium hypochlorite was obtained as 6% solution from Publix (Lakeland, FL).
5.2.2 Preparation of TEMPO derived PG

9 g of NaOH pellets and 1 g of PEG 2000 were dissolved in 90 ml of D.I. water. To it 1 g of freeze dried CNCs were added. Liquid nitrogen was used to freeze the solution solid and the frozen solution was kept in a 0 °C refrigerator for 24 hours. The frozen solution was thawed under vigorous stirring to yield a clear cellulose solution. To it 12.7 g of Sodium bromide, 0.16 g TEMPO were added. After complete dissolution 114 ml of 6% sodium hypochlorite was added to the solution over 10 minutes and the solution was reacted for 6 hours. The reaction was terminated with 5 ml of ethanol. The resulting solution was dialyzed against 500 volumes of DI water till the pH was 7 to remove TEMPO, sodium bromide and sodium hydroxide.

5.2.3 Preparation of Ch and PEG-Ch-PG Membranes

Enough Glacial acetic acid was dissolved in water to yield a pH 3 solution. Ch was dissolved in this pH 3 acidified water as 1% solution. 26 ml of the dialyzed PG-PEG solution was mixed with 24 ml of Ch solution. The solution was stirred for 10 minutes at 200 RPM and then solution cast in a plastic (polyethylene) boat. The films were air dried over the course of 14 days to yield free standing films. 50 ml of only Ch solution was similarly solution casted and dried to yield Ch membranes.

5.2.4 Preparation of Ch-PG Membranes

The dialyzed cellulose solution was mixed with ethanol to precipitate the PG. The precipitate was separated by centrifugation of the suspension at 3000 RPM for 10 minutes. The precipitate was washed with ethanol and the suspension was again centrifuged at the
same conditions. The procedure was repeated thrice to yield pure PG. The PG was then dissolved in water as a 1 wt.% solution. 26 ml of this solution was mixed with 24 ml of the chitosan solution described previously. The resulting PG and Ch solution was then solution casted as described previously to yield free standing Ch-PG films.

5.2.5 Heat treatment of Membranes

PEG-Ch-PG, Ch-PG, and Ch films were then heated in a vacuum oven at 150 °C for 3 hours to yield the corresponding thermally treated films. These conditions are obtained from Sharma et al. [26] sans the vacuum oven.

5.2.6 Characterization of Heat Treated and Untreated Films

The thickness of cast heat treated and untreated films was measured with a micrometer at more than 10 different points on each film. Tensile mechanical properties of heat treated and untreated films were determined by using a high-throughput mechanical characterization (HTMECH) instrument.[27]

The O2 permeability values of the heat treated as well as untreated films were obtained by using a MOCON OXTRAN 1/50 instrument at 50 % RH. The films were humidified as required to soften them using an Electrotech ultrasonic humidification system (Glenside, PA). The humidification facilitated cutting, handling and loading of the Ch-PG and the Ch films into the MOCON without fracturing since they were quite brittle. The films were cut into squares of approximately 9 cm² size and covered with a mask that allowed measurement of 5 cm² of this area. Water vapor transmission values of the free-
standing films were obtained using a MOCON PERMATRAN-W 1/50 instrument at 30-
60 % RH and 23 °C.

5.3 Results & Discussions

Table 5.1 shows the properties of the obtained films. The thickness of the untreated
Ch films are the highest at 229 μm and the untreated Ch-PG films are the thinnest. The
untreated PEG-Ch-PG films have intermediate thicknesses compared to Ch and Ch-PG.
Heat treatment increases the thickness of Ch films to 357 μm, while maintaining the film
thicknesses of the Ch-PG films. PEG-Ch-PG film thicknesses are reduced on heat treatment
to 94 μm.

Table 5.1 Thickness and Oxygen Transmission of films.

<table>
<thead>
<tr>
<th></th>
<th>As Cast Thickness (μm)</th>
<th>Heat Treated Thickness (μm)</th>
<th>O₂ Permeability (cm³-μm/m²/day/kPa)</th>
<th>Heat Treated O₂ Permeability (cm³-μm/m²/day/kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch</td>
<td>229 ± 50</td>
<td>357 ± 63</td>
<td>13.5 ± 2.9</td>
<td>16.2 ± 2.9</td>
</tr>
<tr>
<td>PEG-Ch-PG</td>
<td>135 ± 27</td>
<td>94 ± 14</td>
<td>3.7 ± 0.7</td>
<td>0.2 ± 0.03</td>
</tr>
<tr>
<td>Ch-PG</td>
<td>61 ± 12</td>
<td>73 ± 6</td>
<td>-</td>
<td>0.8 ± 0.07</td>
</tr>
</tbody>
</table>

The oxygen permeability (OP) of the PEG-Ch-PG film is 3.7 cm³-μm/m²/day/kPa. The Ch
film has the highest OP of 13.5 cm³-μm/m²/day/kPa. Heat treatment does not significantly
change the OP of the Ch films with mean values being slightly higher at
16.2 cm³-μm/m²/day/kPa. Heat treatment reduces the OP of the PEG-Ch-PG to
0.2 cm³-μm/m²/day/kPa. The heat treated Ch-PG film had a permeability of
0.8 cm³-μm/m²/day/kPa. The Ch-PG films in general are very fragile and the untreated
films were too fragile to obtain a value of OP.
The neat Ch films have the highest film thicknesses suggesting that they have lower density than other films. They also have the highest OP of all the samples. Removal of PEG from the PEG-Ch-PG films leads to thinner Ch-PG films as evidenced by their lower thicknesses. Correspondingly the OP values of the samples are also significantly lower than Ch. The reduction in thickness of the PEG-Ch-PG films on heat treatment indicates a densification effect. The OP values of the heat treated PEG-Ch-PG films are correspondingly lower. In fact the OP value of the heat treated PEG-Ch-PG films at 50 % RH is at least an order of magnitude lower than most of the commonly used plastics like poly(ethylene terephthalate), polypropylene, polyethylene and are actually similar in permeability to poly(vinylidene chloride) which has OP values in the 0.09-2.9 cm³-μm/m²/day/kPa range.[28] The film thicknesses of the Ch-PG films are not significantly different on heat treatment indicating almost no densification. The OP values of the heat treated Ch-PG films are also comparable to poly(vinylidene chloride).

![Figure 5.1 Water Vapor Transmission Rate of films.](image)
Heat treatment of the Ch films increases their mean thickness indicating that the films are undergoing the reverse process.

Figure 5.1 shows the water vapor transmission rate (WVTR) of the films in response to changing relative humidity (RH). The WVTR of the Ch-PG films increases almost linearly from 3.7 g-mm/m²/day at 35 % RH to 10 g-mm/m²/day at 50 % RH. After 50 % RH, the WVTR was higher than measurable with the PERMATRAN instrument. Heat treatment improves the WVTR response of the Ch-PG films. The WVTR is 4.5 g-mm/m²/day at 50 % RH and increases to 13.2 g-mm/m²/day at 65 % RH after which it cannot be measured. The WVTR of the untreated PEG-Ch-PG films is higher than all the tested films and could not be accurately determined. Heat treatment of the films improves the WVTR. The WVTR of the heat treated PEG-Ch-PG films at 50 % RH is 8.2 g-mm/m²/day and increases to 35 g-mm/m²/day at 70 % RH.

Heat treatment enhances the WVTR of the Ch-PG and PEG-Ch-PG films. The PEG-Ch-PG films have significantly higher WVTR which is of the same order of magnitude as poly(vinyl alcohol) films with WVTR of 30 g-mm/m²/day while the heat treated Ch-PG films had permeability that approached those of polyamide films in the range of 0.5-10 g-mm/m²/day.[28]

Figure 5.2(a) shows the ultimate tensile strength (UTS) of the films. The Ch and the PEG-Ch-PG films have the highest mean UTS values of 21.3 and 17.8 MPa which are not significantly different as indicated by the p values in Table 5.2. The Ch-PG films have the lowest mean UTS value of 9.0 MPa. Heat treatment reduces the UTS of the Ch films
Figure 5.2 (a) Ultimate Tensile Strength and (b) Strain at Break of untreated and treated films

Table 5.2 Two tailed P-values for mean Ultimate Tensile Strength. Values highlighted in red indicate differences in means between the two samples being compared is significant at the level of 0.05.

<table>
<thead>
<tr>
<th></th>
<th>Ch</th>
<th>Ch-HT</th>
<th>PEG-Ch-PG</th>
<th>PEG-Ch-PG-HT</th>
<th>Ch-PG</th>
<th>Ch-PG-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch</td>
<td>1.00</td>
<td>0.04</td>
<td>0.13</td>
<td>0.37</td>
<td>4.45E-03</td>
<td>2.12E-07</td>
</tr>
<tr>
<td>Ch-HT</td>
<td>0.04</td>
<td>1.00</td>
<td>0.37</td>
<td>0.14</td>
<td>0.06</td>
<td>1.35E-04</td>
</tr>
<tr>
<td>PEG-Ch-PG</td>
<td>0.13</td>
<td>0.37</td>
<td>1.00</td>
<td>0.44</td>
<td>0.02</td>
<td>1.00E-07</td>
</tr>
<tr>
<td>PEG-Ch-PG-HT</td>
<td>0.37</td>
<td>0.14</td>
<td>0.44</td>
<td>1.00</td>
<td>0.01</td>
<td>6.36E-08</td>
</tr>
<tr>
<td>Ch-PG</td>
<td>4.45E-03</td>
<td>0.06</td>
<td>0.02</td>
<td>0.01</td>
<td>1.00</td>
<td>0.26</td>
</tr>
<tr>
<td>Ch-PG-HT</td>
<td>2.12E-07</td>
<td>1.35E-04</td>
<td>1.00E-07</td>
<td>6.36E-08</td>
<td>0.26</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Table 5.3 Two tailed P-values for mean strain at break. Values highlighted in red indicate differences in means between the two samples being compared is significant at the level of 0.05.

<table>
<thead>
<tr>
<th></th>
<th>Ch</th>
<th>Ch-HT</th>
<th>PEG-Ch-PG</th>
<th>PEG-Ch-PG-HT</th>
<th>Ch-PG</th>
<th>Ch-PG-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch</td>
<td>1.00</td>
<td>0.93</td>
<td>1.76E-08</td>
<td>3.04E-09</td>
<td>4.85E-09</td>
<td>3.72E-09</td>
</tr>
<tr>
<td>Ch-HT</td>
<td>0.93</td>
<td>1.00</td>
<td>2.97E-04</td>
<td>1.13E-04</td>
<td>1.28E-04</td>
<td>9.73E-05</td>
</tr>
<tr>
<td>PEG-Ch-PG</td>
<td>1.76E-08</td>
<td>2.97E-04</td>
<td>1.00</td>
<td>0.03</td>
<td>0.06</td>
<td>1.24E-04</td>
</tr>
<tr>
<td>PEG-Ch-PG-HT</td>
<td>3.04E-09</td>
<td>1.13E-04</td>
<td>0.03</td>
<td>1.00</td>
<td>0.86</td>
<td>3.33E-02</td>
</tr>
<tr>
<td>Ch-PG</td>
<td>4.85E-09</td>
<td>1.28E-04</td>
<td>0.06</td>
<td>0.86</td>
<td>1.00</td>
<td>0.06</td>
</tr>
<tr>
<td>Ch-PG-HT</td>
<td>3.72E-09</td>
<td>9.73E-05</td>
<td>1.24E-04</td>
<td>0.03</td>
<td>0.06</td>
<td>1.00</td>
</tr>
</tbody>
</table>

...to 15.9 MPa. (p value of 0.04 from Table 5.1). Heat treatment does not significantly reduce the UTS of the PEG-Ch-PG and the Ch-PG films as seen in Table 5.2.

Figure 5.2(b) shows the strain at break of the untreated and treated films. Ch films have significantly (Table 5.2) highest mean strain at break of 60.4 %. PEG-Ch-PG films have a lower mean strain at break (from Table 5.2) of 16.2 %. Ch-PG films have the lowest mean strain at break of 10.8 %. Heat treatment does not alter the strain at break of Ch and Ch-PG films as indicated by Table 5.3. The strain at break of PEG-Ch-PG films is lowered to 10.3 % upon heat treatment. (Table 5.3)

Figure 5.3 shows the FTIR spectrum of all films described above. The spectra were normalized such that the maximum value of the absorbances are 1 for the peaks with the highest magnitude.

The FTIR spectrum for PEG-PG shows the presence of abundant hydrogen bonded OH groups as indicated by the broad peak from 3000-3600 cm\(^{-1}\) [29] with intensity similar to the C-H stretch at 2885 cm\(^{-1}\)[30, 31] that is not seen in PEG.[32] Peaks with wavenumber higher than 3450 cm\(^{-1}\) are attributed to weak hydrogen bonds or are free hydroxyls.[29]
Two peaks of interest are the sharp peaks at 1340 and 1360 cm\(^{-1}\) that are associated with the presence of a crystalline PEG phase in the PEG-PG mixture.\[33\] Additionally, we can

**Figure 5.3 FTIR spectrum of films, PG.**
also see the peak at 1738 cm$^{-1}$ that is associated with free carboxyl groups[34] and the peak at 1592 cm$^{-1}$ that is associated with the stretch of the carboxylate when it is associated with sodium.[35] This indicates the presence free and protonated carboxylate groups, consistent with successful TEMPO oxidation of cellulose. These TEMPO oxidized groups are not present in Ch spectra. Instead we see the characteristic peaks associated with protonated amines, i.e., the symmetric amine bending vibration at 1554 cm$^{-1}$[36] and the antisymmetric amine bending at 1642 cm$^{-1}$ that overlaps with the amide-I band.[36-38] The symmetric and antisymmetric C-H stretching modes are assigned to 2907 cm$^{-1}$ and 2864 cm$^{-1}$ respectively.[36, 37] Like cellulose, chitosan also exhibits hydrogen bonding that shows up as broad overlapped peaks associated with the hydrogen bonds in the N-H and O-H groups present in the 3000-3800 cm$^{-1}$[36] wavenumber range. Specifically, the peak at 3363 cm$^{-1}$ is assigned to N-H stretching and 3451 cm$^{-1}$ is assigned to the O-H group.[38]

On comparison of the PEG-Ch-PG films to the Ch and PEG-PG films the most obvious differences are the disappearance of the free hydrogen bonds and the reduction in intensity of the weak hydrogen bonds (wavenumbers greater than 3450 cm$^{-1}$) of the PEG-PG. The change in bands in the 1500-1700 cm$^{-1}$ range when comparing PEG-Ch-PG films to neat Ch and PEG-PG indicate formation of characteristic amide II and amide I bands at 1586 cm$^{-1}$ and 1649 cm$^{-1}$ respectively.[36] The crystalline PEG phase (1340, 1360 cm$^{-1}$) is still present in the PEG-PG-Ch films.

For the heat treated PEG-Ch-PG films, the FTIR spectra was normalized by using the C-O-C bridge vibrations at 1150 cm$^{-1}$[30, 37] to enable quantitative comparisons between the spectra. The hydroxyl peaks for the heat treated PEG-Ch-PG films (PEG-Ch-PG-HT) indicate a larger fraction of hydrogen bonded -OH, accompanied by the
reappearance of a small peak at 3450 cm\(^{-1}\) corresponding to weak hydrogen bonding. The intensity of bands associated with amides between 1500-1700 cm\(^{-1}\) is increased along with the increase in intensity of the peak at 1320 cm\(^{-1}\) C-N stretch of the amide-III\([36, 37]\) pointing to increased amide bond formation. Another major difference is a decrease in intensity of the C-H vibrations. The C-H vibrations arise from all the components present in the mix and are not expected to undergo any chemical reactions. However, studies of systems with C-H groups and long hydrocarbon chains like alkanoates\([39]\) and PEGs\([40]\) show that the bands in this region are highly sensitive to the chain packing and crystallinity. An important observation is the disappearance of the crystalline PEG-Ch-PG phase on heat treatment indicated by the disappearance of the characteristic crystalline peaks and replacement by a singular peak at 1350 cm\(^{-1}\) associated with amorphous PEG phase. \([33]\)

Ch-PG films are similar to PEG-Ch-PG films where there is a noted absence of free hydrogen bond stretching at wavenumbers greater than 3450 cm\(^{-1}\). The bands corresponding to amides appear in the 1500-1700 cm\(^{-1}\) range similar to PEG-Ch-PG films. The FTIR data for the heat treated Ch-PG films is normalized such that the intensity of the C-O-C bridge vibrations is the same to allow quantitative comparisons. On heat treatment of the Ch-PG, the hydrogen bonding hydroxyl peak intensity is increased but the peak associated with the weak hydrogen bonding is not as distinct as seen in heat treated PEG-Ch-PG. Similarly, the peaks associated with the amides is also increased with heat treatment, as observed for PEG-Ch-PG. There is also a slight increase in the intensity of the signal associated with the C-H stretch indicating a change in the chain packing. The magnitude of change is not as significant as the C-H intensity change associated with heat treatment of PEG-Ch-PG films.
From the FTIR data, we can conclude that mixing the PEG-PG mixture and Ch and also mixing ethanol treated PG with Ch produces films where the peak intensity from free PG –OH groups disappears. At the same time, there is amide bond formation due to reaction between the amine groups from Ch and the carboxylates from PG. The contribution of PEG to the C-H signal in the 2800-3000 cm\(^{-1}\) range is high. The PEG-Ch-PG mixture is rich in CH\(_2\) groups and changes to the crystallinity of PEG upon heat treatment has a high impact on this C-H signal in this region [40] and possibly explains the higher changes in the C-H signal upon heat treatment of the PEG-Ch-PG films compared to the Ch-PG films.

Crosslinking density has been shown to reduce oxygen and water vapor barrier properties in different cases like UV cured coatings[41], cast alginate films[42] and multilayered thin films.[43] Similarly increased hydrogen bonding in multilayered thin films[44], poly(vinyl alcohol) films[45] and cellulose films (through hornification).[26] Thus cross linking and enhancement in inter- and intra-molecular hydrogen bond formation could explain the improvement of the oxygen and water vapor permeability of the heat treated PEG-Ch-PG films.

Heating these PEG-Ch-PG and Ch-PG films causes an increase in hydrogen bonding but also a reorganization of the hydrogen bonds as evidenced by reappearance of the peak associated with weak hydrogen bonds. We also see an increase in intensity of the amide bonds indicating further amide bond formation driven by heating. For the Ch-PG the intensity increase is less significant on heat treatment as compared to the PEG-Ch-PG films. A direct comparison may not be possible between the PEG-Ch-PG and Ch-PG films due to the presence of the PEG, but the dramatic increase in intensity of the amide bands
as well as hydrogen bonding in the PEG based films on heat treatment may point to crystalline PEG hindering the formation of both – hydrogen and amide bonds in the untreated films during film drying. Heat treatment of films drives reorganization of the PEG as expected by the melting of the crystalline PEG upon heating and the formation of amorphous phase on cooling. The melting and reorganization of the PEG chains then allows amide and hydrogen bond formation that is to be expected based on thermodynamics. Crystalline materials in general and PEG in particular is known for its ability to regulate other small molecules and for PEG, the regulatory effect is based on hydrogen bonding interactions[46] pointing to a regulatory effect of PEG in these films. The Ch-PG films on the other hand do not have PEG to regulate hydrogen and amide bond formation during drying thus both bonds have already formed in the untreated.

5.4 Conclusions

In this study we report the first known films made from PG derived from TEMPO oxidation of cellulose and Ch, with and without PEG. The films were heat treated to reduce their OP and WVTR. Heat treatment was found to improve OP value of PEG-Ch-PG films and brought them to 0.2 cm³-μm/m²/day/kPa at 50 % RH which is lower than PET and in the PVDC range. Heat treatment also improved the WVTR of the PEG-Ch-PG films and Ch-PG films. The heat-treated Ch-PG films had WVTR of 13.2 g-mm/m²/day which approached polyamide films and were significantly better than heat treated PEG-Ch-PG films. The treated and untreated Ch-PG films are very brittle and have low average tensile strength values of 5.6 MPa and 9 MPa respectively. Comparatively, both treated and untreated PEG-Ch-PG films have much higher tensile strength values of 17.8 and 19.2 MPa respectively. Heat treatment thus does not change the ultimate tensile strength of the films.
The strain at break values of the PG-Ch and PEG-Ch-PG films had similar values of 16.2 and 10.8 MPa respectively. Heat treatment does not change the strain at break of PG-Ch films but does lower the strain at break of PEG-Ch-PG films to 10.3 %. FTIR studies confirm the presence of carboxylate groups in the PG and the subsequent crosslinking of amines from Ch with carboxylates in PG in the PEG-Ch-PG films and also the Ch-PG films. Heat treatment enhances hydrogen bonding along with an increase in amide bond formation that makes the films that enhances the oxygen and water vapor barrier properties of the PEG-Ch-PG films. For the Ch-PG films there is also enhancement in hydrogen bonding and amide bond formation upon heat treatment, however to a lesser extent than the PEG-Ch-PG films. The PEG-Ch-PG films show indication that the PEG molecules undergo melting and subsequent crystalline to amorphous phase transition during heat treatment that could be regulating hydrogen and amide bond formation in these films. PEG-2000, used in this study, is a biodegradable polymer[20] despite its petrochemical origins. Ch and PG are also biodegradable and the films produced from these are thus expected to be biodegradable. These biodegradable films with high oxygen barrier properties and polymer-like water vapor permeation could form the basis for a new class of biodegradable materials with high content of renewables and desirable barrier properties that could be applicable for packaging applications in various industries.
5.5 REFERENCES


CHAPTER 6.  CONCLUSIONS AND RECOMMENDATIONS

6.1  Summary and Conclusions

The work achieves the goals set forth in Chapter 1 and describes ways to utilize chitin and cellulose based nanomaterials as coatings or blended films. Alongside it also shows the potential to optimize chitin nanofiber content when blended with cellulose nanocrystals. A method for optimized production of nanofibers was also investigated resulting in a path for higher efficiency homogenization. Alongside we optimized the synergy of chitin and cellulose based materials through a novel poly(glucuronic acid)-chitosan based crosslinked film.

6.1.1  Develop Coatings Based on ChNFs and CNCs

A composite multi-layered film consisting of alternating layers of ChNFs and CNCs was fabricated by spray coating the suspensions onto PLA. The nanomaterials were chosen for their opposite charges- CNCs are negatively charged due to the sulfate half ester group and the ChNFs are positively charged due to the amino group. It was found that films with at least two alternating coated layers of ChNFs and CNCs, showed reductions in O2 permeability relative to neat PLA, even at elevated RH (70%). These improvements in O2 barrier properties were not observed for films with single layers of neat ChNF or CNC sprayed from an equivalent volume. The improvement in O2 permeability was correlated with evidence of thin, densely-packed layers of ChNF and CNC. WVTR was not intrinsically improved through the addition of ChNF or CNC layers (but continued to be controlled by the PLA film substrate). The composites were, found to be more brittle than
neat PLA possibly due to mutual interlayer destruction, which could be addressed by minimizing the thickness of coated layers or the use of plasticizers. These films show potential to form the basis for a platform technology of 100% bio renewable barrier packaging.

6.1.2 *Optimize the Utilization of ChNFs in ChNF-CNC Based Composites*

Blends of CNCs and ChNFs were studied for their potential to improve the mechanical and barrier properties of CNC-ChNF films. Two types of chitin were used—LChNF and HChNFs with 73% and 93% DA respectively. The oxygen permeability of neat LChNF films was found to be the lowest of all films, at 1.0 cm$^3$-$\mu$m/m$^2$/d/kPa. Blended CNC-ChNF films had a permeability that was an order of magnitude lower (1.4-1.9 cm$^3$-$\mu$m/m$^2$/d/kPa) than neat CNC films (18.5 cm$^3$ $\mu$m/m$^2$/d/kPa). The addition of either HChNFs or LChNFs to CNCs was found to be able to lower the OP of the blend films nearly equivalently even at concentrations as low as 25% ChNF by weight. The decrease in OP at relatively low concentration of chitin was attributed to aggregation of CNCs with LChNFs or HChNFs, driven by electrostatic attraction (in addition to hydrogen bonding) due to their opposite charge. Structural effects attributed to this aggregation and could reasonably explain the decreased OP in chitin-cellulose blends relative to CNCs. Dense layered lamellar structures were observed in the blend systems in which the diffusion timescale oxygen is expected to be increased relative to neat CNCs. CNC-ChNF aggregates appear to form long diffusion length scales that likely add tortuosity within lamellae, even when ChNF is the minority component due to bridging of chitin fibers by CNCs. LChNFs were found to have better mechanical properties (UTS and strain at break) than HChNF films, which in turn were better than CNC films. Addition of either-LChNF and HChNFs
to CNC films increased UTS and strain at break of the resultant blended films. While cellulose is currently produced all over the world as part of the pulp and paper industry, the industrial processing of chitin lags by orders of magnitude and thus the cost of cellulose-derived materials is expected to be lower than chitin derived materials like ChNFs due to economy of scale being in favor of CNCs. These results show that there may be a way to impart superior mechanical and barrier properties of ChNFs to CNC based materials by blending ChNFs (as additives) with CNCs. Thus, ChNFs were demonstrated to be bio-derived additives that improve the mechanical and barrier properties of CNCs to support renewable materials production.

6.1.3 Increase Production Efficiency ChNFs

The efficiency of HPH was shown to be improved when deacetylation was used to increase cationization of ChNF fibers. The deacetylated ChNFs from commercially available chitin produced suspensions with higher light transmission (74 % and 81 % T) at 30 passes than their untreated counterpart (36 % T). The resultant films from deacetylated ChNFs were more transparent than the 89 % DA ChNFs. Furthermore, deacetylation was also found to improve the mechanical properties of the films. The UTS of the 72 % DA chitin improved to a value of 29 MPa compared to the 89 % DA ChNF sample with value of 18 MPa. The strain at break improved with a decrease in DA with 79 % and 72% DA films having breaking strain values of 28 % and 34 % respectively compared to the untreated ChNF films having a value of 18 %. The barrier properties did not change with the OP of all films being in the 1.9-2.4 cm3 μm/m2/day/kPa range without any obvious trend.
At high deacetylation values (72 % DA) the use of higher pressures did not improve the properties of the films. LP and HP processed ChNF suspensions had similar light transmission values, UTS and breaking strain. The OP values of the LP and HP chitin samples were also found to be similar. This demonstrated that at 72 % DA, the ChNFs could be homogenized at lower pressures than previously reported and still produce comparable films with similar properties to their unacetylated counterparts due to the particle width and lengths being similar. The results were replicated using crab shell chitin, where lower pressures were used and the process was halted at 8 passes. The resultant films were stronger than the non-deacetylated samples, similar to the results with commercial chitin. The results showed that efficiency of HPH can be increased for chitin by deacetylation with corresponding reduction in machine residence time and energy consumption. This would decrease both the capital and operating costs for HPH of chitin. The reduction in homogenization pressure further contributed to energy consumption. These improvements bring the process of ChNF production closer to cost-competitiveness.

6.1.4 Maximize Synergistic Interactions between Chitin and Cellulose based Materials

Films were made from PG derived from TEMPO oxidation of cellulose and Ch, with and without PEG to maximize the synergy between cellulose and chitin derived materials. The films were heat treated to reduce their OP and WVTR. Heat treatment was found to improve OP value of PEG-Ch-PG films (to 0.20 cm$^3$-$\mu$m/m$^2$/day/kPa at 50 % RH) and brought them in the PVDC range. Heat treatment also improved the WVTR of the PEG-Ch-PG films and Ch-PG films. The heat treated Ch-PG films had WVTR of 13.2 g-mm/m$^2$/day which approached polyamide films and were significantly better than heat treated PEG-Ch-PG films. Both treated and untreated Ch-PG films were found to be brittle.
and have low average tensile strength values of 5.6 MPa and 9 MPa respectively. Comparatively, both treated and untreated PEG-Ch-PG films had much higher tensile strength values of 17.8 and 19.2 MPa. Heat treatment did not alter the ultimate tensile strength of the films. The strain at break values of the PG-Ch and PEG-Ch-PG films had similar values of 16.2 and 10.8 MPa respectively. Heat treatment did not alter the strain at break of PG-Ch films but lowered the strain at break of PEG-Ch-PG films to 10.3%. FTIR studies confirmed the presence of carboxylate groups in the PG and the subsequent crosslinking of amines from Ch with carboxylates in PG in the PEG-Ch-PG films and also the Ch-PG films. Heat treatment enhanced hydrogen bonding and amide bond formation that in turn enhanced the oxygen and water vapor barrier properties of the PEG-Ch-PG films. Similar enhancement in hydrogen bonding and amide bond formation upon heat treatment were seen for Ch-PG films, however to a lesser extent than the PEG-Ch-PG films. The PEG-Ch-PG films show indication that the PEG melts during the heating and in doing so regulates hydrogen and amide bond formation in these films. Ch and PG are biodegradable and the films produced from these are thus expected to be biodegradable as PEG-2000, used in this study, is a biodegradable polymer despite its petrochemical origins. These films could form the basis for a new class of biodegradable materials with high content of renewables and desirable barrier properties that could be applicable for packaging applications in various industries.

6.2 Recommendations for future work

In this work we have successfully utilized Chitin and cellulose based materials to prototype new bioderived materials. An essential component of these films is the synergy between chitin and cellulose based materials that drives enhancements in OP and
mechanical properties (UTS, strain at break) which was discovered for our films. The mechanism of synergy is attributed to electrostatic attraction and hydrogen bonding. However, the exact mechanism is still unclear. In order to fully utilize and maximize this synergy the mechanism of synergy must be thoroughly explored.

6.2.1 Simulations for Fiber Assembly and Hydrogen Bonding

Modelling network formation of charged ChNF fibers in aqueous suspensions should be attempted. Electrostatic forces are long ranged forces in such interactions, however there is a need to model short ranged hydrogen bonding interactions in equal detail as these are primarily responsible for premature network formation and thus improper packing of fibers in the matrix. A secondary but important effect is the modification of fibre stiffness and hence deformability on the attachment of smaller (and more crystalline) CNCs to ChNFs. All of these effects influence the final structure of the network, that influence interfiber spacing, and thus the diffusion path for gases in densely packed networks. Apart from this effort, separate molecular dynamic simulations should be undertaken of polymer crystals of α-chitin and Cellulose-I in close proximity. It is hoped that such simulations might reveal a change in hydrogen bonding that changes the crystalline structure of the top surface of the nanoparticles leading to enhancement in hydrogen bonding as seen in FTIR.

6.2.2 Modified Cellulose and Chitin as Platform for Plastic-like Materials

The polymer films described in chapter 5, based on PG, Ch, PEG platform are novel and reveal a potential to upgrade inexpensive petrochemical materials like PEG to valuable products that are biodegradable and also have a high renewable content. Such materials
could help us transition towards a more sustainable economy while better alternatives to conventional plastics like PE, PET, etc are investigated. So far, we have only investigated PG and Ch as cellulose and chitin derived materials. Inclusion of nanomaterials into these matrices could also be investigated, including but not limited to, TEMPO oxidized CNCs/CNFs and ChNFs/ChNWs. Different cross linkers for PG such as propylene glycol or other diols could additionally be investigated. The PEGs, which function as a hydrogen bond regulator, could potentially be replaced with other alternative petrochemicals like biodegradable versions of polyacetal (poly(methylene oxide)) and poly(propylene glycols) along with EO/PO copolymers that are available in myriad forms in order to see the influence of these on hydrogen bonding and interfiber spacing. Alternative, PGs like those derived from deacetylation of carboxymethylcellulose could offer a more economic pathway to commercialization of these films.
APPENDIX A. PARTICLE SIZE AND DIAMETER DISTRIBUTION FOR DEACETYLATION STUDIES

Presented here are the AFM images used for fiber length and width analysis. The analysis methodology can be found in Chapter 4 section 4.2.5. All images are in their original formats, unscaled and with originals scalebars from Bruker Nanoscope analysis software.

A.1 89 % DA ChNFs

![AFM height image of 89 % DA ChNF](image)

Figure A.1 AFM height image of 89 % DA ChNF
Figure A.2 AFM height image of 89 % DA ChNF

Figure A.3 AFM height image of 89 % DA ChNF
A.2 79 % DA ChNFs

Figure A.4 AFM height image of 79 % DA ChNF

Figure A.5 AFM height image of 79 % DA ChNF
Figure A.6 AFM height image of 79% DA ChNF

Figure A.7 AFM height image of 79% DA ChNF
Figure A.8 AFM height image of 79 % DA ChNF

A.3 72 % DA ChNFs

Figure A.9 AFM height image of 72 % DA ChNF
Figure A.10 AFM height image of 72 % DA ChNF

Figure A.11 AFM height image of 72 % DA ChNF
A.4 LP Processed ChNFs

Figure A.12 AFM height image of LP processed ChNF

Figure A.13 AFM height image of LP processed ChNF
Figure A.14 AFM height image of LP processed ChNF

A.5 HP Processed ChNFs

Figure A.15 AFM height image of HP processed ChNF
Figure A.16 AFM height image of HP processed ChNF

Figure A.17 AFM height image of HP processed ChNF