

Facile Preparation of Chitosan/Clay Biocomposites and their Characterizations

*A thesis
submitted to Bodoland University
for the award of the degree*

of

Doctor of Philosophy

by

*Madhabi Bhattacharjee
(FINAL.CHE0001 of 2017-2018)*



*Under the supervision of
Dr. Dhruvajyoti Haloi
Former Associate Professor*

*Department of Chemistry
Bodoland University, Kokrajhar
Assam-783370
24th March-2025*

DECLARATION

I, Madhabi Bhattacharjee, hereby declare that the thesis entitled "*Facile Preparation of Chitosan/Clay Biocomposites and their Characterizations*" submitted in the fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) at Bodoland University is my original work. I affirm that the thesis is based on my own research and has not been previously submitted for any other degree. All sources and references used in this work have been properly cited and acknowledged. I have not engaged in any form of academic misconduct, including plagiarism. Any assistance received in the preparation of this thesis has been acknowledged in the appropriate section. I understand that any violation of academic integrity or false statements in this declaration may have serious consequences.

Date: 24/03/2025

Madhabi Bhattacharjee
Madhabi Bhattacharjee
(FINAL.CHE0001 of 2017-2018)

ACKNOWLEDGEMENT

First of all, I would like to thank my supervisor Dr. Dhruvajyoti Haloi, Former Associate Professor, Department of Chemistry, Bodoland University, Assam, for accepting me as a research student and introducing me to this field. His passion, valuable guidance, constructive feedback, and cooperative spirit throughout this investigation assisted me in overcoming all the challenges encountered during the research process.

I would like to thank Bodoland University, Kokrajhar for giving me an opportunity to pursue my Ph.D. degree.

I want to convey my appreciation to all the faculty members in the Departments of Chemistry, Physics, Botany, and Zoology at Bodoland University for their valuable assistance and encouragement throughout the progression of my research work. Throughout the duration of my time here, the non-teaching staff in the Chemistry Department have been exceptionally kind to me.

I would like to acknowledge the help and co-operation of my lab mates from Department of Chemistry, Mr. Achinta Medhi and Dr. Maromi Roy. I am greatly indebted to Dr. Biswajit Nath for helping me at numerous occasions. I extend my gratitude to all my friends, seniors, and juniors for their timely assistance.

I express my gratitude to Dr. Nikhil K. Singha, Rubber Technology Centre, IIT Kharagpur; Dr. Nabendu B. Pramanik, ICT Mumbai - IOC Bhubaneswar Campus Bhubaneswar, Odisha; Dr. Mousmi Saikia, Department of Herbal Science & Technology, ADP College, Nagaon; Mr. Pranab Borah, Department of Botany, Cotton University, Guwahati; Dr. Jayanta Barman, Department of Physics, ADP College, Nagaon; Dr. Nishant R. M. Hulle, Department of Food Engineering & Technology, Tezpur University, Assam; Dr. Bikash Baishya, CBMR Lucknow; Dr. Manabendra Sarma, Department of Chemistry, IIT Guwahati; Mr. Haobam K. Singh, Department of Chemistry, IIT Guwahati and Mr. Rabu R. Changmai, Department of Chemistry, IIT Guwahati; Mr. Apurba Taye, Department of Applied Sciences, Tezpur University, Mr. Shyam Goswami, Drugs and Narcotics Division, Directorate of Forensic Science, Guwahati, for their help during different experiments related to my research work and cooperation in analysing different samples.

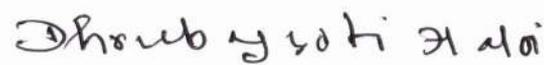
Finally, I want to express my lasting gratitude to my family for their unwavering love, blessings, patience, support, and encouragement.

Date: 24/03/2025

Madhabi Bhattacharjee
Madhabi Bhattacharjee

CERTIFICATE

This is to certify that the thesis entitled "***Facile Preparation of Chitosan/Clay Biocomposites and their Characterizations***" submitted by ***Miss Madhabi Bhattacharjee*** to Bodoland University was carried out under my supervision and is worthy of consideration for the award of the degree of Doctor of Philosophy of the University.


(Dhrubajyoti Haloi)

Dhrubajyoti Haloi, *MSc (GU), MTech & PhD (IIT Kgp)*

Associate Professor (Chemistry)

Dept. of Applied Sciences, Tezpur University

Mobile No: +91-78967-77085 / +91-88223-82803

E-mail: djhaloi@tezu.ernet.in / dhruba2k3@gmail.com

Web: <http://www.tezu.ernet.in/appsc/>

LIST OF ABBREVIATIONS

BIBB	2-Bromoisobutyryl bromide
B3LYP	Becke 3-parameter lee yang parr
BNTN	Bentonite
BPB	2-Bromopropionyl bromide
CS	Chitosan
DMF	Dimethylformamide
DSC	Differential scanning calorimetry
FT-IR	Fourier transform infrared
HOMO	Highest occupied molecular orbital
IRC	Intrinsic reaction coordinates
KAOLIN	Kaolin
LAF	Laminar air flow
LUMO	Lowest unoccupied molecular orbital
MCS	Modified chitosan
MEP	Molecular electrostatic potential
MIC	Minimal inhibitory concentration
NMR	Nuclear magnetic resonance
SEM	Scanning electron microscope
SIO	Silica
TEA	Triethylamine
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
TS	Transition state
UTM	Universal testing machine
UV/Vis	Ultraviolet/Visible
XRD	X-ray diffraction

LIST OF SYMBOLS

Å	Angstrom
cm	Centimeter
°C	Degree Celsius
g	Gram
h	Hours
kg	Kilogram
kV	Kilovolts
mA	Milliampere
MHz	Megahertz
min	Minute
mL	Millilitre
mm	Millimeter
mmol	Millimole
mol	Mole
MPa	Megapascal
µg	Microgram
µL	Microlitre
N	Newton
nm	Nanometer
rpm	Revolutions per minute
T_g	Glass transition temperature
T_{max}	Temperature at which maximum mass loss occurs
T_{onset}	Onset temperature of the degradation process
ppm	Parts per million
%	Percentage
λ	Wavelength

LIST OF TABLES

Table 1.1. Modified CS and their properties/applications.

Table 1.2. CS/clay composites and their properties and applications.

Table 3.1. Thermal characteristics of CS and MCS.

Table 3.2. Zone of inhibition observed in a test involving bacterial strains MTCC-739 and MTCC-441.

Table 3.3. Relative energies for the species of reaction channels 1 and 2 at the DLPNO-CCSD(T)/def2-TZVP//B3LYP/6-31G(d,p) level of theory. Energies are in kcal mol⁻¹.

Table 4.1. The details of the CS/KAO biocomposite films.

Table 4.2. Mechanical properties of the CS film and CS/KAO biocomposite films.

Table 4.3. Thermal properties of CS and CS/KAO biocomposite films.

Table 4.4. Zone of inhibition (diameter in mm) in different concentration against MTCC-739 and MTCC-441 bacterial strain.

Table 4.5. Percentage of swelling for all the biocomposites.

Table 5.1. The preparative data of CS/clay biocomposite films.

Table 5.2. Tensile properties of various CS/clay biocomposite films.

Table 5.3. Thermal properties of CS/clay biocomposite films.

Table 5.4. Zone of inhibition in a test against bacterial strains MTCC-739 and MTCC-441.

Table 5.5. Swelling % of various CS/clay biocomposite films.

LIST OF FIGURES

Figure 1.1. Chemical structure of chitin.

Figure 1.2. Percentage of chitin production from different marine species.

Figure 1.3. Chemical structure of CS.

Figure 1.4. Annual sales amounts of CS in metric tons by different geographic regions/countries.

Figure 1.5. Annual consumption of CS in different field.

Figure 1.6. Chemical conversion of chitin to CS.

Figure 1.7. Functional groups present in CS.

Figure 1.8. Structure of BPB.

Figure 1.9. Structure of KAO clay.

Figure 1.10. Structure of BNTN clay.

Figure 1.11. Structure of SIO clay.

Figure 2.1. Agilent Cary 630 FT-IR spectrometer.

Figure 2.2. UV-2600 UV/Vis spectrophotometer.

Figure 2.3. 600 MHz NMR spectrometer.

Figure 2.4. X-ray diffractometer (Rigaku Ultima IV).

Figure 2.5. Scanning electron microscope.

Figure 2.6. Zwick/Roell UTM.

Figure 2.7. Instrument for TGA and DSC analyses.

Figure 3.1. FT-IR spectra of CS and MCS.

Figure 3.2. UV/Vis spectra of CS and MCS.

Figure 3.3. NMR Spectra of CS and MCS.

Figure 3.4. XRD diffractogram of CS and MCS.

Figure 3.5. SEM micrographs of (a) CS and (b) MCS.

Figure 3.6. TGA plots of CS and MCS.

Figure 3.7. DSC plots of CS and MCS.

Figure 3.8. Antibacterial properties of CS and MCS on (a) *Escherichia coli* and (b) *Bacillus subtilis* bacteria.

Figure 3.9. Antibacterial activities of CS against *Escherichia coli* bacteria via microdilution method for MIC.

Figure 3.10. Optimized geometry of all the species for the reaction of CS with BPB at the B3LYP/6-31G(d,p) level of theory, where (a) and (b) are reactants (CS and BPB); (c) and

(d) are TS1 and P1, respectively for R-1; and (e) and (f) are TS1 and P2, respectively for R-2.

Figure 3.11. Three-dimensional MEP maps of (a) CS (b) BPB. The red and blue regions are the electron-deficient and electron-rich regions, respectively. Hetero atom colour codes: mauve = carbon; red = oxygen; blue = nitrogen; brown = bromine; and grey = hydrogen.

Figure 3.12. Relative energy profile for the reaction of CS and BPB obtained at the DLPNO-CCSD(T)/def2-TZVP//B3LYP/6-31G(d,p) level of theory. Blue colour represents R-1 reaction pathway and red colour represents R-2 reaction pathway.

Figure 3.13. Frontier molecular orbitals (HOMO and LUMO) for the products, (a) P1 and (b) P2 of the reaction pathway, R-1 and R-2.

Figure 4.1. Images of CS and CS/KAO clay biocomposite films.

Figure 4.2. FT-IR spectra of CS and CS/KAO biocomposite films (a–d).

Figure 4.3. UV/Vis spectra of CS and CS/KAO biocomposite films.

Figure 4.4. (a) XRD patterns of CS and CS/KAO biocomposite films.

Figure 4.4. (b) Nelson and Riley plots of CS and CS/KAO biocomposite films.

Figure 4.5. SEM images of (a) CS (b) CS/KAO-1 (c) CS/KAO-2 (d) CS/KAO-3 and (e) CS/KAO-4.

Figure 4.6. (a) TGA and (b) DTG plots of CS and CS/KAO biocomposite films.

Figure 4.7. DSC plots of CS and CS/KAO biocomposite films.

Figure 4.8. (a) Antibacterial activities of CS and CS/KAO clay against gram-negative bacteria.

Figure 4.8. (b) Antibacterial activities of CS and CS/KAO clay against gram-positive bacteria.

Figure 4.9. Swelling test plots of CS and CS/KAO biocomposite films.

Figure 5.1. Biocomposite films made of CS and clay.

Figure 5.2. FT-IR spectra of different biocomposite films composed of CS and clay.

Figure 5.3. UV/Vis spectra of different biocomposite films composed of CS and clay.

Figure 5.4. (a) XRD patterns of different biocomposite films comprising CS and clay.

Figure 5.4. (b) Nelson and Riley graphs for biocomposite films containing CS and clay.

Figure 5.5. SEM micrographs of (a) CS/BNTN-1 (b) CS/BNTN-3 (c) CS/SIO-1 and (d) CS/SIO-3.

Figure 5.6. TGA graphs for different biocomposite films comprising CS and clay.

Figure 5.7. DSC thermograms of different CS/clay biocomposite films.

Figure 5.8. Antimicrobial activities showing zone of inhibition by CS/clay biocomposite films against (a) *Escherichia coli* and (b) *Bacillus subtilis*.

Figure 5.9. Plots of swelling test of various CS/clay biocomposite films.