DEVELOPMENT OF CHITOSAN BASED NANOCOMPOSITE FILM FOR THE WING MEMBRANE OF BIOMIMETIC MICRO AIR VEHICLES (BMAV)

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ABSTRACT

Biomimetic Micro Air Vehicles (BMAV) are unmanned, micro-scaled aircraft that are bioinspired from flying organisms to achieve lift and thrust by flapping their wings. There are still many technological challenges involved with the designing BMAV. One of these is designing ultra-lightweight materials and structures for the wings that have the mechanical strength to withstand continuous flapping at high frequencies (e.g. 30 Hz for a dragonfly). Insects achieve this by using chitin-based, wing frame structures that encompass a thin, film membrane. The overall objective of this research is to develop an innovative wing membrane for a BMAV, bioinspired from actual dragonfly wings. Chitosan was used as a polymer matrix. Chitin nanowhiskers (CNW) and nanocrystalline cellulose (NCC) were prepared in laboratory and used as reinforcement fillers in the design of two types of nanocomposite membranes. In each type, tannic acid was used as crosslinker for the chitosan matrix. Film samples with different ratios of nanomaterials and crosslinking agent were prepared. The chemical changes, structural properties, and mechanical performance of each sample was measured, analyzed, and compared. Following these initial studies, heat treatment was also investigated to assess its potential for improving the chitosan nanocomposite film. Transmission electron microscopy (TEM) and scanning electron microscope (SEM) confirms the nano-scaled size of nanomaterial produced and reveals the dispersion level of the nanomaterials in the chitosan matrix. Fourier-transform-infrared spectroscopy (FTIR) was used to investigate the molecular interaction of film. X-ray diffraction (XRD) results indicated that the nanocomposite films have a rigid structure. Performance analysis using a universal testing machine (UTM) and nanoindentation machine indicates that, the tensile strength and modulus increase significantly for the crosslinker nanocomposite films. Wettability, moisture content and solubility tests show that the film exhibits elevated water resistant when the additives and heat treatment are introduced. A dragonfly wing frame structure

was also bio-mimicked and fabricated using a 3D printer. The membrane was applied to these BMAV wing frames by a casting method. A flapping generator was used to produce static, flapping motion on these BMAV wings and an actual dragonfly wing (for comparison). The aeroelastic properties of both the BMAV and actual dragonfly wings were examined using two high speed frame camera. Bending angle, wing tip deflection and wing tip twist angle were analyzed at the flapping frequencies of 30 Hz, 60 Hz and 120 Hz.

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ABSTRAK

Kenderaan udara Biomimetic mikro (BMAV) adalah pesawat tanpa pemandu, bersaiz mikro. Ia diilhamkan daripada penerbangan serangga yang boleh terbang dan burung untuk mencapai daya angkat dan daya tujahan dengan meniru cara cara atau prinsip penerbangan dengan mengimbas sayap secara semula jadi. Masih terdapat banyak cabaran teknologi yang terlibat dalam merekabentuk BMAV. Salah satu daripadanya adalah merekabentuk bahan-bahan yang sangat ringan dan struktur sayap yang mempunyai kekuatan mekanikal untuk menahan data tujahan dan daya angak secara berterusan pada frekuensi tinggi (contohnya 30 Hz untuk Pepatung). Secara amnya, Pepatung mencapai matlamat ini dengan menggunakan bahan semula jadi sayap iatu Chitin. Sayap Pepatung mempunyai struktur rangka yang yang nipis dan mempunyai lapisan filem membrane yang amat nipis. Objektif keseluruhan kajian ini adalah untuk merekabentuk membran sayap inovatif bagi BMAV, berdasarkan daripada bahan sayap Pepatung sebenar. Chitosan digunakan sebagai matriks polimer. Nanowhiskers chitin (CNW) dan Selulosa nanokristal (NCC) telah disediakan di makmal dan digunakan sebagai pengisi tetulang dalam rekabentuk dua jenis membran nanokomposit. Dalam setiap jenis film, asid tannic digunakan sebagai crosslinker untuk matriks chitosan. Filem dengan nisbah yang berbeza dan unik telah disediakan menggunakan bahan nano dan ejen crosslinking telah disediakan. Perubahan kimia, sifat struktur dan pretasi mekanikal pada setiap sampel telah diuji, dianalisis dan dibandingkan antara satu sama yang lain. Berikutan kajian-kajian awal, rawatan haba juga disiasat untuk menilai potensinya untuk meningkatkan ciri-ciri mekanikal filem nanokomposit chitosan. Mikroskop Transmisi Elektron (TEM) dan Mikroskop Elektron (SEM) mengesahkan saiz nano bahan yang telah disediakan dihasilkan dan mendedahkan tahap penyebarannya dalam matriks chitosan. Spektroskopi inframerah Fourier (FTIR) telah digunakan untuk menyiasat interaksi molekul filem. Keputusan belauan sinar-x (XRD) menunjukkan bahawa filemfilem nanokomposit mempunyai struktur yang tegar. Analisis prestasi menggunakan Mesin nanoindentation dan mesin ujian tensile (UTM) menunjukkan bahawa, kekuatan tegangan dan modulus meningkat secara signifikan untuk filem-filem nanocomposite crosslinker. Ujian wettability, kandungan kelembapan dan keterlarutan menunjukkan bahawa filem ini mempamerkan sifat-sifat tahan air apabila aditifkan dan rawatan haba diperkenalkan. Struktur rangka sayap dragonfly turut bio-mimicked dan fabrikasi menggunakan pencetak 3D. Membran ini dilekatkan pada bingkai sayap BMAV ini melalui kaedah casting. Sebuah penjana penerbangan sayap telah digunakan untuk menghasilkan pergerakan berasakan aliran bersayap. Kajian dilakukan terhadap BMAV dan sayap pepatung sebenar (untuk perbandingan). Sifat-sifat aeroelastic BMAV dan sayap pepatung sebenar diperiksa menggunakan dua kamera video berkelajuan tinggi. Sudut lenturan, pesongan hujung sayap dan putaran sudut sayap dianalisis pada frekuensi-frekuensi flapping 30 Hz, 60 Hz dan 120 Hz.

DEDICATIONS

With the blessings of Almighty, this thesis is dedicated especially to my beloved father and mother, Mr. Viyapuri Muniandy and Ms. Vijiya Periasamy

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TABLE OF CONTENTS

		PAGE
ABS	ΓRACT	iii
DED	ICATION	vii
ACK	NOWLEDGEMENT	viii
TAB	LE OF CONTENT	ix
LIST	OF FIGURES	xiv
LIST	OF TABLES	xvii
LIST	OF SYMBOLS AND ABBREVIATIONS	xviii
LIST OF APPENDICES		
CHAPTER 1: INTRODUCTION		
1.1	Overview	1
1.2	Problem statement	2
1.3	Preliminary studies	4
	1.3.1 Honey bee	4
	1.3.2 Cicada	5
	1.3.3 Dragonfly	5
1.4	Objectives	6
1.5	Procedures	7
1.6	Outline of thesis	8

CHAPTER 2: LITERATURE REVIEW 10			10
2.1	Introduction		10
2.2	Biomi	metic Micro Air Vehicle (BMAV)	10
	2.2.1	Challenges associated with BMAV	11
	2.2.2	BMAV wing structure and materials	12
2.3	Polysa	accharides as a biocomposite film	14
	2.3.1	Chitin	15
	2.3.2	Chitin nanowhiskers (CNW)	16
	2.3.3	Chitosan	18
	2.3.4 Modification of chitosan		19
	2.3.5	Cellulose	20
	2.3.6	Nanocrystalline cellulose (NCC)	22
2.4	Polym	er crosslink	23
	2.4.1	Tannic acid	25
2.5	Polyla	ctic acid (PLA)	26
CHAPTER 3: RESEARCH METHODOLOGY 28			28

CHAPTER 3: RESEARCH METHODOLOGY		28	
3.1	Introduction	28	
3.2	Preparation of nanomaterial		
	3.2.1 Preparation of CNW	28	
	3.2.2 Preparation of nanocrystalline cellulose (NCC)	30	
3.3	Preparation of nanocomposite film	32	

	3.3.1	Preparation of chitosan nanocomposite film	32
	3.3.2	Preparation of heat treated chitosan nanocomposite film	33
3.4 Fil	m nome	enclature	33
3.5 BN	/IAV wi	ng membrane and wing structure	34
3.6 Ch	aracteri	zation and performance test	37
	3.6.1	Morphological studies by Transmission Electron Microscope	38
	3.6.2	Morphological studies by Scanning Electron Microscope	39
	3.6.3	Chemical interaction studies by Fourier-transform-infrared	
		spectroscopy (FTIR)	40
	3.6.4	Chemical interaction studies by X-ray diffraction (XRD)	40
	3.6.5	Performance testing using Universal Testing Machine (UTM)	41
	3.6.6	Performance testing by physicochemical test	42
	3.6.7	Performance testing by water contact angle test	43
	3.6.8	Performance testing by optical transmittance	43
	3.6.9	Performance testing by electromagnetic actuated flapping wing	
	mecha	nism	43
	3.6.10	Performance testing by nanoindentation test	45
CHAP	TER 4:	RESULTS AND DISCUSSION	47
4.1	Introdu	action	47
4.2	Morph	ological studies (TEM)	47
	4.2.1	Chitin nanowhiskers (CNW)	47

	4.2.2	Nanocrystalline cellulose (NCC)	49
4.3	Morpl	nological studies (SEM)	52
4.4	Chem	ical interaction studies by FTIR analysis	54
	4.4.1	Chitosan film with CNW and tannic acid	54
	4.4.2	Heat treated chitosan film with CNW and tannic acid	57
	4.4.3	Chitosan film with NCC and tannic acid	60
	4.4.4	Heat treated chitosan film with NCC and tannic acid	62
4.5	Chem	ical interaction studies by XRD analysis	64
	4.5.1	Chitosan film with CNW and tannic acid	64
	4.5.2	Heat treated chitosan film with CNW and tannic acid	66
	4.5.3	Chitosan film with NCC and tannic acid	69
	4.5.4	Heat treated chitosan film with NCC and tannic acid	70
4.6	Perfor	mance testing by UTM test	72
4.7	Perfor	mance testing by physicochemical studies	84
4.8	Perfor	mance testing by water contact angle (Wettability)	87
4.9	Performance testing by optical transmittance		
	(UV-v	visible spectrophotometer)	91
4.10	Perfor	mance testing by nanoindentation test	93
4.11	Perfor	mance testing by flapping mechanism	97
	4.11.1	Bending angle	98
	4.11.2	Wing tip deflection	100

4.11.3	Wing tip twist angle	101
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CHAI	PTER 5 – CONCLUSION	103
5.1	Summary	103
5.2	Other uses of film beside BMAV	105
5.3	Recommendation for future research	106
REFE	CRENCES	108
LIST	OF PUBLICATIONS CONFERENCE PRESENTATION	
AND	PATENTS	122
APPE	NDICES	124

LIST OF FIGURES

		PAGE
Figure 1.1	Illustration of the overall idea to develop BMAV wing membrane	8
Figure 2.1	Chemical structure of chitin	16
Figure 2.2	Procedure for the preparation of polymer/CNW nanocomposites by casting-evaporating method	18
Figure 2.3	Chemical structure of chitosan	19
Figure 2.4	Arrangement of fibrils, microfibrils and cellulose in cell walls	21
Figure 2.5	Chemical structure of cellulose	22
Figure 2.6	Illustration of crossliking process in a polymer;(1) Crosslinking agent is introduced in polymer matrix,(2) Growth and branching,(3) Fully cured polymer	24
Figure 2.7	Chemical structure of tannic acid	26
Figure 3.1	Schematic diagram of CNW production by hydrolysis process	29
Figure 3.2	Schematic representation of the preparation of NCC starting from MCC showing the surface esterification reaction introducing sulfate groups	30
Figure 3.3	Dark colored solution in the central conical flask depicts the "burned" sample; The most right conical flask contains a correct sample	31
Figure 3.4	3D model of simplified dragon wing structure;(a) Top view fore wing, (b) Iso view forewing,(c) Top view hind wing, (d) Iso view of hind wing,(e) Fabricated dragonfly wing	36
Figure 3.5	Wing structure immersed in chitosan nanocomposite Solution; (a) Fore wing, (b) Hind wing	37
Figure 3.6	SEM film samples coated with gold prior viewing	39
Figure 3.7	Flapping wing mechanism setup	44
Figure 3.8	Graph of indention load P; Penetration depth h; showing the process of loading and unloading of the nanoindenter.	46

Figure 4.1	TEM images of nano-scale chitin whiskers in dilute HCI, (a) Magnified at 500 nm with measured length, (b) Magnified at 200 nm with measured width	49
Figure 4.2	TEM images of NCC in aqueous medium, (a) magnified at 500 nm with measured length, (b) magnified at 500 nm with measured width	51
Figure 4.3	SEM micrograph of surface (a-d): (a) Set I; (b) Set II (B); (c) Set III (C); and (d) Set IV (F); Cross-section (e-h): (e) Set I; (f) Set II (B); (g) Set III (C); and (h) Set IV (F)	54
Figure 4.4	FTIR absorption spectra of pure chitosan film (Set I)	55
Figure 4.5	FTIR spectra of;(a) chitosan matrix with CNW, (b) chitosan matrix with CNW and tannic acid	57
Figure 4.6	FTIR spectra of treated and untreated samples, (a) Set I, (b) Set II (A), (c) Sample III (A), (d) Set IV (A)	60
Figure 4.7	FTIR spectra of: (a) non-crosslinked chitosan film, (b) crosslinked chitosan film (40mg), (c) Comparison of similar dosage between CNW and NCC	62
Figure 4.8	Comparison between CNW and NCC heat treated chitosan film: (a) Without crosslinker, (b) with crosslinker	64
Figure 4.9	XRD diffractograms of selected CNW based chitosan film	65
Figure 4.10	XRD diffractograms of CNW based chitosan film, (a) Set I, (b) Set II (A), (c) Set III (A), (d) Set IV (A)	68
Figure 4.11	Comparison of X-ray diffractograms of chosen sample sets	70
Figure 4.12	XRD diffractograms of : (a) Set III (A) and Set IV (A)	71
Figure 4.13	Average mechanical properties of pure chitosan (Set I) and crosslinked chitosan film (set II), (a) Tensile strength, (b) Elongation at break, (c) Young's Modulus	74
Figure 4.14	Mechanical properties of CNW based chitosan film with and without crosslinker; (a) Tensile strength, Elongation at break, (c) Young's Modulus	76

Figure 4.15	Mechanical properties of NCC based chitosan film with and without crosslinker; (a) Tensile strength, (b) Elongation at break, (c) Young's Modulus, (d) Comparison of tensile strength between CNW and NCC based chitosan film	80
Figure 4.16	Comparison of both heat treated and non-treated nanocomposite film; (a) Tensile strength, (b) Elogation at break, (c) Young's Modulus	83
Figure 4.17	Physicochemical characterization of all sample sets; (a) Moisture content of samples under equilibrium condition at 25 °C and 65% Relative Humidity, (b) Solubility of samples	85
Figure 4.18	Moisture content of both treated and untreated samples ; (a) CNW based chitosan film, (b) NCC based chitosan film	87
Figure 4.19	Water contact angle of; (a) Set I, (b) Set II (A), (c) Set III (A-CNW), (d) Set IV (A-CNW), (e) Set III (A-NCC), (f) Set IV (A-NCC), (g-l) Heat treated counterpart	90
Figure 4.20	Water contact angle of actual dragonfly wing	91
Figure 4.21	Appearance of all sample sets; (a) Set I; (b) Set II (A); (c) Set II (B); (d) Set III (A); (e) Set III (B); (f) Set III (C); (g) Set IV (A); (h) Set IV (B); (i) Set IV (C); (j) Set IV (D); (k) Set IV (E); (l) Set IV (F)	92
Figure 4.22	UV-visible spectrophotometer of chosen sample sets	93
Figure 4.23	Points of nanoindentation test; (a) Actual dragonfly forewing, (b) Actual dragonfly hindwing, (c) BMAV forewing, (d) BMAV hindwing	95
Figure 4.24	Bending angle of actual dragonfly wings and BMAV wing	99
Figure 4.25	Wing tip deflection of actual dragonfly wings and BMAV wing	100
Figure 4.26	Wing tip twist angle of actual dragonfly wings and BMAV wing	102

LIST OF TABLES

		PAGE
Table 3.1	Nomenclature of chitosan nanocomposite for Batch 1	34
Table 3.2	Nomenclature of chitosan nanocomposite for Batch 2	34
Table 3.3	Specification of the wing structure	37
Table 4.1	Fore wing comparison between dragonfly wing and simplified BMAV wing	97
Table 4.2	Hind wing comparison between dragonfly wing and simplified BMAV	97
Table 5.1	Benchmarking of chitosan based nanocomposite film with pure chitosan film and actual dragonfly membrane.	106

LIST OF SYMBOLS AND ABBREVIATIONS

Symbols and Abbreviations	Full name
UAV	Unmanned aerial vehicle
MAV	Micro air vehicle
VTOL	Vertical take-off and landing
BMAV	Biomimetic micro air vehicle
DA	Acetylation
FMAV	Fixed wing
GlcN	2-amine 2-deoxy- D - glucopyranoside
GlcNAc	2-acetamide-2-deoxy-D-glucopyranose
Hz	Hertz
Re	Reynolds number
G	Gravity
PLA	Polylactic acid
CNW	Chitin nanowhiskers
NCC	Nanocrystalline cellulose
UM	Universiti of Malaya
g	Grams
ml	Millilitre
Ν	Normality

HCl	Hydrochloric acid
h	hour
°C	Degree Celsius
RPM	Revolutions per minute
RMAV	Rotary wing
Min	minutes
Cm ³	centimetre cube
Wt %	weight percentage
MCC	Microcrystalline cellulose
H_2SO_4	Sulphuric acid
%	percentage
v/v	volume per volume
mg	milligram
micron	micrometre
Н	heat treated samples
ТА	Tannic acid
CAD	Computer aided design
3D	three dimensional
mm	millimeter
SEM	Scanning electron microscope
TEM	Transmission electron microscope

FTIR	Fourier Transform Infrared Spectroscopy
XRD	X-Ray Powder Diffraction
UTM	Universal Testing Machine
UV	Ultra violet
μm	micrometer
cm ⁻¹	per centimeter
Cu Ka	Copper K-alpha
Å	Angstrom
kV	kilo volt
mA	milliamp
θ	theta
•	Degree
Ν	Newton
ASTM	American Society for Testing and Materials
RH	Relative humidity
Ws	Weight of swollen sample
\mathbf{W}_{i}	Weight of sample after heating
S	Seconds
μL	microliter
nm	nanometer
Tr	Optical transmittance

kW	kilowatt
fps	frames per second
NH_3^+	Amino group
O-H	Oxygen-hydrogen bond
N-H	Nitrogen-hydrogen bond
C-O-C	Carbon-oxygen-carbon
CH ₃	methyl
C-N	Carbon-nitrogen bond
MPa	Mega Pascal
GPa	Giga Pascal
D _{max}	Delfection

LIST OF APPENDICES

PAGE

Appendix A	Force displacement graph of dragonfly fore wing	124
Appendix B	Force displacement graph of dragonfly hind wing	127
Appendix C	Force displacement graph of BMAV fore wing	130
Appendix D	Force displacement graph of BMAV hind wing	133

CHAPTER 1: INTRODUCTION

1.1 Overview

Unmanned air vehicles (UAV) are unmanned, aircraft that is flown remotely by pilot at a ground station. Although basic models were first deployed in 1916 (during World War I), they only came into prominence for military use in the last 20 years (Yahyanejad & Rinner, 2015). They have also become increasingly popular for civil applications (e.g. agricultural modeling, land use planning, industrial site surveys, film production, etc.). Recent advances in rapid development and miniaturization technologies has allowed a new class of micro-scaled UAVs to be developed, called Micro Air Vehicles (MAV). In a Broad Agency Announcement (BAA 97-29), the US Defense Advanced Research Projects Agency (DARPA) defined MAV as being less than 15 cm in any dimension. Later in 2005 (BAA 06-06), DARPA defined nano air vehicles (NAV) as being no larger than 7.5 cm or heavier than 10 g (carrying a 2 g payload) (Ward et al., 2015). Numerous MAV concepts have been proposed since then. These can be categorized into three types of MAV: fixed wing, (FMAV), rotary wing (RMAV), and biomimetic or flapping wing (BMAV).

The focus of this research is BMAV (also called an ornithopter). This concept has been discussed for centuries, most famously in 1485 by Leonardo da Vinci in his theoretical ornithopter designs (Reay, 1977). Despite this long history, a working operational ornithopter (or BMAV) could not be achieved before now, because the level of technology was insufficient. A BMAV is a micro-scaled (or nano-scaled) aircraft that biomimics the flapping wing motion of small biological flying organisms (e.g. insects, bats, or birds) to generate lift and thrust (Dietl & Garcia, 2013). Like their biological counterparts, BMAV are highly maneuverable and ultra-lightweight. Their small size and weight along with their potential flight agility, make them capable of flying in confined areas and even indoors. The vortices produced by flapping wing allow them to generate aerodynamic forces at higher magnitudes than fixed wings. This means that flapping wings are able to produce as much lift as a larger (surface area), fixed wing (Maxworthy, 1981). So BMAV are potentially the smallest of all the MAV types. However, the technological complexities involved with BMAV designs are much greater than FMAV or RMAV. This means that unlike the other two types, there are currently no BMAV in operational service yet.

BMAV are envisioned to carry ultra-lightweight, compact electronics and surveillance detection equipment as a primary payload. They can be deployed by a single operator, and relatively inexpensive to fabricate. BMAVs are envisioned for use on civil and military missions that are of a limited duration, such as (Wu et al., 2014; Nguyen, Ha, & Lee, 2015)

- Remote sensing of hazard sites (i.e. chemical spill, radiation, high voltage area)
- Indoor/Outdoor videography
- Police and military surveillance

1.2 Problem statement

As BMAV is a new generation of miniaturized aircraft, there is an unlimited potential of research can be carried to harness its potential. BMAV bio inspired from flying insects are of great interest since insects have a maneuvering agility and can fly in compact areas. Flying insects have a simpler control processes then birds and bats have a musculature system along the span of their wings. With these advantages, flying insects have been the focus of much attention in the research field.

Some of the challenges associated with bio mimicking flying insects is, generating high flapping frequencies raising from 30 Hz for a dragonfly to 300 Hz for a housefly (depending on the species) in the sensitive Reynolds number (Re) region of less than 10000 (Levy & Seifert, 2010; Rajabi, Moghadami, & Darvizeh, 2011). The wing structure and membrane of the BMAV must be flexible but strong enough to endure the aerodynamic forces produced by flapping motion. During flight, the flying insect wings undergo significant bending and twisting deformations that can alter the direction and magnitude of the aerodynamic forces being generated. Therefore, the artificial wing is a critical component of BMAV.

Research conducted by Combes, (2010) who studied the material and structure of varieties of insects as an inspiration for BMAVs concluded that, although the primary function of most insects wings is to generate aerodynamic forces, their wing design may reflect trade-offs or specialization for other aspects of flight performance, such as efficiency, versatility, maneuverability, or stability. In general, the mechanical properties decline with decreasing volume. Since insect wings are very small compared to birds, mimicking them is a challenging and exciting task. Overall objective of this research is to analyze and study the natural materials that a variety of which flying insects are composed.

Studying this topic in Malaysia is advantages since the biodiversity of Malaysian tropical forest assures abundant insects to investigate. My initial focus was to understand active flying insect's wing materials and structures. Ideal candidates to bio mimic are dragonfly (*Anisoptera*), Honey bees (*Apis*) and Cicada (*Cicadidae*). These active flyers can be found in urban areas as well as the jungle. These insects in particular have shown peculiar ability to navigate in these difficult locations on a regular basis due to their high

agility. They have the flying characteristics that is useful if mimicked for BMAV to navigate through urban environments, building interiors and difficult or dangerous locations.

Besides having a durable wing membrane material, it is envisioned that future BMAV will be deployed using swarm technology in one-way (disposable) missions (Kushleyev et al., 2013). Swarm technology utilizes networking capability to communicate into each other. Payloads such tiny camera and sensor deemed to be very effective by sweeping over a wide area of coverage using this technology. Therefore, this requires the artificial wing structure and membrane to be composed of biodegradable and biocompatible materials so not to pose risk to the biosphere.

1.3 Preliminary studies

Inspired by biomaterial and their unique properties, this study was conducted to produce a high strength bio based film. This film would be used for the application of BMAV wing membrane. From preliminary research, all the potential flying insects wing (Dragonfly, Honey bee and Cicada) are made of chitin material embedded with proteins (Kaya et al., 2015; Sajomsang & Gonil, 2010; Hou et al., 2015). Their wing design and profile is unique to each other and contributes to their flying skills.

1.3.1 Honey bee

Honey bees spend most of their time flying to retrieve nectar and pollen from flowers. The flapping frequency of honey bees is in the range of 180 Hz to 250 Hz (Rittschof & Seeley, 2008). They take off with an explosive buzz sound initially to overcome body weight by sudden boost of flapping frequency. The wings are small in proportion to their size, which is why they need to flap at such a high frequency. Since the flapping frequency of honey bees is very high, it is difficult to artificially replicate their flapping frequency. Furthermore, the wing size is relatively smaller then cicada or dragonflies, so honey bees were not considered as a candidate for bio mimicking in this research.

1.3.2 Cicada

Cicada have prominent eyes set wide apart with short antennae and membranous front wings. They are relatively large insects and flaps its wing in a figure of 8 motion (Jaderic & Huang, 2011). They possess a larger upward protruding wing design that is larger than their body (Luo & Wei, 2015). Cicadas are not an active flyers, rather can better described as long hoppers. Very few researchers have used Cicada as a reference for a BMAV wing design since they have a large weight-to-wing ratio (Jaderic & Huang, 2011; Xinyan, Schenato, & Sastry, 2006). Since the complex flapping mechanism of cicada is challenging, I have opted not to focus on them for bio mimicry.

1.3.3 Dragonfly

Dragonflies are highly agile flyers (Nagai et al., 2009; Thomas et al., 2004). These flying insects are capable of migrating across oceans, moving in any direction, and suddenly changing course. They can hover and accelerate quickly both from dead stop as well from a hovering attitude. Their wings are long, narrow and significantly larger than their body. Dragonfly wings possess a remarkably high power/weight ratio. A study conducted by Chen et al., (2013), reported that a dragonfly can accelerate at 4G (G-Force) linearly and 9G in sharp turns while pursuing prey. Sunada, Zeng and Kawachi 1998; Dickinson, Lehmann and Gotz, (1993) revealed that the wing motion of dragonfly wing is controlled by the wing root muscle only, which is easier to mimic in a BMAV then the wing motions used by other insects. These qualities makes dragonfly a preferred candidate to be bio mimicked in a BMAV.

1.4 Objectives

Overall objective of this research is to develop a bio-based thin film as a membrane for BMAV using natural occurring polysaccharides. A literature review will be carried out on a variety of insects in Malaysia for their flying ability and harnessing their natural material to mimic its mechanical properties for the usage BMAV wing membrane. My driving criteria is that the wing membrane must match or surpass the mechanical properties of an actual dragonfly wing membrane. Chitosan (which is a deacetylation of chitin) was selected as the matrix for the wing membrane. Methods for enhancing chitosan were studied extensively to match the mechanical properties of actual dragonfly membrane. Nanomaterial and crosslinking were incorporated into chitosan matrix and their role in molecular interaction and alteration of properties was examined. Once membrane is created, a Polylactic acid (PLA) based material will be used as a wing frame structure and further tests will be conducted and compared with actual dragonfly wing. Listed below are the primary objectives that must be fulfilled in order to design a new BMAV wing membrane:

- Process and fabricate chitosan nanocomposite membranes for the wing of a dragonfly bio inspired BMAV
- Examine the mechanical, physical and chemical properties of the membrane
- Apply membrane on a dragonfly like wing frame structure and investigate its nanomechanical properties and aeroelasticity

6

1.5 Procedures

Figure 1.1 illustrates the overall idea of the development of wing membrane for BMAV. Each process will be discussed in detail in the methodology section. The summary of procedures conducted in this experiment is listed below:

- 1. Mimicking selected insect's wing membrane material artificially with chitosan as a film matrix.
- Enhancement of chitosan bio film by means physical and chemical reinforcement.
 This is accomplished by:
 - i. Embedding nano material into chitosan matrix
 - (a) 1st batch by using chitin nanowhiskers (CNW)
 - (b) 2nd batch by using nanocrystalline cellulose (NCC)
 - ii. Crosslinking the chitosan matrix using tannic acid
 - iii. Investigating the heat treatment effects of the nanocomposite film
- 3. Effects of CNW, NCC, tannic acid and heat treatment for both CNW and NCC are studied using characterization facilities (available throughout the University of Malaya).
- 4. An actual dragonfly wing will be tested and measurement on nanomechanical properties will be compared to my artificial BMAV wing.
- 5. Aeroelastic properties of an artificial BMAV dragonfly wing will be compared with an actual dragonfly wing using a passive flapping system generator test rig build in Aerospace Laboratory in Department of Mechanical Engineering, Faculty of Engineering, UM.



Figure 1.1: Illustration of the overall idea to develop BMAV wing membrane

1.6 Outline of thesis

There are five chapters; Introduction, Literature Review, Methodology, Result and Discussion and Conclusion. A summary on each topic is listed in the bullet points below:

- Introduction. This chapter introduces BMAV and all their potential applications. It provides an overview of this research and lists the primary objectives. It also lists layout of the procedure for accomplishing these objectives.
- Literature review. This chapter reviews past research to show how this original research builds upon what has been done. Also information gained from past research is used to design experiments discussed later in the methodology chapter.
- **Methodology.** The experiments are discussed extensively in this chapter. Sufficient detail is given so that future readers can replicate this work. It discusses the production of nanomaterials (both CNW and NCC), processing of chitosan thin film, heat treatment procedure, crosslinking method, the fabrication of the

wing structure and flapping mechanism. The characterization and parameters used to assess the nanocomposite film and actual dragonfly wing membrane and structure were also discussed.

- **Result and discussion.** All the data acquired from the experiments are discussed and assessed in this chapter. When possible, these results are compared with data collected by other researcher. Discussion were elaborated and validated by citing previously publish articles by other researcher.
- **Conclusion.** This chapter summarizes this research. It also lists the experienced in the work and suggests improvements that can be done in future studies.

9

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter covers the literature that was reviewed and assisted in designing the experiments. First a review of BMAV is discussed. Following this, is a review of the polysaccharides used in my experiments. Other relevant research work is also discussed in this chapter.

2.2 Biomimetic Micro Air Vehicle (BMAV)

BMAV are bio inspired from the flying insects. Flapping wings generate more lift per wing surface area than FMAV or RMAV. This enables them to be smaller than the other types. They are potentially more agile than FMAV (e.g. hover, forward or reverse flight). This qualities makes researcher to concentrate on developing BMAV. Maxworthy (1981) investigated flapping wing motion of insects, birds and bats and used established aerodynamic principles to calculate the forces resulting from flapping motion. The main challenge lies on the understanding of how insect and birds flies and maneuvers rapidly by investigating on the wing stroke kinematics that alters the forces and moments being generated from the flapping motion. Potential flying organisms studied by researchers includes; humming bird, hawk moths, dragonflies and beetles. Berg and Rayner (1995) have studied wing body relationship by examining diving birds and establish a proportional relationship between wing mass, size, and the torque needed for a desired angular acceleration.

2.2.1 Challenges associated with BMAV

One article surveyed literature regarding MAV by gathering statistical data and made an assessment on the primary challenges facing by future MAV (Pines & Bohorquez, 2006). The challenges described were:

- The need for ultra-lightweight and biologically inspired materials and structures
- BMAV operates in a very sensitive Reynolds number regime (100 to 10000)
- Lack of analysis tools to accurately model the steady and unsteady aerodynamic environments that BMAV encounter while in flight
- The need for micro propulsion systems and power sources
- Miniaturized flight navigation and control
- Lack of system engineering tools to produce very small scale components

This thesis addresses the first challenge (listed above) in developing ultralightweight and biologically inspired materials as a wing membrane for dragonfly-like wing frame structure. Research on new ultra-lightweight wing materials and structures are critical for BMAV, because the largest and most critical structure is the wings. Some of the challenges associated with wing membrane and wing structure design are its ability to generate lift, withstand high flapping frequencies (30 Hz), be ultra-lightweight and possess high tensile and modulus.

A statistical data released by Pennycuick (1969) on the flight velocity versus size relationship of a wide range of birds, the wing flapping frequency versus the wing length (Greenewalt, 1962) and wing flapping frequency versus mass (Azuma, 1992) for birds and insects was given by these following relations:

$$U=4.77m^{1/6} \tag{2.1}$$

$$U_{(upper bound vertical)} = 11.7^{-0.065}$$

$$(2.2)$$

$$U_{(lowerbound vertical)} = 9.6m^{-0.043}$$
(2.3)

By combining Equation (2.1)-(2.3), a plot of wingtip speed and flight speed versus mass of insects and birds was generated (Pornsin-sirirak et al., 2001). It was revealed that, smaller birds and insects fly in an unsteady- state regime as oppose to quasi steady-state as most of the flying organisms do. This means that wing tip speed is faster than their flight speed for insects and small birds. This creates vortices during down stroke generating a high lift coefficient. Thus, the wing structure and membrane must able to withstand the vortices generated from the flapping motion.

2.2.2 BMAV wing structure and materials

Several comprehensive studies have been published on the mechanical properties of an insect's wing. Song et al., (2004) studied the mechanical properties of the forewing Cicada. They use nanoindentation technique to find localized hardness and modulus on the various section of the wings. It was reported that the mean Young's modulus and hardness of Cicada is 3.7 GPa and 0.2 GPa. Kim et al., (2008) uses graphite/epoxy composite as a wing structure and Polyvinyl chloride (PVC) as the wing membrane. They studied the aerodynamic force generated using static flapping device and a load cell. PVC wing membrane have a tensile strength of 25 MPa. Agrawal and Agrawal (2009) have successfully bio-mimicked hawkmoth wings and study the aerodynamic performance. The use commercially available material (carbon, nylon and rubber) as a vein and latex as a membrane. They have measured the mechanical properties of their artificial hawkmoth wing (200 GPa for carbon rod, 6 GPa for nylon and 1.7 MPa for the wing membrane having a thickness of 0.1 mm.) Their results showed an increase in thrust when the wings are flexible compared to rigid wing for all kinematic patterns. In is very important to have a functional artificial veins (structures) on BMAV rather than simple leading edge rod design as a vein. A study conducted by Dirks and Taylor (2012) reported that having membrane only design for BMAV wing will yield a low toughness wing properties. The addition of cross veins increases the wing toughness by 50%. Veins act as a barriers by stopping cracks from propagating.

Research on the wing structures of insects have primarily focused on dragonfly species, since their ability to hover, fly forward or backwards is desired for BMAV application. A study conducted by Sudo et al., (1999) reveals the effect of wing morphology and flapping kinematics on the aerodynamics of dragonfly flights. One article examined the flexibility of a dragonfly's wing as it passively deformed. This is an important feature in generating lift. Data from stress relaxation experiments was used to characterize the biomaterial properties of insect wings (Bao et al., 2006).

Researchers have successfully mimicked several different artificial insect wings that has a wing dimension of less than 2 cm in length. These complex wing structures have been fabricated using photolithography techniques to create a positive relief build up method (Pornsin-sirirak et al., 2001). Research on BMAV wing materials is relatively new. Novel materials based on polysaccharides and other materials are being studied in various institutions around the world. MAV created by Pornsin-sirirak et al., (2001) have titanium alloy (Ti-6Al-4V) as a wing structure and thin Parylene-C, film as a wing membrane. Force generated from this wing were recorded using nano-load cells. Their wing design has a remarkable mechanical properties. The membrane composed of Parylene-C reported to have 70 MPa tensile strength and 3 GPa of modulus. They have successfully flown their MAV. Kumar et al. (2014) created a humming bird inspired wing design made of carbon fiber as a structure and polyethylene as a wing membrane. The polyethylene film with adhesive was laminated on the structure of the wing at 150°C. They conducted a static flapping test to study the damping characteristic.

Research into structures and materials specifically for BMAV is still at an early stage. Few researchers have established a solid building platform on structural and material designs that will improves the efficiency of BMAV wings. However, research on developing new bio-inspired wing membranes and frames is advancing as new materials are synthesized particularly for BMAV applications.

2.3 Polysaccharides as a biocomposite film

Polysaccharides are complex carbohydrate polymers consisting of two or more monosaccharaides linked together covalently by glycosidic linkages in a condensation reaction. Most are insoluble in water. Starch, glycogen, chitin and cellulose are a few examples of polysaccharides abundantly available in nature. Chitin and cellulose are regarded as one of the most abundant biomaterials on earth. They are low cost, environmentally friendly, renewable and biodegradable (Muzzarelli, Mehtedi & Mattioli-Belmonte, 2014). Chitin and cellulose are classified as structural polysaccharides, meaning that these materials act as a natural reinforcement structures. The need for high performance sustainable and biodegradable composite materials has driven many researchers to develop strong and rigid materials for structural and packaging requirements. A review conducted by Vilaplana, Strömberg and Karlsson (2010) listed out raw materials that sustainable and biodegradable as well:

- Biopolymers extracted from renewable natural resources, such as starch, cellulose, proteins, or chitin
- Polymers produced by microbes, such as poly (hydroxyalkanoates)

14
- Polymers synthesized from chemical or enzymatic processes, where the monomers were obtained from renewable resources
- Recycled synthetic polymers that can be upgraded for new applications by the addition of natural reinforcements such as polysaccharides.

Polysaccharide based composites offer the potential to replace non-biocompatible synthetic polymers. (Šimkovic, 2008) expressed the importance of utilizing abundantly available polysaccharides such as chitin and cellulose into a green composite as a successor for synthetic materials. Although some biodegradable synthetic materials exist (such as polyhydroxybutyrate), this materials are more expensive to produce when compared to cellulose or chitin. (Šimkovic, 2013) studied the potential of "allpolysaccharide" composites. They have concluded that composite made solely from polysaccharides are most ecological since blending process between two identical polysaccharides requires less chemicals and work.

2.3.1 Chitin

Chitin is the second most abundant polysaccharide in the world. This structured polysaccharide is usually found in the stiff extracellular coatings on arthropod exoskeletons and insect cuticle and wings (Muzzarelli, 2011). Chitin acts as a natural reinforcement in the form of crystalline nano-sized fibrils embedded in a protein matrix (Salaberria et al., 2015). Figure 2.1 shows the chemical structure of chitin. It is a long chain polymer composed of 2-acetamide-2-deoxy-D-glucopyranose (GlcNAc) units linked by β -(1-4) glycosidic bonds (Möller et al., 2004).

Muzzarelli, (2011) have examined the many potential benefits of chitin for biomedical applications because of its sturdy mechanical properties and biocompatibility for human applications. The polymer forms a highly-structured crystalline network and exists in nature in two allomorphic consisting of: α , β . The α -polymorph is the most abundant and is characterize by antiparallel packing of polysaccharide chains. It is more stable than the β types (Mathew, Laborie, & Oksman, 2009). Despite its wide availability, the utilization of chitin has been restricted by its intractability and insolubility. Pillai, Paul, and Sharma, (2009) reviewed the chemistry, solubility and fiber formation of chitin and mentioned that the difficulty in solubilization of chitin was due to the highly extended hydrogen bonded semi-crystalline structure.



Figure 2.1: Chemical structure of chitin

2.3.2 Chitin nanowhiskers (CNW)

Chitin taking the form of nano-sized fibrils act as a natural reinforcement structure. It intrinsically has the potential to be converted to nanoparticles and nanowhiskers dubbed chitin nanowhiskers (CNW). These nanowhiskers can be prepared from chitin through hydrolysis in a strong acid aqueous medium. Marchessault, Morehead, and Walter (1959) for the first time reported a route for preparing suspension of chitin crystallite particles. In this method, purified chitin was treated in 2.5 Normality (N) hydrochloric acid (HCl) solutions under reflux for 1 h, then the excess acid was removed, and then distilled water was added to obtain the CNW suspension. They found that acid- hydrolyzed chitin spontaneously dispersed into a rod-like particles.

Instead of using chitin, which is insoluble to organic solution, the hydrolyzed chitin (CNW) has potential as a reinforcing material for various application. Numerous articles have been publish about the importance of CNW as a reinforcing material. Zeng et al. (2012) reported that chitin whisker have a very high longitudinal and transverse modulus of 150 and 15 GPa, respectively. Processing techniques have an important influence on the final properties of nanocomposite. CNW can homogeneously disperse in water. Therefore the best processing technique to make a nanocomposites is by dispersing CNW in a polymer aqueous solution or polymer latex to obtain homogeneous dispersion. This can then be casted in a container. By evaporation of water, a dried CNW based nanocomposite can be obtained. Figure 2.2 illustrates the procedure for the preparation of polymer/CNW nanocomposites. Casting evaporation is simple and economical. Most of the recent work reported on the preparation of CNW based nanocomposites were prepared by this method. Paillet and Dufresne (2001) reported using CNW to increase the mechanical properties of thermoplastic. Gopalan Nair and Dufresne (2003a); Gopalan Nair and Dufresne (2003b); Gopalan Nair et al. (2003) use crab shells and hydrolyze it to produce CNW. This nanoparticles then dispersed into natural rubber to improve its properties.



Figure 2.2: Procedure for the preparation of polymer/CNW nanocomposites by casting-evaporating method

2.3.3 Chitosan

Chitosan is a biodegradable and biocompatible natural polysaccharide composed of linear polysaccharide β - 1, 4 -linked 2-amine 2-deoxy- D - glucopyranoside (GlcN) (Birolli, Delezuk & Campana-Filho, 2016). Figure 2.3 shows the chemical structure of chitosan. The difference between chitosan and chitin is that, in chitin, the GlcNAc units are largely predominant (>80%) while chitosan is richer in GlcN (>60%). This difference in contents of GlcNAC and GlcN units is expressed as the average degree of acetylation (DA). This strongly affects the arrangement of polymeric chain as well as the physical and chemical properties (Domard, 2011). When the degree of deacetylation of chitin reaches about 50% (depending on the origin of the polymer), it becomes soluble in aqueous acidic media. The solubilization occurs by the protonation of the –NH₂ function on the C-2 position of the D-glucosamine repeat unit. Chitosan is converted to a polyelectrolyte in acidic media (Rinaudo, 2006).



Figure 2.3: Chemical structure of chitosan

Being soluble in aqueous solutions, it is largely used in applications such as; solutions, gels, films and fibers. Chitosan has excellent film forming capability which gives it great potential for use in many applications. However, the poor mechanical properties of neat chitosan make it unsuitable for applications that needs durability and strength.

2.3.4 Modification on chitosan

Chitosan is much easier to process than chitin, but the stability and mechanical properties is generally lower. Chitosan is inclined to be much hydrophilic than chitin. Various techniques have been developed to control the mechanical and chemical properties. Chitosan can be crosslinked by many reagents. Wei et al., (1992) crosslinked chitosan fibers with epichlorohydrin, Welsh and (2003) used diisocyanate as a crosslinking agent for chitosan to improve the mechanical properties of hydrogels. Roy, Todd and Glasser (1998) also use crosslinking method to enhanced the mechanical properties of hydrogel by crosslinking chitosan with 1,4-butaediol diglycidyl.

Blends and nanomaterials have also been incorporated into chitosan matrix as mentioned by Hirano, 2001. Also recent advances have been made by introducing carbon nanotubes. Composite films exhibit a very significant increase of tensile modulus by incorporating 0.8% of multiwalled carbon nanotubes into chitosan matrix (Wang et al., 2005). Chitosan can be modified to have an improved mechanical properties while retaining its biodegradability. A research conducted by Burger and Fredericksen (1948) use carboxylic anhydrides with different chain lengths on chitosan yield chitosan based derivatives that is totally insoluble in water with lower biodegradability. Besides these blends, there are also reporting of chitosan being blended with cellulose, polyethylene, polyvinyl chloride and polyvinylpyrrolidone(Hosokawa et al., 1990; Mucha, Piekielna, & Wieczorek, 1999; Abou-Aiad et al., 2006).

2.3.5 Cellulose

Cellulose is the most abundant biopolymer in the world. Cellulose can be found as a basic building block in plants, cotton and some marine creatures. Often this naturally occurring cellulose takes the form of microfibrils that act as a natural reinforcing structure (Moon et al., 2011). Since cellulose is the main building blocks for plants, its availability almost inexhaustible and has a fascinating structure and properties. In fact cellulose makes tree trunks strong enough to hold up the tallest trees. Some species of bacteria even secrete cellulose to form a biofilm that act as a protective layer Figure 2.4 shows the arrangement of fibrils, microfibrils and cellulose in cell walls.



Figure 2.4: arrangement of fibrils, microfibrils and cellulose in cell walls

Cellulose is another natural linear carbohydrate polymer. Cellulose consist of long chain polysaccharide composed of β -1, 4 linked 2-amino-deoxy- β -D-glucan (Moon et al., 2011). Figure 2.5 shows the chemical structure of cellulose. Cellulose are known to have an excellent reinforcement, renewable and biodegradable properties. The macrofibers of cellulose in a plant are composed of microfibrils, which are formed by endless of nanofibrils of cellulose. Interestingly, the nanofibrils of cellulose have crystal and amorphous structures. The crystal part of cellulose cannot be broken due to strong hydrogen bond of hydroxyl groups in cellulose. Moon et al. (2011) has gathered data and elucidate that the fibrils can be generally separated into amorphous and/or crystalline components, by mechanical or chemical enzymatic processes.



Figure 2.5: Chemical structure of cellulose

2.3.6 Nanocrystalline cellulose (NCC)

Nano-sized single crystal cellulose (commonly referred as nanocrystalline cellulose (NCC), nanowhiskers cellulose or nanofibrils cellulose) can be extracted from various cellulose sources by the acid hydrolysis process (de Souza Lima & Borsali, 2004). NCC has several polymorphs, the most common are cellulose I and II. Cellulose I is the crystalline cellulose that is naturally produced by living organism, which is sometimes referred to as natural cellulose. Cellulose II is the most stable crystalline structure and can be produced by regeneration. Nanocrystalline cellulose (NCC) exhibits highly crystalline nanometer sized rod-like particle prepared as an aqueous colloidal suspension. Chu et al. (2014) states that, NCC has very high modulus, dimensional stability, low thermal expansion, outstanding reinforcement potential and transparency. Marchessault, Morehead and Walter (1959) produced NCC for the first time in 1959 using sulfuric acid (H₂ SO₄). These high aspect ratio rod-like particles exhibit colloidal behavior. The method was same as preparing CNW. The use of NCC as a reinforcement material has been proven to increase the mechanical and barrier properties. Recent investigation by Wu, Moon and Martini (2013) uses atomistic simulations with both standard uniform

deformation approach and a complementary approach based on nanoscale indentation to find the modulus of NCC. They reported that the modulus value to be 129.5 GPa which is similar to Kevlar material. Another researcher, Dri et at. (2013) published an article on cellulose in tandem arrangement and used quantum mechanics to compute the Young's modulus of NCC. It shows a high modulus value of 206 GPa, which is equivalent to steel.

Research into cellulose based films have gained considerable amount of attention. Šimkovic (2013) mentioned that the colloidal behavior of NCC particles due to carboxyl group introduced by air oxygen oxidation assure uniform and homogeneous settlement of particles in a dry film. Leung et al. (2013) utilizes NCC as a reinforcing agent for plastic and hydrogel by forming a percolation network and hydrogen bonding between the filler material and the polymers. A chitosan-nanocellulose biocomposite film was prepared by Dehnad et al. (2014) yields and enhanced thermal and biodegradable properties. Elanthikkal et al. (2013) studied the effects of cellulose whisker loading into poly(ethylene-co-vinyl acetate) matrix. The development of the composite film showed superior thermal and mechanical properties then the pure counterpart.

2.4 Polymer crosslink

Polymers can be crosslinked to alter the chemical and physical properties. This process initiated by inducing heat, pressure, change in pH and radiation on the polymer matrix with the presence of crosslinking agent. When polymer chains are linked together by these new links, they lose some of their ability to move as an individual polymer chains resulting in compaction of structure. Figure 2.6 shows the illustration of crosslinking process. This chemical process alters the stability and the properties of the polymer. Two different reactions are associated with crosslinking: Schiff base or Michael-type adducts (Kumar et al., 2004).



Figure 2.6: Illustration of crossliking process in a polymer; (1) Crosslinking agent is introduced in polymer matrix, (2) Growth and branching, (3) Fully cured polymer

Crosslinking polymer matrix might give a positive or negative impact to the polymer matrix depending on the dosage and compatibility. Research conducted by Chambi and Grosso (2006) use transglutaminase as crosslinking agent for casein, gelatin and casein-gelatin blend. Transglutaminase only reduce the water vapor barrier properties. There was no changes on the mechanical properties for all the dosage. Sung et al. (1999) studied the cytotoxicity effects of the naturally occurring crosslinker (genipin) on biological tissue fixation. Glutaraldehyde is considered as one of the most used crosslinking agent due to effectiveness. A study conducted by Bigi et al. (2006) on the effects of Glutaraldehyde as a crosslinker for gelatin films. They reported, Glutaraldehyde is very effective in reducing the swelling and thermal behavior of gelatin film even with low amount of dosage. However, researchers tend look for alternative crosslinking agents due to the fact that Glutaraldehyde is considered dangerous and toxic. Usage of this crosslinking agent in a large scale might pose a threat to biosphere. Several chemicals have been studied as alternative crosslinking agents. Muzzarelli (2009) created chitosan hydrogel and examine the effects of genipin as a crosslinker for bio-medical and pharmaceutical application. Some crosslinking agents such as citric acid need catalyst to initiate crosslinking process. A study conducted by Reddy and Yang (2010) reported that

citric acid can be used as a crosslinker for starch film with the aid of sodium hypophosphite.

2.4.1 Tannic acid

Tannic acid is extracted from plants and microorganisms. It is fully biodegradable and less expensive to produce, compared to the other chemical derivatives. It has polyphenolic compound containing a central carbohydrate core that is esterified by gallic acids (Božič, Gorgieva & Kokol, 2012). Figure 2.7 shows the chemical structure of tannic acid. Tannic acid has high molecular weight configuration and possess high antioxidant capacity and can interact with other biological macromolecules (Shutava et al., 2005). Several publisher has studied the effects of tannic acid as a crosslinker on polymer matrix. Cao, Fu and He (2007) uses ferullic acid and tannic acid as a crosslinker gelatin films.Despite its high potential, little has been published on the use of tannic acid as a crosslinking agent for chitosan nanocomposites. Rivero, García and Pinotti (2010) studied crosslinking capacity of tannic acid in chitosan film and stated that the moisture content and solubility decreased. They also mentioned that the mechanical properties of chitosan film increased.



Figure 2.7: Chemical structure of tannic acid

2.5 Polylactic acid (PLA)

PLA is a biodegradable thermoplastic derived from corn, sugar beet and potato starch (Byun et al., 2015). PLA attracted attention mostly because of its sustainable nature and inertness. PLA does not have any active functional groups on the backbone, besides the hydroxyl and carboxylic groups at the chain ends. Several researchers have studied the effects of PLA and chitosan. PLA possess high young's modulus and relatively good strength. Bulota and Budtova (2015) conducted a study on creating a lightweight, highly porous and mechanically strong flax/PLA composites. They concluded that Compression tests revealed a significant influence of PLA concentration on the mechanical properties of the composites. Another research conducted by Arrieta et al. (2015) have developed a flexible materials based on plasticized electrospun PLA/Poly(hydroxybutyrate). It has improved the flexibility of the composite. A study conducted by Chen, Dong and Cheung (2005) mentioned that there is an established

hydrogen bonding occurs between PLA and chitosan. They examined the effects of PLA/chitosan blend using FTIR analysis.

PLA is one of the two most commonly used 3D printing materials (with the other being ABS). This biodegradable material have the virtue of being odorless and doesn't warp significantly than other 3D printing materials. Due to its low melting point, using PLA as a 3D printing material doesn't require a heated bed. 3D printer can be used to fabricate complex structures. Lin et al. (2014) studied the potential of utilizing 3D printing technologies to fabricate bio-inspired shapes and successfully fabricated 3D printed polymer based bio-inspired shapes. They mentioned that geometrically structured shapes and design occurs naturally for a reason and possess enhanced, and often surprising, mechanical properties, and provide inspiration for materials design. Kao et al. (2015) fabricated PLA based scaffolds for bone tissue engineering using 3D printing technique. They have fabricated high resolution scaffolds that is comparable to readily available scaffolds by utilizing the advantages of PLA. Stansbury and Idacavage (2015) have reviewed the possibilities of 3D printing to expand into conventional production method for polymers. They mentioned that 3D printing technologies already altering many industrial and academic operations due to their robustness and simplicity into fabrication any design without the requiring molds or dies. Therefore it will be an interesting things to study the possibilities of producing a 3D printed PLA based wing structure and evaluate the performance of PLA as a BAV wing structure using 3D printer besides its promising mechanical properties.

CHAPTER 3: RESEARCH METHODOGY

3.1 Introduction

This topic is divided into 4 sections. Each section describes the approaches planned and implemented in this research work. The first step involved in the preparation of the nanomaterials. Secondly the nanocomposite films was prepared. The third step involved in fabricating BMAV wing structure and infusion of the film into the wing structure. Finally the film is characterized through different series of test and its installed performance as an artificial dragonfly wing membrane is analyzed.

3.2 Preparation of nanomaterial

Two types of nanomaterial (chitin and cellulose) have been prepared in this work. Both nanomaterials have been incorporated into different batches of chitosan film to investigate the effects of these nanomaterials in the chitosan matrix.

3.2.1 Preparation of CNW

Chitin based material was sourced from crab shell flakes. Chitin nanowhiskers (CNW) were prepared using a acid hydrolysis method, as reported by (Gopalan Nair & Dufresne, 2003). By incorporating this method, the amorphous structure of the chitin flakes will be removed. Figure 3.1 shows the schematic diagram of hydrolysis process involved in producing CNW. The preparation begins by dispersing 5 g of chitin flakes into 150 ml of a 3 N HCI solution in a flat bottom flask. A weight ratio of 1:30 was applied

in preparing CNW (Zhu, Liang & Ji, 2015; Sriupayo et al., 2005; Wongpanit et al., 2007). A heating capable magnetic stirrer was used to stir the solution under reflux for 3 h at 90°C. After acid hydrolysis, a pale brown solution was obtained and transferred into a 50 ml conical tube. This is to prepare the solution for centrifuge process. Six conical tubes were carefully balanced and centrifuged at 10000 rpm for 10 min. This process was repeated three times followed by removal of the HCI solution carefully from the container without disturbing the sediment on the bottom of the conical tube. The samples were diluted with distilled water for each run. Next, the slurry containing nanowhiskers was collected and transferred into a dialysis bag for neutralization process. The slurry was dialyzed in running water for 2 h and then soaked in distilled water for 24 h. Once neutralized, the pH of the chitin whiskers suspension was corrected to 2.5 by adding diluted HCI and adding chloroform to prevent bacterial growth in the suspension Umamaheshwar & Chandra, 2014; Marchessault, Morehead & Walter, 1959). The dispersion was completed by 10 min of ultrasonic treatment for every 30 cm³ aliquot. Finally, the chitin whiskers suspension was refrigerated in an air tight container at 6 °C with a final concentration of 2.5 wt% of CNW in the suspension.



Figure 3.1: Schematic diagram of CNW production by hydrolysis process

3.2.2 Preparation of nanocrystalline cellulose (NCC)

Microcrystalline cellulose (MCC) was hydrolyzed with sulfuric acid (H_2SO_4) as described by (Bondeson, Mathew & Oksman, 2006). Treatment of MCC with sulfuric acid is done to produce isolated cellulose whiskers. Ehmann et al. (2014) reported that hydrolysis of MCC introduce sulfate group. This surface esterification reaction is show in figure 3.2. The first step begins by mixing 10.2 g of MCC with distilled water in a beaker. The mixture was then put in an ice bath until a uniform cold environment is establish within the beaker. Sulfuric acid were carefully added by drops to avoid temperature spike since it is an exothermic process until the desired acid concentration of 63.5 wt% was reached. This optimized values of concentration and MCC mass was reported by (Bondeson, Mathew & Oksman, 2006).



Figure 3.2: Schematic representation of the preparation of NCC starting from MCC showing the surface esterification reaction introducing sulfate groups

Careful attention needed in dropping the acid into MCC/distilled water solution as a sudden increase in temperature will "burn" the cellulose particles resulting in dark brown coloration. In this attempt, a few batches resulted in burned NCC particles due to excess sulfuric acid content or unfavorable temperature conditions. Figure 3.3 shows both "burned" suspension and successful suspension taken in the midst of preparation.

The suspension was heated in a flat bottom flask, fitted with magnetic stirrer for 130 min at 45 °C. After acid hydrolysis, the suspension was centrifuged at 6000 rpm for 10 min. This process was repeated three times, followed by removal of the sulfuric acid solution from the container and diluted with distilled water for each run. Next, the NCC slurry was collected and transferred into a dialysis bag. The slurry was dialyzed in running water for 24 h and then soaked in distilled water for another 24 h to stabilize the pH to neutral state. Once neutralized, the dispersion was completed by 10 min of ultrasonic treatment for every 30 cm3 of aliquot. Then few drops of chloroform was added to prevent bacterial growth. Finally, the suspension was refrigerated in an air tight container at 6 °C with a final concentration of 4.6 wt% of NCC.



Figure 3.3: Dark colored solution in the central conical flask depicts the "burned" sample; The most right conical flask contains a correct sample

3.3 Preparation of nanocomposite film

Chitosan based matrix were modified by two additives by both physical and chemical modification. Physical additions were performed by incorporating nanoparticles and chemical modification was carried out by introducing a crosslinking agent. Four separate batches of chitosan based film were designed for this research work.

- 1. The effects of CNW on the chitosan matrix film (Batch 1)
- 2. The effects on NCC on the chitosan matrix film (Batch 2)
- 3. The effects of heat treatment on CNW based chitosan matrix film (Batch 3)
- 4. The effects of heat treatment on NCC based chitosan matrix film (Batch 4)

3.3.1 Preparation of chitosan nanocomposite film

Chitosan is soluble in acetic acid. The film forming solution was prepared by weighing and adding 2 wt% of chitosan powder into a 2% (v/v) acetic acid solution under rigorous magnetic stirring until chitosan powder completely dissolve into acetic acid solution. A translucent pale yellow colored solution was obtain after 1 hour of stirring. Then, nanomaterials were slowly added according to the ratio intended in the chitosan solution. A high speed homogenizer was used for approximately 5 min to homogenize the nanomaterials and chitosan matrix. Finally the mixture were stirred with magnetic stirrer for an additional 1 h.

For further investigation, a crosslinking agent was introduced in chitosan film. Chemical crosslinking was achieved by incorporating different amounts of tannic acid into the chitosan solution. Tannic acid (20 mg and 40 mg per 1 g of chitosan) was dispersed into the suspensions under vigorous magnetic stirring for another 1 h until it was homogenized. The mixed suspension was cast onto a plastic Petri dish. Upon pouring into the Petri dish, excess bubbles were seen in abundance. The casted suspensions were left overnight in a dry cabinet to remove the bubbles and later evaporated in a drying oven for 48 h to obtain dry composite films. The dry weigh taken from the Petri dish of each samples was approximately ~ 0.4024 g each. All film have an average thickness of 100 micron +/- 10 micron.

3.3.2 Preparation of heat treated chitosan nanocomposite film

Heat treatment process was applied on the film to investigate its effect on enhancing the film. There are two types of heat treatment; moist and dry (Ritthidej, Phaechamud & Koizumi, 2002). The dry heat treatment method was used for our film. Essentially, dry ambient air was simulated in a convection oven at 180°C. Films were heat treated for 30 min at a uniform temperature of 180°C, resulting in a pale brown film at the end of the heat treatment process.

3.4 Film nomenclature

Four sets of film were processed with different additives doses for batch 1 and 2. Table 3.1 shows twelve types of film for Batch 1 (CNW) (Set I: neat chitosan film, serving as the control sample; Set II: crosslinked chitosan film; Set III: chitosan film embedded CNW; Set IV: crosslinked chitosan film embedded with CNW). Table 3.2 shows twelve different types of film for Batch 2 (NCC) (Set I: neat chitosan film, as the control sample; Set II: crosslinked chitosan film; Set III: chitosan film embedded NCC; Set IV: crosslinked chitosan film; Set III: chitosan film embedded NCC; Set IV: crosslinked chitosan film embedded with NCC). For Batch 3 and 4, only Set I, Set II (A), Set III (A) and Set IV (A) were chosen due to their minimal amount of additives in the chitosan matrix. Letter "H" were assigned at the end of their label for heat treated samples.

Sample Set	Sample code	Chitosan (%)	CNW (%)	Tannic acid (mgTA/chitosan)
Set I	Control	100		(ing 171/cintosan)
Set II	А	100		20
	В	100		40
Set III	А	90	10	
	В	80	20	
	С	70	30	
Set IV	А	90	10	20
	В	80	20	20
	С	70	30	20
	D	90	10	40
	E	80	20	40
	F	70	30	40

Table 3.1: Nomenclature of chitosan nanocomposite for Batch 1

 Table 3.2: Nomenclature of chitosan nanocomposite for Batch 2

Sample Set	Sample code	Chitosan (%)	NCC (%)	Tannic acid (mgTA/chitosan)
Set I	Control	100		
Set II	А	100		20
	В	100		40
Set III	Α	90	10	
	В	70	30	
	С	50	50	
Set IV	A	90	10	20
	В	70	30	20
	С	50	50	20
	D	90	10	40
	E	70	30	40
	F	50	50	40

3.5 BMAV wing membrane and wing structure

Dragonfly wings have a very complex and fine structure which is hard to replicate. A simplified wing model was been created. This simplified dragonfly wing (for use on a BMAV) was designed using the Solidworks computer aided design (CAD) software and transferred into 3D printer compatible file. A MakerBot Replicator 2X experimental 3D printer was configured to "fine printing method" for this research since the sample size is in the vicinity of 8 cm with a thickness of 0.5 mm. Both fore and hind wing were fabricated using bio-based Polylactic Acid (PLA). Figure 3.4 shows the 3D model of a simplified dragonfly wing structure and the fabricated wing model.



(a)







(d)



(e)

Figure 3.4: 3D model of simplified dragon wing structure, (a) Top view fore wing;(b) Iso view forewing; (c) Top view hind wing; (d) Iso view of hind wing; (e)Fabricated dragonfly wing

Once the wing structure was printed out, the exposed top surface layer required sanding to remove jagged material remaining on the structure. The structures were then submerged in one of the selected chitosan nanocomposite film solutions in a Petri dish. Figure 3.5 shows the wing structure in the solution. A drying time of 48 h was required. Specifications of the wings are listed in Table 3.3.



Figure 3.5: Wing structure immersed in chitosan nanocomposite solution; (a) Fore

wing, (b) Hind wing

Specification	Fore wing	Hind wing	
Base width	7.00 mm	8.5 mm	
Centre width	12.50 mm 14.4 mm		
Tip width	7.23 mm	8.5 mm	
Length	56.00 mm	48.00 mm	
Mass without membrane	0.12 g	0.08 g	
Mass with membrane	0.18 g	0.15 g	

3.6 Characterization and performance test

The nanocomposite film were characterized in three ways:

1. Morphological studies: Comprising of Transmission Electron Microscope

(TEM) and Scanning Electron Microscope (SEM)

2. Chemical interaction studies: Comprising of Fourier-transform-infrared

spectroscopy (FTIR) and X-ray diffraction (XRD)

3. **Performance testing:** Mechanical test by Universal Testing Machine (UTM), physicochemical performance, Contact angle (wettability), Optical transmittance (UV-visible spectrophotometer) and nanoindentation test

The complete BMAV wings were tested to examine their nanomechanical properties and compared to actual dragonfly wing. Aeroelastic properties were examined using static flapping generator at variable flapping frequency. High speed frame camera was used to capture and analyze behavior pattern of BMAV wings and compared to actual dragonfly wings. Section 3.6.1 to 3.6.10 describes characterization method and parameters used in this research.

3.6.1 Morphological studies by Transmission Electron Microscope (TEM)

Transmission electron microscopy is a microscopy technique in which a beam of electrons is transmitted through thin specimen. The beam of electrons interacts with the specimen to create digital noise. This image can be magnified in a nanometer scale through a charge-coupled device creating a digital image that has a very high resolution. TEM can be utilized to examine the structure of nano-scaled material. For this work, nanoparticles produced from laboratory were analyzed using TEM. Two types of nanomaterials (CNW and NCC) were examined using a Zeis EFTEM Libra 120 TEM. The size and morphological structure of CNW and NCC were examined. The procedure begins by extracting a few drops from the samples that were collected from the preserved nanomaterial storage (in the chiller). The samples were dispersed in distilled water to reduce the opacity of the nanomaterial solution, followed by ultrasonic treatment. The samples were dropped in carbon coated copper grid and stored in dry cabinet 48 h prior of TEM examination. High magnification images were produced to examine the morphological aspects of the nanomaterials.

3.6.2 Morphological studies by Scanning Electron Microscope (SEM)

Scanning electron microscope principle is similar to TEM. The electron beam is generally scanned in a raster scan pattern. This electron microscope scans the surface of a sample to produce samples topography and composition. Generally, SEM requires the samples to be coated with gold powder using sputter machine. This is to ensure the sample is electrically conductive and helps to produce details of samples surface. Dried chitosan nanocomposite films were examined using Zeiss Evo MA10 Scanning Electron Microscope. The dispersion and the blending effects of NCC and tannic acid in the chitosan matrix were studied. Films were cut in a samples of 10 X 10 mm. The film surface and cross sectional area were examined at a magnification of 2.0 K (2 μ m). Liquid nitrogen were used to force fracture the film as described by (Lorevice et al., 2016). Figure 3.6 shows film samples with gold coating. All samples were coated with a fine layer of gold to create an electrical conductive layer on the film surface and thereby produce a sharper and clear image.



Figure 3.6: SEM film samples coated with gold prior viewing

3.6.3 Chemical interaction studies by Fourier-transform-infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) identifies the chemical bonding in a molecule. This is done by emitting infrared radiation through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. The resulting produce a profile of the sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components. FTIR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information. FTIR test were performed for this work using Perkin Elmer Spectrum 400 FT-IR/FT-FIR spectrometer. This test was done to reveal the chemical interactions between the chitosan matrix, nanomaterials and tannic acid. All samples were first cut into 15 X 15 mm and stored in dry cabinet. A total of 32 scans were performed for each sample at a resolution of 4 cm⁻¹. Measurements were recorded at a wavelength range between 4000 and 400 cm⁻¹.

3.6.4 Chemical interaction studies by X-ray diffraction (XRD)

The XRD technique used for identifying the atomic and molecular structure of a crystal. The sample is illuminated with x-rays of a fixed wave-length and the intensity of the reflected radiation is recorded using a goniometer. The crystalline atoms cause a beam of incident X-rays to diffract into many specific directions. This unique representation of angles and intensities used for phase identification of a crystalline material and can provide information on unit cell dimensions. Analysis for all samples were performed using an X-ray diffractometer (Siemens D5000). Samples were cut into 15 x 15 mm and

adhered to a microscope glass side using double-sided tape. This is to ensure all samples have a smooth flat surface prior to test. The X-ray diffractometer machine is equipped with a Cu K α radiation source (k=1.540600 Å) operating at 40 kV and 40 mA (at room temperature). The relative intensity was recorded in the scattering range of (20) 5-80° with a step size of 0.1°.

3.6.5 Performance testing using Universal Testing Machine (UTM)

Mechanical properties were analyzed for all films using a Shimadzu AGS-X series tensile tester machine according to the ASTM D882-02 method. A low capacity load cell (100 N) was used to increase the sensitivity of break due to the film thickness. Films were preconditioned in a dry cabinet 48 h before testing. A crosshead speed of 1 mm/min was used for each test. According to the principle of tensile testing, the material strain can be taken as:

$$\varepsilon = \Delta l/l_0 \tag{3.1}$$

Where Δl is the increase of length in the tensile force direction and l_0 is the original sample gauge length. Correspondingly, the material stress can be calculated as follows:

$$\sigma = F/A_0 \tag{3.2}$$

Where F is the tensile force applied on the sample and A_0 is the cross-sectional area of the sample. By employing Hooke's law, the Young's modulus of the material is:

$$E = \sigma / \epsilon \tag{3.3}$$

Due to the non-linear characteristics of biomaterials, the elastic force and deformation do not exhibit a clearly defined elastic limit. Thus, offset method was used to compensate errors. The amount of 0.02% is set off on the extension axis, and a line is

drawn parallel to the straight line portion of the loading curve. By this principle, Tensile strength, Young's modulus and elongation at the break were calculated. At least 5 samples were tested for each film.

3.6.6 Performance testing by physicochemical test

The chitosan nanocomposite films moisture uptake and solubility were tested using laboratory test rig. Firstly, the film was cut into 15 x 12 mm pieces. Then the film samples were weighed to the nearest 0.0001 g in a dry state after heating in oven at 105 °C for 1.5 h. Moisture uptake was measured by immersing dried film pieces in 75 ml of distilled water (under constant agitation) for 1 h at 25 °C with a relative humidity of 60 RH. Then samples were recovered and dried with filter paper to remove excess surface water and weighed again. The average value of water uptake was calculated using Equation (3.4).

% water uptake =
$$\left[\frac{(W_s - W)_i}{W_i}\right] \times 100$$
(3.4)

Where Ws is the weight of the swollen sample and Wi is the weight of the sample after heating it in a convection oven to make sure the film is in a completely dry state.

Immediately after recording the swollen weight, the samples were dried again in a convection oven at 105 °C for at least 1 h 30 min until it reaches a constant dry weight. The samples were weighted to the nearest 0.0001 g. This dry weight was then used in Equation (3.5) to find the solubility:

% solubility =
$$\frac{\text{initial dry weight} - \text{final dry weight}}{\text{initial dry weight}} \times 100$$
 (3.5)

3.6.7 Performance testing by water contact angle test

Measurement of the water contact angle on the film surface reveals the wettability of the film. Selected film samples were prepared for this test using the Dataphysics Tensiometer Model OCA 15EC. Samples were prepared as described in section 3.3.4 and preconditioned in dry cabinet before test. Needle tip dispersion were set to 2 μ L. Distilled water was used as a liquid medium. The contact angle was measured were captured after 5 s after water contact was established. At least 3 repetitions were performed for each sample to collect the average value.

3.6.8 Performance testing by optical transmittance

Optical transmittance and visible light transparency tests were carried out to investigate the effects of tannic acid and nanomaterials in terms of optical performance. The UV-visible spectrophotometer Cary 50 Probe were used to extract the data of optical transmittance (Tr) for selected film samples. An optical wavelength band of 200 -800 nm was set prior to examining the samples. A generic digital camera with manual mode were set at a minimum focus point distance vertically with a letter 'Samples' written on the table. Film samples were placed evenly on top of the letter and images were captured to analyze the visible light transparency of the film.

3.6.9 Performance testing by electromagnetic actuated flapping wing mechanism

Flapping wing mechanism was powered by a 12 V DC connected to a LM555 crystal clock oscillator integrated circuit that generates a stable oscillatory motion. The flapping frequency can be adjusted with a 22 kW potentiometer. Figure 3.7 shows the mechanism assembly. The wing structure was glued to an iron plate (2 mm long 2.75 mm

thick) at the base part, which mimics the dragonfly joint. The iron plate was then glued to an electromagnetic actuator, which is able to create a linear up- downstroke motion at a variable wing beat frequency up to 250 Hz.

Two high frame rate cameras (Phantom Micro 310 by vision research) were used to precisely capture the flapping wing motion. The resolution of the camera was set at 320 x 240 pixels. This low resolution is needed in order to capture smooth motion at 35000 Frame per Second (FPS). High powered Light Emitting Diode (LED) lighting was used to maximize the video brightness due to a higher lens aperture (Nikon FX lens) and high frame rate. Two cameras were placed perpendicular to each other in order to determine the three- dimensional shape and orientation of the wing surface as reported by (Gui et al., 2010). Data was collected for one complete wing stroke for 30 Hz (dragonfly natural flapping frequency), 60 Hz and 120 Hz (resonant frequency). Wing tip deflection, bending angle and wing tip twist angle were analyzed using Vision Research Phantom Camera Control Software (version 2.6.749.0).



Figure 3.7: Flapping wing mechanism setup

3.6.10 Performance testing by nanoindentation test

Complete wing design (PLA based structure and selected chitosan composite) and actual dragonfly wings were tested using Hysitron TI 750 UBI nanoindenter machine. Five points on a simplified BMAV wing and actual dragonfly wing were examined based on the crucial part in a wing structure as mentioned by (Sun & Bhushan, 2012). Average values of hardness and modulus of actual dragonfly wing and simplified BMAV wing were acquired using a fixed displacement method using Berkovich indenter instead of Vickers pyramid. The Berkovich indenter is a good choice for standard testing because it produces plasticity at very low loads, and minimizes the influence of friction. According to the theory on nanoindentation test (Oliver & Pharr, 1992; Joslin & Oliver, 1990; Saha & Nix, 2002) the reduced modulus is expressed as:

$$Er = \frac{\sqrt{\pi}}{2\beta} \frac{S}{\sqrt{A}}$$
(3.6)

Where *S* is the contact stiffness that is defined by $S=(dP/dh)_{unload}$ and β is coefficient of Berkovich tip $\beta =1.034$. The load and displacement during indentation process are analyzed to obtain the contact area. The contact area, *A* is related to the contact depth h_c as:

$$A = \sum_{n=0}^{8} \left(C_n h_c^{1/2^{n-1}} \right) \tag{3.7}$$

Where C_n are constant depending on the indenter and given by machine calibration. In Equation (3.7) h_c is determined by:

$$h_c = h_{max} - \varepsilon \frac{P_{max}}{s} \tag{3.8}$$

For the Berkovich tip, $\varepsilon = 0.75$ is a constant. The relationship between indentation load, P, and penetration depth, h, is as shown in Figure 3.8. Maximum load P_{max} and maximum penetration depth h_{max} were recorded automatically by the indenter. From equation (3.6)- (3.7) we can obtain the reduced modulus E_r . The effects of a non-rigid indenter on the load displacement behavior can be consider by defining an effective modulus Er, as follows:

$$\frac{1}{E_r} = \frac{1 - v^2}{E} + \frac{1 - v_i^2}{E_i}$$
(3.9)

Where *E* is the Young's modulus and *v* is the Poisson's ratio of the specimen. E_i and v_i are the values from the indenter. Using diamond tip on the indenter the value for E_i is 1141 GPa and v_i as 0.07 respectively. Poisson's ratio of the specimen is taken as 0.3 by default. The hardness of the material is defined by:

$$H = \frac{P_{max}}{A} \tag{3.10}$$

From equation (3.6)-(3.10), the modulus and the hardness of the samples can be obtained.



Figure 3.8: Graph of indention load P; Penetration depth h; showing the process of loading and unloading of the nanoindenter.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Introduction

This Chapter presents an analysis of the data acquired by the experiments performed (as mentioned in chapter 3). This research work establishes fundamental in a progressive manner so that it can be applied to BMAV. There are four parts discussed: Morphological studies, investigation of chemical interactions, performance evaluation and application testing.

4.2 Morphological studies (TEM)

4.2.1 Chitin nanowhiskers (CNW)

As already discussed, the morphology of the chitin nanowhiskers was studied using (TEM). Figure 4.1 shows TEM images of nano-scale chitin whiskers in a carboncoated copper grid. It was found that the chitin suspension is composed of individual nanocrystals. Some of the particles were in aggregated lump state. Higher treatment time in an ultrasonic bath will disperse the aggregated particles. The individual nanocrystals are shaped like long, slender rods. Hydrolyzed chitin produces CNW with an average aspect ratio of 8.8.

Using Soft Imaging Viewer software, measurements of the length and diameter of the particles on at least five areas have been captured. The TEM image in Figure 4.1a at a magnification of 500 nm shows that the chitin whiskers have a length of 200 to 300 nm (with the average value being ~246 nm). Figure 4.1b at a magnification of 200 nm shows that the diameters of the individual chitin whiskers range from 20 to 40 nm (with an average value being ~28 nm). These measurements accord with the dimensions of crab shell chitin whiskers reported in past studies (Zeng et al., 2012; Lu, Weng & Zhang, 2004; Hariraksapitak & Supaphol, 2010).

It is observed that, the chitin whisker suspension in the storage container exhibits colloidal behavior. This observation was made every month after storing the suspension in a chiller. The colloidal behavior can be explained due to the protonation of the amino groups (NH_3^+) which induces positive charges on the surface of the crystallites and promotes the stability of the suspension, as described by (Watthanaphanit et al., 2008).





Figure 4.1: TEM images of nano-scale chitin whiskers in dilute HCI, (a) Magnified at 500 nm with measured length, (b) Magnified at 200 nm with measured width

4.2.2 Nanocrystalline cellulose (NCC)

NCC was prepared in the second phase of this research and intended to compare the improvement over CNW-based chitosan film. Figure 4.2 shows TEM images of NCC in suspension form. The individual nanocrystals were long slender rods having an average aspect ratio of 11. The TEM image in Figure 4.2a shows the length of NCC in a range between 150 to 450 nm (with an average value being ~320 nm). Figure 4.2b shows the diameters of the NCC in a range between 15 to 40 nm (with an average value being ~29 nm).

NCC nanocrystals look similar to the CNW nanowhiskers. The images and nanocrystals dimension are also similar to the results obtained from Voronova et al. (2015) where the average value of NCC derived from microcrystalline cellulose were 300 nm in length and 10 nm in width. This nano-sized material is well dispersed and exhibits

colloidal behavior. The usage of sulfuric acid for hydrolysis process produces NCC with sulfate ester group resulting in electrostatic repulsion among the negatively charged sulfate ester group that promotes good dispersion in the NCC suspension as mentioned by (Zhong et al., 2012; Dong & Gray, 1997).



(a)


Figure 4.2: TEM images of NCC in aqueous medium, (a) magnified at 500 nm with measured length, (b) magnified at 500 nm with measured width

During hydrolysis both amorphous and crystalline structure will be swell towards the preparation of polysaccharide nanocrystals (Habibi, Lucia & Rojas, 2010). The swelling and hydrolysis of amorphous phase in a polysaccharide occurs much faster than crystalline phase due to the tight arrangement of molecular chain in the crystalline domain. Therefore after the removal of amorphous domain, the surface of the crystalline phase will be attacked by excess acid and undergo further hydrolysis. Controlling the concentration of hydrochloric acid for the preparation of CNW and sulfuric acid for the preparation of NCC is very important to prevent the crystalline structure being dissolve completely.

4.3 Morphology studies (SEM)

A scanning electron microscope (SEM) was employed to inspect selected NCC based chitosan nanocomposite film. SEM reveals the homogeneity of the composite, the dispersion level of the nanocomposite film within the chitosan matrix and possible visible changes due to crosslinking effects. Both surface and cross-sectional areas were examined. Figure 4.3(a-d) shows a SEM micrograph of the film surface. Figure 4.3(e-h) shows a SEM micrograph of the fractured cross-section of the chosen films Set I, Set II (B), Set III (C) and Set IV (F) for the NCC based chitosan nanocomposite. Pure chitosan film serves as a control film (Set I) in Figure 4.3a. It appears to be a smooth and have a homogeneous surface, which indicates good miscibility in the aqueous media. However when tannic acid were introduced as a crosslinker, the chitosan film (shown in Figure 4.3b) leads to the formation of a more rough and porous surface. Addition of NCC (in Figure 4.3c) in the film reveals a formation of more ridges and an uneven surface. Also observed, dense, white, rod-like shape structures appear scattered in the Set III (C) film. This structure corresponds to the cellulose nanocrystals as reported by (Azizi Samir, Alloin & Dufresne, 2005). However, the SEM image of the Set IV (F) film which has tannic acid (in Figure 4.3d) shows less roughness and a more homogenized structure, when compared to film Set III (C). This is due to the role played by tannic acid as a crosslinker which interacts with the NCC and chitosan matrix (as seen in FITR spectra) to form a compact formation.

The cross-sections in, Figure 4.3e and 4.3f of Set I and Set II (B) are not much difference, except for differences in roughness and the appearance of cracks. However, both Figures 4.3g and 4.3h show increased surface roughness and fiber-like formations. This indicates aggregation of NCC and phase separation occurrence for the Set III (C) and Set IV (F) films. Similar results have been obtained by (Zhao et al., 2014). They postulated that their sample's SEM image shows that the addition of NCC caused drastic

changes in the film's microstructure. The addition of tannic acid makes the film more rough and porous. SEM analysis further clarifies and supports that the mechanical and water resistivity properties have been improved, due to the introduction of NCC and tannic acid.



(c)

(g)



Figure 4.3: SEM micrograph of surface (a-d): (a) Set I; (b) Set II (B); (c) Set III (C); and (d) Set IV (F); Cross-section (e-h): (e) Set I; (f) Set II (B); (g) Set III (C); and (h) Set IV

(F)

4.4 Chemical interaction studies by FTIR analysis

4.4.1 Chitosan film with CNW and tannic acid

A Fourier transform infrared (FTIR) spectroscopy identifies unknown materials and the chemical components in a mixture by emitting infrared radiation through a sample. The resulting spectrum creates a unique representation of the molecular absorption and transmission. This was used to characterize the chemical interaction of the nanocomposite film in this research. Figure 4.4 shows FTIR spectra of Sample Set I (neat chitosan film). By identifying peaks and valleys, we can establish a chemical interaction in the film. The neat chitosan film exhibited a broad band across the wavenumber of 3000-3600 cm⁻¹ with a maximum absorption recorded at 3246 cm⁻¹. These results was attributed from the O-H and N-H stretching vibrations of the functional group engaged in hydrogen bonding between the chitosan molecules (Zakaria et al., 2012). Bands at 2880 cm⁻¹ and 2960 cm⁻¹ are the characteristic of methyl (CH3) anti-symmetric and symmetric stretching vibrations. This results are in accordance with the past findings of Pradal et al. (2011). Pure chitosan film also exhibits distinctive absorption bands at 1640 cm⁻¹ (C=O stretching in amide group, amide I band) and 1542 cm⁻¹ (NH bending vibration in amide group). An absorption band at 1152 cm^{-1} is the characteristic of anti-symmetric stretching of the C-O-C bridge and 1024 cm⁻¹ is the vibration involving C-O group stretching of the chitosan film as reported in (Pawlak & Mucha, 2003).



Figure 4.4: FTIR absorption spectra of pure chitosan film (Set I)

Figure 4.5a shows the changes occurred when CNW were added into chitosan matrix (Set III A, B and C). Film Set III C were analyzed since it has the highest nanomaterial dosage. This film exhibited a split peaks at 1654 cm⁻¹ and 1618 cm⁻¹. These changes were attributed to the stretching of C=O stretching amide I O=C-NH-CH₃ (signature of α chitin). Traces of chitin validated by the N-H bond of N-acetyl group (amide II) were observed at 1556 cm⁻¹ as observed by (Saravanan, Hemalatha & Sudha, 2011). The peaks at 3424 cm⁻¹ and 3259 cm⁻¹, shows a shift to a higher wave numbers when compared to the neat chitosan (Set I) film spectra. Past reporting relates these peaks with O-H and N-H stretching vibrations (Ifuku, Shervani & Saimoto, 2013). These

observations indicate that there is good miscibility between the chitin whiskers and the chitosan matrix.

The addition of tannic acid reveals some changes on the chemical interaction in the chitosan matrix. Spectra of film Set II (B) (Figure 4.5b) showed a band peak located at 1549 cm⁻¹, which is due to the symmetrical deformation of NH₃⁺ resulted from ionization of the primary amine group in the presence of carboxylic group. This is caused by dissociative interactions between chitosan and tannic acid under acidic conditions. A weak band at 1364 cm⁻¹ replaces the band at 1320 cm⁻¹ from neat chitosan (Set I) spectra. The 1068 cm⁻¹ band (attributed to the C-O-C bonding vibration) and the 1338 cm⁻¹ band both disappear, when compared to the neat chitosan (Set I) film spectra. These changes indicate that, there is an interactions between the chitosan amino group and the carboxyl group located in tannic acid, as reported by (Aelenei et al., 2009). Film Set IV F (Figure 4.5b), which has a blend of CNW and tannic acid, has significant peaks at both 3259 cm⁻¹ and 3424 cm⁻¹, resulting from chitin whiskers in the crosslinked film (this did not appear in Sets I and II). The band shift from 1549 cm⁻¹ to 1555 cm⁻¹ was caused by the addition of chitin whiskers in the crosslinked film.



(a)



Figure 4.5: FTIR spectra of; (a) chitosan matrix with CNW, (b) chitosan matrix with CNW and tannic acid

4.4.2 Heat treated chitosan film with CNW and tannic acid

For further investigation, selected samples were dry heat treated as mentioned in Section 3.3.2. Figure 4.6 shows both heat treated and untreated specimens FTIR spectra for Set I Set II (A), Set III (A) and Set IV (A). Interestingly, the heat treatment process significantly changes the absorbance for certain wavelengths. Figure 4.6a shows pure chitosan film (Set I) having a significant difference within the wavenumber of 3600- 3000 cm⁻¹. (Associated with the O-H and N-H stretching vibration). The heat treated chitosan film produces a narrow and sharper band across the wavenumber. This is due to the dehydration process that the heat treated film undergoes in a high temperature environment (Rivero, García & Pinotti, 2011). In this work all samples that were subjected to heat treatment show a narrower band compared to non-heat treated samples within the wavenumber of 3600- 3000 cm⁻¹. Spectra of crosslinked chitosan film for both treated and untreated samples (Figures 4.6b and d) show more prominent narrowness in the region of 3600- 3000 cm⁻¹. This suggests that tannic acid plays a crucial role in causing chemical changes in the chitosan film which is further accelerated by heat treatment. Other bands located at 1700- 1500 cm⁻¹ show similar modifications due to the heat treatment process.

An absorption peak at 1560 cm⁻¹ is associated with the formation of carboxylate groups. All heat treated samples exhibited a decrease in this wavenumber band intensity. According to Martínez Urreaga and de la Orden (2007) this was due to the gradual disappearance of the chitosan amino groups in a chemical reaction due to heat treatment. Absorptions peak at 1650 cm⁻¹ associated with (C=O stretching in amide group, amide I band) shows a significance increase in absorption for heat treated film with respect to the band of 1158 cm⁻¹ (anti-symmetric stretching of the C-O-C bridge). This is due to an increase of new amide and/or imide groups in the film as reported by (Rivero, García & Pinotti, 2011). For the addition of chitin whiskers, (Figures 4.6c and d) no significant differences were observed for the heat treated film.









Figure 4.6: FTIR spectra of treated and untreated samples, (a) Set I, (b) Set II (A), (c) Sample III (A), (d) Set IV (A)

4.4.3 Chitosan film with NCC and tannic acid

The effects of NCC and tannic acid on chitosan film were also observed in (FTIR) spectroscopy. Figure 4.7 shows FTIR spectra of selected sample sets. The neat chitosan film (Set I, Figure 4.7a) shows similar result as discussed in part II. However, changes were prominent with the addition of NCC (Set III A, B and C). Film Set III (B and C) exhibited an increase in absorbance in a range between 3000-3600 cm⁻¹ with a sharp peak at 3340 cm⁻¹ when compare to the neat chitosan (Set I). Past reporting confirms these peaks to typical O-H vibrations of NCC and occurrence of hydrogen bonding between chitosan and NCC (Khan et al, 2012). Bands at 1160 and 1055 cm⁻¹, had their intensity increased. These bands are related to cellulosic compound and are assigned to C-O, C-C and ring structures. Also observed, increased in peaks on bands between 800 and 650 cm⁻¹ due to O-H out of plane bending vibrations (Nikonenko et al., 2005). These observations indicate that there is good miscibility between the NCC and the chitosan matrix.

FTIR absorbance of chitosan film blended with NCC and tannic acid in film Set II B were show in figure 4.7b. The result of this set show an increment in band peak located at 1549 cm⁻¹, which was not presented in Set I (symmetrical deformation of NH3⁺ group resulted from the ionization of the primary amine group in the presence of carboxylic group). A weak band appeared at band 1452 cm⁻¹ which denotes electrostatic interaction of carboxyl group. The band 1068 cm⁻¹ (attributed to the C-O-C bonding vibration) was attenuated and band 1338 cm⁻¹ disappeared when compared to the neat chitosan (Set I) film spectra. These changes indicate interactions between the chitosan amino group and the carboxyl group located in tannic acid, as discussed in part II. Film Set IV F (Figure 4.7b) has visible peaks at 1160 cm⁻¹ and 1055 cm⁻¹ that does not change. This is due to NCC loading in the crosslinked film. Band at 3340 cm⁻¹ seems to be reduced for the cross-linked film, as can be observe in Figure 4.7c in a comparison of spectra between CNW Set IV (F) and NCC set IV (E), which has an identical amount of nanomaterials and crosslinker dosage. This might be due to the hydrogen bonding between NCC and chitosan that might be interfered by the tannic acid more significantly then CNW and chitosan.





Figure 4.7: FTIR spectra of: (a) non-crosslinked chitosan film, (b) crosslinked chitosan film (40mg), (c) Comparison of similar dosage between CNW and NCC

4.4.4 Heat treated chitosan film with NCC and tannic acid

Heat treatment on NCC based chitosan film revels the same changes as CNW based film. Figure 4.8a shows the comparison between heat treated NCC and CNW based chitosan film without crosslink. Figure 4.8b shows a comparison between the crosslinked

heat treated films. Major difference were seen in the spectra of 3000-3600 cm⁻¹. Not much dehydration occurs on NCC based chitosan film during heat treatment, when compared to CNW based chitosan film. This is due to the lower moisture content presented in NCC based film (as discussed in physicochemical section in this research). The wavenumber at 1549 cm⁻¹ indicates C-N and N-H stretching vibrations for both NCC and CNW. These results are in agreement with past research done (Sionkowska et al., 2014; Pradal et al., 2011). Heat treatment decreases the absorbance of all the sample film in this region, suggesting some bonds were broken due to the heat treatment process.







(b)

Figure 4.8: Comparison between CNW and NCC heat treated chitosan film: (a) without crosslinker, (b) with crosslinker

4.5 Chemical interaction studies by XRD analysis

4.5.1 Chitosan film with CNW and tannic acid

An X-ray diffraction study was conducted to investigate the microstructure and crystalline structure. Figure 4.9 shows X-ray diffractograms for CNW based chitosan film of four samples, one from each of the four sets. The four samples shown were selected because each had the highest concentration of additives within their particular set. These include: Set I (control), Set II (B) 100% chitosan with 40 mg of tannic acid, Set III (C) 30% of chitin whiskers, Set IV (F) 30% of chitin whiskers with 40 mg of tannic acid and pure chitin whiskers. Neat chitosan film exhibited 2 major peaks located at 2θ =22.6° and 11.4° and two other minor peaks located at 18.1° and 9.3°. These two major peaks are associated with the hydrated "tendons" conformation of chitosan as reported by (Ogawa,

Yui & Okuyama, 2004). According to Rivero, García and Pinotti (2010) addition of tannic acid (Set II (B)) raises the peak in the region at 2θ =15° (insert in Figure 10) that exhibits the characteristic of anhydrous ("annealed") crystalline conformation. In present work, all films in Sets II and IV exhibited similar behavior at 2θ =15°.

Sets III and IV both have a significant rise in the peak than Set I due to the introduction of chitin whiskers. Sets III (C) and Set IV (F) exhibits two major peaks located at 2θ =19.3° and 9.3° and minor peaks at 23.5° and 21.1°. According to Kadokawa et al. (2011) this is a typical diffraction pattern observed with chitin powder. It indicates that the crystallinity of α -chitin powder is the same as the embedded chitin whiskers. Addition of chitin whiskers showed strong scattering peak of chitin whiskers being more significant with increasing whisker content. However, with the addition of whisker content, the relative intensity and width of scattering peaks belonging to Set I did not change. This indicates that the addition of chitin whiskers does not affect the apparent degree of crystallinity of the chitosan matrix. As similar trend reported by Sriupayo et al. (2005) where the increasing α -chitin whisker doesn't change the intensity and the width of the scattering peaks of the chitosan matrix and indicated that the presence of α -chitin whisker did not affect the crystallinity of the matrix.



Figure 4.9: XRD diffractograms of selected CNW based chitosan film

4.5.2 Heat treated chitosan film with CNW and tannic acid

Heat treated X-ray diffractograms of selected CNW based chitosan film were shown in Figure 4.10. Neat chitosan film (Set I), both with and without heat treatment, is shown in Figure 4.10a. These results show some changes in the peaks. Non treated film exhibits two major peaks at 2θ = 22.6° and 11.4° and two minor peaks located at 2θ =18.1° and 9.3°. These results were same as previous batch. Heat treatment of the chitosan film diminishes the peak at 11.4° and shifts another peak to higher 20 values in the region of 18° to 24°. These results agree with those published by (Ritthidej, Phaechamud & Koizumi, 2002). Heat treatment of chitosan film changes the structure to anhydrous conformation of chitosan similar to crosslinked samples as in Figure 4.10b. All heat treated samples experience disappearance of the peak around 2θ = 11.4°. Crosslinked chitosan film (Figure 4.10b) shows a peak at 2θ =15° which is associated with the characteristic of anhydrous crystalline conformation. Heat treated samples of crosslinked chitosan film shows flattening effects at 2θ =15°. Similar results were obtained by (Toffey & Glasser 1999). This indicates a synergic effect of tannic acid and heating of the chitosan film.

Figure 4.10c and d show a significant peak rise due to the addition of chitin whiskers. As discussed earlier two major peaks are located at 2θ =19.3° and 9.3° and two minor peaks are located at 23.5° and 21.1° (associated with α -chitin). Heat treatment of this film gives a slight reduction on peak intensity at 23.5°. The change of this peak reflects a change in the mechanical properties of the heat treated films.





Figure 4.10: XRD diffractograms of CNW based chitosan film, (a) Set I, (b) Set II

(A), (c) Set III (A), (d) Set IV (A)

4.5.3 Chitosan film with NCC and tannic acid

The structural analysis by the X-ray diffraction method for NCC based chitosan film were shown in Figure 4.11. Only four samples shown were selected because each had the highest concentration of additives within their particular set. These includes: Set I (control), Set II (B) 100% chitosan with 40 mg of tannic acid, Set III (C) 50% of NCC, and Set IV (F) 50% of NCC with 40 mg of tannic acid. Neat chitosan exhibited a sharp peak at 2θ =11.4° and broader peak at 2θ =22.6° and two other minor peaks located at 18.1° and 9.3° which are identical with previous batch. Sample Set II (B) shows peaks in the region at 2θ =15°, and 21°, which was not available for sample Set I. The addition of tannic acid raises the peak that exhibits the characteristic of anhydrous ("annealed") crystalline conformation.

Sample Sets III and IV have a significant rise in the peak compared to Set I, due to the introduction of NCC. Sets III (C) and Set IV (F) exhibits highest scattering intensity located at 2θ =22.4°, 16.5° and 14.5°, which correspond to the (11⁻⁰), (1 1 0), and (2 0 0) planes of the cellulose I crystal type as according to (Ma et al.,2011). This shows that NCC has higher degree of crystallinity. The addition of NCC resulted in a strong scattering peak of cellulose signature, which becomes more significant with increasing filler content. Characteristics of the amorphous chitosan in the range of 2θ =20-23°, were super-positioned with the peaks of NCC. The increase in peak intensity of the pure chitosan film may due to transcrystallization effect. This effects can be define as orientation of crystals in a semicrystalline matrix perpendicularly to the cellulose nanocrystals (Helbert & Chanzy, 1994). Another research conducted by Gray (2008) reported that crystallization of a polymer matrix were nucleated by cellulose nanocrystals leading to a transcrystalline layer around the cellulose nanocrystals. This shows that the films in Set III and IV exhibited a combination of amorphous and crystalline regions.



Figure 4.11: Comparison of X-ray diffractograms of chosen sample sets

4.5.4 Heat treated chitosan film with NCC and tannic acid

For the heat treated NCC samples, Figure 4.12 shows the XRD diffractograms of both treated and untreated samples of Set III (A) and Set IV (A). Both the treated and untreated film show some significant changes in scattering intensity. All heat treated samples were observed to exhibit a flattening of peak around 11.4° for all samples as observed before on the heat treated CNW based chitosan film. This shows that the heat treatment has an effect on the molecular structure of chitosan. Furthermore heat treated samples show an increased intensity in the region of $2\theta = 19^{\circ}$ to 22° . Previous published result postulate that heat treatment does not increase the crystallinity of the film and there is a possibility of lower crystallinity due to intense crosslinking interaction during heat treatment that would inhibit the formation of the crystalline structure (Thakhiew et al., 2015).



Figure 4.12: XRD diffractograms of : (a) Set III (A) and (b) Set IV (A)

4.6 Performance testing by UTM test

In this section all CNW, Heat treated CNW, NCC and heat treated NCC films were tested and analyzed. Figure 4.13 shows the average mechanical properties of sample Set I and II from 4 separate batches. The control film (Set I) exhibits a low tensile strength of 30.8 MPa, with a maximum elongation of break of 45.6% (Figure 4.13a and b). The addition of tannic acid as a crosslinking agent increases the tensile strength. Set II (A) recorded a tensile strength of 40.9 MPa with an elongation at break of 37.1%. However, the further addition of tannic acid, as done in Set II (B), yielded a slight increase in tensile strength (43.5 MPa). For Young's Modulus (Figure 4.13c), chitosan film recorded the lowest stiffness of 664.8 MPa. Crosslinked chitosan film shows an increment in the stiffness of the film significantly up to 901.4 MPa and 1291.0 MPa for 20 mg and 40 mg of tannic acid addition.

The addition of tannic acid clearly increases the tensile strength and Young's modulus, but decreases the elongation at break. The increment of tensile strength could be link to the formation of a more stable polymer network due to attractive interactions between chitosan and tannic acid. The compaction of polymer network leads to increase in stiffness and reduction in elongation at break. However the tensile difference between 20 mg and 40 mg was just 2.5 MPa, this might be due to excess crosslinking the limits the mobility of the chitosan molecule, leading to lower tensile strength. A similar behavior was observed in past research involving gelatin based film (de Carvalho & Grosso, 2004) and ester crosslinking of cotton fabric by polymeric carboxylic acids and citric acid (Yang, Wang & Kang, 1997).











Figure 4.13: Average mechanical properties of pure chitosan (Set I) and crosslinked chitosan film (set II), (a) Tensile strength, (b) Elongation at break, (c) Young's

Modulus

The effects of CNW and crosslinked CNW on chitosan matrix in terms of mechanical properties were show in Figure 4.14. The addition of chitin nanowhiskers sharply increases the tensile strength and stiffness, but significantly reduces the elongation at break. Set III (C) had the highest tensile strength of 52.2 MPa which is slightly greater than 49.9 MPa measured for Set III (B) and has an elongation at break of 21.3% (Figure 4.14b). By analysing the nanocomposite films, increasing the whiskers content makes the tensile strength increase exponentially until it reaches a threshold. There is a possibility that it will reach a maximum value and gradually decrease with further addition of chitin whiskers. This is due to the less increment in tensile strength between Set III (B) and Set III (C). Similar results was attained by Sriupayo et al. (2005) who studied the effects of α chitin whiskers on chitosan film. The addition of the CNW on Set III clearly increases the Young's Modulus (stiffness) of the chitosan matrix (Figure 4.14c). The Set III films have much greater stiffness than Set I. In fact Set III (B and C)

are both more than twice as stiff as Set I. However, the addition of tannic acid on the nanocomposite decreases the tensile strength and Young's modulus of Set IV films especially for the Set IV (A, B, D and E) as can be seen in Figure 4.14a and c. Addition of tannic acid appears to have no effects on the elongation of the Set IV nanocomposite films.



(b)



Figure 4.14: Mechanical properties of CNW based chitosan film with and without crosslinker; (a) Tensile strength, (b) Elongation at break, (c) Young's Modulus

The results show that the tensile properties and Young's modulus of Sets II, III, and IV are superior to those of neat chitosan film (Set I), due to the reinforcement effect of the crosslinker (Sets II and IV) and chitin whisker fillers (Sets III and IV). From TEM results, chitin nanowhiskers are rod-like short fibres that have a high aspect ratio and possess a high degree of crystallinity (XRD pattern). This acts as contribution factor that increases the mechanical properties of chitosan film. According to past research Ma et al. (2014), hydrogen bonding causes the composite film to exhibit higher modulus and strength. However, Figure 4.14a shows that the difference in tensile strength between Set III (A and B) is higher when compared to the difference between Set III (B and C). This is due to the aggregation of chitin nanowhiskers. The composite film tends to be more rigid, due to higher crystalline structure introduced by chitin whisker, and as a result, the percentage of elongation at break decreases. A similar behaviour was reported by Lu, Weng and Zhang (2004) where the percentage of elongation at break decreases with increasing chitin whisker content for soy protein isolate films. The increase in tensile strength and Young's modulus by crosslinking is probably due to the formation of a more stable network between chitosan and tannic acid. Figure 4.13a shows that a further increase of tannic acid results in an insignificant increase in tensile strength for Set II films. This suggests that a slight phase separation occurs, which will reduce the tensile strength as further increments of tannic acid is added. A similar trend was reported by Peña et al. (2010), reported that a slight phase separation reduced the tensile strength as more tannic acid was added into the gelatin film. The transparency of the film also deteriorated with a higher loading of tannic acid, which agrees well that a slight phase separation took place. The transparency of our film also reduced with the higher loading of tannic acid in Set II (B) and Set IV (D, E and F). Introduction of tannic acid to the Set IV films resulted in a marked decrease in tensile strength and Young's modulus. This is likely due to crosslinking effect of the tannic acid that jeopardises the formation of the chitin network in the chitosan matrix. This phenomenon was explained by past research Gopalan Nair and Dufresne (2003a) found that strength of the chitin network in the crosslinked natural rubber matrix is lower than that in unvulcanized rubber. They concluded that the chemical crosslinking of the matrix most probably interferes with the formation of the chitin network, thereby a lower tensile modulus in vulcanized samples compared to unvulcanized samples.

The addition of NCC based chitosan film with and without crosslinker yields further improvement in mechanical properties (Figure 4.15). The measured mechanical properties of Set I, III and IV shown in Figure 4.15. With the addition of NCC, the tensile strength increased significantly (Figure 4.15a) for Set III B and C and the elongation at break were drastically reduced. Set III (C) records the highest tensile strength of 57.2 MPa for a non crosslinked film, with an elongation-at-break of 15.4%. Set IV (D) had the highest tensile strength which is 61.5 MPa. This is a 99.15% increase when compared to neat chitosan and has an elongation-at-break of 14.0%. The addition of tannic acid appears to have no effect on the elongation of Set IV nanocomposite films. The addition of the NCC and tannic acid (Set III and IV) increases the Young's Modulus (stiffness) of the chitosan matrix (Figure 5.15c). The Set IV films have much greater stiffness than Set I. In fact, films in Set III (C), Set IV (C, D, E and F) are more than twice as stiff as Set I. Figure 4.15d shows the comparison of tensile strength between CNW and NCC based chitosan film. NCC based film is superior to the CNW based film in higher loading content of 30%. Also observed that, the addition of 10 % NCC into the crosslinked film shows an improvement but yields a deteriorating strength for CNW based film. For higher loading of nanomaterials, both NCC and CNW based films shows a downtrend in tensile strength. Among all the samples, a formulation of 10% NCC and 40 mg TA possess the highest tensile strength of 61.49 MPa.













(d)

Figure 4.15: Mechanical properties of NCC based chitosan film with and without crosslinker; (a) Tensile strength, (b) Elongation at break, (c) Young's Modulus, (d) Comparison of tensile strength between CNW and NCC based chitosan film

These results shows that the composite films have superior tensile strength and Young's modulus compared to neat chitosan film (Set I), due to the reinforcement effect of the crosslinker and NCC. The increase in tensile strength caused by the addition of NCC in the chitosan film that can be attributed due to the reinforcing effect through effective stress transfer from the nanocrystals and chitosan polymer interface. Another group of researcher de Mesquita, Donnici and Pereira (2010) postulates that the interaction between anionic sulfate groups of NCC and the cationic amine groups of the chitosan promotes good bonding between them and this may lead to higher tensile strength. The TEM images and XRD diffractograms shows that the NCC were rod-like shape that have a high aspect ratio and possess a high degree of crystallinity. This proves to be another contribution factor that increases the mechanical properties. However, tensile increment difference between films in Set III (A, B) and (B, C) is huge. This shows that increasing the nanocrystals content makes the tensile strength exponentially increase

until it reaches a threshold. There is a possibility that it will reach a maximum value and gradually decrease with additional filler loading. This is due to the aggregation of NCC. This aggregation phenomenon were also observed in CNW based chitosan film. Similar trend was reported in past research by Li, Zhou and Zhang (2009) where the optimum loading of cellulose nanocrystals was 15-20% (w/w) before it reached the percolation threshold. The addition of NCC tends to make the film more rigid due to higher crystalline structure and this resulting in decrease in percentage of elongation-at-break. This behavior was also reported by Rivero, Garcia and Pinotti (2013) where the addition of chitin whiskers in soy protein isolate film decreases the percentage of elongation-atbreak. Using tannic acid as a crosslinker on the nanocomposite in Set IV films increases the tensile strength only for films in Set A and D. Other sample sets resulted in decreased tensile strength, but the Young's modulus increased. The severity of this action gives more effects to CNW based film when compared to NCC based. This can be attributed to two factors. Firstly the higher loading of tannic acid disturbs the nanomaterial network formation in the chitosan matrix. Secondly, as reported in morphological studies a slight phase separation takes place in the nanocomposite material as the loading of tannic acid is increased.

Both CNW and NCC based film formulated with lowest additives were heat treated and tested in UTM machine. Figure 4.16 shows the comparison of mechanical properties of both heat treated and non-heat treated CNW and NCC based chitosan film. In Figure 4.16a, neat chitosan film Set I exhibits the lowest tensile strength, addition of nanomaterials and tannic acid shows an improvement in tensile strength except for Set IV (A). Figure 4.16b shows the percentage of elongation-at-break. The addition of nanomaterials and tannic acid into the chitosan matrix yields a decreases in the elongation-at-break. Figure 4.16c shows Young's Modulus of both treated and untreated

samples. The addition of nanomaterials and tannic acid increases the stiffness of the chitosan matrix.

Heat treatment yields an increase in tensile strength and drastically improve Young's Modulus for all of the samples. The highest difference between untreated and treated films was sample Set IV (A) CNW which gained a tensile strength and stiffness of 12.9 MPa and 270.0 MPa respectively. The increase in tensile strength and stiffness (regardless of physical and chemical additives) was due to the development of a crosslinked matrix and stabilization when being subject to high temperature (Rivero, Garcia & Pinotti, 2013). The addition of tannic acid and chitin whiskers further accelerates the crosslinking process during heat treatment. There is a possibility that the higher crosslinking induced by heat treatment will cause the film to form a highly compact structure that inhibits the formation of a crystalline structure, as seen by XRD diffractograms. The denser structure leads to higher tensile strength and stiffness, due to its greater ability to dissipate energy and restrict chain mobility, leading to a reduction in the percentage-of-elongation of the film (Thakhiew, Devahastin, & Soponronnarit, 2013).











Figure 4.16: Comparison of both heat treated and non-treated nanocomposite film; (a) Tensile strength, (b) Elogation at break, (c) Young's Modulus

4.7 Performance testing by physicochemical studies

Water solubility and moisture content are important factors in determining the application of bio-based films. Figure 4.17 shows the physicochemical properties of both selected CNW and NCC based chitosan film. Set I has a water uptake equal to 545% of its original dry mass (Figure 4.17a). This is unacceptably high for any application that requires water resistivity. (Applying this material as the wing membrane in a BMAV is one such example.) The addition of CNW and NCC drastically reduces the swelling behaviour and solubility of neat chitosan film (Figures 4.17a and b). This is due to the behaviour of CNW that increases the concentration of nanocrystals, which are not involved in binding water molecules in the chitosan matrix and have lower affinity and reactivity to water. This also reduces the number of amino groups that facilitate the interaction with water molecules. NCC tend to be more effective in resisting moisture intake and solubility. Similar trend was observed by Pereda et al. (2014) where the addition of cellulose nanocrystals in polyelectrolyte based chitosan/olive oil film reduces the total solubility matter and moisture sorption of the film. They postulated that, NCC increases the hydrogen bonding between the amino groups and sulfate ester groups, leaving no room for OH group to interact with water molecules. The addition of tannic acid as a crosslinker on Set I and Set II and nanocomposite film Set IV also decreased the water uptake and solubility. According to Kubota et al. (1993), N-acetylation with some crosslinking agents can be formed on chitosan molecules with the presence of water, where the reduction of hydrophilic groups would decrease the solubility and dissolution of the crosslinked film. Another researcher Rivero, García and Pinotti (2012) who studied the heat treatment effects on chitosan based film, observed that (at high temperatures) chitosan film crosslinked with tannic acid showed a reduction in moisture uptake. They postulated that this was due to carboxylic acids reacting with the amine group to form an amide, thus eliminating water. Since this test was conducted after exposing the samples

to a temperature of 105 °C for 1.5 h, the effectiveness of the crosslink increases the hydrophobic and/or hydrogen interaction between chitosan and tannic acid.



(b)

Figure 4.17: Physicochemical characterization of all sample sets; (a) Moisture content of samples under equilibrium condition at 25 °C and 65% Relative Humidity, (b) Solubility of samples

The effects of heat treatment on moisture uptake for both CNW and NCC based chitosan film is shown in Figure 4.18. It have been discussed that the addition of CNW, NCC and tannic acid is known to improve the water resistivity. Heat treatment further reduce the moisture uptake capability of the chitosan film. Water resistivity was significantly enhanced by heat treatment. For Set I, a reduction from 545% to 182% of moisture by weight was recorded for the heat treated sample. This same behavior was recorded for all the other heat treated samples. Similar results were obtained by several researchers (Ghosh, Ali & Dias, 2009; Kim et al., 2002). Heat treatment increases the crosslinking process that led to compaction of film structure. This compaction creates a difficult path for water molecules to pass through (Li et al., 2015). Other research stressed that these changes are due to fact that the chitosan matrix had an increase in its degree of acetylation at elevated temperatures. These changes are due to the release of water from the matrix at higher temperatures (150 °C – 200 °C) Kim et al. (2002). The chemical changes at elevated temperatures converts chitosan into a chitin-like material.



(a)


Figure 4.18: Moisture content of both treated and untreated samples ; (a) CNW based chitosan film, (b) NCC based chitosan film

4.8 Performance testing by water contact angle (Wettability)

Contact angle is another important parameter that can define a materials hydrophobic/hydrophilic properties. It is the measurement of tangent line at the point of contact of the liquid droplets. Selected films were preconditioned in desiccator were tested for a wettability using distilled water. Figure 4.19 shows static water contact angle for film Set I, Set II (A), Set III (A) and Set IV (A) and the heat treated part. The main reason for this selection is to examine the effects on the wettability having a minimum amount of nanomaterial and crosslinker. Pure chitosan film Set I exhibit contact angle value of 90° which is in the range comparable value produced by (Croisier & Jérôme, 2013). Although chitosan is hydrophilic by nature, its wettability could be affected when blending with another material or by heat treatment. Addition of tannic acid as a crosslinker yields an insignificant value of 92°. However introduction of nanomaterials translate the chitosan film to exhibit hydrophobic properties. Films in Set III (A) shows a

contact angle of 99.8° (CNW based) and 100.3° (NCC based). This is due to the hydrophobic nature of nanomaterials that prevents the water molecules to slip into hydrophilic surface of chitosan matrix. Crosslinked nanocomposite chitosan film Set IV (A) exhibits a lower contact angle of 95.9° (CNW based) and 96.0° (NCC based). Although these films shows better water absorption resistivity, it does not prove to be useful in wettability. This is due to the nature of films in Set IV that have a smoother surface when compared to films in Set III as examined in SEM. Rough and porous films usually display a highly hydrophobic character due to the surface roughness and possibilities of air entrapped inside the pores. Similar result was attained by (Teng et al. (2015) who conducted a study to investigate conductive polymer porous film with tunable wettability and adhesion. Heat treatment counterpart shows a slight improvement on the wettability except for Set III which shows some reduction on contact angle. This suggest that hydrophilic nature of chitosan film can be altered with physical and chemical additives.





(e)

(b)

(k)

(h)



Figure 4.19: Water contact angle of 2 μL of dispersion for; (a) Set I, (b) Set II (A),
(c) Set III (A-CNW), (d) Set IV (A-CNW), (e) Set III (A-NCC), (f) Set IV (A-NCC), (g-l) Heat treated counterpart

It is important for a BMAV to have a hydrophobic surface on the film membrane to sustain flight in humid environments. The water contact angle for actual dragonfly wing membrane is 143° (Figure 4.20). This super hydrophobic properties is due to the design of the dragonfly wing surface that have air pockets by the groove of the dorsal and ventral layers. Besides, the membrane is covered with super hydrophobic wax and the membrane has a beads like surface which helps to increase its wettability (Sun & Bhushan, 2012). A study have been conducted by Song et al. (2007) reported actual dragonfly wing have a contact angle of 177°, which very closely approached the theoretical limit of a water contact angle of 180°. Such the super hydrophobicity makes the dragonfly effective in flying in the rain and keep its wings non-wetting and selfcleaning. This properties is also reported to reduce drag during flight.



Figure 4.20: Water contact angle of actual dragonfly wing

4.9 Performance testing by optical transmittance (UV-visible spectrophotometer)

NCC based chitosan film were tested for optical performance to investigate the reason for mechanical properties fluctuation since there is a chance for phase separation and aggregation to occur with high loading content of NCC and tannic acid, as discussed in Section 4.6. The visible light transparency for all NCC based sample sets is shown in Figure 4.21. The printed letter "Sample" can be seen for all sample sets clearly since all the samples were translucent. Generally all sample sets exhibit a slight yellow appearance. The addition of tannic acid tends to make the film appear more yellowish, due to oxidative reactions as discussed by (Rivero, García & Pinotti, 2010). The word "Sample" becomes more blurred with increasing NNC loading especially for sample Set III (C) due to increasing surface roughness.



Figure 4.21: Appearance of all sample sets; (a) Set I; (b) Set II (A); (c) Set II
(B); (d) Set III (A); (e) Set III (B); (f) Set III (C); (g) Set IV (A); (h) Set IV
(B); (i) Set IV (C); (j) Set IV (D); (k) Set IV (E); (l) Set IV (F)

The optical transmittance (Tr) data (for a wavelength interval of 200 - 800 nm) for selected sample sets is shown in Figure 4.22. Film Set I exhibits the highest optical transparency. The addition of tannic acid for Set II reduces the transparency, especially in the range below 700 nm. The transparency significantly decreases as the loading of NCC increases (Set III (C)). There is a significant difference in transparency between 350 and 450 nm with the sample sets having both NCC and tannic acid (Set III(C) and IV (F)). This is due to aggregation of NCC particles in chitosan matrix and influence of oxidative process accelerated by tannic acid, which scatters the light much more for these

films than the control set (Set I) and Set II. This results further concludes the deteriorating mechanical properties for higher loading of nanomaterials and tannic acid.



Figure 4.22: UV-visible spectrophotometer of chosen sample sets

4.10 Performance testing by nanoindentation test

Nanocomposite chitosan films that have been produced were then infused in simplified dragonfly wing. NCC based sample Set IV (D) were chosen due to its superiority in mechanical properties compared to other sample sets. Furthermore NCC sample Set IV (D) closely matches the real dragonfly wing Young's modulus of 1.5 to 2.9 GPa (Song et al., 2007; Kempf, 2000). Due to dragonfly wings small size and complex vein design, it is impossible to analyses the mechanical properties from tensile testing. A nanoindentation test is an alternative way to resolve this issue. The development of the nanoindentation technique allows highly localized hardness and modulus measurements to be performed on a very small material volume. Figure 4.23 shows the points of test being conducted for the actual dragonfly wings and simplified BMAV wing. Specific

points were carefully selected based on their importance. Essentially, point 1; costa, point 2; wing tip, point 3: Nodus, point 4; Postal vein and point 5; Rudius were chosen due to its significant role in retaining flight performance and strength. Approximately the same points on the simplified dragonfly wings were indented for comparison.





Figure 4.23: Points of nanoindentation test; (a) Actual dragonfly forewing, (b) Actual dragonfly hindwing, (c) BMAV forewing, (d) BMAV hindwing

Table 4.1 shows the modulus and hardness of dragonfly forewing and simplified PLA forewing. Table 4.2 shows the modulus and hardness of dragonfly hind wing and simplified PLA hind wing. Different points on the wing bear different loads on actual dragonfly wings, therefore their mechanical properties were different because of adaptation. Relatively speaking, the mechanical properties of a real dragonfly wing vary with the position. Group of researchers Sun et al. (2010) examined the mechanical properties of the costa, rudius and postal veins (Refer to Figure 4.23 for the points) of a dragonfly wing and concluded that variation in modulus were in between 0.5 GPa to 3.5 Gpa. The modulus and hardness we retrieved from different points were comparable to previously published article by (Kesel, Philippi & Nachtigall 1998; Song et al., 2007; C. H. Chang et al., 2009). Costa, Nodus and Rudius (fore wing) and Rudius (hind wing) displayed a very high modulus compared to other points. Almost all points (except point 5 on the hind wing) have a uniform modulus. As suspected, the modulus of both fore wing and hind wing of simplified BMAV wings were highest in the dense 3D printed section. Point 4 in the BMAV hind wing has the lowest modulus and hardness. This is due to a very narrow and small quantity of jagged PLA "vein" structure in point 5 that yields a modulus of 0.9 GPa. The hardness of actual dragonfly wings varies in all the points. A study conducted by Smith et al. (2000) discovered that dragonfly wing veins have an air-filled hollow section. Hardness might be influenced by the diameter of the hollow section in a particular point on the vein. The simplified BMAV wing structure, (3D printed profile) is drawn by fine lines of melted PLA in a course of outer layer flowed by stacking layer by layer until complete. The surface profile is jaggy, this leads to a lower hardness than actual dragonfly veins that grow throughout its lifespan and strengthen key points on the wing profile. In terms of membrane only, dragonfly wing possess a modulus and hardness of 2.6 +/-0.04 GPa and 80.8 +/- 1.56 MPa where else

selected NCC based film Set IV D recorded a modulus and hardness value of 4.6 ± 0.08

GPa and 218. +/- 12.69 MPa. This value surpasses the actual dragonfly wing.

Table 4.1: Fore wing comparison between dragonfly wing and simplified BMAV

Points	Dragonfly fore wing		Simplified BMAV fore wing	
	Modulus (GPa)	Hardness	Modulus (GPa)	Hardness (MPa)
		(MPa)		
1	3.0 (+/-0.28)	102.0 (+/-18.31)	2.2 (+/-0.50)	102.3 (+/-29.9)
2	1.6 (+/-0.04)	139.5 (+/-2.95)	2.7 (+/-0.15)	254.4 (+/-17.30)
3	4.5 (+/-0.01)	344.2 (+/-42.00)	4.9 (+/-0.47)	195.5 (+/-13.85)
4	1.8 (+/-0.02)	228.2 (+/-11.99)	4.8 (+/- 0.47)	213.2 (+/-21.71)
5	4.4 (+/-0.06)	247.2 (+/-20.34)	3.9 (+/-0.05)	201.7 (+/-7.23)

wing

Table 4.2: Hind wing comparison between dragonfly wing and simplified BMAV

Points	Dragonfly hind wing		Simplified BMAV hind wing	
	Modulus (GPa)	Hardness	Modulus (GPa)	Hardness (MPa)
		(MPa)		
1	2.0 (+/-0.16)	284.0 (+/-1.79)	1.9 (+/-0.05)	192.4 (+/-0.48)
2	2.5 (+/-0.41)	157.7 (+/-59.30)	2.2 (+/-0.03)	186.5 (+/-1.17)
3	2.7 (+/-0.14)	234.9 (+/-16.85)	5.7 (+/-0.31)	230.8 (+/-10.74)
4	2.3 (+/-0.09)	243.9 (+/-12.65)	0.9 (+/-52.21)	124.2 (+/- 9.42)
5	7.9 (+/-0.05)	1380 (+/-20.00)	4.3 (+/-0.07)	216.3 (+/-10.80)

wing

4.11 Performance testing by flapping mechanism

Aeroelastic properties of the simplified BMAV wing structure with NCC based Set IV D film as a wing membrane were analyzed using a three dimensional high frame rate imaging system that captures the flapping motion of the wing at a resolution of 320 X 240 pixels with 35000 FPS. Bending angle, wing tip deflection and wing tip twist angle were measured and compared with an actual dragonfly wing. Aeroelastic properties are regarded as an important branch of aerodynamic studies especially for flapping wing aircraft. BMAV gain lift by flapping its wings. This reciprocating motion is significantly more effective than fixed wing aircraft in terms on aerodynamic performance. The flapping motion can be achieved by varieties of mechanism such as staggered crank, outboard wing hinge, dual cranks and transverse shaft. These mechanism is heavy and often used on a larger size BMAV. Electromagnetic actuated flapping mechanism as discussed in section 3.6.9 produce the same reciprocating motion with lesser weight. This mechanism is suitable in this research since mimicking dragonfly wing motion is one of the objectives of this project. Dragonfly doesn't fly in figure of 8 motion, the flapping mechanism is straight forward rather than a complex ones. Static aeroelastic test of an actual dragonfly wing and simplified dragonfly wing would be useful in mimicking the flapping motion of dragonfly. Smith et al. (2000) conducted a study on a tethered desert locust (*Schistocerca gregaria*) concluded that a flapping wing of an insect must undergo an appropriate aeroelastic wing deformation to produce a suitable lift and thrust generation. Several researchers concluded that the flexibility of the wing along with an insect's capability of changing their camber generates higher peak lift forces that rigid and stiff wings (Mountcastle & Daniel, 2009; Young et al., 2009).

As mentioned in the literature review, dragonfly beats its wing in the range of 30 Hz and these fliers have a natural resonant frequency of 120 Hz. Comparisons were made between actual dragonfly wing and simplified BMAV wing in a frequency range of 30 Hz, 60 Hz and 120 Hz. The oscillatory system fluctuates its frequency in approximation of \pm 5 Hz for each steps. Figure 4.24 - 4.26 shows the aeroelastic properties of complete wing design with membrane compared to actual dragonfly wings.

4.11.1 Bending angle

Bending angle in this research was analyzed on the chordwise direction during the maximum dowstroke position (D_{max}). Similar studies have been done by Kang and Shyy (2013) who investigated chordwise flexibility for a simple wing structures. Figure 4.24

shows that for nominal flapping frequencies (30 Hz), Dragonfly wing have an angle of $\theta_{max} = 6.0^{\circ}$ while the BMAV wing recorded an angle of $\theta_{max} = 9.7^{\circ}$ with a percentage of difference of 47%. Increasing the frequency to 60 Hz yields a lower angle for the dragonfly, but the bending angle was observed to increase for the BMAV wing. Further increment of flapping frequency to 120 Hz reveals an opposite trend, whereby the BMAV wing shows a downtrend in angle compared to increasing angle for the dragonfly wing with a difference of 51%. The BMAV results in higher θ_{max} when compared to actual dragonfly values. This is due to the sheer weight of the artificial wing that follows the inertia force exerted when the flapping motion changes its trajectory. Even though bending angle is inversely proportional to the square of the structure thickness (Geiger, Vollertsen & Deinzer, 1993) the inertia force which is directly related to the mass of an object which must be overcome by the flapping force. Similar results were observed by Åke Norberg (1972) who studied the thicker wing section of the beetle elytra and sclerotized sections (pterostigma) of dragonfly wings. They concluded that a heavier wing structure required more inertial power to be exerted by the wing muscles.



Figure 4.24: Bending angle of actual dragonfly wings and BMAV wing

4.11.2 Wing tip deflection

Deflection is another measurement that can be used to assess flapping wing flexibility. Figure 4.25 shows the comparison of wing tip deflection between BMAV wing and actual dragonfly wing. At D_{max}, the magnitude of deflection at 30 Hz is almost similar between BMAW wing and dragonfly wing. Increasing the flapping frequency to 60 Hz shows a slight reduction in deflection for both wings. The trend continues to increase as frequency increased to 120 Hz. The percentage of difference between deflection of actual dragonfly wings is in the region of resonant frequency (at 120 Hz). The changes in wing tip deflection is due to differences in rigidity on the wings by having a complex vein design and corrugation along the wing surface (Richter & Lipson, 2011). The difference in wing tip deflection between BMAV wing and actual dragonfly wing is primarily due to rigidity differences. BMAV flexes more at the tip due to its concentrated PLA structure there. Due to mass centralization on that section is higher, the tip tends to deflect more when there is a sudden changes in path (i.e. during upstroke/downstroke motion).



Figure 4.25: Wing tip deflection of actual dragonfly wings and BMAV wing

4.11.3 Wing tip twist angle

Wing twist angle is an aerodynamic feature that has been show to potentially enhance the thrust on a flapping wing aircraft (Yoon, Kang, & Jo, 2011). Morphing wing design, defined as a technique of the wing to change its shape during flight, improves the wing tip vortex swirling (Wlezien et al., 1998; Lian, & Shyy, 2007). This greatly depends on the wing design. It is important to have an acceptable amount of wing twist angle for a flapping wing efficiency. The maximum twist angle was recorded during the transition of upstroke and downstroke. Figure 4.26 shows the wing tip twist angle of actual dragonfly wing and BMAV wing. The twisting angle for actual dragonfly wing is $\theta =$ 154° for 30 Hz. However at 60 Hz twisting angle increases to 168° and remains at 167° for resonant flapping frequency. The wing tip twist angle of the actual dragonfly wing does not vary significantly as the flapping frequency is varied. For BMAV wing, the wing tip twist angle 136° for 30 Hz with insignificant difference for 60Hz. The angle started to dip significantly at resonant frequency, where the difference between actual dragonfly and BMAV wing was 33%. Untwisted wings have large, drag producing wing surfaces that will reduce the flight efficiency (Ismail et al., 2014). BMAV wing have a thicker more rigid structure as oppose to dragonfly wings. This would prevent wing tip twist further. Besides, the dip in twisting angle at higher frequency can be related to tremendous amount of inertia force being generated on the simplified BMAV wing.



Figure 4.26: Wing tip twist angle of actual dragonfly wings and BMAV wing

CHAPTER 5: CONCLUSION

5.1 Summary

In this concluding chapter of this thesis, all of the experimental and analytical work performed in this thesis is summarized. This chapter also lists the discoveries gained in this original research and suggest improvements that can be done in future studies.

In this thesis, I have demonstrated that all of the objectives of bio-mimicking actual dragonfly wing material and applying it as a membrane for a BMAV wing have been achieved. Chitosan nanocomposite film was created using nanomaterials (CNW or NCC) as a reinforcement and tannic acid as a chemical crosslinker. These additives increases the mechanical properties of the film significantly. Moisture content, wettability and solubility were also reduced. There is a good miscibility between CNW, NCC and the chitosan matrix. The nano-mechanical properties of the BMAV membrane exceeded those in an actual dragonfly wing membrane. The film's excellent aeroelastic properties (e.g. bending angle, wing tip deflection and wing tip twist angle) opens a promising new realm of possibilities for BMAV wing design. Essentially, four different batches of chitosan based nanocomposite film were produced. The first two have 12 sample sets and the remaining last two batches have 8 sample sets. Each of these sample sets contains at least 5 film samples, which have a unique nanomaterial and crosslinking dosage.

For the 1st batch, CNW and tannic acid played a major role in changing the chemical and physical properties of chitosan based matrix. The use of CNW as an additive filler material in the chitosan film matrix improves its mechanical properties. The aspect ratio of CNW under TEM examination was approximately 8.8. Observation of the XRD shows that the presence of CNW did not appreciably affect the apparent degree of

crystallinity of the chitosan matrix. CNW increases the rigidity and stiffness of the chitosan film. The addition of CNW also reduces the water uptake and solubility of the film. Crosslinking the chitosan film with tannic acid increases the rigidity of the film structure and increases the mechanical properties of chitosan film. However, tannic acid reduces these properties in the nanocomposite film. The XRD pattern shows that the introduction of tannic acid changes the film into an anhydrous crystalline conformation. Tannic acid also reduces the swelling behavior and solubility.

Heat treatment (for both CNW and NCC based film) appears to improve the mechanical and physicochemical properties, regardless of the additives studied. FTIR spectra shows significant changes in absorbance for the heat treated samples. This is due to the evaporation of moisture retained in the chitosan matrix. XRD analysis for both CNW and NCC shows different changes in the diffractogram pattern. NCC based films show an increase in intensity peak for the heat treated samples, where CNW based films didn't have any significant changes. However both NCC and CNW based film shows diminishing of peaks at 2θ = 11.4° for the heat treated samples. The heat treated samples showed significant improvements in mechanical strength and reduction in moisture uptake. This was due to accelerated crosslinking process that compacted the structure even further.

For 2nd batch, the addition of NCC improves further the mechanical properties and physicochemical properties of the chitosan based matrix. TEM image confirms that the NCC particles are nanometer-scaled and possess a high aspect ratio of 20.9. The XRD pattern shows that the addition of NCC induces a combination of amorphous and crystalline regions in the chitosan matrix, which elevates the mechanical properties due to the formation of a percolating network and strong filler matrix interaction compared to CNW. The addition of NCC into the chitosan matrix also reduces the water solubility and water uptake better than CNW. Crosslinking the nanocomposite film with tannic acid also improves the mechanical and physicochemical properties.

Bio mimicked dragonfly-like wings requires high strength with an ultralightweight membrane. A flapping frequency of 30 Hz continuously to produce lift creates tremendous amount of stress on the wing for each beat. NCC, film properties are well suited for use in BMAV wing membranes because the mechanical properties are higher than its CNW counterpart. Chitosan film bonds well with the PLA wing frame. Nanoindentation test on a variety of points on the wings of both an actual dragonfly and BMAV revealed a comparable modulus and hardness. Different frequencies show major differences in aeroelastic properties in static flapping test. This is due to the fact that the mass of the wing membrane plays a larger role in creating inertial forces that overcome elastic forces in both dragonfly and BMAV wings.

5.2 Other uses of film besides BMAV

The primary objective of this work is to create a bio-film inspired from dragonfly wing membranes. Performance test on membrane only such as mechanical properties, physiochemical evaluation, wettability, optical transmittance and nanoindentation sets a new benchmark on chitosan based biofilm. The nanocomposite possess superior mechanical properties and significant reduction in moisture uptake and solubility. Table 5.1 shows the improvement of chitosan based nanocomposite film in these test compared to pure chitosan film and dragonfly wing membrane. These film could also be used for different purposes, such as packaging material for food (since it is biocompatible and nontoxic). Furthermore, chitosan nanocomposite film can be used for chelating metals from water treatment plants especially lead ions since water resistivity had been improved significantly.

Table 5.1: Benchmarking of chitosan based nanocomposite film with pure chitosan film

Test	Pure	Nanocomposite	Dragonfly
	chitosan		membrane
Tensile Strength	30.8 MPa	61.5 MPa (Batch 2 Set IV D)	N/A
Young's Modulus	664.9 MPa	2015.0 MPa (Batch 2 Set IV F)	N/A
Moisture uptake	545.4%	94.0% (Batch 2 Set IV E)	N/A
Solubility	21.5%	2.9% (Batch 2 Set IV E)	N/A
Water contact angle	90.7°	100.3°	143.4°
Nanomechanical	N/A	4.6 GPa (Batch 2 Set IV D)	2.6 GPa

and actual dragonfly membrane.

5.3 Recommendation for future research

The established nanocomposite film can be further investigate into in-depth characterization study on structural and chemical analysis. This would definitely give a better understanding on molecular interaction and would open a new realm into modifying chitosan matrix especially on the wettability aspect. Although the hydrophilic nature of chitosan was improved by the addition of nanomaterial and crosslinker, the moisture content of the film is still relatively high. Excess moisture content creates warpage on the film. This issue can be addressed by making the surface of the film less prone to water contact. Super hydrophobicity material can be found on a lotus leaf, where often the contact angle of moisture on the surface is more than 150°. Investigating the wax properties of a lotus plant and incorporating this into a chitosan film would be interesting follow-on work that can be done. Wettability is directly proportional to surface roughness (to an extent). A lot of studies have been conducted on bio mimicking micro beads found on the rough surfaces of lotus leaves and shark fins to create a super hydrophobic physical surface. Preventing the penetration of moisture on the film surface would ensure that the water resistivity is high enough to prevent warpage.

Improvement of the PLA wing thickness should eliminate the unwanted inertial forces that governs the aeroelastic properties of an artificial BMAV wing. I believe that

matching mass of a BMAV wing to that of a dragonfly wing would produce less aeroelastic properties differences. This can be done by fabricating BMAV wings using a higher resolution micro 3D printer or by a photolithography technique that can fabricate micro or nano-sized shapes.

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LIST OF PUBLICATIONS, CONFERENCE PRESENTATION AND PATENTS

First author

- Rubentheren, V., Ward, T. A., Chee, C. Y., & Tang, C. K. (2015). Processing and analysis of chitosan nanocomposites reinforced with chitin whiskers and tannic acid as a crosslinker. *Carbohydrate Polymers*, 115, 379-387. (Q1 Journal: 4.074 IF)
- [2] Rubentheren, V., Ward, T., Chee, C., & Nair, P. (2015). Physical and chemical reinforcement of chitosan film using nanocrystalline cellulose and tannic acid. *Cellulose*, 22(4), 2529-2541. (Q1 Journal:3.573 IF)
- [3] Rubentheren, V., Ward, T., Chee, C., Nair, P., Salami, E., & Fearday, C. (2015). Effects of heat treatment on chitosan nanocomposite film reinforced with nanocrystalline cellulose and tannic acid. *Carbohydrate Polymers*, 140,202-208 (Q1 Journal: 4.074 IF)
- [4] Rubentheren, V., Ward, T. A., Chee, C. Y., Reinforcement of chitosan based nano-composite film using heat treatment for the wing membrane of biomimetic micro air vehicle (BMAV). *Material characterizations* (Submitted to Q1 Journal, 1.845 IF)
- [5] Rubentheren, V., Ward, T., Chee, C., Nair, P., Erfan, S., (2015). Development of heat treated chitosan nano-composite film for the wing membrane of biomimetic micro air vehicle (BMAV). *European conference on organized film ECOF 14*.

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- [1] Ward, T. A., Rezadad, M., Fearday, C. J., & Viyapuri, R. (2015). A Review of Biomimetic Air Vehicle Research: 1984-2014. *International Journal of Micro Air Vehicles*, 7(3), 375-394. (Q3 Journal:0.486 IF)
- [2] Nair, P., Ward, T., Rubentheren, V. (2015). Static Strength Analysis of Dragonfly Inspired wing for Biomimetic Micro Air Vehicles. Chinese Journal of Aeronautics. (Q1 Journal:1.070 IF)
- [3] Nair, P., Ward, T., Rubentheren, V., Johan, M., R. (2015). Experimental analysis of artificial dragonfly wings using black graphite and fibreglass for use in Biomimetic Micro Air Vehicles (BMAV). *MATEC Web of Conferences* 30,03001.
Patents

Two patents have been applied. First round of presentation have been completed.

Ongoing process for next step:

- [1] Rubentheren, V., Ward, T., Chee, C., (2015) Chitosan nanocomposite film with chitin nanowhiskers and tannic acid as a crosslinker
- [2] Rubentheren, V., Ward, T., Chee, C., (2015) Chitosan nanocomposite film with nanocrystalline cellulose and tannic acid as a crosslinker

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APPENDICES

Appendix A: Force displacement graph of dragonfly forewing



A1. Point 1 (Costa)







A4. Point 4 (Postal vein)







Appendix B: Force displacement graph of dragonfly hind wing





B3. Point 3 Nodus



B4. Point 4 (Postal vein)



B5. Point 5 (Rudius)



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Appendix C: Force displacement graph of BMAV fore wing





















Appendix D: Force displacement graph of BMAV hind wing











