Understanding N-containing saccharides, their valorisation by solid acids and HPLC method development for separation and identification of products

by

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10CC16J26009

A thesis submitted to the
Academy of Scientific & Innovative Research
for the award of the degree of
DOCTOR OF PHILOSOPHY
in
SCIENCE

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April - 2022

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This thesis is dedicated to

My family

Acknowledgment

Ph.D. is a unique journey that takes a student through the untraversed new path to achieve the final goal. The path is full of new experiences and new learnings from each and every person around. To achieve the final goal, one needs skill, urge to learn the new, passion and patience as the success is extensive and strenuous in this journey. The journey would not have been possible to complete without the help and support of many people. I am taking this opportunity to express my deepest gratitude to each and every person who has supported me throughout this long journey both professionally and personally. However, mentioning is not sufficient to thank them in the right way but I will be ever grateful to all of you from core of my heart for supporting me to accomplish this biggest dream.

First of all, I would like to express my utmost gratitude to my research supervisor, ***Dr. Paresh Laxmikant Dhepe****. Much of my learnings and success in this journey would not have been possible without his constant assistance, inspiring guidance and huge support. Firstly, I would like to thank him for giving me the opportunity by welcoming me into his research group. I am very much thankful to him for keeping patience and believing me in my initial days while I was suffering from lots of health issues. His constant intellectual and moral support, guidance, constructive discussions and suggestions nourished throughout my doctoral study. He always encouraged me for not only in science but also for becoming a sensible human being to the society. His wide knowledge, tireless attitude and logical way of thinking helped me to know and understand this field in a better way. He taught me the art of making presentation, portraying the work to the audience, communication skills and the skill of writing paper. Working with him was really a great pleasure and fetched me a lot of learning experience. I always remember his words that "Ph. D. is the last platform where someone is here to teach you and you can learn many things which will help in future career." Without his encouragement and constant support, I could not even have finished my thesis. I will always remain a prodigious fan of his excellent communication skill and art of presenting work in front of audience. Lastly, my deepest gratitude and regards are due for him forever for always understanding my personal circumstances and professional needs.*

I am very much thankful to ***Dr. R. Nandini Devi*** *for her valuable suggestions, discussions, constant encouragement and support on professional as well as personal level. She always kept believe in me and encouraged me to do better in my doctoral journey.*

I want to convey my sincere regards to my Doctoral Advisory Committee members, ***Dr. A. K. Bhattacharya****,* ***Dr, T. Raja*** *and* ***Dr. R. Nandini Devi*** *for their constructive comments, valuable suggestions and encouragement time to time during my Ph. D. journey.*

My sincere thanks to ***Dr. Ashish Lele*** *(Director, CSIR-NCL),* ***Prof. A. K. Nangia*** *(Former Director, CSIR-NCL),* ***Dr. D. Srinivas*** *(Former Head, Catalysis and Inorganic Chemistry*

Division), ***Dr. C. S. Gopinath*** *(Former Head, Catalysis and Inorganic Chemistry Division) and* ***Dr. Shubhangi Umbarkar*** *(Head, Catalysis and Inorganic Chemistry Division) for giving me the opportunity and providing all necessary infrastructure and facilities to carry out my research work. My special thanks to* ***Dr. C. S. Gopinath*** *for giving me opportunity to work on a very small part of Oxygen Enrichment Project for COVID-19 as it helped me to understand the analytical technique more and I gathered lots of learnings from that study. I convey my sincere gratitude for all former and present scientists of Catalysis and Inorganic Chemistry Division for their help whenever I need. I would like to acknowledge all the supporting staffs, technical staffs of Catalysis and Inorganic Chemistry Division for their valuable help and co-operation. I wish to convey my sincere thanks to* ***Dr. Sakya S. Sen, Dr. C. V. V. Satyanarayana, Dr. C. P. Vinod, Dr. Vrushali Jadhav, Dr. S. Deshpande, Dr. T. Raja, Dr. B. L. V. Prasad, Dr. C. V. Ramana, Dr. Shatabdi P. Mukherjee, Dr. Savita Shingote, Dr. Tejas Gaydhankar, Mr. R. K. Jha, Ms. Samuel Violet, Mr. Madhu, Mr. Purushothaman, Mrs. Kolhe, Mr. Gati K. Nayak****, all the staffs of Library, Student academic office, AcSIR office, Bills section, Store and Purchase section, Finance and Accounts section, DIRC for their support and help.*

I gratefully acknowledge ***DST-INSPIRE*** *for providing me research fellowship and* ***AcSIR*** *for registering me for the Ph. D. program.*

I would like to thank all my colleagues and lab mates (former and present) for their helpful hand and cheerful attitude as I have spent most of my time with them and learnt many things from them. It is pleasure to thank all my former and present lab mates ***Dr. Anup, Dr. Babasaheb, Dr. Sandip, Dr. Manisha, Dr. Richa, Dr. Nilesh, Dr. Dheerendra, Dr. Shilpa, Shiv, Priya, Jyoti, Lavanya, Tufeil, Ankit, Rohit, Vipul, Akhilendra, Kalyani, Manuraj, Shankar, Prajakta, Yayati, Neethu, Titto, Aswathy, Camey, Shyam, Sheethal, Surabhi, Sivanesh*** *for the lively and cooperative environment in lab during my whole journey.*

I am thankful to many well-wishers and friends form CSIR-NCL who made my journey easier and more comfortable. I like to thank ***Dr. Sourik da, Dr. Shibin, Anurag, Pavan, Priyanka Walko, Shunottara, Seema, Akshay, Dr. Aswathy, Dr. Prabhu, Dr. Pranjal, Dr. Richa, Dr. Govind, Dr. Betsy, Sumanta, Sanjukta, Gargi, Vishal, Dr. Poulomi di, Dr. Abhijit da, Naru, Dr. Paulami, Dr. Samik, Rahul, Susmita, Sreejith, Arunima, Himanshu, Inderjeet, Ravi, Arindam, Kranti, Dr. Dhananjay, Meera, Dr. Rajeshwari di, Dr. Atreyee di, Dr. Sayantan da, Dr. Ujjwal da, Dr. Manoj da, Rohit, Dr. Shailaja*** *from NCL for their kind help and support.*

I am thankful to all my teachers from nursery school, high school, college and university. I have no words to acknowledge my friends circle who supported me at various stages of this journey. I would like to extend my special thanks to ***Sanjukta, Ritu, Sarthak, Pameli, Roshmi, Ankhi, Pratima, Sreejita, Mrinmoyee, Tanika, Bulti, Supriya*** *for their encouragement and love.*

My family is my pillar of strength for pursuing my higher education. God has gifted me very supportive parents and sister who encouraged me whenever I felt low, helped me to get over and start a new day with new energy. I could not complete this journey with their constant support, strength and love. I dedicate my success, my thesis to ***Late Sudha Rani Ghosh (Amma), Late Hara Dayal Nag (Dadu), Late Niharkana Nag (Dida), Sankar Ghosh (Baba), Manika Ghosh (Maa), Nisha Ghosh (Bunu)*** *for nurturing me from my childhood, giving endless love, support and sacrificing many things for my happiness. They always kept believe in me and allowed me to follow my dream of doing doctoral study. I express my gratitude to my new family* ***Asim Kumar Maiti (Baba), Kaberi Maiti (Maa)*** *and* ***Aishwarya Maiti (Bon)*** *for understanding and supporting me to accomplish this journey. My sincere thanks to my other family members (Jethu, Rangama, Pipi, Takai, Bomma, Boro Pisimoni, Mejo Pisimoni, Boro mama, Choto mama, Boro Masi, Boro Mesho, Sona masi, Biman Mesho, Dadabhai, Chordabhai, Bordibhai, Chordibhai, Sneha, Mishtu, Bhai, Gappu, Antu, Binni and my all family members). I am very much indebted to my whole family who supported me in every possible way to accomplish my research work.*

Really, the list of acknowledgements will not be complete if I do not mention the support of my better half, companion and best friend ***Anirban Maiti*** *who have seen me in my all ups and downs, encouraged and supported me in every possible way. My words are not sufficient to thank him for his endless love, support and sacrifice for more than 14 years. I would not have finished this thesis without his suggestions and technical assistance. His inspiration, understanding, constant support and encouragement boosted up my confidence and made this possible.*

Finally, I am thankful to the almighty for giving me this beautiful life. I am grateful to God for gifting me the new life growing inside me and I am thankful to his creation who supported me with good health, strength and boosted me up whenever I felt low. I am really grateful to the almighty for giving me wisdom, health and strength to start this journey and enabling me to its completion.

*.....****Neha***

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List of abbreviations

1D	One dimensional
2D	Two dimensional
3A5AF	3-Acetamido-5-acetylfuran
3D	Three dimensional
5-HMF	5-hydroxymethylfurfural
AA	Acetic acid
ACN	Acetonitrile
ACSC	Aldrich crab shell chitosan
AHM	Ammonium heptamolybdate
AHMWC	Aldrich high molecular weight chitosan
ALMWC	Aldrich low molecular weight chitosan
AMMWC	Aldrich medium molecular weight chitosan
AMT	Ammonium metatungstate hydrate
AR	Analytical reagent
ASSC	Aldrich shrimp shell chitosan
ATR	Attenuated total reflection
BA	Brönsted acidity
BET	Braunauer-Emmett-Teller
BJH	Barrett-Joyner-Halenda
CHA	Chabazite
CHNS	Carbon, Hydrogen, Nitrogen, Sulfur
Chr I	Chromogen I
Chr III	Chromogen III
DA	Degree of acetylation
DAD	Diode array detector

DD	Degree of deacetylation
DMSO	Dimetyl sulfoxide
DSWSC	Dapoli sea water shrimp chitin
EDAX	Energy dispersive X-Ray analysis
ELSD	Evaporative light scattering detector
FA	Formic acid
FAO	Food and Agriculture Organisation of the United Nations
FID	Flame ionization detector
GC	Gas Chromatography
GC-MS	Gas Chromatography Mass Spectrometry
$GlcNH_2$	D-(+)-glucosamine
GlcNAc	N-acetyl-D-glucosamine
HPLC	High Performance Liquid Chromatography
H-MOR	Mordenite (H-form)
H-USY	Ultra Stable Zeolite (H-form)
H-ZSM-5	Zeolite Socony Mobil (H-form)
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
IL	Ionic liquid
IR	Infra-Red
KSWCC	Kerala sea water crab chitin
LA	Levullinic acid
LA	Lewis acidity
LCC	LOBA Chemie chitin
LC-MS	Liquid Chromatography Mass Spectroscopy
MAS	Magic Angle Spinning
MeOH	Methanol
MIBK	Methyl iso-butyl ketone
MPa	Mega Pascal
NMR	Nuclear Magnetic Resonance

PFWSC	Pashan fresh water shrimp chitin
RID	Refractive index detector
RT	Room Temperature
SAPO	Silicoaluminophosphate
TCC	TCI Chemicals chitin
TCHMWC	TCI Chemicals high molecular weight chitosan
TCLMWC	TCI Chemicals low molecular weight chitosan
TCMMWC	TCI Chemicals medium molecular weight chitosan
TGA-DTA	Thermo Gravimetric Analysis-Differential Thermal Analysis
UV-Vis	Ultraviolate-Visible
VWD	Variable wavelength detector
XRD	X-Ray Diffraction

AcSIR	**Synopsis of the thesis to be submitted to the Academy of Scientific and Innovative Research for the award of the degree of Doctor of Philosophy in Chemistry**
Name of the Candidate	**Neha Ghosh**
Name of the Laboratory	**CSIR-NCL, Pune**
Faculty, Enrollment no. and joining session	**Chemical Science, 10CC16J26009, January 2016**
Title of the Thesis	**Understanding N-containing saccharides, their valorisation by solid acids and HPLC method development for separation and identification of products**
Research Supervisor	**Dr. Paresh L. Dhepe**

Introduction

In the past decade, researchers have put lots of effort in the area of renewable feedstocks. Plant biomass, i.e., cellulose, hemicellulose, lignin is well studied and explored as feedstock to produce array of fuels and chemicals.[1-4] However, marine biomass which is also an abundant and renewable resource, is surprisingly overlooked. Marine waste is produced by shellfish processing industry in the countries which have sea-shores. Crustacean species such as crabs, shrimps, lobsters, prawns, squids etc. contributes significantly to waste generation in fishing industries. Shrimp shell powder is cheap and sold at USD 100-120 per ton.[5] Presently, most of the marine wastes are directly disposed of without any valorisation.[5] As marine waste is composed of calcium carbonate (20-50%), protein (20-40%), chitin (15-40%)[6], it may cause environmental issues if it is disposed of directly. Hence, utilization of marine waste has drawn great interest globally now-a-days as it may bring both economic and environmental benefits.[7] Chitin is a homo-polysaccharide of ▪-1,4 linked N-Acetyl-D-Glucosamine (GlcNAc). It is 2nd most plentiful bio-polymer on earth after cellulose with 10^{11} tonnes annual production.[8] Chitin is structural analogue of

cellulose only differing in C2 position, hydroxyl (-OH) group at C2 position of cellulose is replaced by acetamide (-$NHCOCH_3$) group in chitin. Chitin gives chitosan by deacetylation of acetamide group. Chitosan is heteropolymer of D-(+) Glucosamine ($GlcNH_2$) and N-Acetyl-D-Glucosamine (GlcNAc) and has potential applications in various industries i.e., agriculture, water purification, biomedical materials and catalysis.[9-12] Unlike other plant biomass feedstocks, chitin and chitosan contains 7% biologically fixed nitrogen in its structure, which makes them a potential feedstock of nitrogen containing chemicals.[13] Homogeneous catalytic processes have been mainly reported for the direct conversion of chitin/chitosan/chito-monomers to nitrogen containing chemicals and furan derivatives. There is a need to develop stable, reusable catalytic system for conversion of chitosan, chitin and chito-monomers. Development of proper analytical method to separate and quantify the chitin derived products is an important topic to work on. Hence, development of analytical method, conversion of chito-monomers, chitin and chitosan into nitrogen containing chemicals and furan derivatives using various solid acid catalysts and understanding the properties of commercial and extracted chitosan and chitin was undertaken as my Ph.D. work.

Statement of problem

From literature reports, it is suggested that valorisation of chitosan/chitin/chito-monomers using homogeneous acids[14-19], metal salts[20-21], ionic liquids[22-23], supported metal catalysts[24-25] etc. to yield nitrogen contain value added chemicals and furan derivates. However, there are several issues with the current available reports. Those are mentioned below:

- Proper analytical method is not reported to simultaneously separate or quantify reaction products.

- Use of homogenous acid catalysts and metal salts is a serious drawback as the recovery of catalyst is really difficult and possesses environmental issues, toxicity and corrosiveness etc.
- Use of high boiling solvents, mineral acids, mineral bases, organic acids, ionic liquids as reaction medium.
- Few reactions are done in higher temperature and pressure.
- In few cases, substrates were pretreated with mechanical energy (ball milling) or acid treated ball milling was also done.
- Difficulties in effective recycling of catalysts.

Objectives

Based on the above-mentioned drawbacks, it is necessary to develop a solid acid catalyzed system for the conversion of chitin/chitosan/chito-monomers into nitrogen containing chemicals and other value-added chemicals under milder reaction condition (T≤ 200°C) and water as reaction medium in a one-pot process. Catalyst needs to be easily separable and recyclable. It is also essential to develop an analytical method for simultaneous separation and quantification of chitin derived products.

Methodology Used

- Development of HPLC method for separating chitin derived nitrogen containing chemicals and furan derivatives.
- Synthesis of acidic silicoaluminophosphate SAPO-44 by hydrothermal method and amorphous supported metal oxide catalysts (metals- W, Mo and supports SiO_2, SiO_2-Al_2O_3) by wet-impregnation method.
- Detail characterization of synthesized and other procured catalysts using XRD, TPD-NH_3, N_2 sorption, ICP-OES, SEM, CHNS and TGA techniques.
- Use of solid acid catalysts to convert chito-monomers into furan derivatives in water as reaction medium. Reactions were carried out in

batch mode Parr autoclave and analysis of the products was done using HPLC, LCMS, GC and GCMS.

- Understanding the physico-chemical properties of chitosan/chitin by detailed characterization using XRD, SEM-EDAX, CHNS, ICP-OES, IR, ^{13}C NMR, Viscosity measurement etc.
- Collection of shell waste samples from different coastal regions of India and extraction of chitin was done. Understanding the properties of extracted mass by various characterization techniques.
- Development of solid acid catalyzed methodology to convert all types of chitin and chitosan samples into nitrogen containing chemical and furan derivatives in water as reaction medium and at ambient reaction conditions. Reactions were caried out in batch mode Parr reactor an analysis of the products were done using HPLC and LCMS.

Sample Results

HPLC method development for chitin derived products

It is necessary to develop a HPLC methodology for the separation of compounds. Different columns (C18, NH_2, ion exchange lead column) and mobile phases (Water, acetonitrile, methanol) with different polarity were used. From the study, it was observed that Non-polar C18 and mid-polar cation exchange Pb^{2+} columns are best suitable for separation of $GlcNH_2$, GlcNAc, other N-containing chemicals and furan derivatives. Non polar C18 column showed good separation of $GlcNH_2$, GlcNAc and 5-HMF but was unable to separate other sugars like glucose, fructose etc., from each other. Polar NH_2 column showed very strong interaction with compounds due to similar type of functional groups attached (i.e., amino). Due to this, NH_2 column showed peak tailing. On the other hand, Pb^{2+} column could separate most of the compounds from each other because it possesses optimum polarity to allow rapid adsorption-desorption of the compounds which can be seen Figure 1. This

developed HPLC method was demonstrated to be suitable to separate and quantify $GlcNH_2$, GlcNAc and 5-HMF simultaneously within 39 min. The regression equation showed very good linear relationship with R^2= 0.9904-0.9974. Therefore, the developed HPLC method is simple, rapid, accurate, precise, and specific and has the ability to separate and quantify amino sugars ($GlcNH_2$, GlcNAc) and 5-HMF simultaneously.

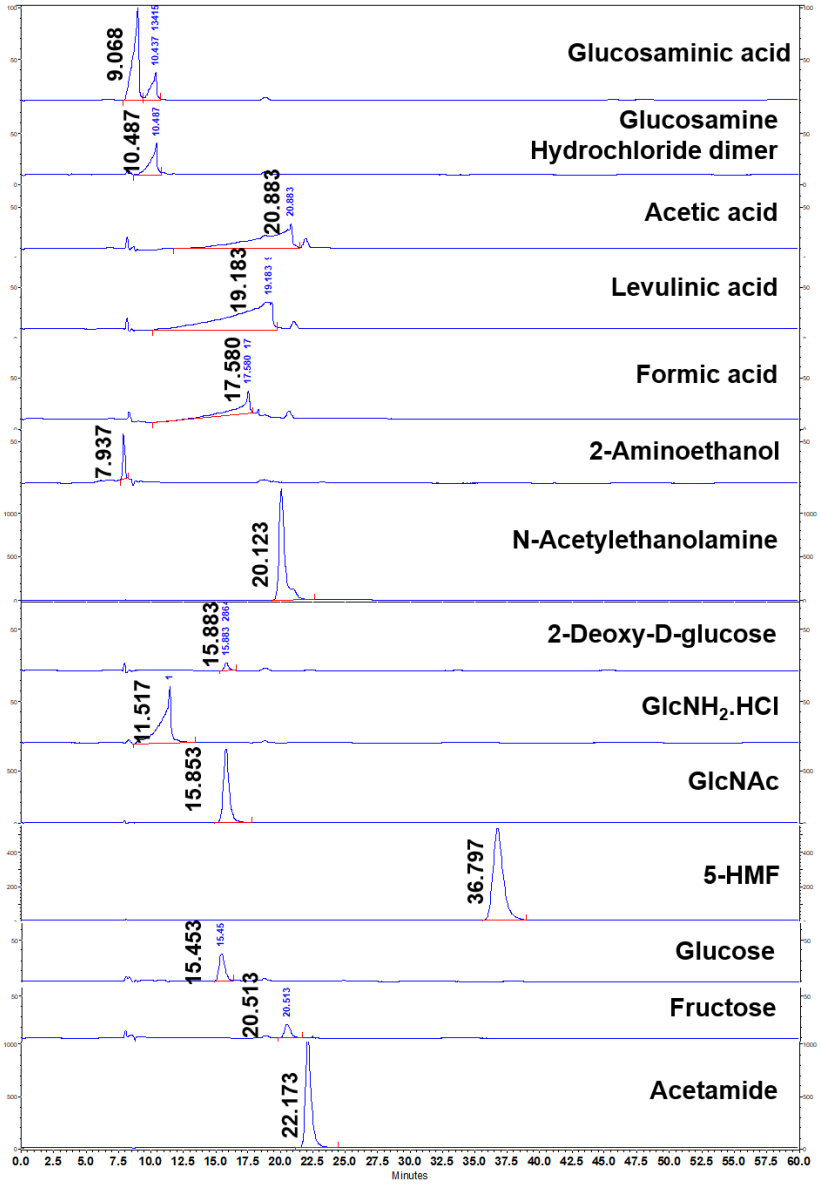

Figure 1. Chromatograms for Amino sugars, other sugars, N-containing chemicals and furan derivatives in Pb^{2+} column

Analysis condition: Column: RPM-Monosaccharide Pb^{2+} (300 mm x 7.8 mm i.d, 8.0 um, 8 % cross linking, RezexTM Phenomenex), mobile phase: water, column temperature: 80°C, flow rate: 0.5 mL/min, VWD wavelength: 195 nm

Conversion of chito-monomers to furan derivatives using solid acid catalysts

Valorisation of marine waste and production of 5-hydroxymethylfurfural (5-HMF) has gained importance now-a-days because 5-HMF is a precursor of many useful chemicals, fuels and fuel additives. Literature suggests there is a need to develop reusable and recyclable heterogeneous catalytic systems. In this work, several structured and amorphous solid acid catalysts were used and they are active to give 5-HMF yield from $GlcNH_2$ at 170 ˚C within 4h (Figure 2). We have not used any mineral acids or organic acids as a reaction media. Only water is used as solvent without any additives. Silicoaluminophosphate, SAPO-44 showed best catalytic activity for the conversion of $GlcNH_2$ to yield 26% 5-HMF but characterization of spent catalyst revealed that the structure of SAPO-44 is not stable under reaction condition. Amorphous catalyst Silica alumina (SA) also showed better activity and able to give 29% 5-HMF by using lower catalyst quantity under same temperature and time. Recycle study with SA catalyst revealed the catalyst is recyclable up to atleast 6^{th} run with similar yield. Catalyst characterization data shows SA is stable under reaction condition.

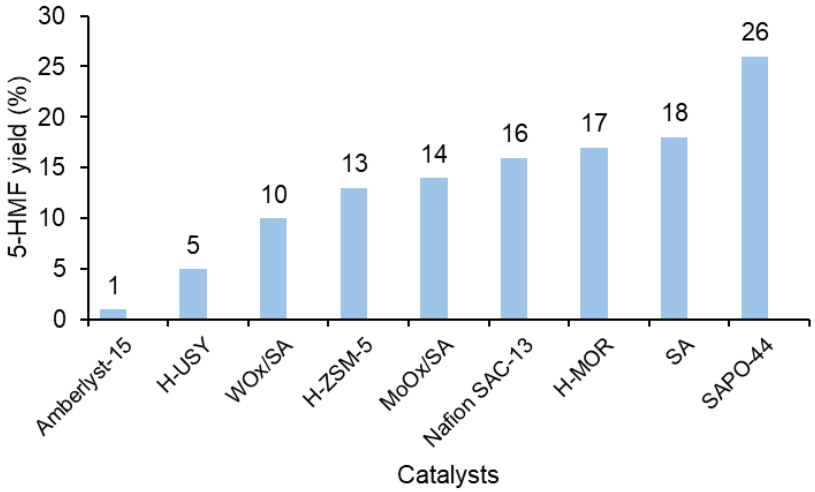

Figure 2. Dehydration of $GlcNH_2$ using various solid acid catalysts. Reaction conditions: $GlcNH_2$ (311 μmol), catalyst (0.4 g), Water (30 mL), 170 °C, 0.5 MPa N_2 pressure, 4 h

Understanding the properties of chitin/chitosan and their valorisation using solid acid catalysts

Different types of chitin and chitosan samples were procured from different suppliers. Various physio-chemical characterizations were done for all the commercially procured chitosan and chitin samples to know their physical and chemical properties. Structural and functional properties can be different for chitin and chitosan samples depending on their source and method of extraction. Hence, complete characterization of all chitosan and chitin samples were performed before starting the catalytic runs using various techniques like CHNS, ICP-OES elemental analysis, SEM-EDAX, ATR, 13C NMR, XRD analysis. Degree of deacetylation (DD %) was calculated and it is found that chitosan samples are 54-86% deacetylated. Morphology of chitosan and chitin samples were found in SEM analysis and EDAX analysis revealed that Ca and Na are present in the samples as impurity. Bulk level ICP-OES analysis was performed to quantify the metal content. ICP analysis shows the presence of Ca which proves that demineralization was not complete and presence of Na can be attributed to the use of NaOH in the deproteinization and deacetylation step. ATR and 13C

NMR spectroscopic measurements were performed to know the backbone and functional groups present in the samples. All the chitosan samples have similar type of functional groups. A comparative study on structure of chitosan and chitin was done and it is found that major structural changes happened during deacetylation process. Interchain hydrogen bonding network was disturbed during deacetylation. DTA analysis revealed that chitosan samples are thermally stable upto 300 °C and chitin has greater thermal stability that chitosan, i.e., 330 °C. The stability of chitin is higher than chitosan is due to H-bonding network in chitin which leads to structural rigidity in chitin. This proves that H-bonding network got disturbed during deacetylation of chitin as strong base is used during this process. Morphology of chitosan and chitin samples were studied using XRD analysis. XRD patterns of chitin samples shows intense diffraction peaks which are characteristic peaks for α-chitin. In case of chitosan, decrease in intensity of diffraction peaks was observed and few peaks were disappeared. The decrease in crystallinity is due to NaOH treatment during deacetylation of chitin to chitosan. Inter chain H-bonds may be broken during base treatment. Characterization data of all chitin and chitosan samples revealed that chitosan will be easier to depolymerize than chitin due to its lower crystallinity, lesser interchain hydrogen bonding network, lower structural rigidity, lower molecular weight.

For the exploration of an efficient catalytic system to depolymerize chitosan and chitin, batch mode reactions were performed at 170 °C for 24 h in presence of solid acid catalysts and water as reaction medium. It can be seen from Figure. 3a, structured solid acid catalysts (zeolites, clay, Nafion resins, silicoaluminophosphates) were used first and among all the catalysts, SAPO-44 gave 3.4% 5-HMF yield because it has higher acidity.

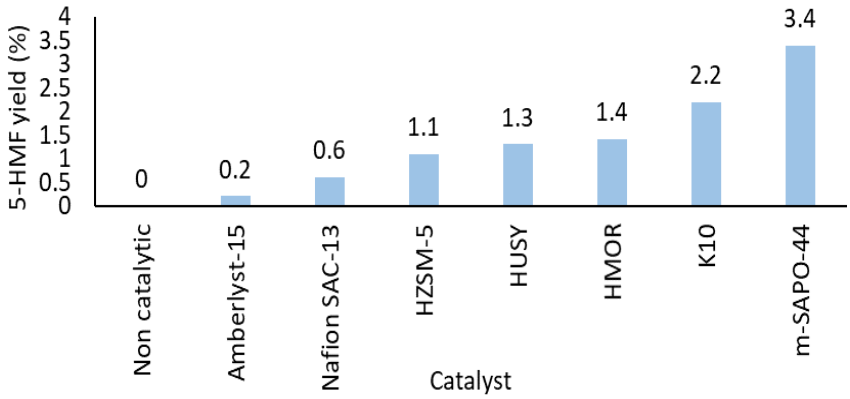

Figure 3a. Conversion of chitosan using various solid acid catalysts. Reaction conditions: Chitosan (0.31 mmol), catalyst (400 mg), Water (30 mL), 170 °C, 2 MPa N_2 pressure, 24 h

All types of chitin and chitosan can be converted using SAPO-44. LCMSMS study was performed to know the other products form and it was found that nitrogen containing chemicals are formed, i.e., M.W of 287, 185. Characterization of spent SAPO-44 proves that the catalyst is not stable under the reaction condition as it underwent morphological changes and pore structure and acidity was also altered after the reaction.

As structured catalysts showed morphological changes, activity of amorphous solid acid catalysts was also explored and silica alumina was found to be the best catalyst with 2.2% 5-HMF yield at 170 °C under 1MPa N_2 pressure within 24h (Figure 3b). Supported metal oxides on silica alumina also showed better 5-HMF yields but they showed very high metal leaching in reaction medium which is proved by ICP-OES. 3.6% 5-HMF yield was achieved in MIBK/water (1/2 *v/v*) within 4h. Characterization data of spent and fresh catalyst proves that the catalyst is stable under the reaction condition and the catalyst is recyclable. Silica alumina is also able to convert all types of chitosan and chitin samples. In this work, solid acid catalyzed green process for converting chitosan and chitin to value added chemicals were developed for the first time without using any mineral acids, organic acids and mineral bases.

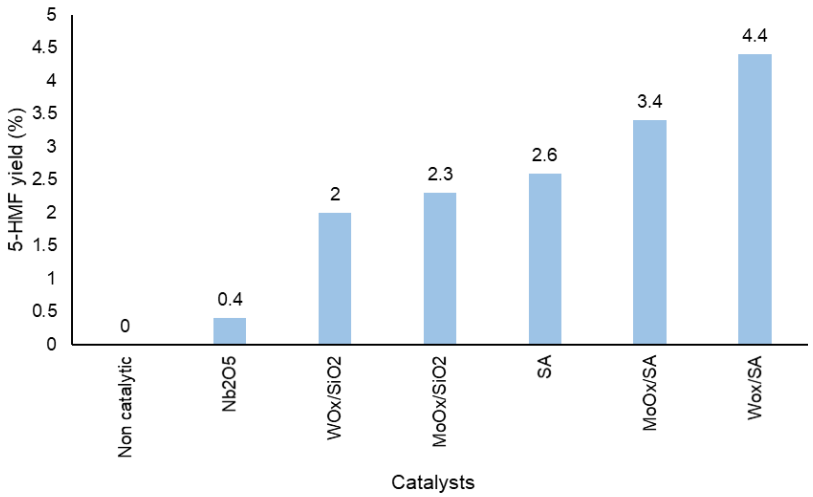

Figure 3b. Conversion of chitosan using various solid acid catalysts. Reaction conditions: Chitosan (0.31 mmol), catalyst (400 mg), Water (30 mL), 170 ˚C, 1 MPa N_2 pressure, 24 h

Conclusion and future directions

In this work, a simple, rapid and precise HPLC method has been developed to separated and quantify chitin derived chemicals. Ion exchange lead column with medium polarity is best for separating these compounds. Commercially available chitosan and chitin samples are thoroughly characterized using various characterization techniques and it is found that the samples have structural similarities but they have difference in degree of crystallinity, molecular weight, degree of deacetylation etc. and chitosan will be easier to depolymerize than chitin due to its lower crystallinity, lesser interchain hydrogen bonding network, lower structural rigidity, lower molecular weight. Solid acid catalyzed green process for converting chito-monomers, chitosan and chitin into nitrogen containing value added chemicals and furan derivatives was developed and it is found that amorphous catalyst silica alumina is the most stable catalyst which is reusable and recyclable. More work is needed in future for analysis and identification of reaction products which can help to understand mechanistic pathway more clearly.

References

1. Bhaumik, P.; Dhepe, P. L., Solid acid catalyzed synthesis of furans from carbohydrates. *Catalysis Reviews* **2016,** *58* (1), 36-112.

2. Deepa, A. K.; Dhepe, P. L., Lignin depolymerization into aromatic monomers over solid acid catalysts. *ACS Catalysis* **2015,** *5* (1), 365-379.

3. Dhepe, P. L.; Fukuoka, A., Cellulose conversion under heterogeneous catalysis. *ChemSusChem: Chemistry & Sustainability Energy & Materials* **2008,** *1* (12), 969-975.

4. Pfaltzgraff, L. A.; Cooper, E. C.; Budarin, V.; Clark, J. H., Food waste biomass: a resource for high-value chemicals. *Green Chemistry* **2013,** *15* (2), 307-314.

5. Kerton, F. M.; Liu, Y.; Omari, K. W.; Hawboldt, K., Green chemistry and the ocean-based biorefinery. *Green Chemistry* **2013,** *15* (4), 860-871.

6. Yan, N.; Chen, X., Sustainability: Don't waste seafood waste. *Nature News* **2015,** *524* (7564), 155.

7. Chen, X.; Yang, H.; Yan, N., Shell biorefinery: dream or reality? *Chemistry–A European Journal* **2016,** *22* (38), 13402-13421.

8. Jardine, A.; Sayed, S., Challenges in the valorisation of chitinous biomass within the biorefinery concept. *Current Opinion in Green and Sustainable Chemistry* **2016,** *2*, 34-39.

9. Nivethaa, E.; Martin, C. A.; Frank-Kamenetskaya, O. V.; Kalkura, S. N., Chitosan and chitosan based nanocomposites for applications as a drug delivery carrier: A review. *Processes and Phenomena on the Boundary Between Biogenic and Abiogenic Nature* **2020**, 23-37.

10. Elwakeel, K. Z., Environmental application of chitosan resins for the treatment of water and wastewater: a review. *Journal of dispersion science and technology* **2010,** *31* (3), 273-288.

11. Katiyar, D.; Hemantaranjan, A.; Singh, B., Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review. *Indian Journal of Plant Physiology* **2015,** *20* (1), 1-9.

12. El Kadib, A., Chitosan as a sustainable organocatalyst: a concise overview. *ChemSusChem* **2015,** *8* (2), 217-244.

13. Kim, S.-K., *Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications*. CRC Press: 2010.

14. Kurita, K.; Tomita, K.; Ishii, S.; Nishimura, S. I.; Shimoda, K., β-chitin as a convenient starting material for acetolysis for efficient preparation of N-acetylchitooligosaccharides. *Journal of Polymer Science Part A: Polymer Chemistry* **1993,** *31* (9), 2393-2395.

15. Tømmeraas, K.; Vårum, K. M.; Christensen, B. E.; Smidsrød, O., Preparation and characterisation of oligosaccharides produced by nitrous acid depolymerisation of chitosans. *Carbohydrate Research* **2001,** *333* (2), 137-144.

16. Mascal, M.; Nikitin, E. B., Dramatic advancements in the saccharide to 5-(chloromethyl) furfural conversion reaction. *ChemSusChem: Chemistry & Sustainability Energy & Materials* **2009,** *2* (9), 859-861.

17. Pierson, Y.; Chen, X.; Bobbink, F. D.; Zhang, J.; Yan, N., Acid-catalyzed chitin liquefaction in ethylene glycol. *ACS Sustainable Chemistry & Engineering* **2014,** *2* (8), 2081-2089.

18. Zhang, J.; Yan, N., Formic acid-mediated liquefaction of chitin. *Green Chemistry* **2016,** *18* (18), 5050-5058.

19. Kalane, N. D.; Krishnan, R. A.; Yadav, V. D.; Jain, R.; Dandekar, P., Synergistic effect of hetero-and homo-catalysts on the 'green'synthesis of 5-hydroxymethylfurfural from chitosan biomass. *Cellulose* **2019,** *26* (4), 2805-2819.

20. Omari, K. W.; Besaw, J. E.; Kerton, F. M., Hydrolysis of chitosan to yield levulinic acid and 5-hydroxymethylfurfural in water under microwave irradiation. *Green Chemistry* **2012,** *14* (5), 1480-1487.

21. Yu, S.; Zang, H.; Chen, S.; Jiang, Y.; Yan, B.; Cheng, B., Efficient conversion of chitin biomass into 5-hydroxymethylfurfural over metal salts catalysts in dimethyl sulfoxide-water mixture under hydrothermal conditions. *Polymer Degradation and Stability* **2016,** *134*, 105-114.

22. Zhang, Z.; Li, C.; Wang, Q.; Zhao, Z. K., Efficient hydrolysis of chitosan in ionic liquids. *Carbohydrate polymers* **2009,** *78* (4), 685-689.

23. Li, M.; Zang, H.; Feng, J.; Yan, Q.; Yu, N.; Shi, X.; Cheng, B., Efficient conversion of chitosan into 5-hydroxymethylfurfural via hydrothermal synthesis in ionic liquids aqueous solution. *Polymer Degradation and Stability* **2015,** *121*, 331-339.

24. Bobbink, F. D.; Zhang, J.; Pierson, Y.; Chen, X.; Yan, N., Conversion of chitin derived N-acetyl-D-glucosamine (NAG) into polyols over transition metal catalysts and hydrogen in water. *Green Chemistry* **2015,** *17* (2), 1024-1031.

25. Kobayashi, H.; Techikawara, K.; Fukuoka, A., Hydrolytic hydrogenation of chitin to amino sugar alcohol. *Green chemistry* **2017,** *19* (14), 3350-3356.

Nehal Ghosh
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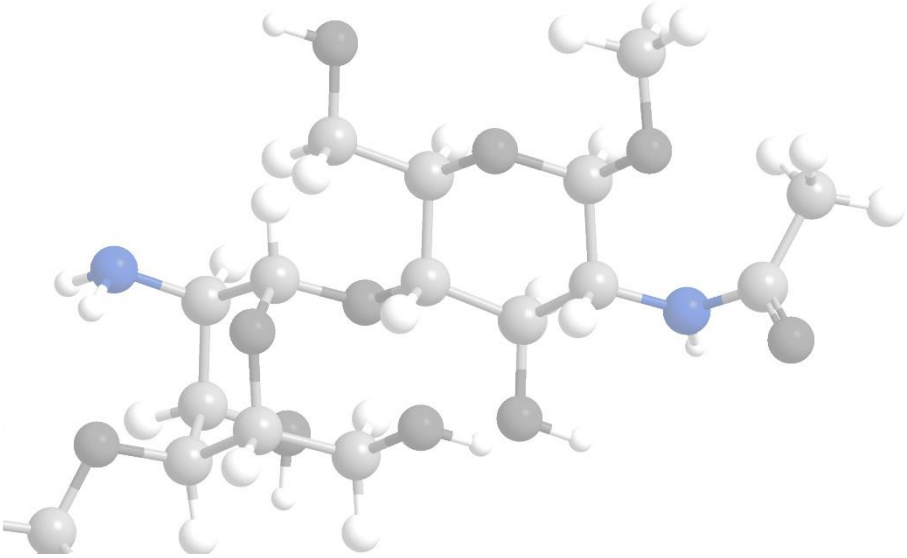
Signature of Supervisor
Dr. Paresh Dhepe

Chapter 01

General introduction and literature review



1.1. General introduction

Fossil feedstocks (crude oil, coal and natural gas) are non-renewable resources which are derived from dead organisms under high pressures and temperatures. Presently, most of the chemicals manufactured by chemical industry are derived from fossil feedstocks in spite of its diminishing availability.[1-2] The reserves of fossil feedstocks are diminishing drastically, oil prices are increasing day by day and global warming is becoming an alarming issue now-a-day. These problems are forcing scientific community to look for other alternatives.[3] Deriving fuels and chemicals from renewable resources becomes an important and interesting research area from last decade in order to reduce global dependency on fossil feedstocks. Biomass is considered as a promising alternative for fossil feedstocks as it is abundantly available and comparatively cleaner raw material to produce chemicals and fuels.[4-5] Biomass can be defined as an organic matter which is derived from any living or recently living organism. Several kinds of biomass are converted to chemicals and fuels which include agricultural waste, waste from food processing operation, wood and wood waste, sludge from water treatment plants etc.[6] Plant based non edible biomass which are not used as food are commonly called as lignocellulosic biomass and it consists of three components, i.e., cellulose, hemicellulose and lignin.[7] In last decades, researchers have taken efforts to convert non-edible lignocellulosic biomass into chemicals and fuels as it is abundantly available, relatively cheaper that food material feedstock like starch and sugar rich resources.[8-10]

1.2. Introduction to the biomass and its classification

Biomass can be defined as "biological organic matter derived from living and recently living organism." It is a source of carbon, hydrogen, oxygen and nitrogen and a renewable and promising feedstock for chemicals and fuels. Biomass feedstocks include crop or agricultural waste, industrial wastes from food

processing industry, municipal solid waste, sewage waste, animal waste and residues, wood waste etc. (Figure 1.1).[11]

Figure 1.1. Major sources of biomass

Biomass has numerous advantages over conventional fossil feedstocks which can be pointed below as,

- ✓ Plentiful, cheap and locally available renewable resource
- ✓ Can be an alternative for diminishing crude oil resources
- ✓ Sustainability
- ✓ Recycling of greenhouse gas (CO_2) by bio-refinery concept
- ✓ Economic and environmental benefits
- ✓ Waste to wealth concept which can improves economy

Biomass can be classified as plant derived biomass (e.g., starch, sugars, lignocellulosic biomass) and animal derived biomass (e.g., cow dung, chitin). Plant biomass can be further classified as edible (starch and sugars) and non-edible

biomass (lignocellulosic biomass).[12] Classification of biomass is represented in Figure 1.2.

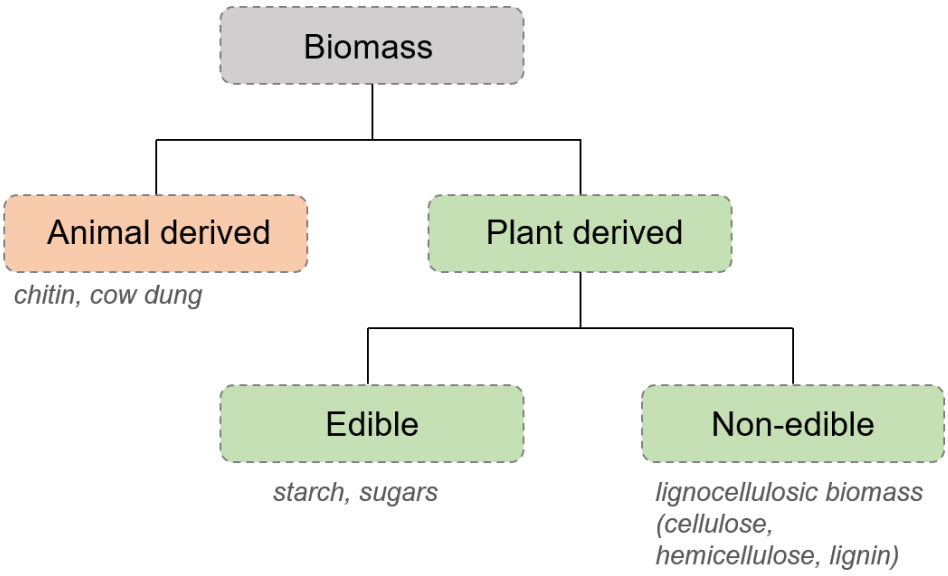


Figure 1.2. Classification of biomass

1.2.1. Plant derived biomass

Plants generate biomass by utilizing carbon dioxide, water and sunlight in presence of chlorophyll and produce oxygen as by-product. The primary products are C6 and C5 sugars which further polymerised to give carbohydrates (starch, cellulose and hemicellulose).[13] The plant biomass can be further classified into edible and non-edible biomass depending on the edibility to humans.[14] The edible biomass is a food material such as starch which can be found in rice, potato, corn, wheat, cereals etc. Starch is made up of α-glucose units connected together via α-1,4 glycosidic linkages to form amylase and via α-1,4 glycosidic linkages along with α-1,6 glycosidic linkages to form amylopectin (Figure 1.3).[14]

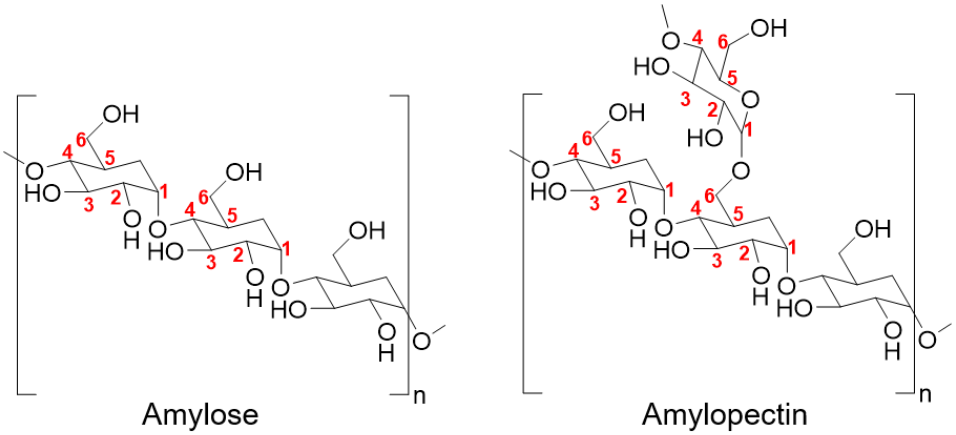


Figure 1.3. Structure of starch

Another category of plant biomass is non-edible biomass which is not edible to human. This is commonly known as lignocellulosic biomass. The lignocellulosic biomass is mainly made up of 40-50% cellulose, 25-35% hemicellulose, 10-25% lignin and some other minor components like micro and macro nutrients, proteins and wax (Figure 1.4).[7, 15] Cellulose is a homo-polysaccharide made up of β-glucose units connected together via β-1,4-glycosidic linkages.[16] Hemicellulose is a hetero-polymer of C5 sugars (xylose, arabinose) and C6 sugars (glucose, galactose, glucuronic acid).[17] Lignin is a three-dimensional polymer made up of aromatic monomers (p-coumeryl alcohol, coniferyl alcohol and sinapyl alcohol) connected together via β-O-4, α-O-4, 4-O-5, β-β, β-5, 5-5, β-1 linkages.[10] Cellulose, hemicellulose and lignin can be converted to various important chemicals as they are source of C, H and O.[10, 17-20]

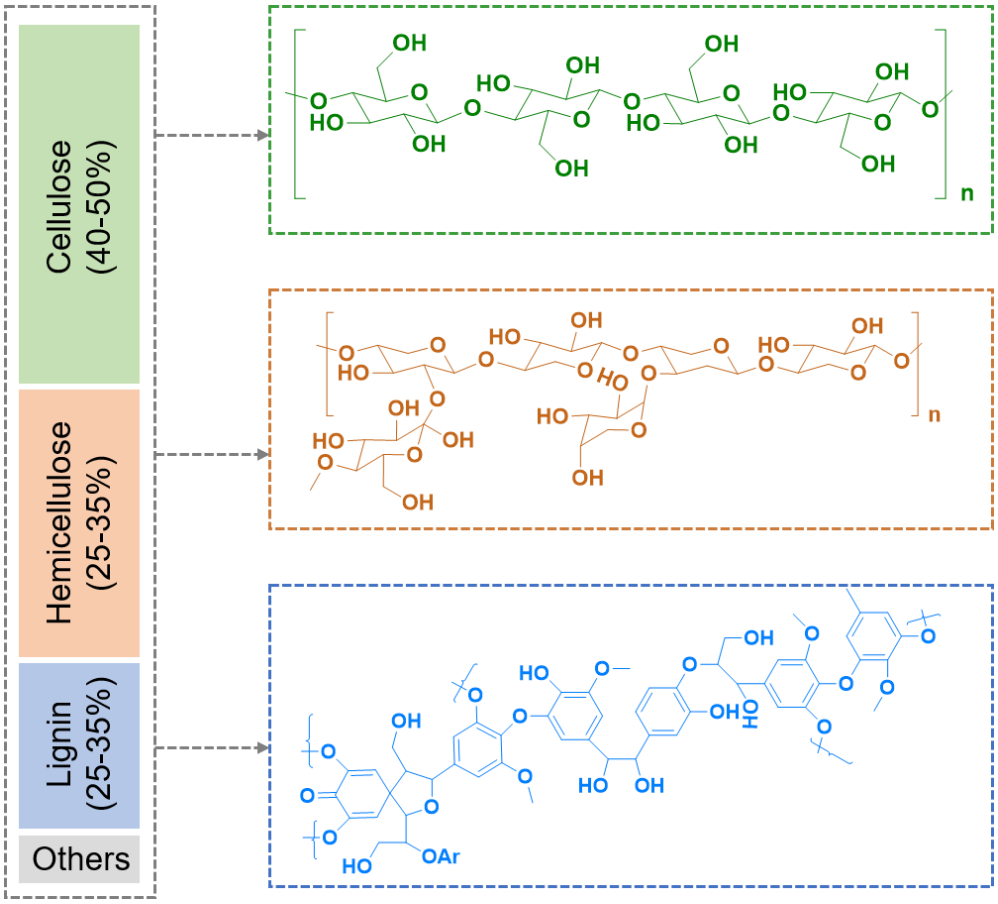


Figure 1.4. Composition of lignocellulosic biomass and the structures of different components

Currently, biomass utilization research is mainly focused on lignocellulosic biomass and very less attention is given to valorisation of animal derived biomass. Exploring new types of biomass resources is highly beneficial to expand current biorefinery system.[21]

1.2.2. Animal derived biomass

Biomass that is generated from animal waste, their non-edible body parts and exoskeletons is called animal derived biomass. Most abundantly available animal derived biomass is chitin and it is also known as marine or oceanic biomass.[21] Chitin is 2nd most plentiful bio-polymer on earth after cellulose and it is easily available and promising feedstock for chemicals but it is surprisingly largely overlooked.[22] Chitin is a major component found in exoskeletons/shells of

crustaceans and molluscs which gives strength to these exoskeletons.[23] Crustaceans are diverse arthropod taxon which have a hard exoskeleton to protect their body. Crabs, lobsters, crayfish, shrimps, krill, prawns are the examples of crustaceans. Molluscs are the second largest phylum of invertebrate with approximately 50,000 species. Not all the molluscs have chitin present in their body. Squid and cuttlefish are the example of molluscs which have chitin.[24] Chitin is a homo-polymer of N-acetyl-D-glucosamine (GlcNAc) connected together via β-1,4 glycosidic linkages. Chitin has structural similarities with cellulose (Figure 1.5) and only difference in C2 position. Hydroxyl group (-OH) at C2 position in cellulose is replaced by acetamido group (-$NHCOCH_3$) in chitin.[25] Presence of 7% biologically fixed nitrogen in chitin makes it promising and valuable feedstock for nitrogen containing chemicals which is not possible to produce from other biomass materials like cellulose, hemicellulose and lignin as they do not contain nitrogen in their structures.[22, 26]

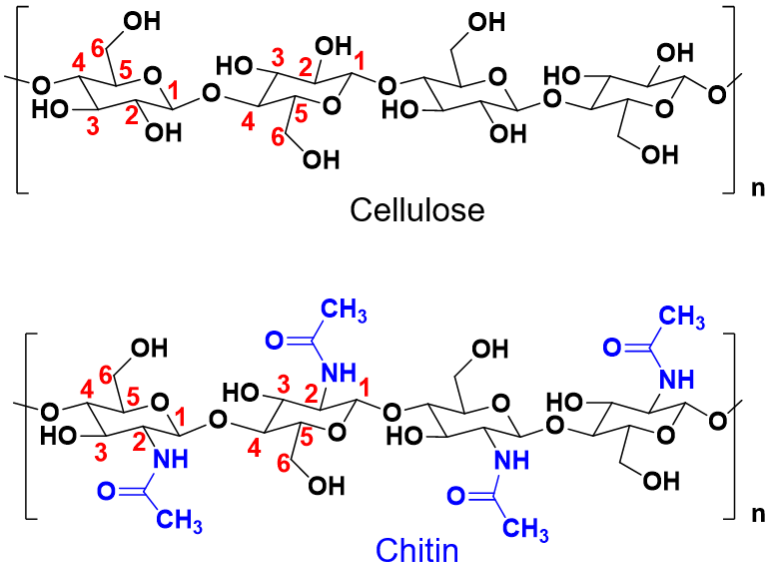


Figure 1.5. Comparative structures of cellulose and chitin

1.3. Global scenario for fisheries production and waste generation

According to the "The State of World Fisheries and Aquaculture" report published by FAO (Food and Agriculture Organisation of the United Nations) in 2020, global

fisheries (including fish, crustaceans, molluscs) production is increasing day by day and reached about 179 million tonnes (both marine and inland) in 2018.[27] 156 million tonnes were used for human consumption and remaining were used for non-food uses like fish oil or fish meal production. Major fish producing country is China with 35% of global fish production in 2018. Apart from China, a significant contribution came from Asia (34%), Americas (14%), Europe (10%), Africa (7%) and Oceania (1%). The main reason for increasing global fisheries production is fisheries consumption by humans. Per capita fisheries consumption has increased from 9.0 kg (live weight) in 1961 to 20.5 kg in 2018 as it is a major source of animal protein.[27] According to FAO 2020 report, 6 million tonnes crustaceans (prawns, shrimps, lobsters, krill, crab etc.) were produced in marine water and 9.4 million tonnes crustaceans were produced annually by aquaculture in 2018. Furthermore, production of crustacean farming has significantly increased in Asia in 2018.[27] The total marine and inland crustacean annual production was 8.4 million tonnes in Asian countries which is 89.6% of the total world production of crustaceans (Table 1.1).[27-28]

Table 1.1. Aquaculture production of crustaceans[27]

Category	Asia	World
	(Million tonnes, live weight)	
Inland aquaculture	3.6	3.7
Marine aquaculture	4.8	5.7
Total	8.4	9.4

India also has long coastline of about 8118 km which includes West Bengal (WB), Orissa (OD), Andhra Pradesh (AP), Tamil Nadu (TN), Pondicherry (PD), Andaman and Nicobar Islands (AN) as east coast and Gujarat (GJ), Maharashtra (MH), Goa (GA), Karnataka (KA), Kerala (KL), Lakshadweep (LD) as west coast (Figure 1.6).[29]

According to "Handbook of Fisheries Statistics 2020" published by Ministry of Fisheries, Animal Husbandry and Dairying, Government of India, total annual fisheries production (marine and inland) is 13.5 million tonnes in 2018.[30] Table 1.2 shows species wise marine fish production in coastal states of India.

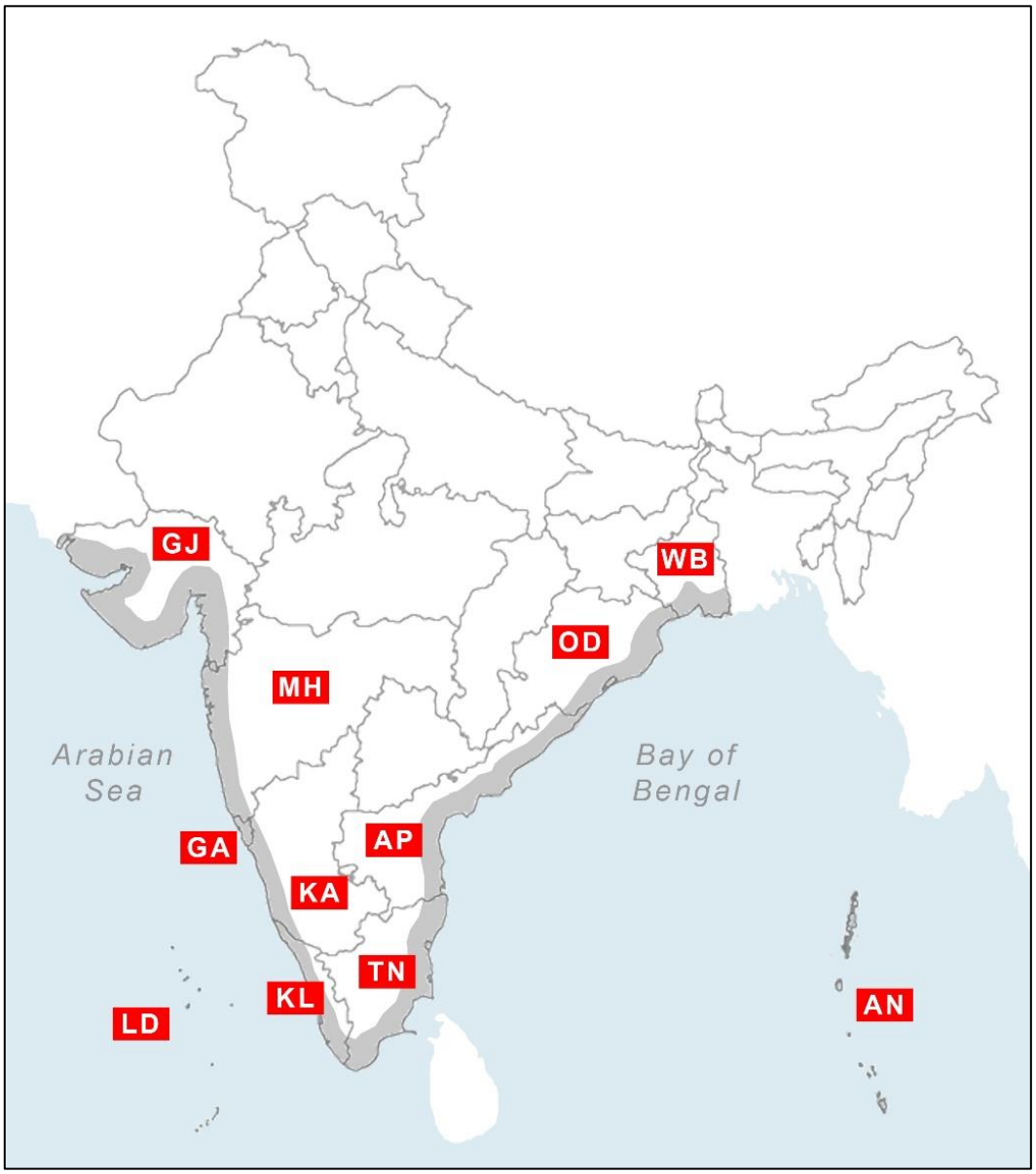


Figure 1.6. Coastline of India

Table 1.2. Species-wise marine fish production in coastal states of India[30]

Production in coastal regions in India	Species wise marine shells production in 2019-20 (in Lakh Tonnes)[30]			
	Penaeid prawns (Penaeidae)	Non-penaeid prawns (Sergestidae)	Lobsters (Nephropidae)	Crabs (Brachyura)
East coast (AP, OD, TN, WB, AN, PD)	1.6	0.63	0.04	0.13
West coast (GA, GJ, KA, KL, MH)	1.22	0.93	0.03	0.19

Based on the production rate and consumption demand of crustaceans, copious amount of shell wastes is generated by farming and food processing industries.[27] Shell (exoskeleton), head and legs of shrimps, prawns and crabs were discarded as waste after using the edible part. The disposal of the shell waste produced creates a practical challenge.[31] Shell wastes were openly disposed of in landfills or sea in majority.[11] After dumping in the landfill, shell wastes were burned or left out to spoil. Sea water gets polluted and hardness of water increases due to calcium carbonate content in shell waste which can harm aquatic animals due to drastic change in mineral content in water. As a consequence of burning the shell wastes in landfill grounds, harmful gases like CO, CO_2, NO_2, NO can be formed which cause environmental pollution. Otherwise, the shell waste disposal lands produce bad odour, dust and gases while the process of decomposition. Moreover, infectious diseases can be caused and transmitted by rodents, flies, mosquitoes and other pests at the nearby locality. Soil, water and air pollution can happen and livelihood of people can be affected as a consequence of improper disposal of shell waste.[28] Moreover, in developed countries like in some parts of UK, the proper disposal at landfill ground could cost over £80 per tonne.[32] Hence, the improper disposal of shell waste can be a nuisance and polluting if it

is not controlled properly.[32] Shell waste contains several useful components including the second most plentiful biopolymer, chitin. Hence, fractionation of shell waste by separating the components, upgradation of each fraction and conversion of them into useful chemicals can be a solution to the shell waste management and can contribute to the existing biorefinery system. The concept of shell biorefinery was recently proposed and it has gained immense interest in research community because utilization and valorisation of shell waste can bring both economic and environmental benefits.[33-34]

1.4. Composition of shell waste and their potential uses

Crustacean shells are composed of unique components which are distinctively different from lignocellulosic biomass sources. Shells are made up of three major components, calcium carbonate (20-50%), protein (20-40%) and chitin (15-40%) along with minor amounts of lipids, minerals and pigments like astaxanthin (Figure 1.7).[34] This composition varies depending on the species, habitat, season and geographical location.[28, 31]

1.4.1. Calcium carbonate

Calcium carbonate is found as common substance in rocks and shells of various organisms like snail, egg shell. Three polymorphs of $CaCO_3$, that is, β-$CaCO_3$ (calcite), λ-$CaCO_3$ (aragonite), and μ-$CaCO_3$ (vaterite) are found in nature.[35] These three polymorphs can co-exist in several marine organisms. In shrimps shells, calcite and aragonite forms both are present in prismatic layer and nacreous layer respectively.[36] $CaCO_3$ has wide applications in construction and building industry. It is also used as extender in the paint industry. In addition to this, $CaCO_3$ is used for health and dietary applications such as gastric antacid, phosphate binder etc. It has an important application in soil treatment in the agricultural industry.[33] Shell waste can be a reliable and potential source of $CaCO_3$ as it contains 20-50% of $CaCO_3$.

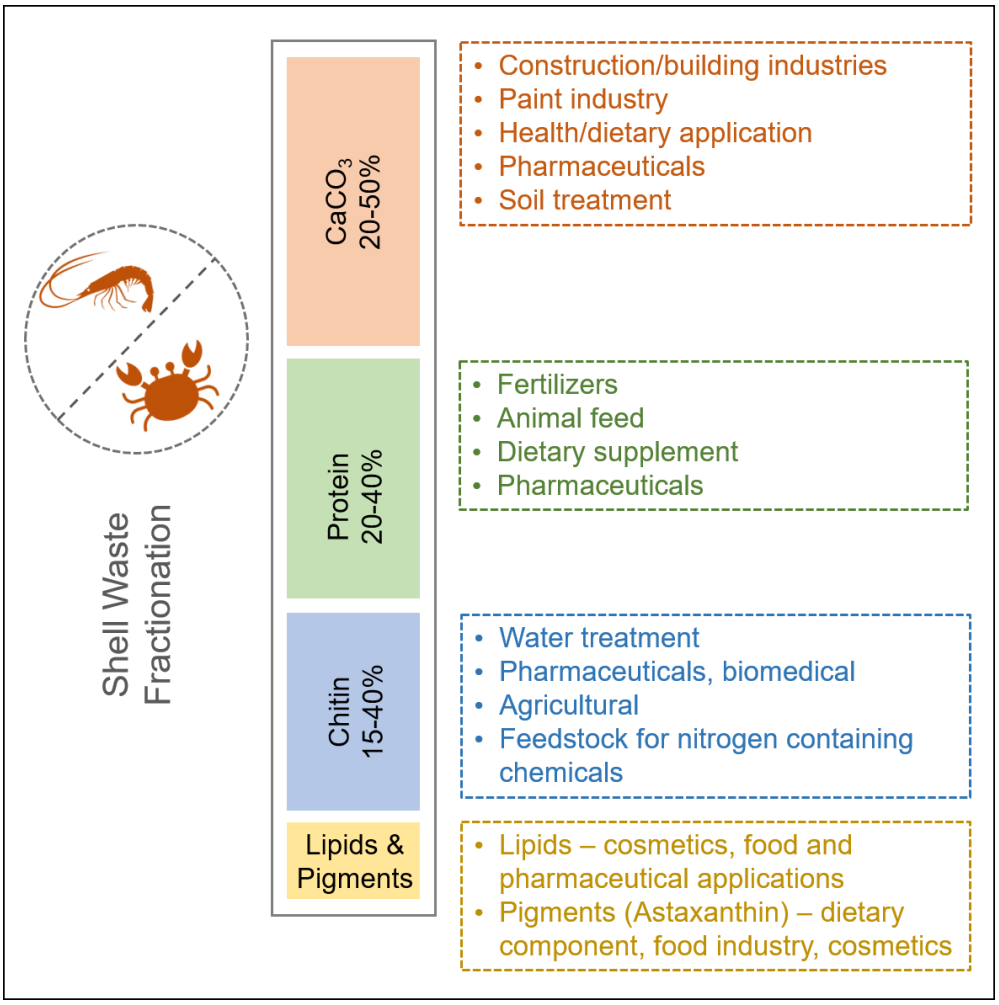


Figure 1.7. Fractions of shells and their applications

1.4.2. Protein

Proteins are macromolecules which have 3-dimensional structures and usually contains one or more polypeptides comprising up to 20 different L-α-amino acid residues. 20 natural amino acids can be synthesized by most microorganisms and plats. In animals, amino acids are obtained by consuming proteins as component of daily diet and proteins are breaking down to amino acids through digestion. Human body needs protein in daily diet to help the body repair cells and make new ones. The protein hydrolysate which is found in some shrimp shells can be used in industries including human nutrition, animal feed, pharmaceuticals and cosmetics.[37] The percentage of essential amino acids in protein hydrolysate determines the nutritional value of a food protein. Several species shrimp shells

found to have proteins constituting amino acids glutamic acid, lysine, histidine, and valine which represent shell waste can be used as a potential nutritious protein source as animal feed and food supplement.[38]

1.4.3. Chitin

Chitin is the second most plentiful natural polysaccharide on earth after cellulose with a global production rate exceeding 100 billion tonnes per year. Chitin is found in the shells/exoskeletons of crustaceans (crabs, shrimps, prawns, lobsters), molluscs (squid, cuttlefish), insects, fish scales and also in some fungi and bacteria.[39] Chitin plays the role of protective support in the cells of these organisms where chitin fibrils are embedded in a matrix of calcium carbonate and phosphate along with protein (Figure 1.8a).[40-41] The main source of chitin is shell waste as it contains 15-40% of chitin. Chitin content varies from species to species, e.g.- 14-27% chitin in shrimp and 13-15% chitin in crab shell wastes were found. Chitin is a linear polymer composed of N-acetyl-D-glucosamine units connected via β-1-4 glycosidic linkages. Chitin has a structural similarity with cellulose. Hydroxy group at C2 position of the glucose units are replaced by acetamido group in chitin. Three polymorphic forms of chitin occur in nature, namely, α-chitin, β-chitin and γ-chitin.[42] They differ in the packing and polarities of adjacent chains in successive layers (Figure 1.8b). Among all the polymorphs, α-chitin is the most abundant and thermodynamically form with an antiparallel arrangement of polymeric chain. The β-chitin has a parallel arrangement of chains while γ-chitin has a parallel and antiparallel structure, which is considered as a variant of α-chitin family by recent studies.[40, 43] The structural transformation of α-chitin to β-chitin is possible while the reverse cannot be done. α-chitin is found in fungal and yeast cells as well as crab, lobster, shrimp shells and β-chitin are mainly found in squid or loligo pens.[40, 44-45] The crystallinity of α-chitin is due to the presence of intra chain C3′-OH to O5, C6′-OH to acetamido carbonyl O7 and C6-OH to C6′) and interchain (between NH and the acetamido carbonyl O7′ and C6′-OH to O6)

hydrogen bonding between the polymeric chains.[46] Due to this extensive hydrogen bonding network and structural rigidity, chitin is insoluble in most of the common solvents which limits its utilization. Chitin is soluble only in some environment unfriendly solvents like hexafluoro isopropanol, hexafluoroacetone sesquihydrate, methanesulfonic acid, inorganic salts such as LiCNS, Ca $(CNS)_2$, CaI_2, $CaCl_2$, concentrated mineral acids like trichloroacetic acid, dichloroacetic acid, mineral bases like NaOH. Moreover, Lithium Chloride (LiCl) can form a complex with chitin which is soluble in dimethylacetamide (DMA), N,N-dimethylpropionamide (NMP) and 1,3-dimethyl-2-imidazolldione. Hence chitin can be solubilised in above mentioned binary mixtures through complex formation with LiCl.[47] Chitosan is derived from chitin by deacetylation using base, with a minimum deacetylation of 70%. Thus, chitosan is a heteropolymer made up of β-1,4 linked D(+)-glucosamine ($GlcNH_2$) and N-acetyl-D-glucosamine (GlcNAc) units.[48] Chemical modification to chitin is done to produce chitosan which is more utilizable derivative.[48] Chitin and chitosan contain nitrogen along with C, H and O in their structure offering an excellent resource to produce a series of valuable chemicals especially nitrogen-containing chemicals, which cannot be derived from cellulose, hemicellulose and lignin. Moreover, the manufacture of N-containing chemicals is typically laborious and energy exhaustive starting from high temperature, high-pressure ammonia synthesis.[22] Hence, chitin can be a potential resource which is inexpensive and sustainable to produce nitrogen-containing chemicals.[33] Chitin and its derivatives have many applications in wastewater treatment, cosmetics, textile, paper, agriculture industry and they are very much important materials for biomedical applications.[49] The biological properties of chitin and chitosan such as antibacterial, antioxidative, biocompatibility, biodegradability, etc. have made chitin as a versatile material for variety of applications biomedical, pharmaceutical fields etc.[50-51] Chitin biomass represents a bio-renewable resource for the synthesis of nitrogen self-doped carbon materials.[52-53]

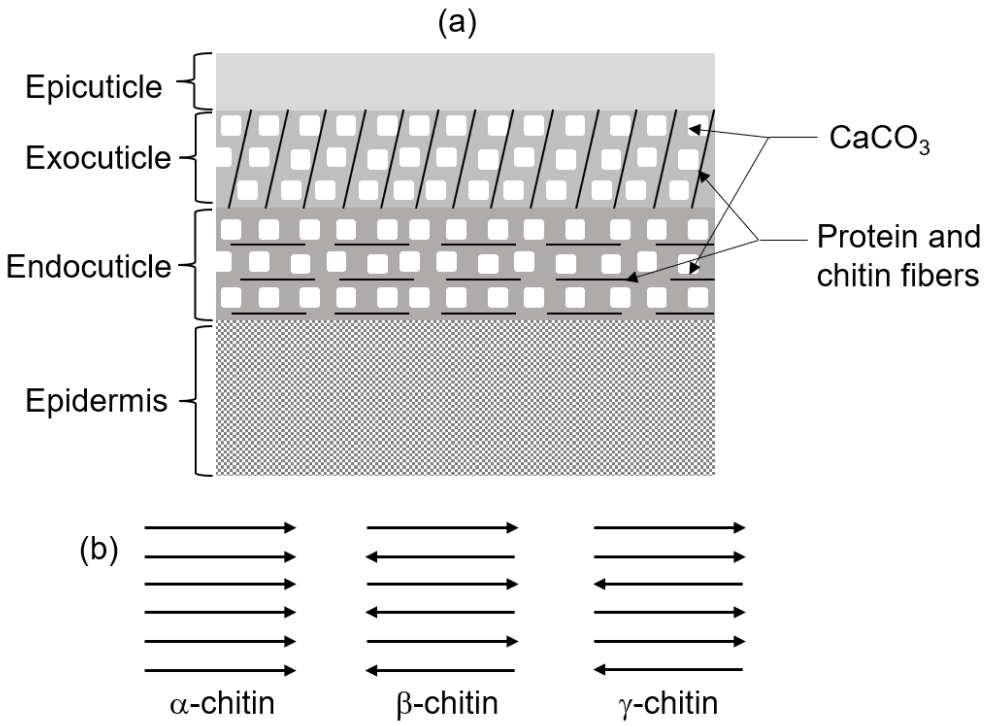


Figure 1.8. (a) Schematic representation of special arrangement of shell components in crustacean shells, (b) Chain arrangement of three polymorphs of chitin (α-chitin, β-chitin, γ-chitin)

1.4.4. Lipids and pigments

Lipids contain hydrocarbons and plays vital role as building blocks for the structure and functioning of cells. Examples of lipids are fats, waxes, oils, hormones, certain vitamins and cell membrane etc. Shrimp shells majorly contains fatty acids, with 30-50% saturated and 50-70% unsaturated fatty acids.[33] C20:0 Eicosanoic acid is the most abundantly available fatty aid in shrimp shells. Here 20 refers to the carbon chain length, and 0 refers to the number of C=C bonds).[54] Oleic acid (C18:1) is the major monounsaturated component and linoleic acid (C18:2) is the major polyunsaturated fatty acid found in crustacean shells. Several n-3 long chain polyunsaturated fatty acids, such as eicopentaenoic acid {EP, 20:5 (n-3)}, docosahexaenoic acid {DHA, 22:6 (n-3)} and other essential fatty acids are also present in crustacean shells. Chemical structures of these fatty acids are

represented in Figure 1.9. These lipids are used in cosmetics, food and pharmaceutical applications.[33, 55]

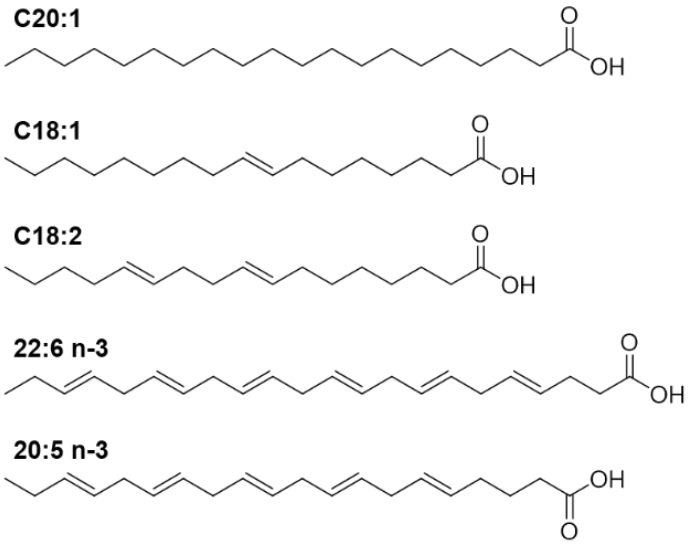


Figure 1.9. Structures of fatty acids present in crustacean shells

Pigments like astaxanthin, astathin, lutein, canthaxanthin and β-carotene etc, are found in crustacean shells.[43] Astaxanthin is a red coloured pigment which is soluble in lipids and its colour is due to the extended chain of conjugated double bonds.[56] As astaxanthin has anti-oxidant, anti-inflammatory, anti-immunomodulating effects, it has a wide application in pharmaceuticals, food and cosmetics industry. Astaxanthin is very beneficial to human health and it is used as dietary component and colour additive in food industry. Crustaceans are found to be transparent or in different colours rather than red because the pigments are bound to the protein in the shell. After denaturation and unfolding of protein, astaxanthin becomes free and turns red. That's why, seafood turns red when cooked.[33]

1.5. Fractionation methods of shell waste

There are several methods for separating the fractions of shell wastes which are reported in literature. Those are industrial method/chemical method, solvent extraction method and bioprocessing methods (Figure 1.10).

1.5.1. Industrial method

In early 1800, scientists showed interest in shell waste fractionation due to their curiosity for chitin. In 1811, Henri Braconnot has found that chitin is present in cell wall of fungi and it is resistant to chemicals. Chitin remained unaltered after the treatment of alkali and acid.[36] In 1970s, the extraction of chitin from marine waste was kickstarted due to regulations on the dumping of untreated marine waste into the sea.[57] Well established protocol for chitin extraction from shell waste is available which is followed by industries for chitin extraction. The process has three steps starting from demineralization followed by deproteinization and decolouration steps.[58-59] First of all, shell wastes were cleaned and powdered. Then, demineralization is carried out in presence of acids such as hydrochloric acid (HCl), sulphuric acid (H_2SO_4), nitric acid (HNO_3), acetic acid or formic acid. However, HCl is mainly used for this treatment with a concentration varied from 0.2 to 2M. Processing time can be varied from few hours to two days and temperature is kept between room temperature to 100 °C. During the acid treatment, calcium carbonate and other minerals forms chloride salts which are soluble in water and carbon dioxide is removed as gas.[33] (Eq. 1)

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + H_2O + CO_2 \uparrow \quad \text{..... Eq. 1}$$

Deproteinization is mainly performed with the treatment of alkali. Typically, sodium hydroxide is used with concentration varies from 0.1 to 2M at the temperatures of 65-100 °C for 1-72 h. Shell wastes generally contains 20-40% protein which in presence of NaOH gets solubilized after breakage of peptide bonds. In addition to deproteinization, base treatment also leads to partial deacetylation of chitin.[60] To remove the pigments and to get colourless chitin for commercial applications, oxidative bleaching with sodium hypochlorite solution (NaOCl) and hydrogen peroxide (H_2O_2) is carried out.[59] Sometimes, depigmentation step is carried out before demineralization to extract high value astaxanthin. Even though the commercial method is widely used in industries as

it is easy and able to fractionate effectively, it has several drawbacks like costly, destructive, generation of neutralisation waste and environment unfriendly.[61] Since it involves corrosive mineral acids and bases, corrosion resistant equipment is required and furthermore, a huge amount of wastewater is generated from the neutralization process which brings extra treatment expenses.[61] However, this is a very important research area to find out comparatively green and cost-effective fractionation method.

1.5.2. Solvent extraction method

Chitin is insoluble in many of the common solvents but ionic liquids (ILs) like [BMIM][Cl], [AMIM][Br], [BMIM][OAc], [EMIM][OAc] etc. and deep eutectic solvents, such as choline chloride, thiourea, choline-chloride-urea can dissolve chitin.[62-64] The dissolution of other biopolymers such as cellulose, lignin, keratin etc. in several ILs is well explained in literature. The interaction of free and strongly coordinating anions in the ionic liquids with the hydroxyl group of chitin helps to reduce the extend of hydrogen bonding in the structure and aid the dissolution of chitin. This dissolution ability of ILs has be used for chitin extraction from shell waste.[65] The chitin extraction using IL is reported by using [EMIM][OAc] (1-ethyl-3-methylimidazoliumacetate). In this report, 0.4 g shell waste with 10 g IL was loaded under microwave heating for 2 minutes. Then the dissolved chitin was precipitated by adding water and chitin is obtained with high purity and molecular weight.[66] Calcium carbonate was also dissolved along with chitin. To eliminate calcium contamination, an additional step of demineralization using citric acid can be employed.[67] The shells treated with IL at 100 $^{\circ}$C for 24 hours is followed by demineralization with the citric acid solution for 3 h to yield chitin. Besides ILs, deep eutectic solvents, such as choline chloride-thiourea, chlorocholine chloride-urea and betaine hydrochloride-urea were used to dissolve chitin upon microwave heating.[64] Solvent extraction method along with

demineralization can extract pure chitin but costly ILs are needed in high amount which can increase the cost of extracted chitin.

1.5.3. Bioprocessing methods

1.5.3.1. Enzymatic method

Proteolytic enzymes that found in plants, microbes and animals such as proteases are used for deproteinization of shell waste. Protease enzymes like papain, trypsin, pepsin, devolvase, alcalase and pancreatin are reported in literature to remove proteins from shells with minimal deacetylation.[68] Enzymatic deproteinization of shell waste after demineralization has yielded chitin and protein hydrolysate.[38] Deproteinization can be done before or after the demineralization step. Crude proteases can also be used and they are obtained from bacteria and fish viscera. Even though enzymatic methods offer high-quality products and remain as an eco-friendly process, they are costly, and the efficiency is lower in comparison to the chemical processes.[69] Around 5 to 10% of residual proteins are found associated with the isolated chitin. Additional alkali treatment possibly will increase the purity of isolated chitin.

1.5.3.2. Fermentation method

Deproteinization using fermentation method can reduce the enzyme cost by adding selected microbial strains and endogenous microbes.[58] Deproteinization and demineralization both steps can be done simultaneously in fermentation process. Water soluble Ca^{2+} enriched protein hydrolysates after fermentation which can be utilised as animal feed supplements. Usually, different types of microorganisms and fermentation conditions could lead to significant differences in efficiency of deproteinization and demineralization.[33] Fermentation can be classified into two classes: lactic acid fermentation and non-lactic acid fermentation. For lactic acid fermentation, *Lactobasillus paracasei* is used for fermentation of shells which produce proteases and lactic acid. Lactic acid is obtained by alteration of glucose which brings about a decrease in pH of fodder

thereby suppress the growth of spoilage microbes. The productivity of lactic acid fermentation depends on various factors like the quantity and microbial composition of inoculum, initial pH, pH progression during fermentation, carbon source and its concentration, temperature, and duration of fermentation.[70] Bacillus, Pseudomonas, Aspergillus were used for fermentation of crustacean shells in non-lactic acid fermentation method. The protease producing bacteria, Pseudomonas aeruginosa gave the highest deproteinization and demineralization efficiencies. Biological methods are efficient and green but they are very sensitive to pH, temperature, duration, concentration etc.

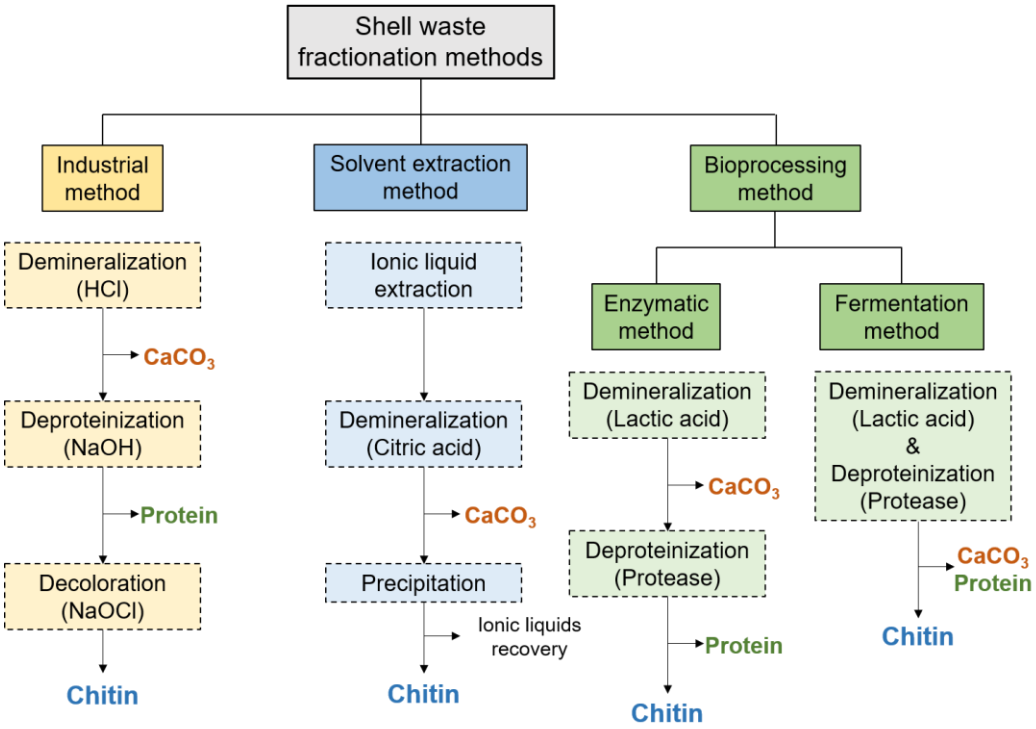


Figure 1.10. Flow chart of the fractionation methods for crustacean shells

1.6. Chitosan

Chitosan can be derived from chitin by deacetylation using sodium or potassium hydroxide solution. Chitosan is the heteropolymer of N-acetyl-D-glucosamine and D (+)-glucosamine (Figure 1.11). N-acetyl groups cannot be deacetylated by acidic reagents as hydrolysis of the polysaccharide may happen in acidic medium. Thus, alkaline methods were used for deacetylation. The temperature was kept in

the range of 100-140 ˚C and the reaction time varies from 1-24 h. Sodium hydroxide solution is used typically with 35-60% concentration. The extent of deacetylation depends upon concentration of alkali, temperature and time. The alkali concentration and reaction condition should be optimum to prevent the degradation of molecular chain.[71]

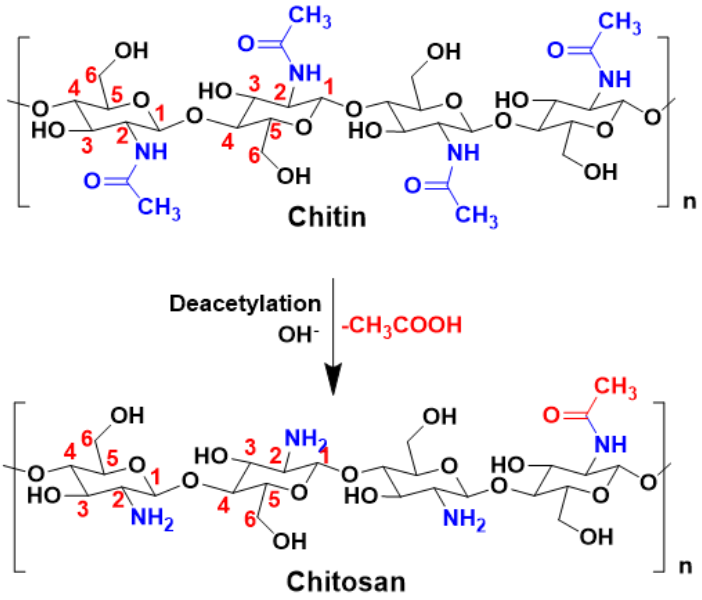


Figure 1.11. Schematic representation of deacetylation of chitin to form chitosan

1.7. Applications of chitin and chitosan

Presence of N-acetyl group in the polymeric backbone increases the dimension of chitin for various applications. Chitin and chitosan both have various applications in different sectors which make them more important than its structural analogue, cellulose. Chitin and chitosan both can absorb dyes, proteins, toxic metals and metal cations, aromatic hydrocarbons.[72] Due to these adsorbing properties of polycationic polymer chitosan, it is used widely in waste water treatment for coagulation and flocculation.[49, 73] Hydrogels, films, fibres, blends and composites can be formed from chitin and chitosan.[51] Furthermore, chitin and chitosan have various pharmaceutical, physiological and biological applications due to their biocompatibility, non-toxicity and biodegradability. They

are utilized in hypolipidemic enhancement, eliciting biological responses, antimicrobial activity, wound healing, mucoadhesion and immunity enhancement.[50] Chitosan is immensely used in tissue engineering, cell culture and gene delivery.[74] Additionally, chitin and chitosan are promising feedstock for a wide range of chemicals. Nitrogen containing chemicals can be synthesized from chitin and chitosan which is not possible form other biomass sources.[34] Moreover, due to inherent nitrogen content, they can be used as efficient catalysts or catalyst support.[33]

1.8. Recent developments on chitin and chitosan valorisation

Chitin biomass (includes chitin, chitosan, N-acetyl-D-glucosamine, D(+)-glucosamine) is a potential feedstock for several value-added chemicals as chitin is the second most abundant biopolymer on earth. Presence of 7% biologically fixed nitrogen in its structure along with C, H and O offers an excellent source to produce nitrogen containing chemicals which are not possible from lignocellulosic biomass. Conversion of chitin and chitosan to value added chemicals has gained immense attention of the researchers and a lot of efforts have been made for the valorisation of chitin within a decade. Chitosan is more suitable for the chemical transformation because of its enhanced solubility due to the weakening of crystallinity in the structure. Both chitin and chitosan are excellent feedstock for production of important chemicals.[22, 33] Acids are well known for hydrolysing the polysaccharide chain and give depolymerised products. It is also well known from cellulose chemistry that acids were used to depolymerise cellulose to give D-glucose and further dehydration can give furan derivatives. As chitin has a similar structure as cellulose, Chitin and chitosan can also be depolymerized in presence of acidic medium to give monomers and further dehydration can give valuable furan derivatives (with or without nitrogen) and organic acids. Transformations of chitin and chitosan in acidic medium is represented in Figure 1.12.

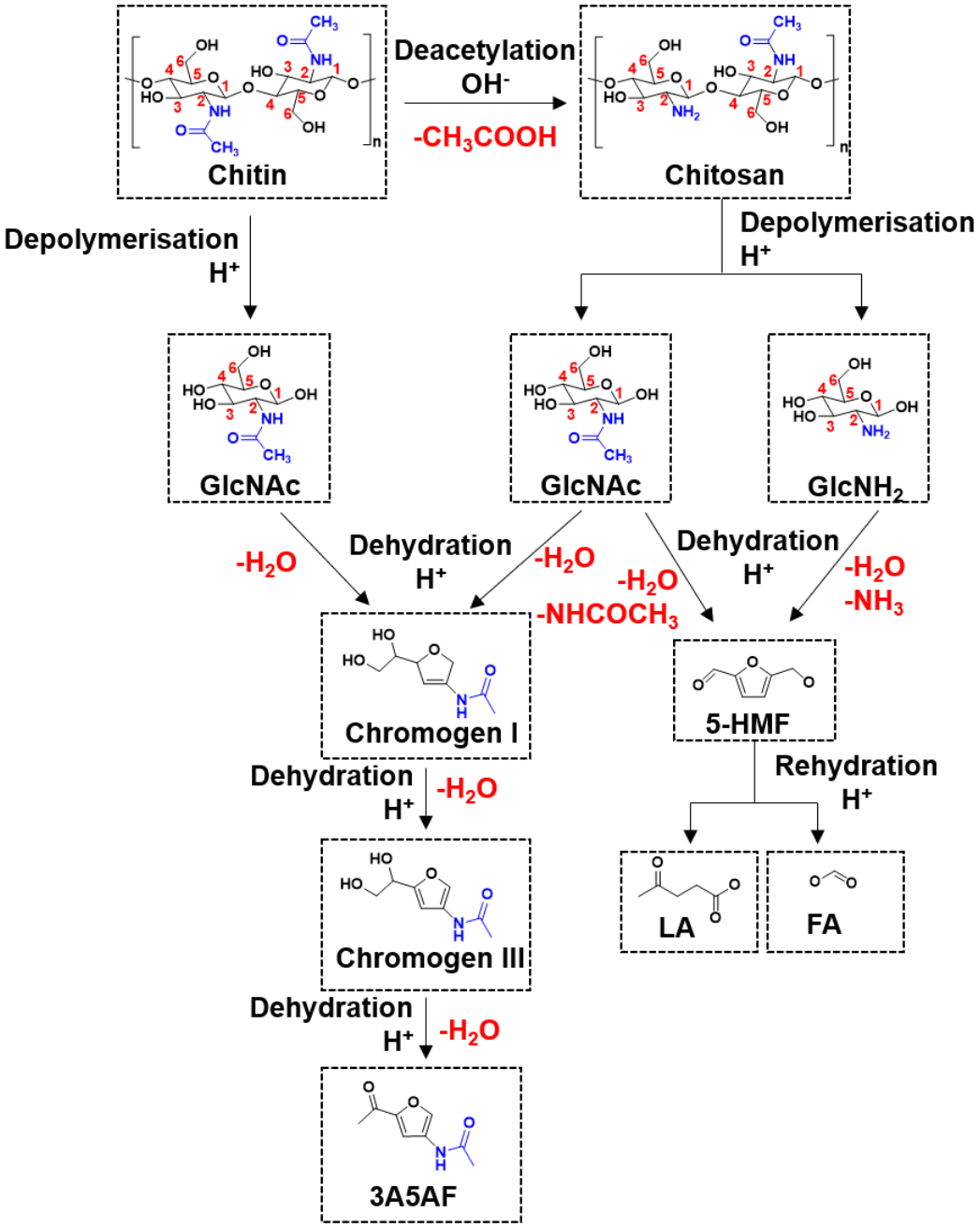


Figure 1.12. Transformation of chitin and chitosan in acidic medium

(GlcNAc: N-acetyl-D-G-glucosamine, $GlcNH_2$: D(+)-glucosamine, 5-HMF: 5-hydroxymethylfurfural, LA: Levullinic acid, FA: Formic acid, 3A5AF: 3-Acetamido-5-acetylfuran)

1.8.1. Depolymerisation of chitin biomass to oligomers and amino sugar monomers

The first step in the chemical conversion of chitin and chitosan is their depolymerisation into monomeric units N-acetyl-D-glucosamine (GlcNAc) and D (+)-glucosamine ($GlcNH_2$) respectively. The depolymerization methods can be chemical, physical, enzymatic and combinations of any of these methods as well. These amino sugars are used in biomedical, pharmaceutical and food industry for various applications.[75-76] The depolymerization methods can be chemical, physical, enzymatic and combinations of any of these methods as well. Chemical methods include the hydrolysis of chitin and chitosan in the presence of strong acids,[77] oxidative degradation using hydrogen peroxide[78-79] and ozone treatment[80] at elevated temperatures. Specific enzymes e.g. chitinase, chitosanase and lysozyme, some non-specific enzymes like cellulose, protease, lipase and pepsin are also effective for enzymatic hydrolysis.[81] Microwave and ultrasound techniques also found to be useful along with chemical and enzymatic methods by reducing the reaction time and solubility of chitin. Recently ionic liquid mediated chitin and chitosan hydrolysis has also emerged with promising results, HCl promoted hydrolysis of Chitosan in [BMIM][Cl] ionic liquid formed a mixture of total reducing sugars(TRS) with 60% yield within 7 hours at 100 °C in the presence of 6.0 *wt%* concentrated HCl.[82] Sulfonic acid functionalized imidazolium ILs (SFIL) have been used for microwave-assisted hydrolysis of chitosan.[83] 1-(3-sulfonic acid) propylpyridinium hydrogen sulfate showed the best activity in DMSO because of higher Brönsted acidity. The highest yield of 90% of TRS obtained within 2 minutes at 640 W microwave power. Mechano-force assisted depolymerization of chitin using a catalytic amount of H_2SO_4 was reported by Kobayashi et al. ball milling of acid impregnated chitin under solvent-free conditions yielded soluble short-chain oligomers, further hydrolysis of the ball-milled sample at 170 °C for 1 hour gave 53% of GlcNAc and methanolysis afforded 1-O-methyl-N-acetylglucosamine (MeGlcNAc) with 70% yield.[84] Ning Yan and

Co-workers have employed a similar strategy for hydrolysis of chitin, instead of water medium co-solvent systems are used. Various polar aprotic solvents were screened in which a 4:1 diethylene glycol diethyl ether (DGDE)/water co-solvent system in the presence of sulfuric acid 80% yield of $GlcNH_2$ was achieved at 175 °C in 1h from ball-milled chitin.[85] Depolymerisation and functionalization of ball-milled chitin and proto-chitin directly from shrimp shells were achieved during formic acid-mediated chitin liquefaction. An overall yield of 60% of monomeric products was obtained at 100 °C in 12h.[86] With longer reaction times, these monomeric products underwent dehydration to generate 5-(formyloxymethyl) furfural as the final product. H_2SO_4 catalysed chitin liquefaction in ethylene glycol (EG) medium have been reported by the same group, 75% of chitin was liquefied at 165 °C within 90 minutes by using 8 *wt*% of acid. Hydroxyethyl-2-amino-2-deoxyhexopyranoside (HADP) and hydroxyethyl-2-acetamido-2-deoxyhexopyranoside (HAADP) were obtained as the major products in approximate yield of 30%.[87] A glucose-based solid acid catalyst (GSA) synthesized by hydrothermal carbonization was employed for hydrolysis of chitosan to yield $GlcNH_2$. 98.1% yield of $GlcNH_2$ is obtained at 110 °C in 6 h and the catalyst found to be stable up to 6 catalytic cycle.[88] HCl is used for solubilizing chitosan before reaction. The monomers or oligomers of chitin and chitosan are also used for chemical transformations to various compounds. Reports on conversion of chitin biomass to oligomers and amino sugars are tabulated in Table 1.3.

Table 1.3. Summary of reports on oligomers and amino sugar monomers from chitin biomass

Substrate	**Catalyst**	**Reaction Conditions**			**Product (% Yield)**
		Solvent	**Temp.(°C)**	**Time (min)**	
Chitosan	HCl	[BMIM][Cl]	100	420	TRS (60)
Chitosan	SFIL	DMSO	640W(MW)	2	TRS (90)

Chitin (ball milled with H_2SO_4)	H_2SO_4	Water	170	60	GlcNAc (53)
		Methanol	190	60	MeGlcNAc (70)
Chitin (ball milled)	H_2SO_4	DGDE/Water (4:1 *v/v*)	175	60	$GlcNH_2$ (80)
Chitin (ball milled)	Formic acid	Formic acid	100	720	Monomeric products (60)
Chitin	H_2SO_4	EG	165	90	HADP+HAADP (30)
Chitosan	GSA	HCl	110	360	$GlcNH_2$ (98.1)

(TRS: total reducing sugars, SFIL: Sulfonic acid functionalized imidazolium IL, DGDE: diethylene glycol diethyl ether, HADP: Hydroxyethyl-2-amino-2-deoxyhexopyranoside, HAADP: hydroxyethyl-2-acetamido-2-deoxyhexopyranoside, GSA: glucose-based solid acid)

1.8.2. Synthesis of furan derivative without nitrogen, 5-hydroxymethylfurfural (5-HMF)

5-Hydroxymethylfurfural (5-HMF) is one of the valuable platform chemicals derived from biomass.[89] 5-HMF production from cellulosic biomass materials is well known and the same from chitin biomass is also possible. The earlier methods were based on mineral acids catalysts like HCl, H_2SO_4 and acetic acid etc. Chitin was transformed to 5-chloromethylfurfural (5-CMF) and Levulinic acid (LA) in HCl/dichloroethane (1/2 *v/v*) biphasic system at 150 °C in 3h.[90] 45% of 5-CMF and 29% of LA was obtained, while cellulose yielded 85% of 5-CMF, hence, proven the difficulty of chitin transformation due to its structural rigidity. 5-CMF can be easily converted into 5-HMF by hydrolysis and reaction of 5-CMF with ethanol and hydrogen can give 5-(ethoxymethyl)furfural and 5-methylfurfural respectively. In another HCl catalysed the degradation of chitosan at 200 °C for 30 min under microwave heating obtained 2.2% 5-HMF.[91] Degradation of chitosan at 174 °C

for 36.9 min yielded 12.1% 5-HMF in a low-temperature hydrothermal process using dilute (2.2%) H_2SO_4,[92] while $GlcNH_2$ conversion at similar condition yielded an 5-HMF yield of 1.8% in 5 min and higher amounts of LA was obtained.[93] LA, which is the rehydration product of 5-HMF, often formed along with 5-HMF. 5-HMF was formed during ultrasound-assisted chitosan degradation using 0.5% (v/v) acetic acid solution.[94] Chitosan sonicated at 40 °C for 30 min yielded a high concentration of 5-HMF in the soluble fraction. In a formic acid-mediated liquefaction of chitin produced 5-(formyloxymethyl)furfural (5-FMF) with up to 35% yield after elongated reaction time (168 h at 80 °C). The formylation of the hydroxyl group is followed by the formation of soluble polymeric products, which undergo hydrolysis to yield monomeric products and rehydrated products.[86] $GlcNH_2$ conversion in presence of 0.1M methanesulfonic acid (MSA) was carried out at 160 °C for 40 min by Jeong et al. produced 2.3% of 5-HMF along with LA and formic acid (FA).[95] The same group have reported chitosan conversion with MSA at 200 °C for 15 min, and the 5-HMF yield was 15.0%. They have used 2% (*w/w*) chitosan and 0.2M methanesulfonic acid as catalyst.[96] The use of mineral Brönsted acids offer high output and simple operation at the same time they often require in excess concentration which leads to expensive post-treatment, possibility of by-product formation, large amount of neutralisation waste and the inherent corrosiveness may affect the reactor parts to corrode. Reports on formation of 5-HMF from chitin biomass are tabulated in Table 1.4. These drawbacks pushed the researchers to investigate the chitin biomass conversion using other acid catalysts such as Lewis acidic metal chlorides and ionic liquids.

Table 1.4. Summary of reports on conversion of chitin biomass to 5-HMF using Brönsted acid catalysts

Substrate	Catalyst	Reaction Condition			Product
		Solvent	Temp.(°C)	Time (h)	(% Yield)
Chitin	HCl	DCE	150	3	5-CMF (45)
Chitosan	HCl	H_2O	200 (Microwave)	0.5	5-HMF (2.2)
Chitosan	H_2SO_4	H_2O	174	0.6	5-HMF (12.1)
Chitosan	CH_3COOH	H_2O	40 (Ultrasound)	0.5	5-HMF (12.1)
Chitin	HCOOH	HCOOH	80	168	5-FMF (35)
$GlcNH_2$	MSA	H_2O	160	0.67	5-HMF (2.3)
Chitosan	MSA	H_2O	200	0.25	5-HMF (15)

(5-CMF: 5-chloromethylfurfural, 5-FMF :5-(formyloxymethyl)furfural, DCE: dichloroethane, MSA: methanesulfonic acid)

Hydrolysis of chitosan using $SnCl_4{\cdot}5H_2O$ at 200 °C for 30 minutes under microwave irradiation was found to produce 5-HMF.[91] 21 catalysts were screened in the study and metal-containing catalysts found to be effective. At lower concentration of $SnCl_4{\cdot}5H_2O$ 10 wt% of 5-HMF was formed and a higher concentration of the catalyst produced LA (21.6%). At the same time, chitin hydrolysis in similar conditions did not form 5-HMF, but LA was formed 11.5-12.6% yield from chitin obtained from various sources. Without the catalysts and under conventional heating there was no 5-HMF or LA formation. $GlcNH_2$ was treated in similar conditions to explain the mechanism of hydrolysis, under optimised conditions 32% of LA was obtained without no 5-HMF formation. The coordination of amino group with metal centre or proton aided the cleavage of glycosidic bonds in chitosan to yield glucosamine which on subsequent

dehydration and deamination forms 5-HMF, which on rehydration by 2 molecules of water produces LA according to the proposed mechanism.[91] The $SnCl_4$ often forms SnO_2 and HCl in aqueous solution and under similar conditions using SnO_2 and HCl, chitosan formed 27.4% LA. They have also checked the effect of microwave heating by keeping same reactions in conventional heating. 12.08% LA was found after 9 days of conventional heating under similar condition, no 5-HMF formation was found to form after 9 days under conventional heating. In another report, 67 *wt%* aqueous solution of $ZnCl_2$ found to catalyse the hydrolysis of chitosan, $GlcNH_2$, and GlcNAc to 5-HMF.[97] At 120 °C in 90 minutes chitosan yielded 10.1% of 5-HMF and $GlcNH_2$ and GlcNAc produced 21.6% and 2.8% of 5-HMF, respectively. Yield of 5-HMF and conversion of substrate decreases with decrease in $ZnCl_2$ concentration. Eight Lewis acidic metal chlorides were also screened as cocatalysts, out of which H_3BO_3 and $AlCl_3$ were having promotional effects in 5-HMF formation. The complex formation of Zn^{2+} with $-NH_2$ and -OH groups weakened the polymeric backbone and eased the hydrolysis step to form monomers. The zinc-monomer complex in pyranose form isomerises into open form and forms cyclic furanose form unlike in the case of tin chloride catalyst, further dehydration, and deamination led to the formation of 5-HMF. 5-HMF formation from GlcNAc was poor because acetamido group is bulky group, hence, it is a bad leaving group. Zang et al. have screened 16 metal salts in DMSO-water system for the conversion of chitin biomass under hydrothermal conditions.[98] $FeCl_2{\cdot}4H_2O$ showed best catalytic activity in conversion of $GlcNH_2$, GlcNAc, chitosan, and chitin to 5-HMF. The yield of 5-HMF obtained was 24.5, 37.9 (at 180 °C in 5h) 26.6, and 19.3% (at 190 °C in 6h) respectively from $GlcNH_2$, GlcNAc, chitosan, and chitin. Hydrogen bonds are formed through $-NH_2$ and -OH, co-ordination with Cl^- helps to break the hydrogen bonding network in chitosan and chitin. Metal cations helps to weaken the β-glycosidic bond, therefore promotes hydrolysis. Sulfamic acid is used as an alternative for Lewis and Brönsted acid catalysts due to its unique properties. It is non-volatile, odourless, non-corrodible,

moderately acidic (pKa =1.0), non- hygroscopic, and low cost. Its catalytic properties are due to the intrinsic zwitterionic nature ($H_3N^+SO_3^-$), which occurs by its tautomeric structure.[99] 3% (*w/w*) chitosan was converted to yield 21.48% of 5-HMF at 200 °C and 2 min, in presence of 0.7M sulfamic acid.[100] Compared to previous studies 5-HMF was obtained in higher yields and shorter time by using sulfamic acid. The same group have reported $GlcNH_2$ conversion to LA, where at low sulfamic acid concentration 5-HMF was produced in less amount. 0.14% 5-HMF was obtained at 200 °C in 15 min with 0.3M sulfamic acid and 33.76% LA was observed.[99] Table 1.5 shows the literature reports on conversion of chitin biomass to 5-HMF.

Table 1.5. Reports on formation of 5-HMF from chitin biomass using Lewis acid catalysts

Substrate	**Catalyst**	**Reaction Condition**			**5-HMF**
		Solvent	**Temp.(°C)**	**Time (h)**	**(% Yield)**
Chitosan	$SnCl_4·5H_2O$	H_2O	200 (Microwave)	0.5	10
Chitosan	$ZnCl_2$	H_2O	120	1.5	10.1
GlcNAc					2.8
$GlcNH_2$					21.6
Chitin	$FeCl_2·4H_2O$	DMSO	190	6	19.3
Chitosan			180	5	26.6
GlcNAc					37.9
$GlcNH_2$					24.5
Chitosan	Sulfamic acid	H_2O	200	0.03	21.5
$GlcNH_2$	Sulfamic acid	H_2O	200	0.25	0.14

Heterogeneous catalysts are superior to homogeneous catalysts in terms of separation, recovery, recyclability, and they are environmentally benign and does not corrode reaction equipment. There are many types of solid acid and base catalysts, conversion of chitin biomass to 5-HMF using heterogeneous catalysts is not a well-explored area but recently few studies were reported. H-β-zeolite is used in combination with acetic acid, to produce 5-HMF from chitosan by Dandekar et al.[101] Different catalysts were screened where H-β-zeolite and Amberlyst-15 were found to have the best performance along with 10% acetic acid as solvent and co-catalyst. Under the optimized reaction conditions (180 °C and 60 minutes in presence of 5% catalyst concentration) chitosan (20kDa), GlcNAc, and $GlcNH_2$·HCl yielded 25.16 ± 0.751%, 12.42 ± 0.25%, and 23.78 ± 0.15% of 5-HMF. The synergistic effect using the solid acid and acetic acid was explained as, Lewis acid sites (provided by the solid catalysts) assisted Brönsted acid sites (hydronium ions provided by aqueous acetic acid) helps in faster isomerization of the pyranose ring into furanose ring, followed by hydrolysis and the presence (10% *v/v*) acetic acid enhanced the surface catalyst coverage (of solid acid) with low molecular weight chitosan and 5-HMF. The poor yield of 5-HMF was obtained while using solid acid alone and the low molecular weight of the chitosan was found to produce a higher yield of 5-HMF compared to higher molecular weight substrates. Poisoning of the H-β-zeolite was observed at higher acid loading and the recovered H-β-zeolite gave 5-HMF yield of 7.43%, which was about half of that obtained during the first catalytic cycle.[101] Recently Chung et al. have reported 5-HMF production from chitosan using Nafion®50 resin in a co-solvent system of methyl isobutyl ketone (MIBK) and water (V/V=2) at 180 °C for 2 h.[102] 32.6 ± 4.2% of 5-HMF was obtained which is comparable to the yields obtained from homogeneous acid systems. The reactivity of catalyst for chitosan conversion could be attributed to interactions of the chitosan with the perfluorinated surface of the resin and its super-acidic properties. However,

recyclability of the catalyst was not shown. Reports on formation of 5-HMF using heterogeneous catalysts are summarized in Table 1.6.

Table 1.6. Summary of reports on conversion of chitin biomass to 5-HMF using heterogeneous acid catalysts

Substrate	Catalyst	Reaction Condition			5-HMF
		Solvent	Temp.(°C)	Time (h)	(% Yield)
Chitosan	H-β-Zeolite + CH_3COOH	H_2O	180	1	25.2
GlcNAc					12
$GlcNH_2$					23.8
Chitosan	Nafion®50	MIBK +H_2O	180	2	32.6

Ionic liquids are used as solvents and catalysts for biomass dissolution and conversion and extensively employed for conversion of lignocellulosic biomass into platform chemicals.[103] The properties of the ionic liquids can be tuned by modifying the cation or anion in it, based on the desirable properties expecting for the catalyst. In last decade, ILs are used as catalysts in several reports for valorisation of lignocellulosic biomass e.g.- cellulose[104], hemicellulose[105] and lignin[106] to form platform chemicals. Degradation of chitin using ILs has gained the attention of researchers and there are several attempts made for the chitin biomass valorisation to 5-HMF using ionic liquids. Zang et al. reported conversion of chitosan to 5-HMF using 5 *wt.%* 1-butyl-3-methylimidazolium hydrogen sulfate, [BMIM][HSO_4] aqueous solution and 100 *mol%* $AlCl_3{\cdot}6H_2O$ as co-catalyst, at 180 °C for 5h under hydrothermal condition.[107] 25.2% of 5-HMF yield was obtained in these work. Catalysts were recyclable and the activity was found to be nearly same till the first five cycles. In this study, they have screened 5 different ILs along with 5 different Lewis acids and $AlCl_3{\cdot}6H_2O$ was showing remarkable

activity among them. Chitosan was converted into 5-HMF using different acidic ionic liquids via hydrothermal reaction by Zang and co-workers.[108] 4 *wt.%* N-methyl imidazolium hydrogen sulfate, [MIM][HSO_4] aqueous solution at 180 °C for 5 h produced 29.5% and 19.3% of 5-HMF from chitosan and chitin respectively. Catalyst is recoverable after simple post-processing and recyclable upto 5th run. They have screened 9 ILs in their work. The coordination of anions with hydroxyl and amino group helps in weakening hydrogen bonding and the proton and IL cation weakens the glycosidic bonding and aids the formation of monomers. The isomerisation followed by cyclisation to furan form and the subsequent dehydration leads to 5-HMF formation. Later, A series of Brönsted-Lewis acidic ionic liquids were employed for hydrothermal synthesis of 5-HMF from chitosan.[109] 1.25 *wt.%* aqueous solution of [HMIM][HSO_4]-0.5$FeCl_2$ yielded 44.11% of 5-HMF at 180°C in 4h. Catalyst is found to show remarkable activity for the first four catalytic cycles. Similarly, GlcNAc conversion to 5-HMF was reported in DMSO-water system. During the hydrothermal synthesis at 180 °C for 6h, 64.6 mol% of 5-HMF was obtained from GlcNAc using the [HMIM][HSO_4] ionic liquid.[110] The effect of different imidazolium and thiazolium cation based ILs were also evaluated, the presence of HSO_4^- anion was necessary for higher yields of 5-HMF. $GlcNH_2$, chitosan, and chitin produced 54.9, 34.7, and 25.7% of 5-HMF under similar conditions. The reduced accessibility of HSO_4^- anions to chitosan and chitin decreases the hydrolysis of the polymers to form monomers which account for the lower yield of 5-HMF from the chitosan and chitin. The catalyst was recovered along with DMSO and used as such for the recyclability studies. Reasonable activity was obtained for five catalytic cycles. Benzimidazole and NMP based ionic liquids also were studied for the chitosan degradation by the same group. Benzimidazolium chloride, [HBIM][Cl] ionic liquid showed the best catalytic activity.[111] 30.8% of 5-HMF was obtained when only aqueous IL was used, while 34.9 mol% of 5-HMF was formed when the reaction was carried out in 10% DMSO-water system. Use of DMSO enhanced the solubility and reduced the side

product formation. The catalyst was reusable up to five times without compromising the activity. Acidic ionic liquids were found to show remarkable activity in 5-HMF production from chitin biomass as compared to conventional acid catalysts in terms of conversion, yield and recovery and recyclability of catalysts. The careful tuning of the properties of IL can provide better activities alone and in combination with co-catalysts also. Ionic liquid catalysed reports for the conversion of chitin biomass into 5-HMF is summarized in Table 1.7.

Table 1.7. Summary of reports on ionic liquid catalysed conversion of chitin biomass to 5-HMF

Substrate	**Catalyst**	**Reaction Condition**			**5-HMF**
		Solvent	**Temp.(°C)**	**Time (h)**	**(% Yield)**
Chitosan	[BMIM][HSO_4] + $AlCl_3 \cdot 6H_2O$	H_2O	180	5	25.2
Chitosan	[MIM][HSO_4]	H_2O	180	5	19.3
Chitosan	[HMIM][HSO_4]+0.5$FeCl_2$	H_2O	180	4	44.1
Chitin	[HMIM][HSO_4]	H_2O	180	6	25.7
Chitosan					34.7
GlcNAc					64.6
$GlcNH_2$					54.9
Chitosan	[HBIM][Cl]	H_2O+ DMSO	180	3	34.9

1.8.3. Synthesis of nitrogen containing furan derivative, 3-acetamido-5-acetylfuran (3A5AF)

The conversion of cellulosic biomass to furan derivatives like 5-HMF, furfural, etc are well explored. Similarly, the chemical transformation of chitin, chitosan and

chitin derived chemicals like $GlcNH_2$ and GlcNAc were studied to obtain nitrogen-containing furan derivatives which are valuable counterparts and they cannot be obtained from cellulose. 3-acetamido-5-acetylfuran (3A5AF) is the first reported N-containing furan compound from animal biomass sources. It was first obtained as a pyrolysis product of GlcNAc with a yield of 2% along with 3-acetamidofuran (5%) and acetamidoacetaldehyde (3%).[112] Later in thermal degradation of GlcNAc at 200 °C for 30 min, 3A5AF was found to be the major degradation product, with 2-acetylfuran, 3-acetamidofuran, pyrazine, pyridine, ethylpyrazine, methylpyrazine, 2-ethyl-6-methylpyrazine, 2,3-dimethylpyrazine, and acetamide.[113] Kerton et al. were the first to report chemical conversion of GlcNAc to 3A5AF in ILs. At 180 °C in the presence of [BMIM][Cl] under microwave heating for 3 mins yielded 25.5% of 3A5AF.[114] The use of boric acid as an additive at 180 °C in 1h, yield increased to 60% whereas when NaCl was used as an additive, 38.3% of 3A5AF was obtained. Freely bound chloride ions were found to be essential for the higher yields of the product. They have studied effect of 7 different ionic liquids and 15 additives.[114] In a similar work by the same group has reported that the use of 2:1 NaCl-H_3BO_3 produces 58% yield 3A5AF in dimethylacetamide (DMA) under microwave irradiation (220 °C, 15 min). The yield of 3A5AF could be maximized to 62% in the presence of 4 equivalent NaCl.[115] The direct conversion of chitin to 3A5AF was reported by N. Yan, et al., where they screened 6 organic solvents and 27 additives, including organic/inorganic acids, metal chlorides, bases, and heteropolyacids as additives for the reaction. At 215 °C for 1h, a 7.5% yield of 3A5AF obtained with 50% chitin conversion by using N-methyl-2-pyrrolidone (NMP) as solvent and H_3BO_3, LiCl and HCl as additives.[22] Along with the 3A5AF, other products, such as levoglucosenone, acetic acid, 4-(acetylamino)-1,3-benzenediol and humins were also identified and the existence of parallel reaction pathways have been proposed. Later the same group reported chitin and GlcNAc conversion to 3A5AF in the presence of 10 different ILs and 25 additives. In presence of [BMIM][Cl] at 180 °C, 1 h reaction by using boric acid

and HCl as additives significant amount of product was formed.[116] Enhanced reaction rate was observed while using ILs instead of organic solvents. To improve the yield different pre-treatment methods were employed for chitin such as ball mill grinding, steam explosion, alkaline treatment, phosphoric acid, and ionic liquid (IL) dissolution/reprecipitation. Ball milling in dry condition was found to be effective in reducing the crystallinity and H-bonding network in chitin, ball-milled chitin yielded 28% 3A5AF where boric acid and HCl were used as additives and [BMIM][Cl] as solvent at 180 °C and 1 h reaction.[117] Zang. et al. studied the amino acid ionic liquid (AAIL) catalyzed conversion of GlcNAc to 3-A5AF. Glycine chloride ionic liquid, [Gly][Cl] without any additive yielded 43.22% 3A5AF in 10 min at 200 °C and when $CaCl_2$ was used as the additive higher yield of 3A5AF (52.61%) and conversion was obtained. Moreover, AAILs were reusable for 8 catalytic cycles and afforded 43.22-36.59% yield over eight cycles.[118] Recently Fukuoka. et al. reported dehydration of GlcNAc in the presence of $AlCl_3 \cdot 6H_2O$ and N, N-dimethylformamide (DMF) solvent at 120 °C, which was the lowest reported temperature for the conversion of GlcNAc by conventional heating.[119] 30% yield of 3A5AF was obtained within 30 min and they have demonstrated a simple isolation procedure for high purity 3A5AF by using column chromatography. Recently, Zang et al. has reported use of pyridinium based ionic liquids to yield 3A5AF from GlcNAc.[120] They have achieved 37.49% 3A5AF with 1-carboxymethyl pyridinium chloride, [CMPy][Cl] as catalyst. The yield is increased to 67.37% at 180 °C in 20 min with the use of B_2O_3 and $CaCl_2$ as additive. Ethanolamine ionic liquids were used for conversion of GlcNAc to 3A5AF. 62% yield was obtained using triethanolamine hydrochloride, [TEA][Cl] at 170°C within 20 min. The catalyst is recyclable upto 5th run.[121] Recently, Zang et al. reported 36.94 3A5AF from GlcNAc in presence of pyrazine hydrochloride, [Pyz][Cl]. Twelve catalysts were investigated for the conversion of GlcNAc to 3A5AF. Further, boric acid and $CaCl_2$ enhanced the activity and 3A5AF yield was increased to 69.54% in DMA at 190 °C

within 60 min.[122] Literature reports on conversion of chitin biomass to 3A5AF are summarized in Table 1.8.

Table 1.8. Reports on conversion of chitin biomass to 3A5AF

Substrate	Catalyst	Reaction Conditions			3-A5AF (% Yield)
		Solvent	Temp.(°C)	Time (min)	
GlcNAc	[BMIM][Cl]	H_2O	180 (Microwave)	3	25.5
GlcNAc	H_3BO_3 + NaCl	DMA	220 (Microwave)	15	58
Chitin	H_3BO_3 + LiCl+ HCl	NMP	215	60	7.5
Chitin (Ball milled)	H_3BO_3 + HCl	[BMIM][Cl]	180	60	28
GlcNAc	[Gly][Cl] + $CaCl_2$	DMA	200	10	52.6
GlcNAc	$AlCl_3$. $6H_2O$	DMF	120	30	30
GlcNAc	[CMPy][Cl] + B_2O_3 + $CaCl_2$	NMP	180	20	37.49
GlcNAc	[TEA][Cl]	DMA	170	20	62
GlcNAc	[Pyz][Cl] + B_2O_3 + $CaCl_2$	DMA	190	60	69.54

The potential of 3A5AF as an important organic compound and building block for other chemical has been explored by several groups. Hydrolysis of amido group and reduction acetyl group in 3A5AF have resulted in 2-acetyl-4 aminofuran and 3-acetamido-5-(1-hydroxyethyl)furan.[123] Sperry, et al. have studied the synthesis scopes for 3-amidofuran from 3A5AF by various ring and keto group functionalization of 3A5AF.[124] Proximicins are excellent

chemotherapeutic drug leads. Sperry and co-workers have attempted the synthesis of proximicin A from the chitin-derived 3A5AF. The synthetic steps were greener and sustainable.[125] Oxidative ring expansion of 3A5AF to 2-amino sugars like N-acetyl-L-rednose (RedNAc) was reported with excellent yield and enantiopurity.[126] 3A5AF undergoes a Piancatelli rearrangement to give a 3-aminocyclopentenone which is readily converted into a variety of chemicals like 4-aminocyclopentanones, 4-aminocyclopentene-1,3-diones and these compounds are useful in making many important nitrogen-containing heterocycles.[127] The reaction between 3A5AF and aliphatic ketones resulted in the formation of a dihydrodifuropyridine scaffold.[128] Therefore, 3A5AF is a promising platform chemical which is derived from chitin biomass.

1.8.4. Synthesis of chromogen I and chromogen III

Derivatives of GlcNAc such as chromogen I (2-acetamido-2,3-dideoxy-D-erythro-hex-2-enofuranose), chromogen III (3-acetamido-5-(1',2'-dihydroxyethyl)furan), 2-acetamido-3,6-anhydro-2-deoxy-D-glucofuranose, and 2-acetamido-3,6-anhydro-2-deoxy-D-mannofuranose are having potent biological applications and could be used in medicine and food industry as additives. Chromogen I was reported as a major product obtained from Morgan–Elson reaction and later alkaline and borate treatment of N-acetyl-D-mannosamine (NAM) and GlcNAc have found to give Chromogen I and III.[129] Osada and co-workers tried the dehydration of GlcNAc and they were able to obtain chromogen I (23%) and chromogen III (23.1%) in high-temperature water(HT-water) at 120–220 ºC and 250 bar pressure with a reaction time of 39 seconds.[130] GlcNAc exists in both furan ring and open-chain form in water the epimerisation to N-acetyl-D-mannosamine is possible via keto-enol tautomerization. Dehydration happens from open-chain (keto) form and electron-withdrawing effect of the N-acetyl group helps in the elimination of proton (H-2) easily. The ring closure of dehydrated open-chain leads to chromogen I and it is further dehydrated to get chromogen III. High-

temperature water dehydration media is non catalytic and greener. It is found that higher ion product constant of water at 180–220ºC than that at ambient temperature which helps in dehydration. In similar conditions dehydration of a chitin dimer (N,N′-diacetylchitobiose) resulted in poor yields of chromogens.[131] Zheng et al. reported the formation of two nonanuclear lanthanide oxo/hydroxo complexes by introducing chromogen I as the supporting organic ligand. Optically pure chromogen I was later obtained in high yield using direct digestion of the crystals of the complex with 1M HCl in methanol followed by titration with a saturated aqueous solution of $NaHCO_3$ led to the precipitation of lanthanide carbonate while leaving in the supernatant-free Chromogen I ligand with a yield of 40%.[132] An updated work on the non-catalytic conversion of GlcNAc in high-temperature water by Osada et al. has reported 37% and 34.5% of chromogen I and III respectively at 180–280 °C and 250 bar with a reaction time of 5–34 seconds. 3A5AF was obtained in less than 1% yields from dehydration of chromogen III.[133] Less yield of 3A5AF is because the dehydration of chromogen III is difficult in absence of a catalyst. The effect of acidic and basic catalysts was studied, acid catalysts suppressed the chromogen I formation while the promotional effect was observed for dehydration of chromogen I to chromogen III. Base catalyst found to show the opposite effect on the reactions. The same group has reported the direct conversion of chitin to chromogen I in a similar fashion at 290–390 °C and 250 bar with a reaction time of 0-180 min, about 90% of water-soluble chitin is formed and yield of chromogen I was 2.6%.[134] Reports on formation of chromogen I (Chr I) and chromogen III (Chr III) are tabulated in Table 1.9.

Table 1.9. Summary of reports on formation of chromogen I and chromogen III from chitin biomass

Substrate	Reaction Conditions				(% Yield)
	Solvent	**Temp.(°C)**	**Time (s)**	**Pressure (bar)**	
GlcNAc	HT-H_2O	220	39	250	Chr I (23) Chr III (23.1)
GlcNAc	HT-H_2O	280	34	250	Chr I (37) Chr III (34.5)
Chitin	HT-H_2O	390	3600	250	Chr I (2.6)

(HT: high temperature)

1.8.5. Synthesis of levulinic acid (LA)

Levulinic acid is a versatile building block for various value-added chemicals which is formed as a by-product in biomass valorisation of 5-HMF.[135] 5-HMF undergo rehydration under acidic conditions at elevated temperature to form LA and FA. LA formation was often observed along with 5-HMF in most of the reactions discussed in the previous section. Microwave-assisted hydrothermal conversion of chitin using 2M H_2SO_4 at 190 °C for 30 min yielded 37.8% of LA while using HCl 32.7% of LA was formed at same conditions.[136] $GlcNH_2$, GlcNAc, and different chitosan samples were also used for the study and 19.3-37.0% LA was observed with both HCl and H_2SO_4 within 10-30 min reaction time under similar reaction conditions. In another study 4 *wt%* of H_2SO_4 was able to convert $GlcNH_2$ into LA (25.3 wt.%) at 188 °C in 49 min.[93] In a Lewis acid catalysed system, $SnCl_4{\cdot}5H_2O$ catalysed hydrothermal conversion of chitosan at 200 °C for 30 minutes under microwave irradiation gave 23.9% LA and chitin gave 11.5% LA.[91] In case of chitin, less yield of LA is due to more structural rigidity and hydrogen bonding network and crystallinity present in chitin. 0.48% of LA was formed in a sulfamic acid catalysed hydrothermal degradation of chitosan at 200 °C within 2 min with 0.7M

sulfamic acid.[100] At the same temperature 0.3M sulfamic acid catalysed hydrolysis of glucosamine yielded 33.76% of LA in 15 min. 39.03 % FA and 26.48% humins (insoluble products) are also detected.[99] Methanesulfonic acid (MSA) is an eco-friendly and green catalyst. It is a strong acid (pka= -1.9) but non-oxidising, non-foaming, less corrosive and bio-degradable. At 200 °C in 30 min. 0.5M MSA catalysed the conversion of glucosamine to 49.9% LA and 50.8% FA.[95] In the same reaction conditions, 28.2% of LA was formed from 2% chitosan when 0.2M MSA was used.[96] Zirconium oxychloride ($ZrOCl_2$) was able to catalyse the hydrothermal degradation of chitosan. At 200 °C and 20 min, 21.29% of LA was obtained from 5 *wt%* chitosan using 15 mol% catalyst.[137] Solid acid, Amberlyst-15 was able to transform $GlcNH_2$ into 36.9% LA at 180 °C in 60 minutes.[138] The catalyst is not recyclable because it is known that amberlyst-15 is stable up to 140 °C. Sulfonic acid-functionalized imidazolium ionic liquids were used for the conversion of chitosan to LA, various anion combinations were tried by keeping the cation same.[139] Among them, $[C_3SO_3HMIM][HSO_4]$ (1-methyl-3-(3-sulfopropyl)-imidazolium hydrogen sulphate) showed the best activity. At 170 °C and 5 h, 1g of the catalyst was able to convert 250 mg of chitosan in water (4g) to 49% LA. The dilution effect of water played a major role in reaction, in the absence of water, LA yield is reduced and high amount of humins were formed. By reducing the substrate amount to 50 mg the LA yield increased to 64%. The selective conversion of chitosan to LA was because of the highly acidic nature of the IL. Chitosan undergo quaternization and depolymerizes to glucosammonium salt, followed by dehydration and deamination to form 5-HMF and then further rehydration results in the formation of LA, which is proven by proton NMR studies. The catalytic activity of IL found to be decreasing after 2 cycles due to the reduction in the acidity of IL caused by ammonia released during the reaction. They have also quantified the amount of NH_4^+ in reaction mixture using ion chromatography analysis. When 1 equivalent of H_2SO_4 was added after recovery, the catalyst was found to be reusable up to five catalytic cycles.[139] Liu et al. have

recently reported chitin conversion to LA using the acidic ionic liquid, $[C_3SO_3HMIM][HSO_4]$.[140] The effect of IL structure on the chitin conversion and the role of acetylamino group were investigated in detail. At 180 °C and 5 h, 56.5% of LA was obtained from 3.4 *wt.%* of chitin. Remarkable increase in LA yield (67%) was observed when *0.7 wt.%* of chitin was used. $(C_3SO_3Hmim)Cl$ was found to have remarkable efficiency in LA (54%) formation despite of its less acidity compared to $[C_3SO_3HMIM][HSO_4]$. Catalyst was found to show decrease in activity in recycle experiments and the LA yield decreased to 34% in fifth catalytic cycle. It is probably because of the reduction in acidity due to interaction with NH_3 released during the reaction. In presence of 1 equivalent of sulfuric acid supplement, consistent catalytic activity was observed. A two-way approach to the depolymerisation and formation of LA was proposed. It can be either via deacetylation followed by depolymerisation to $GlcNH_2$ and then dehydration-rehydration to form LA or by direct depolymerisation to GlcNAc and its conversion to $GlcNH_2$ and further formation of LA via dehydration-rehydration. The mechanistic studies were elucidate using proton NMR experiments. Literature reports on conversion of chitin biomass to LA are summarized in Table 1.10.

Table 1.10. Reports on Levulinic acid formation from chitin biomass

Substrate	**Catalyst**	**Reaction Condition**			**LA**
		Solvent	**Temp.(°C)**	**Time (h)**	**(% Yield)**
Chitosan	HCl	H_2O	190 (MW)	0.5	32.7
	H_2SO_4				37.8
$GlcNH_2$	H_2SO_4	H_2O	188	0.82	25.3
Chitin	HCl	DCE	150	3	29
Chitosan	Sulfamic acid	H_2O	200	0.25	33.8
Chitosan	MSA	H_2O	200	0.5	28.2

$GlcNH_2$	MSA	H_2O	200	0.5	49.9
Chitosan	$SnCl_4{\cdot}5H_2O$	H_2O	200 (MW)	0.5	23.9
Chitosan	$ZrOCl_2$	H_2O	200	0.3	21.3
$GlcNH_2$	Amberlyst-15	H_2O	180	1	36.9
Chitosan	$[C_3SO_3HMIM][HSO_4]$	H_2O	170	5	49
Chitin	$[C_3SO_3HMIM][HSO_4]$ $[C_3SO_3HMIM][Cl]$	H_2O	180	5	56.5 54

(MSA: methanesulfonic acid, MW: microwave heating)

1.9. Drawbacks of earlier reported methods and gap analysis

Although, several reports are available on valorisation of chitin and chitosan discussed above. Mineral acids (HCl, H_2SO_4), organic acids (HCOOH, AcOH), other homogeneous acids (methanesulfonic acid, sulfamic acid), ionic liquids, metal chlorides were used for the transformation. In few reports, chitin and chitosan was pre-treated using mechanical energy (ball milling) before the reaction. For the production of chromogen I and chromogen III, high temperature water was used for the reaction. These reports are associated with several drawbacks like non-recyclability of the catalyst, corrosion or handling issues while using mineral acids, use of very high temperature, use of energy extensive methods (ball milling, microwave heating, ultrasonication), use of mineral acids, organic acids and high boiling solvents (DMSO, NMP, DMA) as solvent. Very less reports are available on use of heterogenous catalysts for the conversion of chitin and these reports are also associated with few drawbacks like use of organic acid as solvent system, instability and non-recyclability of the catalysts.

After doing thorough literature survey, it was understood that there is a need to develop a catalytic system where catalyst is easily recoverable, stable and

recyclable. Green solvent needs to be used as the reaction medium and the reactions should be done in ambient temperature and pressure. Solid acids are known to convert cellulose by breaking β-1,4 glycosidic linkages and they are easily separable and recyclable. As chitosan and chitin has a very similar structure like cellulose, they can also be depolymerized using solid acid catalysts. Solid acid alone was not previously reported as catalyst along with water as solvent. From the literature study, it was also found that proper analytical techniques for separating amino sugars and furan derivatives are not available in previous literature reports. So, developing proper analytical technique for simultaneous separation and quantification of the amino sugars and furan derivatives is an important topic to work.

1.10. Introduction to catalysis (homogeneous and heterogeneous)

A catalyst is a substance or mixture of substance that alters the rate of a chemical reaction by providing a faster reaction pathway without altering the thermodynamic factors. The catalyst remains unchanged at the end of the reaction. The process in which a catalyst speeds up the rate of chemical reaction without being altered is called as catalysis.[141] Catalysis can be classified into two types according to the phases involved in the process i.e., homogeneous and heterogeneous catalysis.

When the reactants, products and catalysts are present in the same phase, it is called as homogeneous catalysis. Soluble acids, bases, salts and organometallic compounds are the example of homogeneous catalysts.[142] Homogeneous catalysis has several drawbacks like difficulty in separation and recovery of catalysts, corrosion when mineral acids/bases were used, costly treatment for toxic liquid wastes obtained after the separation, possibility of contamination of desired products by catalysts.

In case of heterogeneous catalysis, the reactants, products and catalysts are present in different phase. Generally, the reactant and products are in the liquid

or vapour phase while the catalyst is in the solid phase. Metal oxides, zeolites, clays, resins, supported metal catalysts can be used as heterogeneous catalysts.[141] The advantages associated with heterogeneous catalysts are as follows:

- ✓ Catalysts are easily separable from the reactants and products
- ✓ Elimination of corrosion problems
- ✓ No liquid waste generation
- ✓ Regeneration of catalysts are very simple.

Here, I am giving brief introduction of solid acid catalysts since those are used in this thesis for depolymerisation of chitosan and chitin.

1.11. Solid acid catalysts

Homogeneous acids are known for depolymerisation of chitin and chitosan as discussed in section 1.8. From the knowledge from acid catalysed hydrolysis or chitin/chitosan and use of solid acid in cellulose chemistry, it was believed that solid acids alone can catalyse help to break β-1,4 glycosidic linkages. Moreover, sugars can undergo dehydration under acid medium and give furan derivatives. Acid catalysed dehydration of chitin, chitosan and its monomers are discussed in section 1.8.2, 1.8.3 and 1.8.4. Solid acids were widely used in literature for dehydration of glucose, fructose.[143] Hence, it was believed that solid acids can convert chito-monomers to furan derivatives. Solid acid catalysts can effectively give the products and minimize the problems associated with use of homogeneous acids. Solid acid catalysts can have Brönsted and/or Lewis acidity which can cleave the β-1,4 glycosidic linkages in chitosan and chitin. Based on the pore structure, solid acid catalysts can be classified into two categories, i.e., structured and amorphous catalysts.

Zeolites were discovered in 1756 and the term "zeolites" came from a Greek word *zeo* (to boil) and *lithos* (stones) and Swedish minerologist Cronstedt first used it.[144] Zeolites are aluminosilicates with fully cross-linked framework structures made

up of corner sharing SiO_4 and AlO_4 tetrahedra, which are primary structural units. They are formed when some of the Si^{4+} is replaced by Al^{3+}. When Al^{3+} replaces Si^{4+}, the charge is balanced by other positive ions such as Na^+, K^+, Ca^{2+}, NH_4^+ ions. The structural formula of zeolite is $(M^{n+})_{x/n}\{(AlO_2)_x(SiO_2)_y\}.zH_2O$. Here, 'M' represents the exchangeable cation of valance n and M is a cation generally belongs to group I and II. Cations can balance the negative charge created by Al.[145] When 'M' is replaced by NH^{4+} ions, calcination was done at 550 °C to remove NH_3 and H^+ is left behind. This hydrogen ions give Brönsted acidity in zeolites (Figure 1.13).[146] Lewis acid sites are related to the formation of positively charged oxide ions within the structures of zeolite. The cationic species present in the catalyst framework can act as Lewis acid site (Figure 1.13).

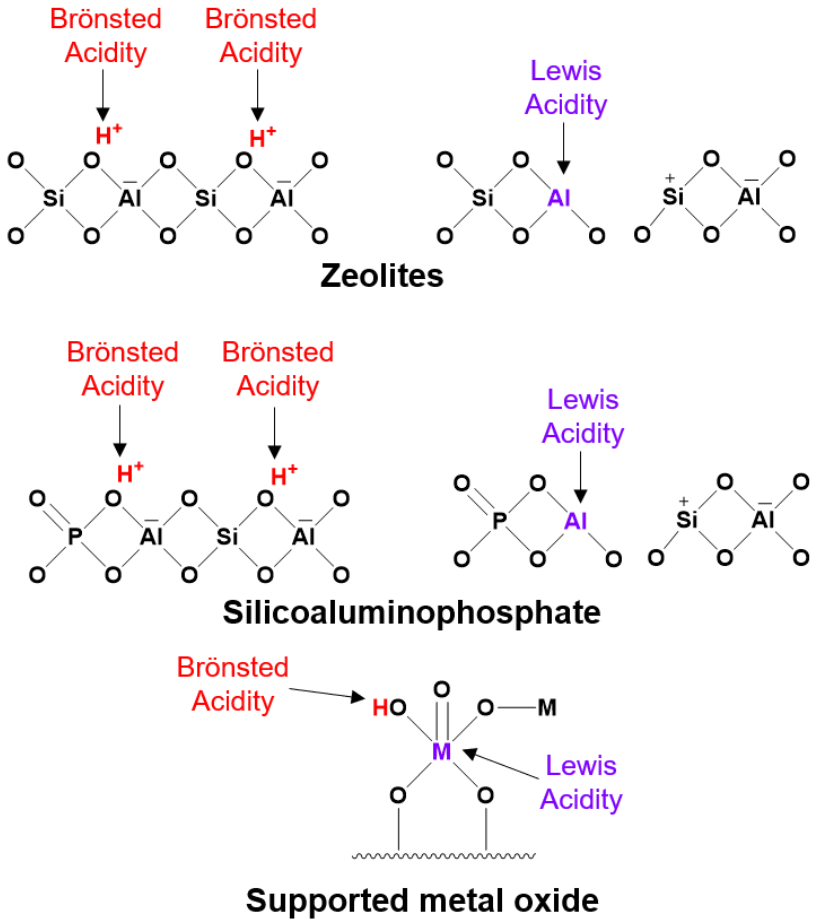


Figure 1.13. Brönsted acid sites and Lewis acid sites in zeolite, silicoaluminophosphate and supported metal oxide

Silicoaluminophosphate (SAPO) catalysts are well known in the literature for biomass conversion for its high hydrothermal stability (600 °C under 20% steam). They are hydrothermally more stable due to incorporation of stronger P-O bonds in aluminosilicate framework. SAPO materials can have various pore channel structure depending upon structure directing reagent and synthesis procedure. In my work, I will be using SAPO-44 which have 'Chabazite (CHA)' morphology. The T atom ring size in CHA morphology contains 8,6 and 4 membered rings. CHA has a framework structure consisting of stacked sequence of 6 ring with AABBCC order. It has pseudo-rhombic unit cell. CHA single crystals looks like almost cube shaped morphology. SAPO-44 contains both Brönsted and Lewis acid sites arising similar like zeolites (Figure 1.13).[147-148]

Clays are another type of structure solid acid catalysts used in this work. Clays are defined as phyllosilicate consisting of 2D tetrahedral sheet fused with octahedral sheet having charge balancing cations for charge neutralisation. Montmorillonite K10 contains sheets of aluminosilicate in which aluminate layer is surrounded by two silicate layers. The space between two aluminosilicate sheets is generally occupied by exchangeable interlayer cations and water which influences the acidity of clay catalyst.[149-150]

Amorphous solid acids were also used in this work because there can be a possibility for the breakage of catalyst pore structure after reaction. Amorphous solid acid catalysts do not have definite channel structure, pore volume and pore diameter. Silica alumina (SA) and supported metal oxides (MoO_x/SA, WO_x/SA) will be used for chitosan and chitin valorisation. SA catalyst has both Brönsted and Lewis acid sites.[145] Moreover, in case of supported metal oxide, they may contain M=O, M-OH, M-O-M and M-O bonds with support. Metal centre can act as Lewis acid site due to its available vacant orbitals and -OH group attached to metal centre can give H^+ and act as Brönsted acid centre (Figure 1.13).[145, 147]

1.12. Statement of problem

Homogeneous acids, metal salts, ionic liquids, metal chlorides were reported to valorise chitin/chitosan/chito-monomers to yield nitrogen containing value added chemicals and furan derivatives. However, there are several issues associated with the available literature reports. Those are listed below:

- Proper analytical method is not reported to simultaneously separate or quantify reaction products.
- Use of homogenous acid catalysts and metal salts is a serious drawback as the recovery of catalyst is really difficult and possesses environmental issues, toxicity and corrosiveness etc.
- Use of high boiling solvents, mineral acids, mineral bases, organic acids, ionic liquids as reaction medium.
- Few reactions are done in higher temperature and pressure.
- In few cases, substrates were pretreated with mechanical energy (ball milling) or acid treated ball milling was also done.
- Difficulties in effective recycling of catalysts.

1.13. Objectives and scope of the thesis

By considering the above discussions regarding literature on chitin valorisation and importance of the utilisation of shell waste and chitin, I have set objectives of my work as follows:

- ***To develop a proper analytical method to simultaneously separate and quantify the products formed in the reaction.***

  It is known that by subjecting chitin and chitosan to acid catalysed hydrolysis reaction, those are reported to yield oligomers and monomers, glucosamine ($GlcNH_2$) and N-acetyl glucosamine (GlcNAc) as products. These monomers can further undergo dehydration reactions to produce

5-Hydroxymethylfurfural (5-HMF) and other furan derivatives. From literature, it was observed that the analysis of the reaction mixture is restricted to either oligomers formation or monomer formation of furan formation. Since $GlcNH_2$, GlcNAc and other N-containing chemicals are sugar derivatives which have complete solubility in water. Thus, it is essential to develop and use proper analytic method(s) to separate and quantify these compounds to take work in this area on next level. Otherwise, it was often observed that few reaction products gone undetected or not separated or identified due to lack of optimum analytical conditions. High performance liquid chromatography (HPLC) is extensively used and prime technology for the separation and detection of water-soluble compounds.

- ✓ To achieve this objective, it was decided to develop a proper HPLC methodology to separate and quantify all the chitin derived products (oligomer, monomers, N-containing chemicals and furan derivatives). A simple, rapid, precise and specific HPLC method needs to be developed which can separate and quantify chitin derived products simultaneously.

- ***To develop reusable and recyclable catalytic system for conversion of chito-monomers ($GlcNH_2$ and GlcNAc) into 5-HMF.***

  In the bio-refinery concept, lot of attention has been diverted to the synthesis of furan derivatives like furfural, 5-hydroxymethyl furfural (5-HMF due to their unique properties such as, heterocyclic structure, reactive functional groups (-CHO and $-CH_2OH$) etc, which otherwise are difficult to achieve through petro-refinery route. Additionally, 5-HMF can act as a platform chemical which can be further converted into various value-added and important chemicals like; 2, 5-furandicarboxyl acid (potential replacement to terephthalic acid in polymer synthesis), 2,5-dimethylfuran (potential fuel), 2,5-dihydroxymethylfuran (raw materials for polymers and

intermediate of drugs and crown ethers), 2,5-bis(hydroxymethyl)tetrahydrofuran (use in manufacture of polyester), 2,5-bis(hydroxymethyl)furan (use in manufacture of polyurethane foam) etc. Though, lots of efforts were already put in the synthesis of 5-HMF from cellulose/glucose/fructose through acid catalysed dehydration reaction, it can also be produced from chitin, chitosan, its oligomers and monomers, as cellulose being touted as one the alternatives to synthesize bio-ethanol and thus availability of the same for making other chemicals may be in jeopardy. A handful of reports are available in literature for synthesis of 5-HMF from monomers but those are associated with difficulties in recovery and recycling of catalysts, neutralization of reaction mixture, suppression of side/further reactions, instability of the catalysts, use of high boiling solvents etc. In view of this, it is very much essential to design and develop water tolerant, substrate tolerant and recyclable catalysts.

- ✓ To achieve this objective, I have decided to use solid acid catalysts (both structured and amorphous) for the conversion of $GlcNH_2$ and GlcNAc to 5-HMF. Catalysts needs to be easily recoverable, reusable and recyclable. Exploration of various solid acids and optimization of reaction conditions needs to be done to achieve maximum yield of 5-HMF. Recycle run will be carried out.

- ***Understanding the structural, physical and functional properties of different commercial chitosan and chitin substrates before employed them in the depolymerisation reactions.***

  As discussed in previous sections, different structural and physical properties can be found in different chitosan and chitin substrates depending on source and extraction methods. Literature also suggests that the molecular weight, structure, crystallinity, ash content etc. can be varied from source to source. Therefore, it is very essential to characterize various

chitosan and chitin substrates systematically before the starting the depolymerisation study.

- ✓ It is decided to characterize eight different samples of chitosan and two different samples of chitin before employing the substrates for depolymerisation reactions using various physico-chemical techniques like XRD analysis (to know the morphology, diffraction peaks, structure of chitosan and chitin), SEM-EDAX analysis (to know morphology, elements present), TG-DTA (to study thermal stability and thermal degradation behaviour of chitosan and chitin), CHNS analysis (to measure carbon, hydrogen, nitrogen content, monomeric molecular formula and degree of deacetylation), ICP-OES analysis (to quantify of metals present in the samples), ATR and solid ^{13}C NMR analysis (to identify the presence of functional groups), absolute viscosity measurement (to measure absolute viscosity of the samples).

- ***Extraction of chitin from different shell waste samples (raw biomass collected from local markets) and understanding their physico-chemical properties through detailed characterization.***

  As per previous discussion, shell waste is dumped to sea or landfill after consuming the edible part which can cause several environmental issues. By considering the very low monetary value of shell waste and the environmental impact due to their disposal, it is preferable to use abundant and inexpensive shell waste material directly for the extraction of high value chitin which is a potential source of nitrogen containing value added chemicals. After following the steps for chitin extraction, extracted mass needs to be thoroughly characterized to know their physical and structural properties. Moreover, complete demineralization and deproteinization can also be confirmed by doing detailed characterization.

- ✓ At first, I decided to collect different shell waste samples from local markets in different coastal states of India. As industrial/chemical method is well established, effective to extract chitin and used widely in industries, it is decided to follow conventional protocol. Then extracted samples were characterized using different analytical techniques like XRD analysis (to know the morphology, diffraction peaks, predict the structure of extracted mass), SEM-EDAX analysis (to know morphology, elements present), TG-DTA (to study thermal stability and thermal degradation behaviour of extracted samples), CHNS analysis (to measure carbon, hydrogen, nitrogen content, monomeric molecular formula, presence of left over protein), ICP-OES analysis (to quantify of metals present in the samples, presence of left over calcium), ATR and solid ^{13}C NMR analysis (to identify the presence of functional groups).

- ***To develop an efficient catalytic method the depolymerisation of chitosan and chitin into value added chemicals using solid acid catalysts.***

  As discussed previously, chitin is 2nd most abundant biopolymer on earth and it is a potential feedstock of value-added chemicals, especially nitrogen containing ones as chitin contains 7% biologically fixed nitrogen in its structure. Presence of nitrogen makes it different from other biomass sources. As per literature reports, mineral acids, organic acids, metal salts were used for depolymerisation which have several drawbacks like difficulties in recovery and recycling of catalysts, neutralization of reaction mixture etc. Therefore, it is necessary to develop an efficient catalytic system for depolymerisation using easily recoverable, reusable and recyclable catalysts.

  - ✓ To achieve this objective, it was decided to use solid acid catalysts for the depolymerisation reaction. Effects of various structured and

amorphous solid acids will be checked. Catalysts need to be easily recoverable, reusable and recyclable. The reactions will be done in water as reaction medium. Optimization of reaction conditions will be done to achieve the maximum yield of products.

1.14. Outline of the thesis

This thesis is divided into seven chapters. In the present chapter, brief introduction on biomass, its classification is discussed. Further details on animal biomass, i.e., chitin is described. Scenario of global fisheries production along with the production status of India are also discussed. Further details on chitin extraction and conversion methods along with drawbacks associated with available literature reports were discussed in detail. Detailed introduction of solid acids and their properties were discussed in detail. Lastly, motivation of the work, scope and objective of the thesis are portrayed.

In the second chapter, development of HPLC method for simultaneous separation of chitin derived compounds is discussed. In this work, a HPLC method with a variable wavelength and refractive index detector is optimised for simultaneous separation and quantification of amino sugars (D (+)-glucosamine, N-acetyl-D-glucosamine, Glucosamine hydrochloride dimer), other sugars (D (+)-glucose, D-(+)-fructose, 2-Deoxy-D-glucose), N-containing chemicals (D-glucosaminic acid, 2-Aminoethanol, N-acetylethanolamine, Acetamide) and furan derivative (5-Hydroxymethylfurfural). Different columns along with different mobile phase systems were screened and a simple, rapid, precise and accurate method is achieved.

In the chapter three, synthesis of 5-HMF from chito-monomers (GlcNAc and $GlcNH_2$) using solid acid catalysts is represented. Synthesis and characterization of solid acids (fresh and spent) are also described. Analysis of the reaction mixture is discussed with the help of HPLC, GC analysis. Furthermore, mechanistic pathway of the reaction was discussed. Catalyst recycle study was also shown.

In the fourth chapter, detailed characterization of eight chitosan and two chitin substrates are described using various physico-chemical techniques.

Chapter five comprises the collection of shell waste samples from different parts of India and extraction of chitin. Moreover, extracted samples were thoroughly characterized by using various analytical techniques.

In the chapter six, depolymerisation studies of different chitosan and chitin samples using various solid acid catalysts were represented. Analysis of the products were carried out by using HPLC and LC-MS. Formation of products and plausible mechanistic pathway were discussed with the help of HPLC analysis results.

In the seventh chapter, main results and novelty of this work are summarized in the form of conclusion.

1.15. References

1. Goswami, D. Y.; Kreith, F., *Handbook of energy efficiency and renewable energy*. Crc Press: 2007.

2. Simonetti, D. A.; Dumesic, J. A., Catalytic strategies for changing the energy content and achieving C▪ C coupling in biomass-derived oxygenated hydrocarbons. *ChemSusChem: Chemistry & Sustainability Energy & Materials* **2008,** *1* (8-9), 725-733.

3. Octave, S.; Thomas, D., Biorefinery: Toward an industrial metabolism. *Biochimie* **2009,** *91* (6), 659-664.

4. Claassen, P.; Van Lier, J.; Lopez Contreras, A.; Van Niel, E.; Sijtsma, L.; Stams, A.; De Vries, S.; Weusthuis, R., Utilisation of biomass for the supply of energy carriers. *Applied microbiology and biotechnology* **1999,** *52* (6), 741-755.

5. Zhang, S.; Maréchal, F.; Gassner, M.; Périn-Levasseur, Z.; Qi, W.; Ren, Z.; Yan, Y.; Favrat, D., Process modeling and integration of fuel ethanol production from

lignocellulosic biomass based on double acid hydrolysis. *Energy & fuels* **2009,** *23* (3), 1759-1765.

6. Sahu, R., Solid acid and supported metal catalyst for biomass conversion into value added chemicals. **2011**.

7. Kobayashi, H.; Fukuoka, A., Synthesis and utilisation of sugar compounds derived from lignocellulosic biomass. *Green Chemistry* **2013,** *15* (7), 1740-1763.

8. Sahu, R.; Dhepe, P. L., A one-pot method for the selective conversion of hemicellulose from crop waste into C5 sugars and furfural by using solid acid catalysts. *ChemSusChem* **2012,** *5* (4), 751-761.

9. Fukuoka, A.; Dhepe, P. L., Catalytic conversion of cellulose into sugar alcohols. *Angewandte Chemie International Edition* **2006,** *45* (31), 5161-5163.

10. Deepa, A. K.; Dhepe, P. L., Lignin depolymerization into aromatic monomers over solid acid catalysts. *ACS Catalysis* **2015,** *5* (1), 365-379.

11. Xu, Y.; Bajaj, M.; Schneider, R.; Grage, S. L.; Ulrich, A. S.; Winter, J.; Gallert, C., Transformation of the matrix structure of shrimp shells during bacterial deproteination and demineralization. *Microbial Cell Factories* **2013,** *12* (1), 90.

12. Chaudhary, R., Employment of heterogeneous base catalysts in the depolymerization of lignin and upgradaion of lignin model compounds. **2017**.

13. Murphy, J.; McCarthy, K., Ethanol production from energy crops and wastes for use as a transport fuel in Ireland. *Applied Energy* **2005,** *82* (2), 148-166.

14. Gundekari, S.; Mitra, J.; Varkolu, M., Classification, characterization, and properties of edible and non-edible biomass feedstocks. In *Advanced Functional Solid Catalysts for Biomass Valorization*, Elsevier: 2020; pp 89-120.

15. Maki-Arvela, P.; Salmi, T.; Holmbom, B.; Willfor, S.; Murzin, D. Y., Synthesis of sugars by hydrolysis of hemicelluloses-a review. *Chemical reviews* **2011,** *111* (9), 5638-5666.

16. Zhou, C.-H.; Xia, X.; Lin, C.-X.; Tong, D.-S.; Beltramini, J., Catalytic conversion of lignocellulosic biomass to fine chemicals and fuels. *Chemical Society Reviews* **2011,** *40* (11), 5588-5617.

17. Luo, Y.; Li, Z.; Li, X.; Liu, X.; Fan, J.; Clark, J. H.; Hu, C., The production of furfural directly from hemicellulose in lignocellulosic biomass: A review. *Catalysis Today* **2019,** *319*, 14-24.

18. Van de Vyver, S.; Geboers, J.; Jacobs, P. A.; Sels, B. F., Recent advances in the catalytic conversion of cellulose. *ChemCatChem* **2011,** *3* (1), 82-94.

19. Chaudhary, R.; Dhepe, P. L., Solid base catalyzed depolymerization of lignin into low molecular weight products. *Green Chemistry* **2017,** *19* (3), 778-788.

20. Singh, S. K.; Dhepe, P. L., Ionic liquids catalyzed lignin liquefaction: mechanistic studies using TPO-MS, FT-IR, RAMAN and 1D, 2D-HSQC/NOSEY NMR. *Green Chemistry* **2016,** *18* (14), 4098-4108.

21. Lucas, N.; Athawale, A. A.; Rode, C. V., Valorization of oceanic waste biomass: a catalytic perspective. *The Chemical Record* **2019,** *19* (9), 1995-2021.

22. Chen, X.; Chew, S. L.; Kerton, F. M.; Yan, N., Direct conversion of chitin into a N-containing furan derivative. *Green Chemistry* **2014,** *16* (4), 2204-2212.

23. Elieh-Ali-Komi, D.; Hamblin, M. R., Chitin and chitosan: production and application of versatile biomedical nanomaterials. *International journal of advanced research* **2016,** *4* (3), 411.

24. Santos, V. P.; Marques, N. S.; Maia, P. C.; Lima, M. A. B. d.; Franco, L. d. O.; Campos-Takaki, G. M. d., Seafood waste as attractive source of chitin and chitosan production and their applications. *International journal of molecular sciences* **2020,** *21* (12), 4290.

25. Jardine, A.; Sayed, S., Challenges in the valorisation of chitinous biomass within the biorefinery concept. *Current Opinion in Green and Sustainable Chemistry* **2016,** *2*, 34-39.

26. Ghosh, N.; Dhepe, P. L., HPLC method development for chitin and chitosan valorisation chemistry. *Carbohydrate Polymer Technologies and Applications* **2021,** *2*, 100139.

27. FAO. The state of world fisheries and aquaculture 2020. https://www.fao.org/documents/card/en/c/ca9229en.

28. Mathew, G. M.; Mathew, D. C.; Sukumaran, R. K.; Sindhu, R.; Huang, C.-C.; Binod, P.; Sirohi, R.; Kim, S.-H.; Pandey, A., Sustainable and eco-friendly strategies for shrimp shell valorization. *Environmental Pollution* **2020,** *267*, 115656.

29. Marine fisheries, Department of fisheries. https://dof.gov.in/marine-fisheries.

30. Department of fisheries, Ministry of fisheries, animal husbandry and dairying, Govt. of India, Handbook on fisheries statistics 2020. https://dof.gov.in/sites/default/files/2021-02/Final_Book.pdf.

31. Sachindra, N. M.; Bhaskar, N.; Mahendrakar, N. S., Carotenoids in different body components of Indian shrimps. *Journal of the Science of Food and Agriculture* **2005,** *85* (1), 167-172.

32. Morris, J. P.; Backeljau, T.; Chapelle, G., Shells from aquaculture: a valuable biomaterial, not a nuisance waste product. *Reviews in Aquaculture* **2019,** *11* (1), 42-57.

33. Chen, X.; Yang, H.; Yan, N., Shell biorefinery: dream or reality? *Chemistry–A European Journal* **2016,** *22* (38), 13402-13421.

34. Yan, N.; Chen, X., Sustainability: Don't waste seafood waste. *Nature* **2015,** *524* (7564), 155-157.

35. Al Omari, M.; Rashid, I.; Qinna, N.; Jaber, A.; Badwan, A., Calcium carbonate. *Profiles of drug substances, excipients and related methodology* **2016,** *41*, 31-132.

36. Khoushab, F.; Yamabhai, M., Chitin research revisited. *Marine drugs* **2010,** *8* (7), 1988-2012.

37. Ravichandran, S.; Rameshkumar, G.; Prince, A. R., Biochemical composition of shell and flesh of the Indian white shrimp Penaeus indicus (H. milne Edwards 1837). *American-Eurasian Journal of Scientific Research* **2009,** *4* (3), 191-194.

38. Synowiecki, J.; Al-Khateeb, N. A. A. Q., The recovery of protein hydrolysate during enzymatic isolation of chitin from shrimp Crangon crangon processing discards. *Food chemistry* **2000,** *68* (2), 147-152.

39. Yadav, M.; Goswami, P.; Paritosh, K.; Kumar, M.; Pareek, N.; Vivekanand, V., Seafood waste: a source for preparation of commercially employable chitin/chitosan materials. *Bioresources and Bioprocessing* **2019,** *6* (1), 1-20.

40. Kim, S.-K., *Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications*. CRC Press: 2010.

41. Sahoo, D.; Nayak, P., Chitosan: The most valuable derivative of chitin. John Wiley & Sons, Inc.: Hoboken, NJ, USA: 2011; pp 129-166.

42. Wan, A. C.; Tai, B. C., Chitin—a promising biomaterial for tissue engineering and stem cell technologies. *Biotechnology advances* **2013,** *31* (8), 1776-1785.

43. Aranaz, I.; Mengíbar, M.; Harris, R.; Paños, I.; Miralles, B.; Acosta, N.; Galed, G.; Heras, Á., Functional characterization of chitin and chitosan. *Current chemical biology* **2009,** *3* (2), 203-230.

44. Lavall, R. L.; Assis, O. B.; Campana-Filho, S. P., β-Chitin from the pens of Loligo sp.: Extraction and characterization. *Bioresource Technology* **2007,** *98* (13), 2465-2472.

45. Kim, S. S.; Kim, S. H.; Lee, Y. M., Preparation, characterization and properties of β-chitin and N-acetylated β-chitin. *Journal of Polymer Science Part B: Polymer Physics* **1996,** *34* (14), 2367-2374.

46. Cárdenas, G.; Cabrera, G.; Taboada, E.; Miranda, S. P., Chitin characterization by SEM, FTIR, XRD, and 13C cross polarization/mass angle spinning NMR. *Journal of Applied Polymer Science* **2004,** *93* (4), 1876-1885.

47. Pillai, C. K.; Paul, W.; Sharma, C. P., Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Progress in polymer science* **2009,** *34* (7), 641-678.

48. Ibitoye, E.; Lokman, I.; Hezmee, M.; Goh, Y.; Zuki, A.; Jimoh, A., Extraction and physicochemical characterization of chitin and chitosan isolated from house cricket. *Biomedical Materials* **2018,** *13* (2), 025009.

49. Thome, J.; Jeuniaux, C.; Weltrowski, M., Applications of chitosan for the elimination of organochlorine xenobiotics from wastewater. *Applications of Chitan and Chitosan* **2020**.

50. Kurita, K., Chitin and chitosan: functional biopolymers from marine crustaceans. *Marine biotechnology* **2006,** *8* (3), 203-226.

51. Rinaudo, M., Chitin and chitosan: Properties and applications. *Progress in polymer science* **2006,** *31* (7), 603-632.

52. Chu, M.; Zhai, Y.; Shang, N.; Guo, P.; Wang, C.; Gao, Y., N-doped carbon derived from the monomer of chitin for high-performance supercapacitor. *Applied Surface Science* **2020,** *517*, 146140.

53. Zhai, Z.; Ren, B.; Xu, Y.; Wang, S.; Zhang, L.; Liu, Z., Nitrogen self-doped carbon aerogels from chitin for supercapacitors. *Journal of Power Sources* **2021,** *481*, 228976.

54. Chow, C. K., *Fatty acids in foods and their health implications*. CRC press: 2007.

55. Heu, M.-S.; Kim, J.-S.; Shahidi, F., Components and nutritional quality of shrimp processing by-products. *Food Chemistry* **2003,** *82* (2), 235-242.

56. Guerin, M.; Huntley, M. E.; Olaizola, M., Haematococcus astaxanthin: applications for human health and nutrition. *TRENDS in Biotechnology* **2003,** *21* (5), 210-216.

57. http://www.rsc.org/chemistryworld/2013/08/chitin-biopolymer-chitosan-podcast.

58. Arbia, W.; Arbia, L.; Adour, L.; Amrane, A., Chitin extraction from crustacean shells using biological methods–a review. *Food Technology and Biotechnology* **2013,** *51* (1), 12-25.

59. No, H. K.; Meyers, S. P.; Lee, K. S., Isolation and characterization of chitin from crawfish shell waste. *Journal of Agricultural and Food Chemistry* **1989,** *37* (3), 575-579.

60. Percot, A.; Viton, C.; Domard, A., Optimization of chitin extraction from shrimp shells. *Biomacromolecules* **2003,** *4* (1), 12-18.

61. Beaney, P.; Lizardi-Mendoza, J.; Healy, M., Comparison of chitins produced by chemical and bioprocessing methods. *Journal of Chemical Technology & Biotechnology* **2005,** *80* (2), 145-150.

62. Xie, H.; Zhang, S.; Li, S., Chitin and chitosan dissolved in ionic liquids as reversible sorbents of CO2. *Green Chemistry* **2006,** *8* (7), 630-633.

63. Yamazaki, S.; Takegawa, A.; Kaneko, Y.; Kadokawa, J.-i.; Yamagata, M.; Ishikawa, M., An acidic cellulose–chitin hybrid gel as novel electrolyte for an electric double layer capacitor. *Electrochemistry Communications* **2009,** *11* (1), 68-70.

64. Sharma, M.; Mukesh, C.; Mondal, D.; Prasad, K., Dissolution of α-chitin in deep eutectic solvents. *RSC Advances* **2013,** *3* (39), 18149-18155.

65. Swatloski, R. P.; Spear, S. K.; Holbrey, J. D.; Rogers, R. D., Dissolution of cellose with ionic liquids. *Journal of the American chemical society* **2002,** *124* (18), 4974-4975.

66. Qin, Y.; Lu, X.; Sun, N.; Rogers, R. D., Dissolution or extraction of crustacean shells using ionic liquids to obtain high molecular weight purified chitin and direct production of chitin films and fibers. *Green Chemistry* **2010,** *12* (6), 968-971.

67. Setoguchi, T.; Kato, T.; Yamamoto, K.; Kadokawa, J.-i., Facile production of chitin from crab shells using ionic liquid and citric acid. *International Journal of Biological Macromolecules* **2012,** *50* (3), 861-864.

68. Gortari, M. C.; Hours, R. A., Biotechnological processes for chitin recovery out of crustacean waste: a mini-review. *Electronic Journal of Biotechnology* **2013,** *16* (3), 14-14.

69. Kaur, S.; Dhillon, G. S., Recent trends in biological extraction of chitin from marine shell wastes: a review. *Critical reviews in biotechnology* **2015,** *35* (1), 44-61.

70. Prameela, K.; Mohan, C. M.; Smitha, P.; Hemalatha, K., Bioremediation of shrimp biowaste by using natural probiotic for chitin and carotenoid production an alternative method to hazardous chemical method. **2010**.

71. No, H. K.; Meyers, S. P., Preparation and Characterization of Chitin and Chitosan—A Review. *Journal of Aquatic Food Product Technology* **1995,** *4* (2), 27-52.

72. Roberts, G. A., *Chitin chemistry*. Macmillan International Higher Education: 1992.

73. Muzzarelli, R. A.; Rocchetti, R., The determination of molybdenum in sea water by hot graphite atomic absorption spectrometry after concentration on p-aminobenzylcellulose or chitosan. *Analytica Chimica Acta* **1973,** *64* (3), 371-379.

74. Kumar, M. N. R., A review of chitin and chitosan applications. *Reactive and functional polymers* **2000,** *46* (1), 1-27.

75. Liu, L.; Liu, Y.; Shin, H.-d.; Chen, R.; Li, J.; Du, G.; Chen, J., Microbial production of glucosamine and N-acetylglucosamine: advances and perspectives. *Applied Microbiology and Biotechnology* **2013,** *97* (14), 6149-6158.

76. Muzzarelli, R., Human enzymatic activities related to the therapeutic administration of chitin derivatives. *Cellular and Molecular Life Sciences CMLS* **1997,** *53* (2), 131-140.

77. Mourya, V.; Inamdar, N.; Choudhari, Y. M., Chitooligosaccharides: Synthesis, characterization and applications. *Polymer Science Series A* **2011,** *53* (7), 583-612.

78. Seo, S.; King, J.; Prinyawiwatkul, W., Simultaneous depolymerization and decolorization of chitosan by ozone treatment. *Journal of food science* **2007,** *72* (9), C522-C526.

79. Tian, F.; Liu, Y.; Hu, K.; Zhao, B., The depolymerization mechanism of chitosan by hydrogen peroxide. *Journal of Materials Science* **2003,** *38* (23), 4709-4712.

80. Tanioka, S.; Matsui, Y.; Irie, T.; Tanigawa, T.; Tanaka, Y.; Shibata, H.; Sawa, Y.; Kono, Y., Oxidative depolymerization of chitosan by hydroxyl radical. *Bioscience, biotechnology, and biochemistry* **1996,** *60* (12), 2001-2004.

81. Xia, W.; Liu, P.; Liu, J., Advance in chitosan hydrolysis by non-specific cellulases. *Bioresource technology* **2008,** *99* (15), 6751-6762.

82. Zhang, Z.; Li, C.; Wang, Q.; Zhao, Z. K., Efficient hydrolysis of chitosan in ionic liquids. *Carbohydrate polymers* **2009,** *78* (4), 685-689.

83. Chen, Q.; Xiao, W.; Zhou, L.; Wu, T.; Wu, Y., Hydrolysis of chitosan under microwave irradiation in ionic liquids promoted by sulfonic acid-functionalized ionic liquids. *Polymer degradation and stability* **2012,** *97* (1), 49-53.

84. Yabushita, M.; Kobayashi, H.; Kuroki, K.; Ito, S.; Fukuoka, A., Catalytic Depolymerization of Chitin with Retention of N-Acetyl Group. *ChemSusChem* **2015,** *8* (22), 3760-3763.

85. Zhang, J.; Yan, N., Production of Glucosamine from Chitin by Co-solvent Promoted Hydrolysis and Deacetylation. *ChemCatChem* **2017,** *9* (14), 2790-2796.

86. Zhang, J.; Yan, N., Formic acid-mediated liquefaction of chitin. *Green Chemistry* **2016,** *18* (18), 5050-5058.

87. Pierson, Y.; Chen, X.; Bobbink, F.; Zhang, J.; Yan, N., ACS Sustainable Chem. *Eng* **2014,** *2*, 2081-2089.

88. Zhang, H.; Lu, Y.; Wang, Y.; Zhang, X.; Wang, T., d-Glucosamine production from chitosan hydrolyzation over a glucose-derived solid acid catalyst. *RSC advances* **2018,** *8* (10), 5608-5613.

89. Werpy, T.; Petersen, G. *Top value added chemicals from biomass: volume I--results of screening for potential candidates from sugars and synthesis gas*; National Renewable Energy Lab., Golden, CO (US): 2004.

90. Mascal, M.; Nikitin, E. B., Dramatic advancements in the saccharide to 5-(chloromethyl) furfural conversion reaction. *ChemSusChem: Chemistry & Sustainability Energy & Materials* **2009,** *2* (9), 859-861.

91. Omari, K. W.; Besaw, J. E.; Kerton, F. M., Hydrolysis of chitosan to yield levulinic acid and 5-hydroxymethylfurfural in water under microwave irradiation. *Green Chemistry* **2012,** *14* (5), 1480-1487.

92. Lee, S.-B.; Jeong, G.-T., Catalytic conversion of chitosan to 5-hydroxymethylfurfural under low temperature hydrothermal process. *Applied biochemistry and biotechnology* **2015,** *176* (4), 1151-1161.

93. Jeong, G.-T., Production of levulinic acid from glucosamine by dilute-acid catalyzed hydrothermal process. *Industrial Crops and Products* **2014,** *62*, 77-83.

94. Savitri, E.; Juliastuti, S. R.; Handaratri, A.; Roesyadi, A., Degradation of chitosan by sonication in very-low-concentration acetic acid. *Polymer degradation and stability* **2014,** *110*, 344-352.

95. Park, M.-R.; Kim, H. S.; Kim, S.-K.; Jeong, G.-T., Thermo-chemical conversion for production of levulinic and formic acids from glucosamine. *Fuel Processing Technology* **2018,** *172*, 115-124.

96. Kim, H. S.; Park, M.-R.; Kim, S.-K.; Jeong, G.-T., Valorization of chitosan into levulinic acid by hydrothermal catalytic conversion with methanesulfonic acid. *Korean Journal of Chemical Engineering* **2018,** *35* (6), 1290-1296.

97. Wang, Y.; Pedersen, C. M.; Deng, T.; Qiao, Y.; Hou, X., Direct conversion of chitin biomass to 5-hydroxymethylfurfural in concentrated ZnCl2 aqueous solution. *Bioresource technology* **2013,** *143*, 384-390.

98. Yu, S.; Zang, H.; Chen, S.; Jiang, Y.; Yan, B.; Cheng, B., Efficient conversion of chitin biomass into 5-hydroxymethylfurfural over metal salts catalysts in dimethyl sulfoxide-water mixture under hydrothermal conditions. *Polymer Degradation and Stability* **2016,** *134*, 105-114.

99. Kim, H. S.; Kim, S.-K.; Jeong, G.-T., Efficient conversion of glucosamine to levulinic acid in a sulfamic acid-catalyzed hydrothermal reaction. *RSC advances* **2018,** *8* (6), 3198-3205.

100. Kim, H. S.; Park, M. R.; Jeon, Y. J.; Kim, S. K.; Hong, Y. K.; Jeong, G. T., Valorization of Chitosan as Food Waste of Aquatic Organisms into 5-Hydroxymethylfurfural by Sulfamic Acid-Catalyzed Conversion Process. *Energy Technology* **2018,** *6* (9), 1747-1754.

101. Kalane, N. D.; Krishnan, R. A.; Yadav, V. D.; Jain, R.; Dandekar, P., Synergistic effect of hetero-and homo-catalysts on the 'green'synthesis of 5-hydroxymethylfurfural from chitosan biomass. *Cellulose* **2019,** *26* (4), 2805-2819.

102. Tzeng, T.-W.; Bhaumik, P.; Chung, P.-W., Understanding the production of 5-hydroxymethylfurfural (HMF) from chitosan using solid acids. *Molecular Catalysis* **2019,** *479*, 110627.

103. Zhang, Z.; Song, J.; Han, B., Catalytic transformation of lignocellulose into chemicals and fuel products in ionic liquids. *Chemical reviews* **2017,** *117* (10), 6834-6880.

104. Zhou, L.; Liang, R.; Ma, Z.; Wu, T.; Wu, Y., Conversion of cellulose to HMF in ionic liquid catalyzed by bifunctional ionic liquids. *Bioresource technology* **2013,** *129*, 450-455.

105. Matsagar, B. M.; Hossain, S. A.; Islam, T.; Alamri, H. R.; Alothman, Z. A.; Yamauchi, Y.; Dhepe, P. L.; Wu, K. C.-W., Direct production of furfural in one-pot fashion from raw biomass using Brønsted acidic ionic liquids. *Scientific reports* **2017,** *7* (1), 1-7.

106. Singh, S. K.; Dhepe, P. L., Ionic liquids catalyzed lignin liquefaction: mechanistic studies using TPO-MS, FT-IR, RAMAN and 1D, 2D-HSQC/NOSEY NMR. *Green chemistry* **2016**.

107. Feng, J. X.; Zang, H. J.; Yan, Q.; Li, M. G.; Cheng, B. W. In *Conversion of chitosan into 5-hydroxymethylfurfural via hydrothermal synthesis*, Advanced Materials Research, Trans Tech Publ: 2015; pp 411-414.

108. Li, M.; Zang, H.; Feng, J.; Yan, Q.; Yu, N.; Shi, X.; Cheng, B., Efficient conversion of chitosan into 5-hydroxymethylfurfural via hydrothermal synthesis in ionic liquids aqueous solution. *Polymer Degradation and Stability* **2015,** *121*, 331-339.

109. Jiang, Y.; Zang, H.; Han, S.; Yan, B.; Yu, S.; Cheng, B., Direct conversion of chitosan to 5-hydroxymethylfurfural in water using Brønsted–Lewis acidic ionic liquids as catalysts. *RSC advances* **2016,** *6* (105), 103774-103781.

110. Zang, H.; Yu, S.; Yu, P.; Ding, H.; Du, Y.; Yang, Y.; Zhang, Y., Hydrothermal conversion of N-acetyl-d-glucosamine to 5-hydroxymethylfurfural using ionic liquid as a recycled catalyst in a water-dimethyl sulfoxide mixture. *Carbohydrate research* **2017,** *442*, 1-8.

111. Zhang, M.; Zang, H.; Ma, B.; Zhang, X.; Xie, R.; Cheng, B., Green Synthesis of 5-Hydroxymethylfurfural from Chitosan Biomass Catalyzed by Benzimidazole-Based Ionic Liquids. *ChemistrySelect* **2017,** *2* (32), 10323-10328.

112. Franich, R. A.; Goodin, S. J.; Wilkins, A. L., Acetamidofurans, acetamidopyrones, and acetamidoacetaldehyde from pyrolysis of chitin and n-acetylglucosamine. *Journal of analytical and applied pyrolysis* **1984,** *7* (1-2), 91-100.

113. Chen, J.; Wang, M.; Ho, C.-T., Volatile compounds generated from thermal degradation of N-acetylglucosamine. *Journal of agricultural and food chemistry* **1998,** *46* (8), 3207-3209.

114. Drover, M. W.; Omari, K. W.; Murphy, J. N.; Kerton, F. M., Formation of a renewable amide, 3-acetamido-5-acetylfuran, via direct conversion of N-acetyl-D-glucosamine. *Rsc Advances* **2012,** *2* (11), 4642-4644.

115. Omari, K. W.; Dodot, L.; Kerton, F. M., A simple one-pot dehydration process to convert N-acetyl-D-glucosamine into a nitrogen-containing compound, 3-acetamido-5-acetylfuran. *ChemSusChem* **2012,** *5* (9), 1767-1772.

116. Chen, X.; Liu, Y.; Kerton, F. M.; Yan, N., Conversion of chitin and N-acetyl-d-glucosamine into a N-containing furan derivative in ionic liquids. *Rsc Advances* **2015,** *5* (26), 20073-20080.

117. Chen, X.; Gao, Y.; Wang, L.; Chen, H.; Yan, N., Effect of treatment methods on chitin structure and its transformation into nitrogen-containing chemicals. *ChemPlusChem* **2015,** *80* (10), 1565-1572.

118. Wang, J.; Zang, H.; Jiao, S.; Wang, K.; Shang, Z.; Li, H.; Lou, J., Efficient conversion of N-acetyl-D-glucosamine into nitrogen-containing compound 3-

acetamido-5-acetylfuran using amino acid ionic liquid as the recyclable catalyst. *Science of The Total Environment* **2020,** *710*, 136293.

119. Padovan, D.; Kobayashi, H.; Fukuoka, A., Facile Preparation of 3-Acetamido-5-acetylfuran from N-Acetyl-d-glucosamine by using Commercially Available Aluminum Salts. *ChemSusChem* **2020,** *13* (14), 3594-3598.

120. Zang, H.; Lou, J.; Jiao, S.; Li, H.; Du, Y.; Wang, J., Valorization of chitin derived N-acetyl-D-glucosamine into high valuable N-containing 3-acetamido-5-acetylfuran using pyridinium-based ionic liquids. *Journal of Molecular Liquids* **2021,** *330*, 115667.

121. Zang, H.; Li, H.; Jiao, S.; Lou, J.; Du, Y.; Huang, N., Green Conversion of N-Acetylglucosamine into Valuable Platform Compound 3-Acetamido-5-acetylfuran Using Ethanolamine Ionic Liquids as Recyclable Catalyst. *ChemistrySelect* **2021,** *6* (16), 3848-3857.

122. Du, Y.; Zang, H.; Feng, Y.; Wang, K.; Lv, Y.; Liu, Z., Efficient catalytic system for converting N-acetyl-d-glucosamine into valuable chemical 3-acetylamino-5-acetylfuran. *Journal of Molecular Liquids* **2022,** *347*, 117970.

123. Liu, Y.; Stähler, C.; Murphy, J. N.; Furlong, B. J.; Kerton, F. M., Formation of a Renewable Amine and an Alcohol via Transformations of 3-Acetamido-5-acetylfuran. *ACS Sustainable Chemistry & Engineering* **2017,** *5* (6), 4916-4922.

124. Pham, T. T.; Lindsay, A. C.; Kim, S. W.; Persello, L.; Chen, X.; Yan, N.; Sperry, J., Two-Step Preparation of Diverse 3-Amidofurans from Chitin. *ChemistrySelect* **2019,** *4* (34), 10097-10099.

125. Sadiq, A. D.; Chen, X.; Yan, N.; Sperry, J., Towards the shell biorefinery: sustainable synthesis of the anticancer alkaloid proximicin A from chitin. *ChemSusChem* **2018,** *11* (3), 532-535.

126. Pham, T. T.; Gözaydın, G.; Söhnel, T.; Yan, N.; Sperry, J., Oxidative Ring-Expansion of a Chitin-Derived Platform Enables Access to Unexplored 2-Amino Sugar Chemical Space. *European Journal of Organic Chemistry* **2019,** *2019* (6), 1355-1360.

127. Pham, T. T.; Chen, X.; Söhnel, T.; Yan, N.; Sperry, J., Haber-independent, diversity-oriented synthesis of nitrogen compounds from biorenewable chitin. *Green Chemistry* **2020,** *22* (6), 1978-1984.

128. Pham, T. T.; Chen, X.; Yan, N.; Sperry, J., A novel dihydrodifuropyridine scaffold derived from ketones and the chitin-derived heterocycle 3-acetamido-5-acetylfuran. *Monatshefte für Chemie-Chemical Monthly* **2018,** *149* (4), 857-861.

129. Kuhn, R.; Krüger, G., Das Chromogen III der Morgan-Elson-Reaktion. *Chemische Berichte* **1957,** *90* (2), 264-277.

130. Osada, M.; Kikuta, K.; Yoshida, K.; Totani, K.; Ogata, M.; Usui, T., Non-catalytic synthesis of Chromogen I and III from N-acetyl-D-glucosamine in high-temperature water. *Green chemistry* **2013,** *15* (10), 2960-2966.

131. Osada, M.; Kikuta, K.; Yoshida, K.; Totani, K.; Ogata, M.; Usui, T., Non-catalytic dehydration of N, N′-diacetylchitobiose in high-temperature water. *RSC advances* **2014,** *4* (64), 33651-33657.

132. Zheng, X.-Y.; Peng, J.-B.; Livera, M. V. S.; Luo, Y.; Wang, Y.-Y.; Kong, X.-J.; Long, L.-S.; Zheng, Z.; Zheng, L.-S., Selective formation of chromogen I from N-acetyl-D-glucosamine upon lanthanide coordination. *Inorganic Chemistry* **2017,** *56* (1), 110-113.

133. Osada, M.; Shoji, S.; Suenaga, S.; Ogata, M., Conversion of N-acetyl-d-glucosamine to nitrogen-containing chemicals in high-temperature water. *Fuel Processing Technology* **2019,** *195*, 106154.

134. Osada, M.; Kobayashi, H.; Miyazawa, T.; Suenaga, S.; Ogata, M., Non-catalytic conversion of chitin into Chromogen I in high-temperature water. *International journal of biological macromolecules* **2019,** *136*, 994-999.

135. Fang, Z.; Qi, X., *Production of platform chemicals from sustainable resources*. Springer: 2017.

136. Szabolcs, Á.; Molnár, M.; Dibó, G.; Mika, L. T., Microwave-assisted conversion of carbohydrates to levulinic acid: an essential step in biomass conversion. *Green Chemistry* **2013,** *15* (2), 439-445.

137. Park, M.-R.; Kim, S.-K.; Jeong, G.-T., Production of levulinic acid from glucosamine using zirconium oxychloride. *Journal of industrial and engineering chemistry* **2018,** *61*, 119-123.

138. Park, M.-R.; Kim, H. S.; Kim, S.-K.; Jeong, G.-T., Production of Levulinic Acid from Glucosamine Using Solid Acid Catalyst. *Korean Chemical Engineering Research* **2018,** *56* (1), 61-65.

139. Hou, W.; Liu, L.; Shen, H., Selective conversion of chitosan to levulinic acid catalysed by acidic ionic liquid: intriguing NH2 effect in comparison with cellulose. *Carbohydrate polymers* **2018,** *195*, 267-274.

140. Hou, W.; Zhao, Q.; Liu, L., Selective conversion of chitin to levulinic acid catalyzed by ionic liquids: distinctive effect of N-acetyl groups. *Green Chemistry* **2020,** *22* (1), 62-70.

141. Catalysis. https://en.wikipedia.org/wiki/Catalysis.

142. Homogeneous catalysis. https://en.wikipedia.org/wiki/Homogeneous_catalysis#:~:text=Homogeneous%20catalysis%20refers%20to%20reactions,typically%20solid%2Dgas%2C%20respectively.

143. Bhaumik, P.; Dhepe, P. L., Solid acid catalyzed synthesis of furans from carbohydrates. *Catalysis Reviews* **2016,** *58* (1), 36-112.

144. Zeolite. https://en.wikipedia.org/wiki/Zeolite.

145. Corma, A., Inorganic solid acids and their use in acid-catalyzed hydrocarbon reactions. *Chemical reviews* **1995,** *95* (3), 559-614.

146. Ma, Y.; Tong, W.; Zhou, H.; Suib, S. L., A review of zeolite-like porous materials. *Microporous and mesoporous materials* **2000,** *37* (1-2), 243-252.

147. Bhaumik, P., Efficient ways to volorize biomass into sugars & furans: study on the properties of sapo's & metal oxide catalysts. **2014**.

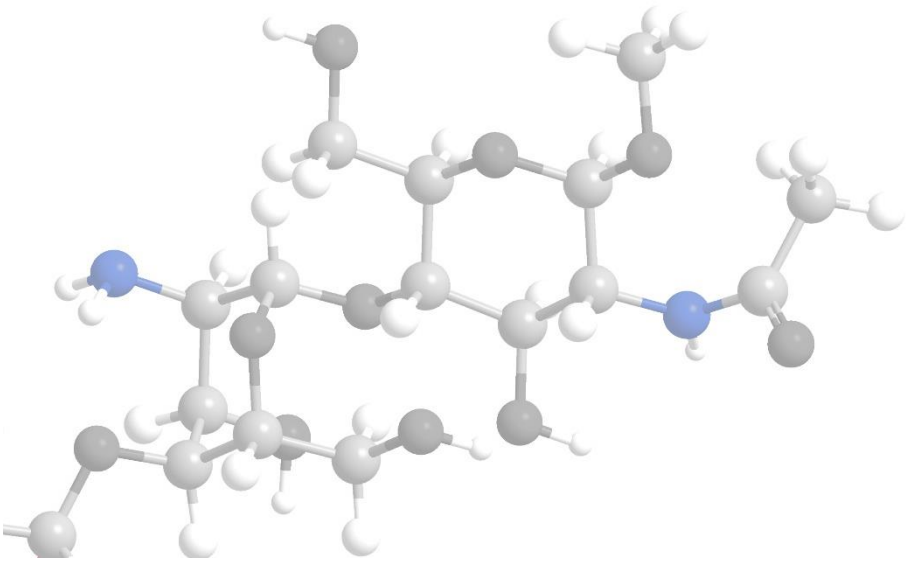
148. Ashtekar, S.; Chilukuri, S. V.; Chakrabarty, D. K., Small-pore molecular sieves SAPO-34 and SAPO-44 with chabazite structure: a study of silicon incorporation. *The Journal of Physical Chemistry* **1994,** *98* (18), 4878-4883.

149. Mott, C., Clay minerals-an introduction. *Catalysis today* **1988,** *2* (2-3), 199-208.

150. Uddin, F., Clays, nanoclays, and montmorillonite minerals. *Metallurgical and Materials Transactions A* **2008,** *39* (12), 2804-2814.

Chapter 02

HPLC method development for simultaneous separation & analysis of monomers, furans and other reaction products



2.1. Introduction

In the last decade, due to apparent drawbacks associated with the fossil feedstocks, researchers have directed a lot of efforts to develop methodologies for the synthesis of chemicals and fuels from sustainable biomass feedstock such as lignocellulosic biomass. Typically, lignocellulosic biomass, a part of plant derived biomass is at forefront since it is rich in polysaccharides (cellulose, hemicellulose) and phenolic polymer (lignin).[1-5] However, another sustainable biomass namely; marine biomass or also called as oceanic biomass which is available in abundance is surprisingly overlooked by researchers. One of the marine biomasses is the waste generated from the crustacean species i.e., crabs, shrimps, lobsters, prawns, squids etc. Though the shell waste is readily and cheaply available for e.g., shrimp shell powder is sold at USD 100-120 per ton, is not evaluated for its valorisation to synthesize chemicals and fuels[6] but rather is directly disposed of (land filling or disposed in seas).[6] As this waste is mainly composed of calcium carbonate (20-50%), protein (20-40%), and chitin (15-40%)[7], it may cause environmental issues if it is disposed of directly and also at the same time, the world is losing huge potential feedstock for the chemicals and fuels synthesis. Hence, in recent times, the utilization of marine waste has been looked into to bring both economic and environmental benefits to industries, including the fishery industry.[8] Chitin, one of the important constitute of crustacean waste, is a homo-polysaccharide made up of several N-Acetyl-D-Glucosamine (GlcNAc) units liked via β-1,4 linkages. From abundance point of view, it is the 2nd most plentiful bio-polymer available on earth after cellulose with 10^{11} tonnes of annual production.[9] As observed, chitin is structural analogue of cellulose with only a difference in structure at C2 position, where in place of hydroxyl (-OH) group, acetamide ($-NHCOCH_3$) group is present (Figure 2.1). Upon base treatment native chitin yields chitosan. To be precise, chitosan is a heteropolymer consisting of D-(+) Glucosamine ($GlcNH_2$) and N-Acetyl-D-

Glucosamine (GlcNAc) units and has potential applications in various industries i.e., agriculture, water purification, biomedical materials and catalysis.[10-13] Unlike other plant biomass feedstocks, the importance of chitin and chitosan is that they contain 7% biologically fixed nitrogen in their structure, which can be harnessed to make nitrogen-containing chemicals, otherwise very tedious to prepare.[14-15] It is known that by subjecting chitin and chitosan to acid or base catalysed hydrolysis reaction, those are reported to yield oligomers and monomers, glucosamine ($GlcNH_2$) and N-acetyl glucosamine (GlcNAc) as products. These monomers can further undergo dehydration reactions to produce 5-Hydroxymethylfurfural (5-HMF) and other furan derivatives (Figure 2.1). Although Figure 2.1. shows a very simplistic illustration of products formed, but in reality, various products are bound to form as several functional groups are present. Those can undergo various reactions in the presence of acid/base or even upon thermal treatment.

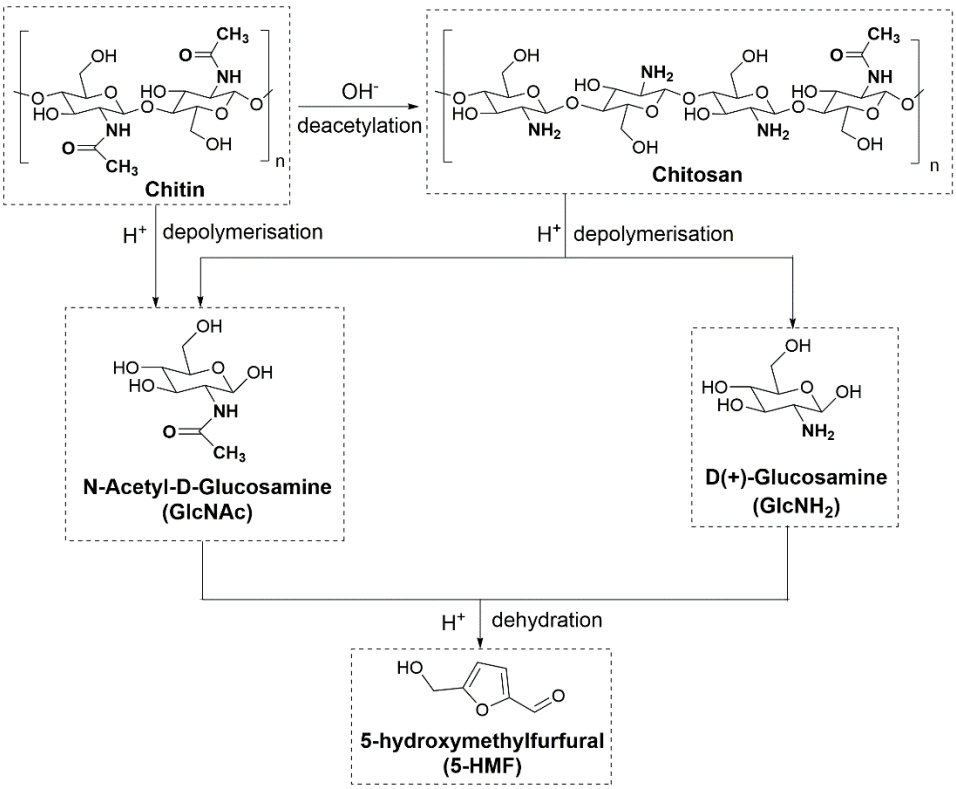


Figure 2.1. Depolymerisation of chitosan and chitin into $GlcNH_2$ and GlcNAc and further dehydration to 5-HMF

While, researchers are developing various catalytic systems to yield monomers, N-containing chemicals and furan derivatives from chitin and chitosan via biochemical, catalytic or hydrothermal routes, it is often observed that the analysis of the reaction mixture is restricted to either oligomers formation or monomer formations or furan formation. Since $GlcNH_2$, GlcNAc and other N-containing chemicals are sugar derivatives which have complete solubility in water. Thus, it is essential to develop and use proper analytic method(s) to separate and quantify these compounds to take work in this area on next level. Moreover, besides these reported products, it is often seen that catalytic or thermal reactions may yield few other products which have until now gone undetected or simply not separated or identified due to lack of optimum analytical conditions.

Literature reports, several analytical methods such as chromatographic (gas chromatography (GC),[16-18] column chromatography, high performance liquid chromatography (HPLC),[17, 19-20] chemical method (Fehling's test, gravimetric analysis)[21] and colorimetric method (DNS method)[19-20, 22-23] for the separation and quantification of chitin/chitosan derived chemicals.

Gas chromatography (GC), as is known, requires compounds to be volatile or having boiling points less than their degradation temperature(s) for their successful analysis. This method is suitable for the detection and quantification of organic compounds having the above properties. It is seen that it can be very well used to detect and quantify 5-HMF and other volatile compounds formed in chitin/chitosan chemistry.[17-18] Nonetheless, amino sugars such as $GlcNH_2$ and GlcNAc do not have clear boiling points and are also non-volatile and thus cannot be detected by the GC method unless those are derivatized. Moreover, these amino sugars also undergo degradation at higher temperatures employed during GC analysis and thus make GC method unsuitable for their separation and quantification. Additionally, chitin/chitosan hydrolysis reactions are carried

out in water media and water-based samples are not recommended to be injected in the GC system since water can extinguish the flame (in flame ionization detector) and water at higher temperature may dissolve silica in capillary column. Therefore, simultaneous analysis and separation of $GlcNH_2$, GlcNAc, N-containing chemicals, furan derivatives and 5-HMF is not probable using GC technique.[24]

Column chromatography is another good analytical technique which is used for the analysis of products in most of organic reactions. Amino sugars, N-containing chemicals, and furan derivatives have very comparable polarity due to their structural and functional groups similarity. Thus, this can lead to difficulty in the separation of these compounds. Additionally, this technique requires tedious process to follow and hence running a column for every reaction/sample is also time-consuming process and is not recommended for fast and quantitative analysis. In consideration of this, column chromatography may not be a good technique for separating these compounds.[24]

Another method by which reducing sugars can be quantified is a gravimetric analysis method by measuring the weight of CuO_2 precipitate formed in the reaction of reducing sugar with copper(II) sulfate in an alkaline medium.[21] However, the drawback of this method is that only reducing sugars can be quantified. Thus, organic compounds such as furan derivatives and any other N-containing chemicals cannot be detected. Moreover, as the main drawback of this method, the total amount of reducing sugars can be calculated. Still, the separation of two or more different reducing sugars and their separate quantification is not possible. Also, gravimetric analysis is very sensitive to reaction time, temperature, pH and concentration of both sugars and copper (II) sulfate.

Reducing sugars can also be quantified by the colorimetric technique that consists of a redox reaction between 3,5-dinitrosalicylic acid and the reducing

sugars present in the sample, then quantified by UV-Visible spectroscopy at 540 nm. The intensity of the colour is proportional to the concentration of sugars and thus the total concentration of sugars can be easily quantified. Nevertheless, this method too has the same drawbacks as like previously discussed chemical method. Colorimetric method is also used by researchers in chitin/chitosan field for quantifying reducing sugars. [20, 23, 25]

However, those are often associated with several drawbacks as summarized in Table 2.1.

Table 2.1. Summary on the analytical methods used in sugar chemistry and their drawbacks

Analytical method	Drawbacks
Gas Chromatography (GC)	Sugar compounds being non-volatile and undergo degradation at elevated temperatures cannot be analyzed unless derivatized.
Column Chromatography (CC)	Amino sugars, N-containing chemicals and furan derivatives have comparable polarity, this can lead to the difficulty in the separation of these compounds. This technique also a tedious and time-consuming process and cannot be applied on routine basis for quantitative and qualitative analysis.
Chemical method (gravimetric analysis)	Only reducing sugars can be detected. Total amount of reducing sugars can only be calculated but separate quantification of two or more reducing sugars is not possible.
Colorimetric method (DNS method)	Same as chemical methods

For a very long time, HPLC has been a well-known technique used to separate and quantify sugar compounds. HPLC is also an easy, rapid, sensitive analytical technique that can separate water soluble sugar compounds as well as organic compounds.[24]

While researchers are working on the chitin/chitosan chemistry, they have extensively used HPLC as a prime methodology to detect the products formed in their reactions, nevertheless with few restrictions. A quick observation from Table 2.2 suggests that, in most of the works, HPLC system is used along with refractive index detector (RID)/variable wavelength detector (VWD) detector and a variety of columns are used. However, from the sugar chemistry originated from plant biomass, it is known that non-polar columns cannot separate sugar compounds very well as the non-polar stationary phase cannot have strong interaction with polar sugar compounds. It is acknowledged from the sugar chemistry originated from plant biomass that the best HPLC column for the separation and quantification of glucose, fructose, xylose, arabinose etc., along with furfural, 5-hydroxymethylfurfural (5-HMF) etc. are ion-exchanged columns such as Pb^{2+}, H^{+}, Ca^{2+} etc. These columns are preferred due to their tolerance towards a wide range of pH (1-8) and solvents (<10% organic solvent with water as base eluent). However, it is seen from Table 2.2 that in chitin/chitosan chemistry, rarely ion-exchange columns are used for their efficiencies to detect and quantify amino sugars along with N-containing chemicals and furan derivatives. Additionally, in most of the works (Table 2.2), simultaneous analysis of all the chemicals is not reported due to the lack of efforts put in the direction for the separation of these compounds by altering analytical parameters.[17, 26-27]

Table 2.2. Summary on the analysis of chitin/chitosan based chemistry reported in the literature

Substrate	Products formed	Catalytic technique	Solvent	Analytical technique used	HPLC column	Additional information	Products detected	Ref.
Chitin	GlcNAc, Oligomers of GlcNAc, 5-HMF	Mechano-catalytic hydrolytic	Data not available	**HPLC-RID, HPLC-VWD**	Rezex RPM-Monosaccharide Pb^{2+} (300 mm x 7.8 mm I.D)	Not available	GlcNAc, Oligomers of GlcNAc, 5-HMF	28
	ADS	hydrogenation	N.A	GC-MS	HR-20M	Silylation to derivatize hydroxyl groups	ADS	
Chitosan	5-HMF, FA, AA, LA	Catalytic	0.01N H_2SO_4	**HPLC-RID**	Biorad Aminex HPX-87H H^+ column (300 mm x 7.8 mm I.D)	RID at 50°C Flow rate- 0.6 mL/min	FA, LA, AA 5-HMF	17

				GC-FID	SH-RXI 5MS (30m x 0.25 mm)	Initial temperature- 70 °C, 200 °C- 5min hold (10 °C/min ramp), detector at 250 °C	5-HMF	
Chitosan	5-HMF	Catalytic	1% aqueous CH_3CN/2 % aqueous CH_3COOH (50/50 *v/v*)	**HPLC-DAD**	C18 column (250 mm x 4.6 mm I.D, 5 mm)	Wavelength- 283 nm Flow rate- 1.0 mL/min	5-HMF	25
Chitosan	5-HMF, some unidentified products	Catalytic	Methanol /water (2.3/7.7 *v/v*)	**HPLC-VWD**	Kromasil C18 column (250 mm x 4.6 mm I.D, 5 mm)	Wavelength- 284 nm, Flow rate- 0.5 mL/min	5-HMF	23
	Reducing sugar	Catalytic	N.A	Colorimetric method (DNS method)	Not applicable	Using DNS method, total concentration of reducing sugar is calculated	Reducing sugar total concentration	

Chitin	GlcNAc and its oligomers	Catalytic and enzymatic	0.005M H_2SO_4	**HPLC- VWD**	Aminax HPX-87H H^+ column	Wavelength- 210 nm, Flow rate- 0.7 mL/min	GlcNAc and its oligomers	26
Chitin and GlcNAc	3A5AF	Catalytic	CH_3CN/ water (1.7/8.3 *v/v*)	**HPLC- VWD**	Agilent ZORBAX eclipse C18	Wavelength- 230 nm, Flow rate- 0.5 mL/min	3A5AF	15, 29
Chitin	HADP, HAADP	Liquefaction	Water	**HPLC- VWD**	Agilent Hi-Plex Ca^{2+} sugar column	Wavelength- 210 nm	HADP, HAADP	27
			N.A	GC-MS, triple axis detector	HP-5 GC column	Silylation to derivatize hydroxyl groups	HADP, HAADP identification	
			N.A	GC-FID	HP-5 GC column	Silylation done to derivatize hydroxyl group, n-dodacane as internal standard	HADP, HAADP quantification	
Chitosan/ Chitin	5-HMF, LA	Catalytic	N.A	GC-MS, triple axis detector	DB-5 Column	50 °C (hold 1 min), 25 °C/min to 150	5-HMF, LA	30

						°C, 20 °C/min to 170 °C and 80 °C/min to 250 °C for 3 min		
	Oligomers	Catalytic	N.A	MALDI TOF/TOF analyzer	Not applicable	DHB used as matrix	Oligomers	
Chitosan	Oligomers	Mineral acid hydrolysis	0.25M AcOH/ 0.25M Sodium acetate	Size exclusion chromatography with RID detector	TosoHaas-TSK gel column (GMPWXL, 300 mm × 7.8mm)	Flow rate- 0.4 mL/min	Oligomers	31
GlcNAc	C2/C3/C4/C6 polyols	Hydrogenation	N.A	GC-MS with triple axis and GC-FID	Data not available	After silylation and adding n-dodecane as internal standard	C2/C3/ C4/C6 polyols	32
Chitin	$GlcNH_2$ GlcNAc	Catalytic hydrolysis followed by deacetylation	N.A	GC-FID	HP-5 (30m x 0.25 mm)	After acetylation and addition of sorbitol as internal standard	Total yield of GlcNAc, $GlcNH_2$	18

			0.005 M H_2SO_4	**HPLC**	Hi-Plex H^+ column		GlcNAc quantification	
Chitosan	$GlcNH_2$, 5-HMF	Catalytic	Methanol /water (2.3/7.7 v/v)	**HPLC- VWD**	C18 column (250 mm x 4.6 mm I.D, 5 mm)	Wavelength- 283 nm, Flow rate- 0.5 mL/min	5-HMF	19
			N.A	Colorimetric method (Schales method)	Not applicable	Using Schales reagent and UV-Vis measurement at 420 nm	$GlcNH_2$	
Chitin	GlcNAc	Acidified molten salt hydrates	0.005 M H_2SO_4	**HPLC-RID**	Agilent Hi-Plex H^+ column	Flow rate- 0.6 mL/min	GlcNAc	33
Chitosan	$GlcNH_2$, Glucosaminic Acid	Hydrolytic oxidation	0.005 M H_2SO_4	**HPLC- VWD /RID**	Zorbax NH_2 Column	Flow rate- 0.6 mL/min	$GlcNH_2$, Glucosaminic Acid	34
Chitin	GlcNAc, $(GlcNAc)_{2-5}$	Biochemical conversion	CH_3CN/ water (3/7 *v/v*)	**HPLC-ELSD**	Cosmosil C18(250 mm x 4.6 mm I.D)	Flow rate- 0.5 mL/min	GlcNAc, $(GlcNAc)_{2-5}$	35
Chitin	GlcNAc, oligomers	Biochemical conversion	Data not available	**HPLC- VWD**	TSK gel amide column (250 mm x 4.6 mm I.D)	Wavelength- 200 nm	GlcNAc, oligomers	36

Chitin	GlcNAc	Mechanical and enzymatic	Water	**HPLC-RID**	Benson polymeric Pb^{2+} column (300 mm x 7.8 mm I.D)	Flow rate- 0.8 mL/min	GlcNAc	37
Chitin	GlcNAc and its oligomers	Biochemical	Data not available	**HPLC-RID**	KS-802 sugar column (250 mm x 4.6 mm I.D)		GlcNAc and its oligomers	38

N.A: not applicable, HADP: hydroxyethyl-2-amino-2-deoxyhexopyranoside, HAADP: hydroxyethyl-2-acetamido-2-deoxyhexopyranoside, DHB: 2,5-dihydroxybenzoic acid, 3A5AF: 3-acetamido-5-acetylfuran, LA: levulinic acid, AA: acetic acid, FA: formic Acid

Table 2.3. Summary on the HPLC methods demonstrated by column manufactures

Manufacturer	**Column used**	**Dimensions**	**Solvent**	**Detector**	**Observation**	**Remark**
Shodex	Shodex Asahipak NH2P-50 4E	4.6 mm I.D. x 250 mm	CH_3CN/H_2O (8/2 *v/v*)	ELSD	Separation of $GlcNH_2$ and GlcNAc is reported along with Galactosamine, Galactose, Glucose	No separation of 5-HMF and other sugars like fructose, xylose etc. is reported

Sielc Technologies	Primesep 100	4.6 mm I.D x 150 mm, 5 um	$CH_3CN/H_2O/H_2SO_4$ (0.8/9.2/0.003 *v/v*)	VWD (205 nm)	Analysis of $GlcNH_2$ is only shown	Separation of amino sugars, other sugars and 5-HMF are not shown.
Agilent Technologies	Agilent Hi-Plex H^+	7.7 mm I.D x 300 mm, 8 um	0.005 M H_2SO_4	RI	Detection of GlcNAc, 5-HMF, xylose, fructose, glucose is shown	Detection of $GlcNH_2$ is not shown
Phenomenex	Luna® 5 µm NH_2 100 Å	4.6 mm I.D x 250 mm, 5 um	CH_3CN/H_2O (8/2 *v/v*)	RI	Separation of xylose, fructose, glucose is shown	Separation of GlcNAc, $GlcNH_2$, 5-HMF is not shown
Phenomenex	Luna® 5 µm C18(2) 100 Å	4.6 mm I.D x 150 mm, 5 um	CH_3CN/H_2O with 0.1% H_3PO_4 (1/9 *v/v*)	VWD	Only detection of $GlcNH_2$ is shown	Separation with other amino sugars and sugars are not shown
Hamilton	RCX-10 anion exchange column	4.1 mm I.D x 250 mm	10 mN NaOH solution	RID	Separation of $GlcNH_2$ and GlcNAc is reported along with Galactosamine	No separation of 5-HMF and other sugars like fructose, xylose etc. is reported

Besides researchers working in this area, HPLC column manufacturing companies also report the use of several methods for the analysis of amino sugars and 5-HMF. The details on those are given in Table 2.3. As observed from Table 2.3, even the HPLC column manufacturers have not put much effort into separating chemicals obtained in chitin/chitosan chemistry. Most of the manufacturers have typically used amino or C18 columns except by Agilent and Hamilton; those have shown the possibility of using ion-exchange columns. Additionally, in sugar chemistry originated from plant biomass, few researchers have used C18, amino (-NH_2) and cyano (-CN) columns for the separation and quantification of products, but the main drawback of these columns is overlapping of peaks for few of sugar compounds. Thus, those may possibly give wrong results. From the above, it is clear that a detailed study on the separation of various chemicals that can form in chitin/chitosan chemistry is not done.

In view of the constraints observed to detect, separate and quantify products formed in the recently developing chitin/chitosan chemistry, this work aimed to investigate and optimize various HPLC parameters, which must be suitable for simultaneous separation and quantification of sugars, N-containing chemicals and furan derivatives. In this work, columns with varying properties such as polarity, length, stationary phase, and the mobile phase of different polarity and detectors were evaluated to separate these compounds better. A simple, rapid and reproducible method is developed, which can give simultaneous separation and quantification of the compounds formed from chitin and chitosan.

2.2. Experimental section

2.2.1. Materials and reagents

D-(+)-glucosamine ($GlcNH_2$·HCl, 99%) and dimer of D-(+)-glucosamine (99%) were purchased as pure hydrochloride salt from Sigma-Aldrich, USA. N-Acetyl-D-glucosamine (GlcNAc, 99%) 5-hydroxymethylfurfural (5-HMF, 99%), Levulinic

acid (98%), 2-Aminoethanol (>98%), N-Acetylethanolamine (technical grade), 2-Deoxy-D-glucose (99%) Acetamide (99%) were also purchased from Sigma-Aldrich, USA. D-Glucosaminic acid (98%) is procured from TCI chemicals. D-(+)-glucose (99%), D-(+)-fructose (99%), Formic acid (98%) and Glacial acetic acid were purchased from Loba chemie. HPLC grade acetonitrile, methanol and Millipore Milli-Q water (Millipore, Milford, USA) were used for the separation and sample preparations. HPLC grade acetonitrile and methanol were purchased from Merck. Methanol (AR grade), isopropyl alcohol (AR grade), ethyl acetate (AR grade), dichloromethane (AR grade), methyl-iso-butyl ketone (AR grade), acetone (AR grade), hexane (AR grade) are procured from Loba chemie and used for solubility study. Ethanol (99.9% AR) is procured from Analytical CSS reagent and used for solubility study.

2.2.2. Preparation of standards

2.2.2.1. Preparation of $GlcNH_2$.HCl, GlcNAc, 5-HMF

Standard solutions of all the chemicals used in this study were prepared in Milli-Q water. Standards were prepared by dissolving a known quantity of compounds in water. The details of prepared samples are summarized in Table 2.4a-2.4c. To avoid any degradation, these solutions were used immediately after their preparations or otherwise stored in the refrigerator at 4°C to avoid any degradation. For their further use, those were allowed to warm up to room temperature (32±3°C). All the samples were injected three times to check the accuracy and precision of results.

Table 2.4a. Summary on the standards prepared for $GlcNH_2$·HCl

Sr. no.	Sample code	Water taken (g)	Weight of $GlcNH_2$·HCl taken (mg)	$GlcNH_2$·HCl (*wt*%)	$GlcNH_2$·HCl concentration (mg/L)
1	GA-1	30.00±0.2	6.32±0.05	0.02105	212
2	GA-2	30.00±0.2	13.78±0.05	0.04590	459
3	GA-3	30.00±0.2	20.13±0.05	0.06707	672
4	GA-4	30.00±0.2	35.71±0.05	0.11919	1192

Table 2.4b. Summary on the standards prepared for GlcNAc

Sr. no.	Sample code	Water taken (g)	Weight of GlcNAc taken (mg)	GlcNAc (*wt*%)	GlcNAc concentration (mg/L)
1	NAG-1	30.00±0.2	5.55±0.05	0.01848	185
2	NAG-2	30.00±0.2	10.86±0.05	0.03627	363
3	NAG-3	30.00±0.2	16.68±0.05	0.05558	556
4	NAG-4	30.00±0.2	27.72±0.05	0.09225	926

Table 2.4c. Summary on the standards prepared for 5-HMF

Sr. no.	Sample code	Water taken (mL)	Weight of 5-HMF taken (mg)	5-HMF *(wt*%)	5-HMF Concentration (mg/L)
1	HMF-1	30.00±0.2	5.13±0.05	0.01735	174
2	HMF-2	30.00±0.2	8.76±0.05	0.02908	291
3	HMF-3	30.00±0.2	12.36±0.05	0.04113	411
4	HMF-4	30.00±0.2	21.9±0.05	0.07336	733

2.2.2.2. Preparation of standard mixture ($GlcNH_2$.HCl + GlcNAc + 5-HMF)

Standard mixture solution of $GlcNH_2$.HCl, GlcNAc and 5-HMF was prepared by dissolving 13.78 mg $GlcNH_2$.HCl, 10.86 mg GlcNAc and 8.76 mg 5-HMF in Milli-Q water (30.02 g). Standard was used immediately after its preparations or otherwise stored at 4°C in the refrigerator to avoid degradation. For their further use, those were allowed to warm up to room temperature (32±3 °C).

2.2.2.3. Preparation of other standards for checking the effectiveness of the developed method

Standard solutions of D-glucosaminic acid, Glucosamine Hydrochloride dimer, Acetic acid, Levulinic acid, Formic acid, 2-Aminoethanol, N-Acetylethanolamine, 2-Deoxy-D-glucose, D-(+)-glucose, D-(+)-fructose and Acetamide were prepared by dissolving 5.00±0.05 mg compound in 10.00±0.2g water. These solutions were used immediately after their preparations or otherwise stored at

4°C in the refrigerator or to avoid degradation. For their further use, those were allowed to warm up to room temperature (32±3 °C).

2.2.3. Solubility study

To develop optimum analytical conditions, the solubility of the standard compounds was checked in different solvents, e.g., water, methanol, ethanol, isopropyl alcohol, ethyl acetate, dichloromethane, methyl-iso-butyl ketone, acetone, hexane. In a typical solubility experiment, 50±0.05 mg ('X') compound was weighed and to it 30±0.02 g solvent was added at room temperature (32±3 °C). The resultant mixture was stirred for 4 h with 200 rpm to ensure enough time is given for the solubility of compounds in the solvents. Subsequently, solution was filtered through Whatman filter paper (grade no. 41). Filtrate was taken in a round bottom flask and solvent was evaporated using rotary-evaporator. Further, to remove the solvent completely, RB was evacuated at 50±3 °C under vacuum for 2 h. Solubility of compounds was calculated based on the weight of the compound observed in the RB ('Y'). Following formulae were used for the calculation.

$$\text{Solubility of compound (\%)} = \frac{\text{Initial weight of compound taken (X)} - \text{Weight of compound observed in RB (Y)}}{\text{Intial weight of compound taken (X)}} \times 100$$

Weight of compound observed in RB(Y) = (Weight of RB after removal of solvent - Weight of blank RB)

2.2.4. Thin layer chromatography (TLC)

TLC was carried out to understand the polarity trend of the compounds. Methanol/Dichloromethane (DCM) system was used as mobile phase in different *v/v* ratio to achieve satisfactory separation of compounds. Following formula was used for calculation retention factor of the compounds.

$$\text{Retention factor} = \frac{\text{Distance travelled by the compound from origin (mm)}}{\text{Distance travelled by the solvent from origin (mm)}}$$

2.2.5. HPLC equipment

For the work, two separate HPLC systems were used viz. Shimadzu LC-9A HPLC system equipped with variable wavelength detector (SPD-6A) and Agilent 1260 Infinity HPLC equipped with G1310 quaternary pump, G1367 sample autosampler, G1314 variable wavelength detector, G1362 refractive index detector and OpenLab software. For sample injection in Shimadzu make HPLC, Rheodyne manual injector 7725i equipped with 20 μL loop was used. For the sample injection in Agilent HPLC, autosampler G1367 with 10 μL sample injection volume was chosen.

2.2.6. Chromatographic conditions

For chromatographic separation, three different types of columns were used based on their packing and polarity. The details of different columns are summarized in Table 2.5.

Table 2.5. Details on the columns used in this study

Column	Dimensions (l x d in mm)/packing/particle size (μm)/pH range	Polarity and phase	Analysis conditions	Detector used
Agilent Zorbax C18	250 mm x 4.6 mm I.D/ODS/5/2-9	Non-polar and reverse	Column temp: 25 °C, Acetonitrile: Water (1:9 *v/v*), Flow rate: 0.5 mL/min	VWD
Agilent C18	100 mm x 4.6 mm I.D/ODS/5/2-9	Non-polar and reverse	Column temp: 25 °C, Acetonitrile: Water (1:9 *v/v*), Flow rate: 0.5 mL/min	VWD
Rezex Phenomenex	300 mm x 7.8 mm I.D/Sulfonated Styrene	Mid-polar and	Column temp: 80 °C, Water,	VWD, RID

Pb^{2+}	Divinyl Benzene/8/ Neutral	reverse	Flow rate: 0.4-0.7 mL/min	
Machery-Nagel Amino	250 mm x 4.6 mm I.D/Silica/5/2-8	Polar and normal	Column temp: 30 °C, Acetonitrile: Water (8:2 *v/v*), Flow rate: 0.5-1.0 mL/min	VWD, RID

As summarized in the Table 2.5, columns used for optimization were normal phase polar column, i.e, amino (250 mm x 4.6 mm i.d., 5.0 µm, Nucleosil, Macherey-Nagel), mid-polar ion exchange column, i.e., RPM-Monosaccharide Pb^{2+} (300 mm x 7.8 mm i.d., 8.0 µm, 8 % cross linking, Rezex™ Phenomenex), and non-polar reversed phase column C18 (250 mm x 4.6 mm i.d., 5.0 µm, Agilent Zorbax). The separation of investigated compounds was tested by changing the ratio of acetonitrile and methanol with water. Mobile phase was prepared by mixing acetonitrile/methanol and water with varying *v/v* ratio followed by sonication to remove air-bubbles and proper mixing of two solvents. Column temperature was also an important factor to achieve better separation and minimize the viscosity of mobile phase and column pressure, column temperature was set to 80°C while using Pb^{2+} column and 25°C while using C18 column. Flow rate of the pump was varied from 0.4-0.7 mL/min. Injection volume was kept at 10 uL for Agilent HPLC autosampler G1367 and 20 uL sample was manually injected using Rheodyne manual injector 7725i equipped with 20 uL loop in case of Shimadzu LC-9A HPLC system. Wavelength of variable wavelength detector (VWD) was selected after doing UV-Vis spectrophotometric experiment. Temperature of refractive index detector was kept at 40°C.[24]

2.2.7. Calibration curves

The standard solutions tabulated in Table 2.4a-2.4c were prepared and injected for the construction of calibration curves. This process was repeated thrice to

get reproducible results and then standard deviation was calculated. The calibration curves were plotted by the peak area versus concentration of each compound.

2.2.8. Repeatability and precision

Repeatability of the optimized method was evaluated by injecting same sample ($GlcNH_2$: 1192 mg/L, GlcNAc: 926 mg/L and 5-HMF: 733 mg/L) three times. Then percentage of relative standard deviation (%RSD) was calculated. The precision of injection was demonstrated by three replicative injections of the standard solutions. Measurements of retention time and peak areas were used to check the repeatability and reproducibility of the developed method.

2.3. Results and discussion

2.3.1. Understanding structural and chemical properties of the investigating compounds

Understanding their properties is very important before initializing the analytical method development studies for these compounds (Table 2.6a and Table 2.6b). Kinetic dimensions of these compounds were calculated using Chem3D software after energy minimization and the results are presented in Table 2.6a. Kinetic diameter of these compounds were also derived using theoretical formula using molecular weight (Eq. 1).[39]

$$\text{Kinetic diameter } (\sigma) = 1.234 \times (\text{Molecular weight})^{1/3} \quad \ldots\ldots (1)$$

Data on the size of these compounds clearly shows a minimal difference in the size of these molecules, which suggests that size-based separation (size exclusion or typical HPLC column) of these compounds is not possible.

Solubility data of these compounds also shows that those are completely soluble in water and are partially soluble in methanol. This indicates that all the compounds are polar in nature and thus only based on polarity those may not be able to separate from each other.

Table 2.6a. Properties of the $GlcNH_2$, GlcNAc and 5-HMF

Properties	$GlcNH_2$	GlcNAc	5-HMF
Width (Å) (from Chem 3D)	5.039	5.341	3.179
Length (Å) (from Chem 3D)	7.139	8.388	7.321
Kinetic diameter (Å) (from M.W)	6.954	7.460	6.186
M.W (g/mol)	179	221	126
Melting Point (°C)	150	211	34
Boiling point (°C)	-	-	115
Degradation temperature (°C)	190	230	-
Solubility (%)	Water (100) Methanol (35)	Water (100) Methanol (44)	Water (100) Methanol (95)

Table 2.6b. Properties of the different chitin/chitosan derived compounds

Compound name	Width (Å) (from Chem 3D)	Length (Å) (from Chem 3D)	Kinetic diameter (Å) (from M.W)	M.W (g/mol)	M.P (°C)	B.P (°C)
2-aminoethanol	3.113	5.252	4.859	61.08	10	170
N-acetylethanolamine	3.732	7.210	5.787	103.12	16	136
Acetic acid	3.318	3.149	4.832	60.05	16	118
D-Glucosaminic acid	4.277	8.623	7.158	195.17	235	633
Glucosamine hydrocholaride dimer	8.968	10.482	8.613	340	-	-
Levulinic acid	3.087	6.651	6.020	116.12	34	245
Formic acid	2.011	2.968	4.422	46.03	8	101
2-deoxy-D-	4.935	7.393	6.757	164.16	147	-

glucose						
D (+)-Glucose	3.624	7.540	6.969	180.16	133	-
D (+)-Fructose	4.367	8.527	6.969	180.16	103	-
Acetamide	3.128	4.218	4.806	59.07	80	221

Since both, GlcNAc and $GlcNH_2$ does not have clear boiling points and degrade very close to their melting points, those will be impossible to analyse using GC as in this technique typically higher temperatures (injector, oven and detector) are used to vaporize the samples. Moreover, their restricted solubility in organic solvents restricts their injection in GC as with water as a solvent (in which 100% solubility is observed), FID cannot be used for their detection. In view of these restrictions, it would be essential to find out a technique, which gives fast results and thus, HPLC seems to be the most suitable technique.[24]

Properties of other N-containing chemicals and furan derivatives in chitin/chitosan chemistry were also tabulated in Table 2.6b. Sugar derivatives like D-(+)-Glucose, D-(+)-Fructose, 2-deoxy-D-glucose, Glucosamine hydrochloride dimer do not have boiling points, thus they will degrade and are impossible to analyse using GC technique. D-Glucosaminic acid has very high boiling point and hence GC will not be a good method to analyse all these compounds which are derived from chitin and chitosan.

Polarity trend of main three compounds, $GlcNH_2$, GlcNAc and 5-HMF is understood from TLC experiment (Figure 2.2). As amino sugars are only soluble in methanol solvent (at least partially), methanol/DCM solvent system was used as a running solvent.

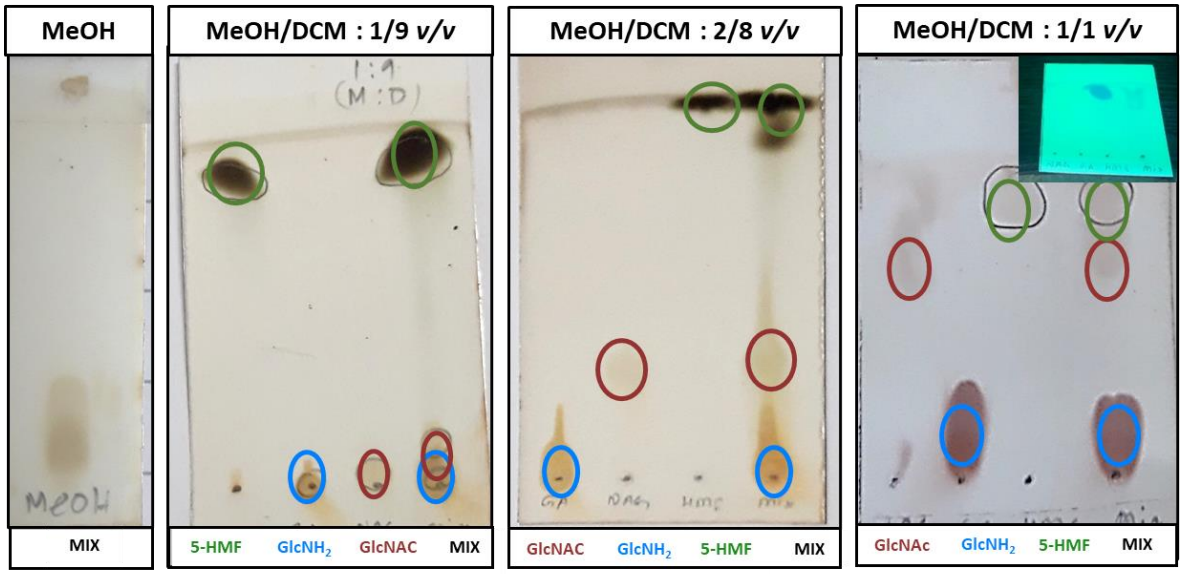


Figure 2.2. TLC experiment

TLC plate of pure methanol shows that compounds were not moving from the origin. For changing the polarity of methanol, DCM was added. The ratio of methanol/DCM was varied from 1/9 *v/v* to 1/1 *v/v* to check under which solvent system best separation of these compounds is possible. 5-HMF is found to be least polar as the spot of 5-HMF runs along with the solvent system. As the polarity of solvent system was increased by the addition of methanol, the spot of GlcNAc got separated from $GlcNH_2$. From the TLC plate, it is understood that $GlcNH_2$ is most polar, GlcNAc is mid-polar and 5-HMF is least polar. Retention factor of each compound in different solvent systems were tabulated in Table 2.7.

Table 2.7. Retention factor (R_f) of the compounds

Solvent system	$GlcNH_2$.HCl	GlcNAc	5-HMF
MeOH	0	0	0
MeOH/DCM (1/9 *v/v*)	0	0.106	0.957
MeOH/DCM (2/8 *v/v*)	0	0.286	0.971
MeOH/DCM (1/1 *v/v*)	0.114	0.628	0.8

2.3.2. HPLC method development

2.3.2.1. Wavelength selection for VWD and validation for separating compounds

The mixture of $GlcNH_2$, GlcNAc and 5-HMF (sample details given in section 2.2.2.2) was first injected in HPLC using previously reported method for analysing 5-HMF {C18 column (250 mm x 4.6 mm, 5.0 um, Agilent Zorbax) and methanol/water: 2.3/7.7 *v/v* ratio, VWD wavelength was kept at 284 nm}.[20] The chromatogram for the analysis is shown in Figure 2.3. As seen, only one peak for 5-HMF, confirmed by injecting standard for 5-HMF was observed in the chromatogram. The absence of peaks for $GlcNH_2$ and GlcNAc showed that this method may not be suitable for the analysis of all the compounds.

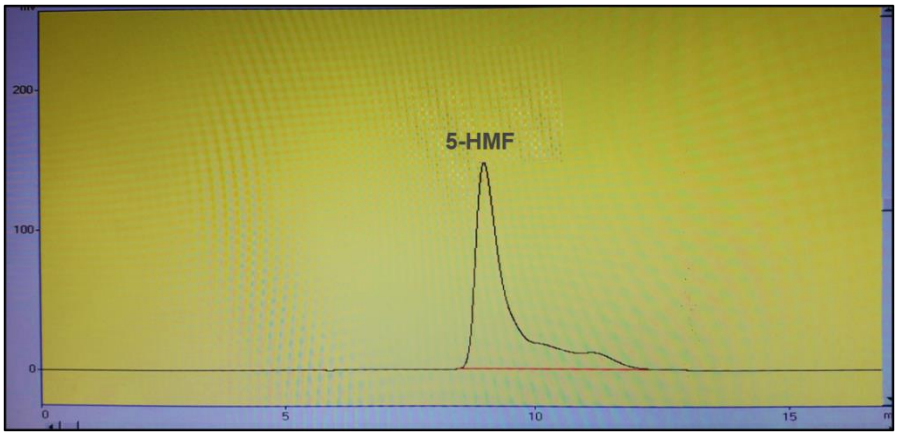


Figure 2.3. Chromatogram for Mixture ($GlcNH_2$+ GlcNAc+5-HMF) at 284 nm wavelength of VWD

Analysis condition: Column; C18 (250 mm x 4.6 mm i.d., 5.0 μm, Agilent Zorbax), mobile phase; methanol/water, 2.3/7.7 *v/v*, column temperature; 25°C, Flow rate; 0.5 mL/min, VWD wavelength; 284 nm

To understand the reason behind absence of peaks for $GlcNH_2$ and GlcNAc, UV-Visible spectra of these three compounds were recorded to find out the λ_{max} for all the compounds (Figure 2.4).

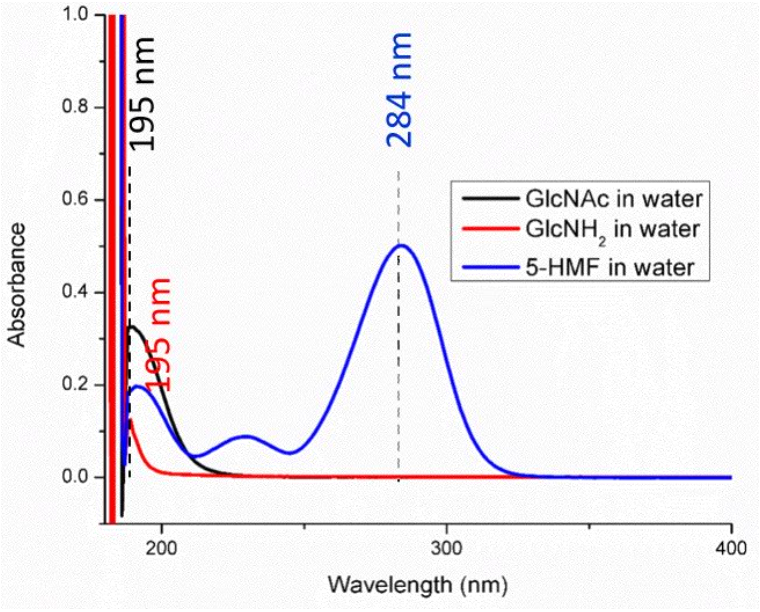


Figure 2.4. UV spectra for investigating compounds

UV-Visible spectra of these compounds clearly shows that $GlcNH_2$ and GlcNAc have λ_{max} at 195 nm and 5-HMF has λ_{max} at 284 nm. As lower wavelength corresponds to higher energy, more energy is required to excite the electrons in case of amino sugars, so, they are not detected at higher wavelength, i.e., 284 nm. Then, the detector wavelength was set at 195 nm keeping other conditions same as previous and three peaks were found in the chromatogram (shown in Figure 2.5). Peaks were identified by injecting separate standard samples. Energy associated with lower wavelength at 195 nm is able to excite electrons for all the compounds. In an another report, formation of 5-HMF from chitosan was analyzed using VWD as a detector with fixed wavelength at 283 nm and hence compounds which are not UV active at this wavelength can be missed [25]. Recently, formation of $GlcNH_2$ (calorimetric) and 5-HMF (HPLC) from chitosan using two separate methods is reported, which eventually increases the time required for the analysis [19]. In the work, VWD wavelength was fixed at 283 nm, which would hamper detection of $GlcNH_2$, if formed in the reaction. The colorimetric method is also a specific method to quantify total reducing sugars,

which cannot quantify 5-HMF. Thus, it is essential to use correct wavelength to detect all the compounds in the sample.

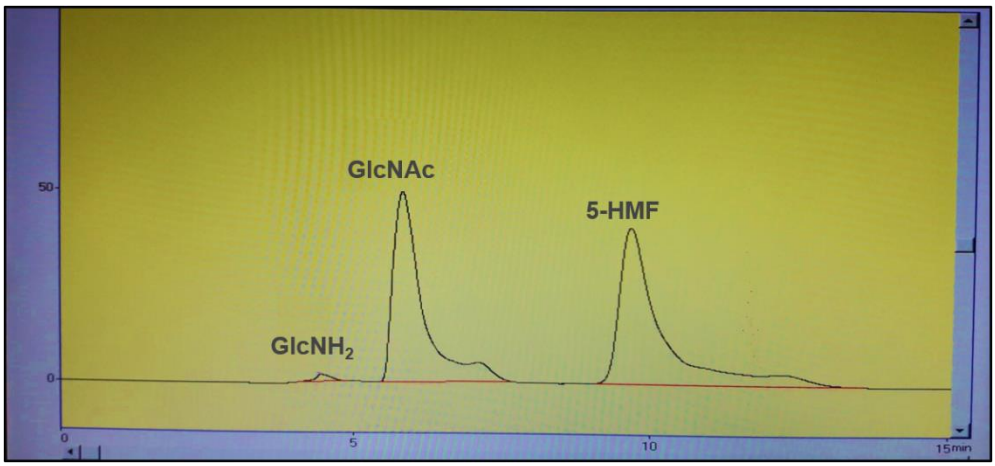


Figure 2.5. Chromatogram for Mixture ($GlcNH_2$+ GlcNAc+5-HMF) at 195 nm wavelength of VWD

Analysis condition: Column; C18 (250 mm x 4.6 mm i.d., 5.0 μm, Agilent Zorbax), mobile phase; methanol/water, 2.3/7.7 v/v, column temperature; 25°C, Flow rate; 0.5 mL/min, VWD wavelength; 195 nm

2.3.2.2. Separation in non-polar C18 column

2.3.2.2.1. Selection of mobile phase

Previously researchers have used methanol/water systems for the analysis of reaction mixtures and hence they required to keep the VWD above 205 nm wavelength since methanol has a UV cut-off at 205 nm [19-20, 23]. From the literature, it was found that every solvent has their specific UV cut off. UV cut off value of methanol is 205 nm.[40] If measurement wavelength is less than this cut off value, then mobile phase also has some contribution in absorbance. As detector wavelength must be kept at 195 nm to detect all the compounds, methanol has to be replaced by another solvent which has less UV cut off than 195 nm. Acetonitrile was found to have less UV cut off, i.e., <190 nm. Then acetonitrile/water-2.3/7.7 *v/v* was used as mobile phase keeping column and

VWD wavelength same as section 3.2.1. The chromatogram (Figure 2.6) shows clear separation for all the compounds in acetonitrile/water- 2.3/7.7 *v/v*.

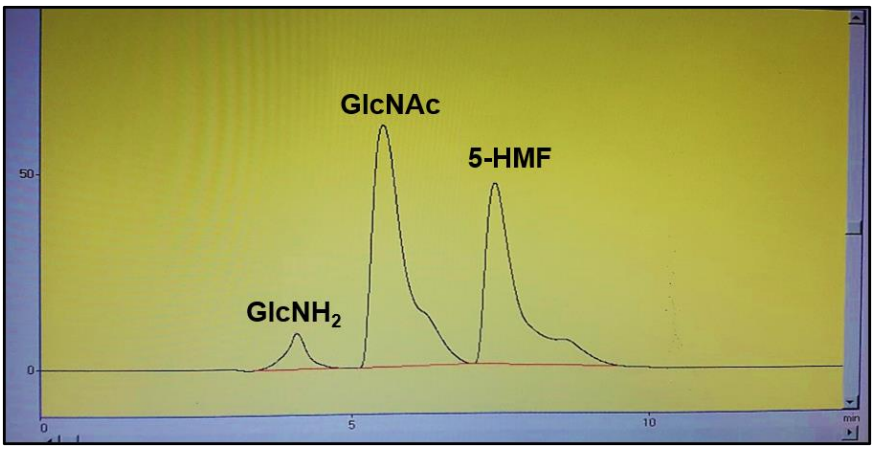


Figure 2.6. Chromatogram for Mixture ($GlcNH_2$+ GlcNAc+5-HMF) in acetonitrile/water: 2.3/7.7 *v/v* mobile phase

Analysis condition: Column; C18 (250 mm x 4.6 mm i.d., 5.0 μm, Agilent Zorbax), mobile phase; acetonitrile/water, 2.3/7.7 v/v, column temperature; 25°C, Flow rate; 0.5 mL/min, VWD wavelength; 195 nm

2.3.2.2.2. Effect of acetonitrile and water ratio

Acetonitrile/water (2.3/7.7 *v/v*) mobile phase system shows less difference in retention time of GlcNAc and 5-HMF. Polarity of the mobile phase system was then altered by addition of more polar solvent i.e., water. Ratio of acetonitrile/water was varied from 1/1 *v/v* to 1/9 *v/v* and the separation profiles are shown in Figures 2.7a-2.7c. It was observed that as the polarity of the mobile phase increased by the addition of water, separation of the compounds is better. As, 5-HMF is least polar in nature, 5-HMF showed strong interaction with non-polar stationary phase. In case of least polar solvent system, i.e., acetonitrile/water (1/1 *v/v*), 5-HMF eluted fast. Because of this, peaks of GlcNAc and 5-HMF were observed to be merging with each other. Acetonitrile/water (1/9 *v/v*) mobile phase system shows best separation of three compounds.

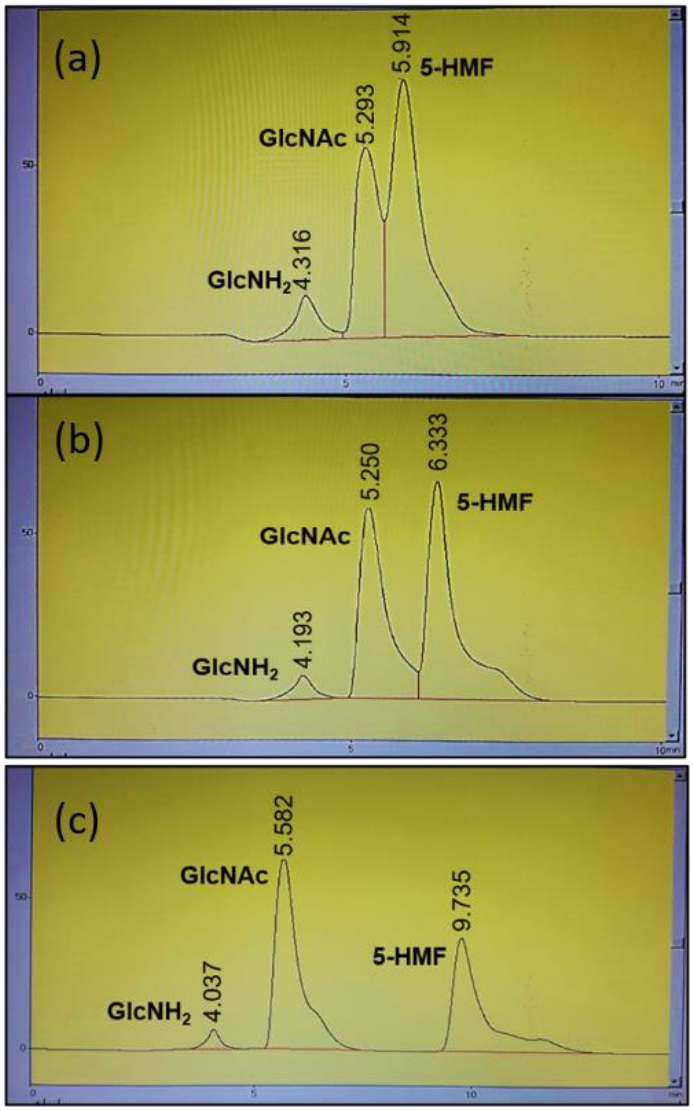


Figure 2.7. Chromatogram for Mixture ($GlcNH_2$+ GlcNAc+5-HMF) in acetonitrile/water

Analysis condition: Column; C18 (250 mm x 4.6 mm i.d., 5.0 μm, Agilent Zorbax), column temperature; 25°C, Flow rate; 0.5 mL/min, VWD wavelength; 195 nm mobile phase; (a) acetonitrile/water 1/1 *v/v*, (b) acetonitrile/water: 3/7 *v/v*, (c) acetonitrile/water: 1/9 *v/v*

2.3.2.2.3. Effect of column length

To reduce the analysis time, separation of the compounds was checked in shorter column. C18 column of 100 mm with 4.6 mm i.d., 5 um particle size was used for the purpose. But with the decrease of column length, compounds had

shorter time to interact with stationary phase and that resulted in the merging of peaks of $GlcNH_2$ and GlcNAc (Figure 2.8). These results imply that for a better separation, longer columns are better.

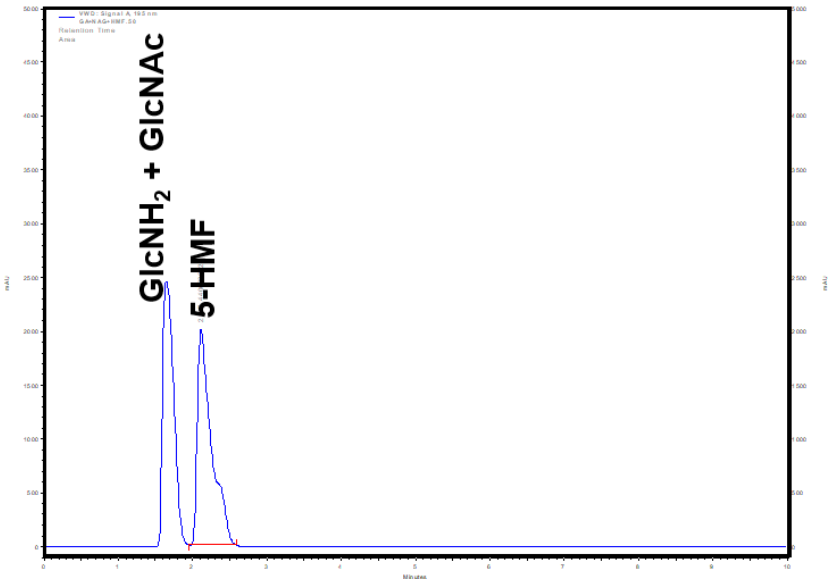


Figure 2.8. Chromatogram for Mixture ($GlcNH_2$+ GlcNAc+5-HMF) in shorter column

Analysis condition: Column; C18 (100 mm x 4.6 mm i.d., 5.0 μm, Agilent Zorbax), mobile phase; acetonitrile/water, 2.3/7.7 v/v, column temperature; 25°C, Flow rate; 0.5 mL/min, VWD wavelength; 195 nm

2.3.2.2.4. Separation of other sugars, N-containing chemicals, furan derivatives in C18 column

Detection and separation of glucose and fructose were also checked using optimized HPLC condition, i.e., C18 column (250 mm x 4.6 mm i.d., 5.0 um, Agilent Zorbax), Mobile phase: acetonitrile/water-1/9 *v/v*, column temperature: 25°C, Flow rate: 0.5 mL/min, VWD wavelength: 195 nm. There is very small difference in the retention times of glucose, fructose, GlcNAc and acetic acid. Peaks of glucosaminic acid, $GlcNH_2$ and glucosamine hydrochloride dimer are also merging with each other under this analytical condition. The difference in retention time of N-acetylethanolamine and 2-deoxy-D-glucose is also very less

(Figure 2.9). So, it is very obvious that this method will be unable to separate these compounds.

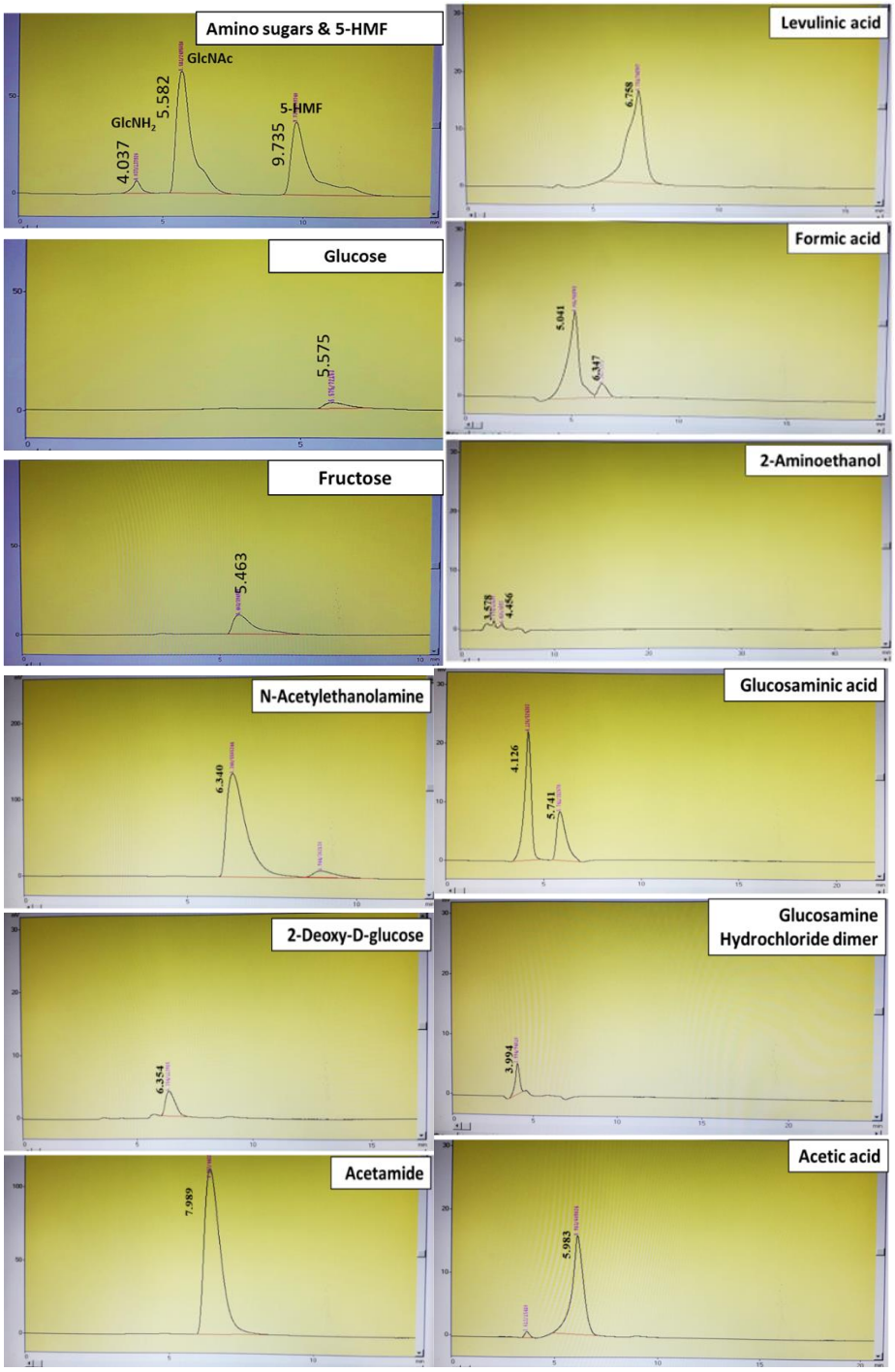


Figure 2.9. Chromatograms of chitin/chitosan derived compounds in C18 column

Analysis condition: Column; C18 (250 mm x 4.6 mm i.d., 5.0 μm, Agilent Zorbax), mobile phase; acetonitrile/water, 2.3/7.7 v/v, column temperature; 25°C, Flow rate; 0.5 mL/min, VWD wavelength; 195 nm

2.3.2.3. Separation in polar amino column

It was thought that polar stationary phase can give better separation as polar compounds will have strong interactions with polar stationary phase. In view of this, polar amino column was used for potential separation of the investigating compounds. The chromatogram (Figure 2.10a) shows that polar compounds have very strong interaction with the stationary phase of the column, leading to peak tailing. Nevertheless, these compounds retained on the stationary phase strongly that peak tailing was observed even when flow rate was increased from 0.5 mL/min to 1.0 mL/min (Figure 2.10b) with an increment of 0.1 mL/min. Further increase in flow rate would merge the peaks with each other as with 1.0 mL/min flow too peaks were not resolved completely (as observed from the baseline) and hence, amino column is not a good choice for separating amino sugars.

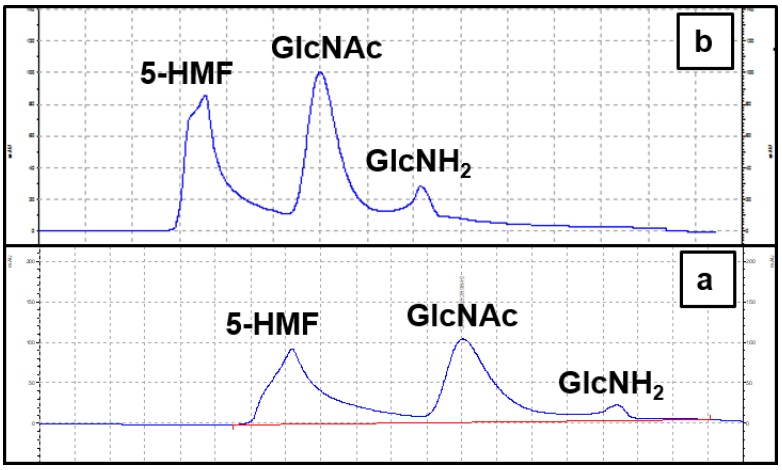


Figure 2.10. Chromatogram for mixture ($GlcNH_2$+ GlcNAc+5-HMF) in amino column

Analysis conditions: Column; Amino (250 mm x 4.6 mm i.d, 5.0 µm, Nucleosil, Macherey-Nagel), Mobile phase; acetonitrile/water, 8/2 *v/v*, column temperature; 25°C, VWD wavelength; 195 nm, Flow rate; (a) 0.5 mL/min, (b) 1.0 mL/min

Separation of other sugars and chitin/chitosan derived various products were checked which is shown in Figure 2.11. It was found that peak of Glucosaminic acid, acetamide and 5-HMF is merging with each other under employed conditions. Peak of fructose and GlcNAc were also merged with each other.

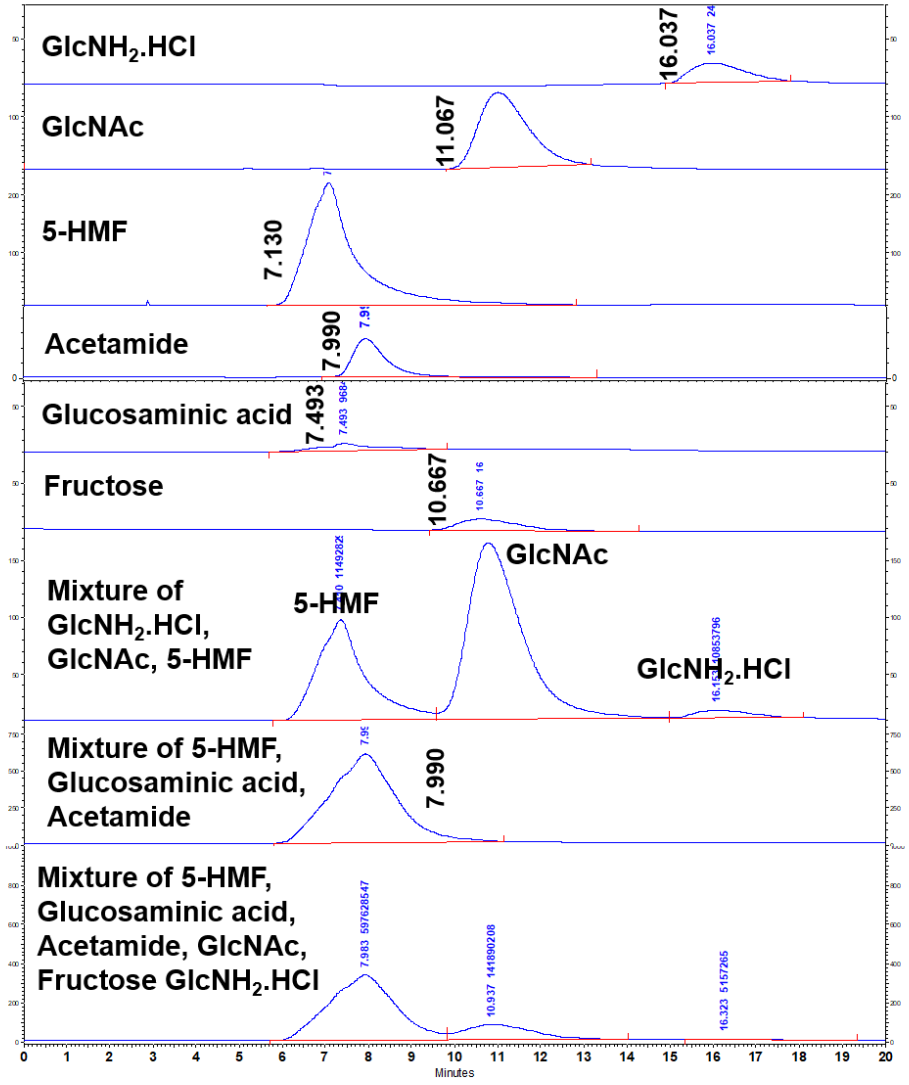


Figure 2.11. Chromatograms of other chitin/chitosan derived compounds and sugar compounds

Analysis conditions: Column; Amino (250 mm x 4.6 mm i.d, 5.0 μm, Nucleosil, Macherey-Nagel), Mobile phase; acetonitrile/water, 8/2 *v/v*, column temperature; 25°C, VWD wavelength; 195 nm, Flow rate; (a) 0.5 mL/min

In view of peak merging and peak tailing in amino column, it is considered that this column is not suitable for separating these compounds.

2.3.2.4. Separation in mid-polar ion-exchange column

Ion-exchange column, monosaccharide Pb^{2+} column has mid-polarity as the matrix of the column is a sulfonated styrene divinyl benzene polymer. It is a cation exchange column with Pb^{2+} ions. Typically for separation of compounds on this column, water is used as mobile phase and in our study 0.5 mL/min flow was observed as best flow to achieve better separation. In this work, column temperature was kept at 80 °C (which is also recommended by manufacturers as optimum for separation of various sugar compounds) to minimize the viscosity of the mobile phase and column pressure. Mixture of $GlcNH_2$, GlcNAc and 5-HMF was injected and good separation was observed as seen in Figure 2.12.

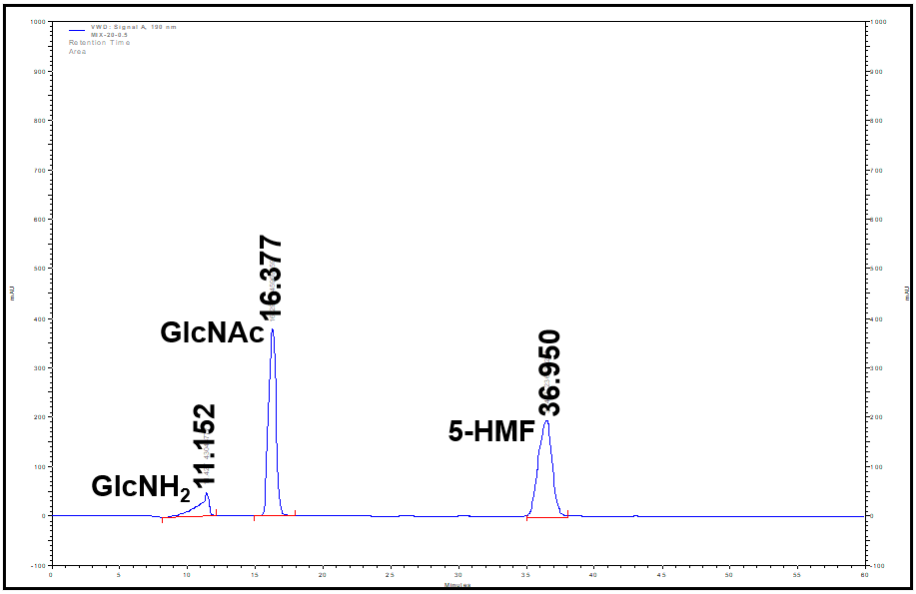


Figure 2.12. Chromatogram for mixture ($GlcNH_2$+ GlcNAc+5-HMF)
Analysis condition: Column; RPM-Monosaccharide Pb^{2+} (300 mm x 7.8 mm i.d, 8.0 µm, 8 % cross linking, Rezex™ Phenomenex), Mobile phase; water, column temperature; 80°C, Flow rate; 0.5 mL/min, VWD wavelength; 195 nm

With the use of this column, better separation of $GlcNH_2$, GlcNAc and 5-HMF was observed in comparison to other columns used. Also except for $GlcNH_2$, sharp peaks were observed for 5-HMF and GlcNAc. Subsequent to this observation, other sugars such as glucose and fructose were injected and clear separation of all the compounds was seen with the analytical conditions employed (Figure 2.13). Since the packing material in this column has mid-polarity, it has played a key role in the optimized interaction of different compounds with the phase, which has yielded clear separation of the chemicals.

Since, separation of typical sugars and monomers of chitin/chitosan was better on this column, evaluation of separation of other chitin and chitosan derived compounds was also checked and the chromatograms are illustrated in Figure 2.13.

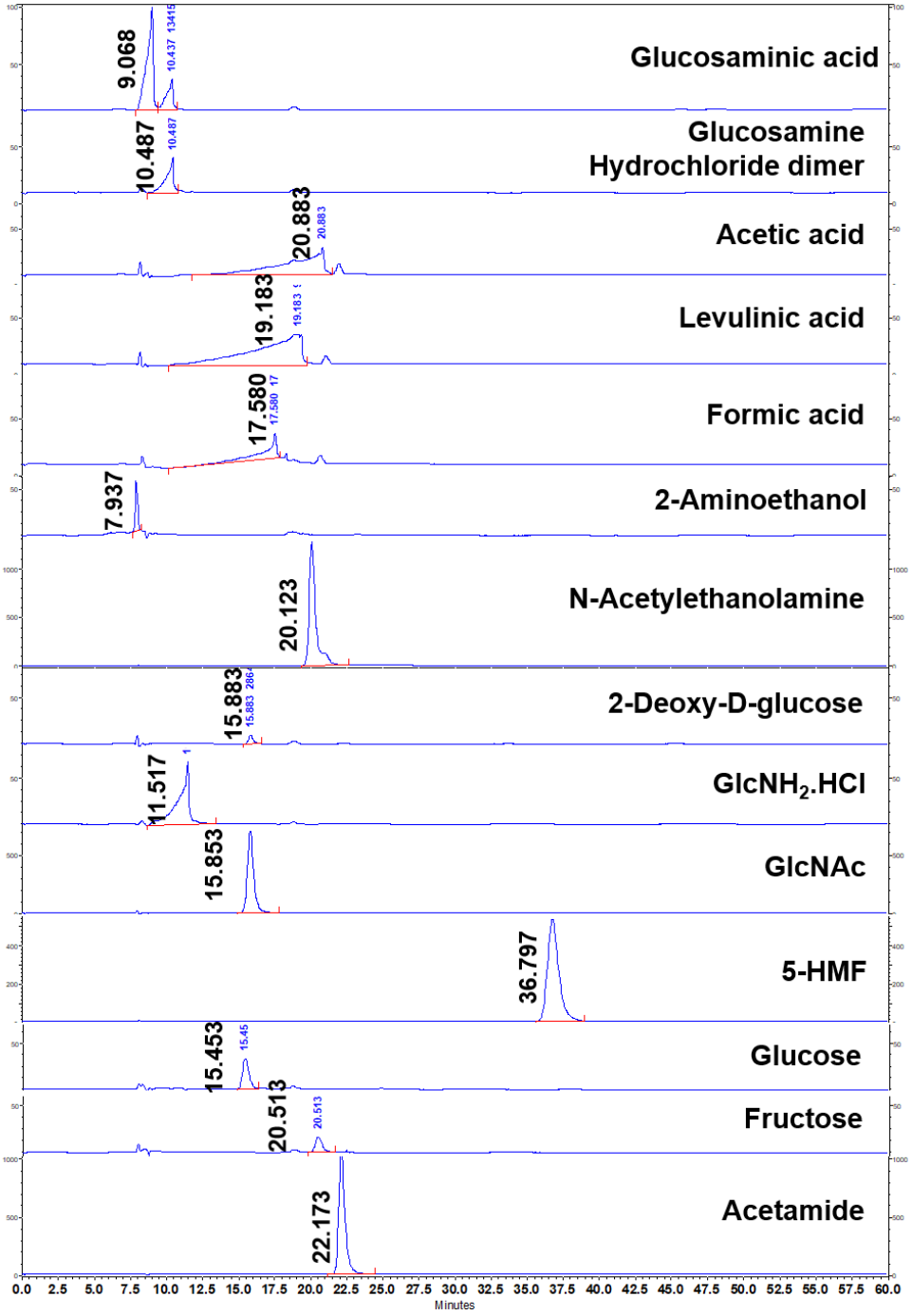


Figure 2.13. Chromatogram for various chitin derived N-containing chemicals, amino sugars, other sugars and furan derivatives

Analysis condition: Column; RPM-Monosaccharide Pb^{2+} (300 mm x 7.8 mm i.d, 8.0 µm, 8 % cross linking, Rezex™ Phenomenex), Mobile phase; water, column temperature; 80°C, Flow rate; 0.5 mL/min, VWD wavelength; 195 nm

As seen from Figure 2.13, within 39 min run time, separation of all compounds can be completed and quantification can be done by using calibration curve. Nevertheless, it is seen that few of the peaks can overlap in actual analysis but this is the best possible separation achievable using this column and also best with any other system. In order to reduce the time of analysis, flow rate of mobile phase was increased from 0.5 mL/min to 0.7 mL/min (Figure 2.14). The analysis could be completed within 30 min with sharp peaks for the compounds if the flow rate is kept at 0.7 mL/min. Although this result is good but with increase in flow, separation of other compounds can hamper as some peaks may overlap. Higher flow rate also creates high column pressure, which is detrimental for this column as maximum pressure for this column is 1000 psi.

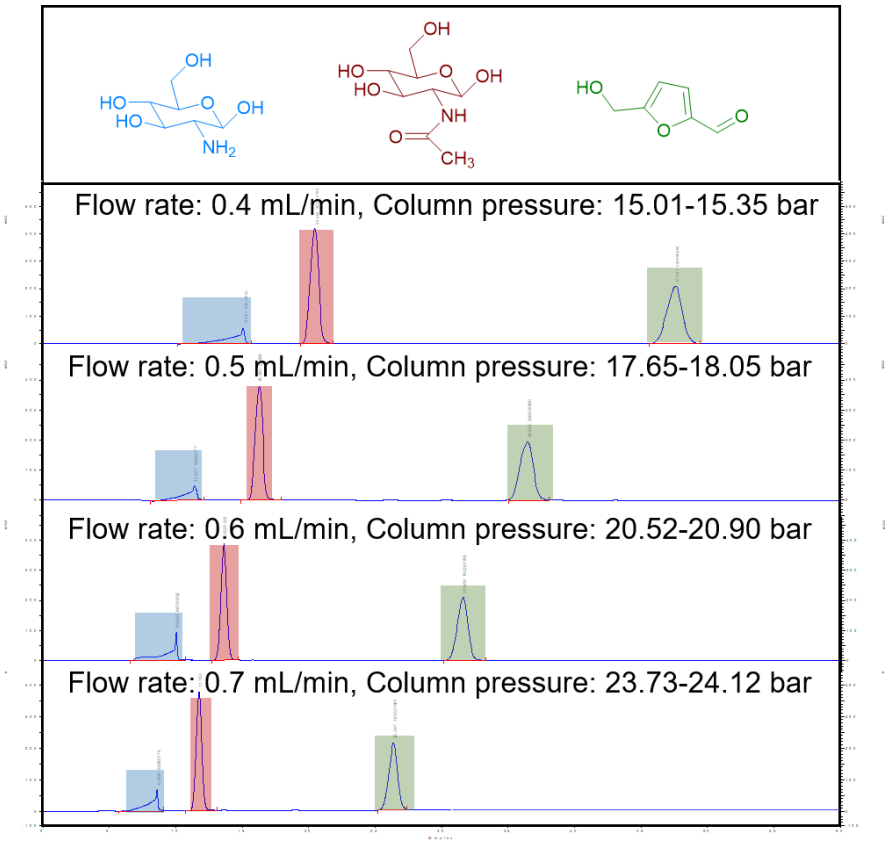


Figure 2.14. Chromatogram for mixture in different flow rates

Analysis condition: Column; RPM-Monosaccharide Pb^{2+} (300 mm x 7.8 mm i.d, 8.0 µm, 8 % cross linking, Rezex™ Phenomenex), Mobile phase; water, column temperature; 80°C, Flow rate; 0.5 mL/min, VWD wavelength; 195 nm

2.3.3. Method Validation

The reliability of the method in terms of precision was evaluated by injecting 4 samples for each compound. All calibration curves showed good linear regression. R^2 values and regression equation is tabulated in Table 2.8.

Day-to-day variations of retention times were determined on three days using the same standard sample mixture and it was observed that results are satisfactory (Table 2.9). Therefore, the developed HPLC method is precise, accurate and sensitive enough for simultaneous separation and quantification of $GlcNH_2$, GlcNAc, 5-HMF and other compounds.

Repeatability of the optimized method is evaluated by analysing the same sample (GlcNH2: 1190 mg/L, GlcNAc: 920 mg/L and 5-HMF: 700 mg/L) four times. Peak areas of the four replicated injection fall in the same range, proving that the results are reproducible. %RSD is also calculated and tabulated in Table 2.10. Low value of %RSD also confirms that the method can quantify precisely.

Data for another detector, i.e., RID was also checked and same trend was followed like VWD. (Tables 2.8-2.10)

Table 2.8. Regression equation, R^2 value for $GlcNH_2$, GlcNAc, 5-HMF

	VWD			RID		
Standard compound	**Regression equation**	**R^2**	**Linear range (mg/L)**	**Regression equation**	**R^2**	**Linear range (mg/L)**
$GlcNH_2$	28059x	0.9904	0-1190	5469.2x	0.9982	0-1190
GlcNAc	502651x	0.9974	0-920	17205x	0.9907	0-920
5-HMF	571659x	0.9952	0-700	14490x	0.9941	0-700

Table 2.9. Retention times obtained for $GlcNH_2$, GlcNAc and 5-HMF analysis under optimized conditions for 3 days

Standard compound	Concentration (mg/L)	VWD			RID		
		Retention time (min)			Retention time (min)		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
$GlcNH_2$	1190	11.15	11.21	11.15	11.42	11.40	11.42
GlcNAc	920	16.38	16.36	16.37	16.65	16.63	16.65
5-HMF	700	36.95	36.95	36.95	37.22	37.22	37.22

Table 2.10. Repeatability test

Standard compound	$GlcNH_2$-1190 mg/L		GlcNAc- 920 mg/L		5-HMF- 700 mg/L	
	VWD	RID	VWD	RID	VWD	RID
Area of injection 1	33879745	6609407	462838950	12930598	408592560	12358591
Area of injection 2	33879289	6610025	462865982	12925459	408542659	12355548
Area of injection 3	33878995	6609856	463001236	12932658	408356982	12362589
Area of injection 4	33880020	6609265	462829248	12925149	408600258	12360241
%RSD	0.0014	0.0055	0.0172	0.0290	0.0278	0.0239

2.3.4. Lead leaching

According to organometallic chemistry, lead has a tendency to form chelate complexes with nitrogen containing ligands and thus it becomes essential to check whether it is possible to use Pb^{2+} column for this analysis. To check the leaching of lead, Mixture of $GlcNH_2$ and GlcNAc was injected 72 times on the column. Elution of these two compounds was completed within 20 min. At the end of the analysis, HPLC waste was collected and ICP-OES was done to check the presence of lead. This experiment is done for two mixture samples.

a. Dilute sample (0.031 *wt*% of amino sugars)

b. Concentrated sample (0.30 *wt*% of amino sugars)

For dilute sample amount of lead was not detected but concentrated sample showed 0.1152 ppm leaching of lead at the end of 72 samples. This shows that although amino groups can bind with Pb^{2+}, no much change of Pb^{2+} concentration was seen on the column and that the reproducibility of the results was achieved.

2.4. Conclusion

To assist researchers working in the area of chitin and chitosan chemistry, it was necessary to develop new HPLC methodology for separation of compounds. From the study, it was observed that Non-polar C18 and mid-polar cation exchange Pb^{2+} columns are best suitable for separation of $GlcNH_2$, GlcNAc, other N-containing chemicals and furan derivatives. Non-polar C18 column although showed better separation than NH_2 column but was unable to separate other sugars like glucose, fructose etc. from each other and other chemicals injected. On the other hand, Pb^{2+} column could separate most of the compounds from each other because it possesses optimum polarity to allow rapid adsorption-desorption of the compounds. This developed HPLC method was demonstrated to be suitable to separate and quantify $GlcNH_2$, GlcNAc and 5-HMF simultaneously within 39 min. The regression equation showed very good linear relationship with R^2= 0.9904-0.9974. Therefore, the developed HPLC method is simple, rapid, accurate, precise, and specific and has the ability to separate and quantify amino sugars ($GlcNH_2$, GlcNAc) and 5-HMF simultaneously.

2.5. References

1. Bhaumik, P.; Dhepe, P. L., Solid acid catalyzed synthesis of furans from carbohydrates. *Catalysis Reviews* **2016,** *58* (1), 36-112.

2. Deepa, A. K.; Dhepe, P. L., Lignin depolymerization into aromatic monomers over solid acid catalysts. *ACS Catalysis* **2015,** *5* (1), 365-379.

3. Dhepe, P. L.; Fukuoka, A., Cellulose conversion under heterogeneous catalysis. *ChemSusChem: Chemistry & Sustainability Energy & Materials* **2008,** *1* (12), 969-975.

4. Lin, C. S. K.; Pfaltzgraff, L. A.; Herrero-Davila, L.; Mubofu, E. B.; Abderrahim, S.; Clark, J. H.; Koutinas, A. A.; Kopsahelis, N.; Stamatelatou, K.; Dickson, F., Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. *Energy & Environmental Science* **2013,** *6* (2), 426-464.

5. Pfaltzgraff, L. A.; Cooper, E. C.; Budarin, V.; Clark, J. H., Food waste biomass: a resource for high-value chemicals. *Green Chemistry* **2013,** *15* (2), 307-314.

6. Kerton, F. M.; Liu, Y.; Omari, K. W.; Hawboldt, K., Green chemistry and the ocean-based biorefinery. *Green Chemistry* **2013,** *15* (4), 860-871.

7. Yan, N.; Chen, X., Sustainability: Don't waste seafood waste. *Nature News* **2015,** *524* (7564), 155.

8. Chen, X.; Yang, H.; Yan, N., Shell biorefinery: dream or reality? *Chemistry–A European Journal* **2016,** *22* (38), 13402-13421.

9. Jardine, A.; Sayed, S., Challenges in the valorisation of chitinous biomass within the biorefinery concept. *Current Opinion in Green and Sustainable Chemistry* **2016,** *2*, 34-39.

10. Nivethaa, E.; Martin, C. A.; Frank-Kamenetskaya, O. V.; Kalkura, S. N., Chitosan and chitosan based nanocomposites for applications as a drug delivery carrier: A review. *Processes and Phenomena on the Boundary Between Biogenic and Abiogenic Nature* **2020**, 23-37.

11. Elwakeel, K. Z., Environmental application of chitosan resins for the treatment of water and wastewater: a review. *Journal of dispersion science and technology* **2010,** *31* (3), 273-288.

12. Katiyar, D.; Hemantaranjan, A.; Singh, B., Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review. *Indian Journal of Plant Physiology* **2015,** *20* (1), 1-9.

13. El Kadib, A., Chitosan as a sustainable organocatalyst: a concise overview. *ChemSusChem* **2015,** *8* (2), 217-244.

14. Kim, S.-K., *Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications*. CRC Press: 2010.

15. Chen, X.; Chew, S. L.; Kerton, F. M.; Yan, N., Direct conversion of chitin into a N-containing furan derivative. *Green Chemistry* **2014,** *16* (4), 2204-2212.

16. Matsagar, B.; Munshi, M.; Kelkar, A.; Dhepe, P., Conversion of concentrated sugar solutions into 5-hydroxymethyl furfural and furfural using Brönsted acidic ionic liquids. *Catalysis Science & Technology* **2015,** *5* (12), 5086-5090.

17. Tzeng, T.-W.; Bhaumik, P.; Chung, P.-W., Understanding the production of 5-hydroxymethylfurfural (HMF) from chitosan using solid acids. *Molecular Catalysis* **2019,** *479*, 110627.

18. Zhang, J.; Yan, N., Production of Glucosamine from Chitin by Co-solvent Promoted Hydrolysis and Deacetylation. *ChemCatChem* **2017,** *9* (14), 2790-2796.

19. Pandit, A.; Deshpande, C.; Patil, S.; Jain, R.; Dandekar, P., Mechanistic insights into controlled depolymerization of Chitosan using H-Mordenite. *Carbohydrate polymers* **2020,** *230*, 115600.

20. Yu, S.; Zang, H.; Chen, S.; Jiang, Y.; Yan, B.; Cheng, B., Efficient conversion of chitin biomass into 5-hydroxymethylfurfural over metal salts catalysts in dimethyl sulfoxide-water mixture under hydrothermal conditions. *Polymer Degradation and Stability* **2016,** *134*, 105-114.

21. Erb, C.; Zerban, F., Determination of total reducing sugars and of dextrose and levulose in cane molasses. *Industrial & Engineering Chemistry Analytical Edition* **1938,** *10* (5), 246-250.

22. Garriga, M.; Almaraz, M.; Marchiaro, A., Actas de Ingeniería. *Actas de ingeniería* **2017,** *3*, 173-179.

23. Li, M.; Zang, H.; Feng, J.; Yan, Q.; Yu, N.; Shi, X.; Cheng, B., Efficient conversion of chitosan into 5-hydroxymethylfurfural via hydrothermal synthesis in ionic liquids aqueous solution. *Polymer Degradation and Stability* **2015,** *121*, 331-339.

24. Ghosh, N.; Dhepe, P. L., HPLC method development for chitin and chitosan valorisation chemistry. *Carbohydrate Polymer Technologies and Applications* **2021,** *2*, 100139.

25. Kalane, N. D.; Krishnan, R. A.; Yadav, V. D.; Jain, R.; Dandekar, P., Synergistic effect of hetero-and homo-catalysts on the 'green'synthesis of 5-hydroxymethylfurfural from chitosan biomass. *Cellulose* **2019,** *26* (4), 2805-2819.

26. Ma, X.; Gözaydın, G.; Yang, H.; Ning, W.; Han, X.; Poon, N. Y.; Liang, H.; Yan, N.; Zhou, K., Upcycling chitin-containing waste into organonitrogen chemicals via an integrated process. *Proceedings of the National Academy of Sciences* **2020,** *117* (14), 7719-7728.

27. Pierson, Y.; Chen, X.; Bobbink, F. D.; Zhang, J.; Yan, N., Acid-catalyzed chitin liquefaction in ethylene glycol. *ACS Sustainable Chemistry & Engineering* **2014,** *2* (8), 2081-2089.

28. Kobayashi, H.; Techikawara, K.; Fukuoka, A., Hydrolytic hydrogenation of chitin to amino sugar alcohol. *Green chemistry* **2017,** *19* (14), 3350-3356.

29. Chen, X.; Liu, Y.; Kerton, F. M.; Yan, N., Conversion of chitin and N-acetyl-d-glucosamine into a N-containing furan derivative in ionic liquids. *RSC Advances* **2015,** *5* (26), 20073-20080.

30. Omari, K. W.; Besaw, J. E.; Kerton, F. M., Hydrolysis of chitosan to yield levulinic acid and 5-hydroxymethylfurfural in water under microwave irradiation. *Green Chemistry* **2012,** *14* (5), 1480-1487.

31. Kasaai, M. R.; Arul, J.; Charlet, G., Fragmentation of chitosan by acids. *The Scientific World Journal* **2013,** *2013*.

32. Bobbink, F. D.; Zhang, J.; Pierson, Y.; Chen, X.; Yan, N., Conversion of chitin derived N-acetyl-D-glucosamine (NAG) into polyols over transition metal catalysts and hydrogen in water. *Green Chemistry* **2015,** *17* (2), 1024-1031.

33. Gözaydın, G.; Song, S.; Yan, N., Chitin hydrolysis in acidified molten salt hydrates. *Green Chemistry* **2020,** *22* (15), 5096-5104.

34. Dai, J.; Gözaydın, G. k.; Hu, C.; Yan, N., Catalytic conversion of chitosan to glucosaminic acid by tandem hydrolysis and oxidation. *ACS Sustainable Chemistry & Engineering* **2019,** *7* (14), 12399-12407.

35. Fu, X.; Guo, Y.; Jin, Y.; Ma, M., Bioconversion of chitin waste using a cold-adapted chitinase to produce chitin oligosaccharides. *LWT* **2020,** *133*, 109863.

36. van Munster, J. M.; van der Kaaij, R. M.; Dijkhuizen, L.; van der Maarel, M. J., Biochemical characterization of Aspergillus niger CfcI, a glycoside hydrolase family 18 chitinase that releases monomers during substrate hydrolysis. *Microbiology* **2012,** *158* (8), 2168-2179.

37. Liu, Y.; Jiang, Z.; Ma, J.; Ma, S.; Yan, Q.; Yang, S., Biochemical characterization and structural analysis of a β-N-acetylglucosaminidase from Paenibacillus barengoltzii for efficient production of N-acetyl-d-glucosamine. *Journal of agricultural and food chemistry* **2020,** *68* (20), 5648-5657.

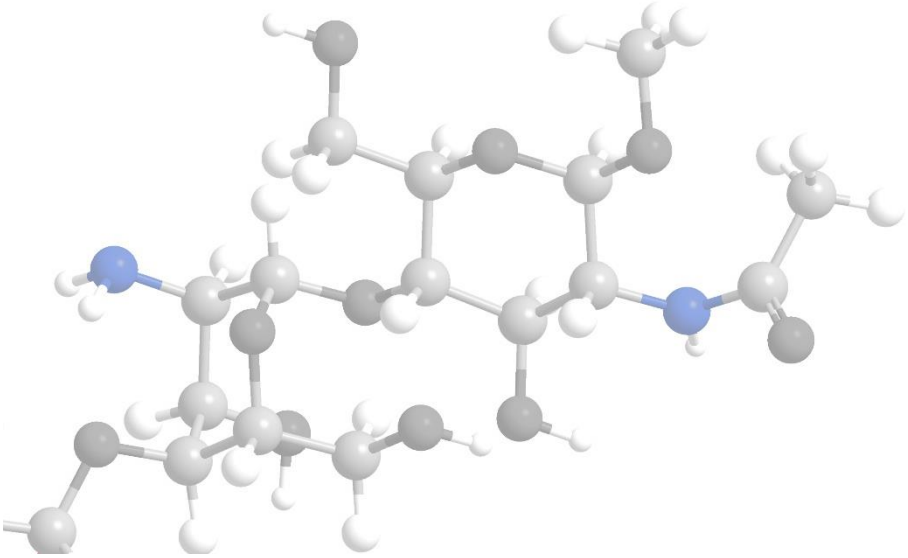
38. Zhang, W.; Liu, Y.; Ma, J.; Yan, Q.; Jiang, Z.; Yang, S., Biochemical characterization of a bifunctional chitinase/lysozyme from Streptomyces sampsonii suitable for N-acetyl chitobiose production. *Biotechnology letters* **2020,** *42* (8).

39. Wang, H.; Frenklach, M., Transport properties of polycyclic aromatic hydrocarbons for flame modeling. *Combustion and flame* **1994,** *96* (1-2), 163-170.

40. Corradini, D., *Handbook of HPLC* **2016**, 155.

Chapter 03

Synthesis of 5-HMF from chito-monomers using solid acid catalysts



3.1. Introduction

In the past decades, accelerated increase in global warming and diminishing fossil feedstocks have pushed chemical researchers to find an alternative and sustainable source for producing fuels and chemicals.[1-2] In this context, plant biomass has been extensively studied in the last couple of years as an alternative to fossil feedstock.[3-7] Comparatively, animal biomass, i.e., chitin which is also known as marine or oceanic biomass has acquired less attention.[8] To note, chitin is the second most plentiful bio-polymer on earth (ca. 1.5 x 10^5 tons per year) after cellulose and it is found in exoskeleton of prawns, shrimps, crab, lobsters, squids etc.[8] In the bio-refinery concept, lot of attention has been diverted to the synthesis of furan derivatives (furfural, 5-hydroxymethyl furfural (5-HMF)) due to their unique properties such as, heterocyclic structure, reactive functional groups (-CHO and -CH_2OH) etc, which otherwise are difficult to achieve through petro-refinery route.[9-13] Additionally, 5-HMF can act as a platform chemical which can be further converted into various value-added and important chemicals like; 2, 5-furandicarboxyl acid (potential replacement to terephthalic acid in polymer synthesis), 2,5-dimethylfuran (potential fuel), 2,5-dihydroxymethylfuran (raw materials for polymers and intermediate of drugs and crown ethers), 2,5-bis(hydroxymethyl)tetrahydrofuran (use in manufacture of polyester), 2,5-bis(hydroxymethyl)furan (use in manufacture of polyurethane foam) etc.[9, 11, 14] Though, lot of efforts are put in the synthesis of 5-HMF from cellulose/glucose/fructose through acid catalysed dehydration reaction, it can also be produced from chitin, chitosan, its oligomers and monomers, as cellulose being touted as one the alternatives to synthesize bio-ethanol and thus availability of the same for making other chemicals may be in jeopardy.[15] Moreover, structure of chitin, a homo-polymer of β-1,4 linked monomer, N-Acetyl-D-glucosamine (GlcNAc) is analogues to cellulose. Further, commercially, deacetylation of chitin gives chitosan, a heteropolymer of D(+)-Glucosamine

($GlcNH_2$) and N-Acetyl-D-glucosamine (GlcNAc).[16] As shown in Figure 3.1, $GlcNH_2$ and GlcNAc can upon dehydration and removal of amino or acetamide group, yield 5-HMF.

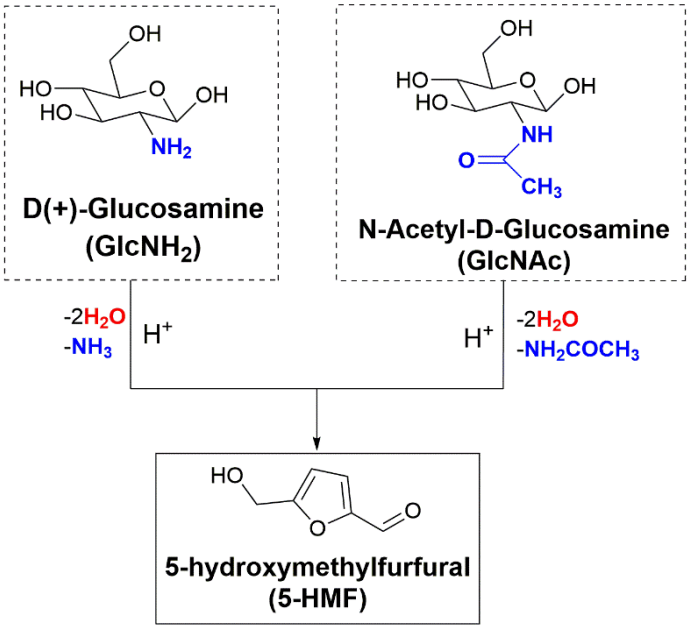


Figure 3.1. Scheme for the synthesis of 5-HMF from $GlcNH_2$ or GlcNAc upon dehydration (loss of two water molecules) and deamination (loss of NH_3)/ removal of acetamide (NH_2COCH_3)

Conventionally, it is shown that dehydration of chitin, chitosan, chito-oligomers and monomers to furan derivatives is achieved using mineral acids (HCl, H_2SO_4 etc.) as catalysts. Report with HCl/dichloroethane biphasic system shows synthesis of furan derivative, 5-chloromethylfurfural (5-CMF) from chitin with 45% yield.[17] Synthesis of 5-HMF with 12.5% yield is possible from chitosan in the presence of H_2SO_4 however, with $GlcNH_2$ as a substrate only 1.8% of 5-HMF is formed as it is further converted into levulinic acid (LA). This suggests that it is very much important to optimize the reaction conditions to achieve 5-HMF as a selective product.[18] Yield of 2.3% (5-HMF) along with LA and formic acid (FA) formation from $GlcNH_2$ at 160 °C in presence of methanesulfonic acid is also reported.[19] Although, homogeneous catalysts show possibility of synthesis of 5-HMF from chitin/chitosan/monomers, the methodology is not free of corrosiveness,

handling hazards etc. and thus, few other catalysts are also evaluated for the synthesis of 5-HMF. It is shown that $SnCl_4.5H_2O$ was able to produce 5-HMF from chitosan at 200 °C under microwave irradiation, yet with monomer as a substrate, no 5-HMF formation was seen as reaction yielded LA as major product.[8] Use of 67% aqueous solution of $ZnCl_2$ or $FeCl_2.4H_2O$ could also impart conversion of $GlcNH_2$, GlcNAc to 5-HMF with varying yields.[20-21] Sulfamic acid is also used by researchers to convert $GlcNH_2$ into 5-HMF (0.14 mol%) and LA (33.76%).[22] Besides this, ionic liquids (IL) such as [BMIM] $[HSO_4]$ [23] $[MIM]HSO_4$ [24] and $[Hmim]HSO_4$[25] too are shown to yield 5-HMF from chitosan with varying yields (19.3-25.7 %). Dehydration of $GlcNH_2$ and GlcNAc over heterogeneous catalyst such as, H-β zeolite in presence of 10% acetic acid yielded 23.78% and 12.42% 5-HMF respectively at 180 °C. Nevertheless, detailed investigation of the spent catalyst showed that H-β catalyst undergoes structural deformations and hence in recycle runs, decrease in 5-HMF yield was seen.[26] 5-HMF synthesis from $GlcNH_2$ (30% yield) and GlcNAc (29%) using Nafion®50 in a biphasic solvent system at 180 °C is shown yet, report does not describe reusability of the catalyst.[27]

As seen, handful of works report synthesis of 5-HMF from chitin/chitosan or monomers but those are associated with difficulties in recovery and recycling of catalysts, neutralization of reaction mixture, suppression of side/further reactions, instability of the catalysts, use of high boiling solvents etc. In view of this, it is very much essential to design and develop water tolerant, substrate tolerant and recyclable catalysts. In the current work, I have designed and developed stable water tolerant catalyst which work effectively to produce 5-HMF.

3.2. Experimental section

3.2.1. Materials and reagents

D-(+)-glucosamine ($GlcNH_2$, 99%), N-Acetyl-D-glucosamine (GlcNAc, 99%), and 5-hydroxymethylfurfural (5-HMF, 99%) were purchased from Sigma-Aldrich and used as received. Zeolites, H-USY (CBV-720, Si/Al = 15), NH_4-ZSM-5 (CBV-2314,

Si/Al= 11.5), NH_4-MOR (CBV21A, Si/Al = 10) were obtained from Zeolyst International. Prior to use, zeolites (except H-USY) were calcined at 550°C for 12 h in muffle furnace under air flow to achieve H form of the same. SiO_2–Al_2O_3 (Aldrich, Si/Al=5.3), K10 clay (Sigma-Aldrich), Nafion SAC-13 (Sigma-Aldrich), Amberlyst-15 (Fluka) were also purchased and used as received. Pseudoboehmite (Marathwada Chemical Industries Pvt. Ltd., India, 65-78%), Fumed silica (Sigma-Aldrich, USA, 99%), Ortho phosphoric acid (Loba Chemie, 85%), Cyclohexyl amine (Sigma-Aldrich, 99%), Ammonium heptamolybdate (Loba chemie, 99%), Ammonium metatungstate hydrate (Sigma-Aldrich, USA, >66.5%), Phosphorus Acid (Loba Chemie, 98%) were procured and used for catalyst preparation. Methyl-iso-butyl ketone (MIBK, 99% AR) was purchased form Loba Chemie and Toluene (99 % AR) was procured from Rankem.

3.2.2. Synthesis and Characterization of catalysts

3.2.2.1. Synthesis of catalysts

3.2.2.1.1. Synthesis of modified SAPO-44 (m-SAPO-44)

Crystalline modified silicoaluminophosphate (m-SAPO-44) material was synthesized as mentioned in literature.[28-29] Crystallization time was increased to get more hydrothermal stability. Increase in crystallization time gives intense characteristic peak for CHA morphology. Molar gel composition, Al, Si and P source, structure directing agent, crystallization time, temperature, calcination time, temperature are detailed in Table 3.1.

Table 3.1. Synthesis condition of m-SAPO-44 material

Molar gel composition	1.0 Al_2O_3:1.0 SiO_2:1.0 P_2O_5: 1.0 CHA:60.0 H_2O
Al source	Pseudoboehmite (Marathwada Chemical Industries Pvt. Ltd, grade: MCI-1524, 65-78% Al_2O_3)
Si source	Fumed silica (Sigma-Aldrich, USA, 99%)
P source	Orthophosphoric acid (Loba Chemie, 85%)
Structure directing agent	Cyclohexylamine (Sigma-Aldrich, USA, 99%)

Crystallization time and temperature	176 h, 200 ˚C
Calcination time and temperature	6 h, 550 ˚C (ramp – 1 ˚C/min), in a tubular furnace, O_2 (10 mL/min)

In a typical procedure, 4.60 g pseudoboehmite was added slowly (within 2h) to the phosphoric acid solution (7.28 g H_3PO_4 + 12.50 g water) to form *gel A*. In a beaker, silica slurry was prepared by addition of 2.05 g fumed silica in 23.5 g water under vigorous stirring. Lumps should not be formed. The 3.33 g of structure directing agent, Cyclohexylamine was added dropwise into the mixture is denoted as *gel B*. Then, *gel B* is added to *gel A* under continuous stirring and the stirring is continued to 6h to make it homogeneous. The solution was transferred to Teflon lined autoclave and kept for crystallization for 176 h at 200 ˚C under atmospheric pressure without agitation. Solid materials were separated through filtration and washed thoroughly. The material is kept for drying at 60 ˚C for 16 h followed by at 150 ˚C for 6 h under vacuum. Then the material was calcined at 550 ˚C at a heating rate of 1 ˚C/min in a tubular furnace for 6h in presence of O_2 (10 mL/min).

3.2.2.1.2. Synthesis of wet-impregnated supported metal oxides

Silica and silica-alumina support were used in the synthesis of supported metal oxide catalysts by wet-impregnation method. 10 *wt%* metal on support material was prepared using typical procedure.[30] 1.0 g of activated support i.e., silica alumina (SA) was dispersed in 8 mL distilled water by stirring it using magnetic stirred for 30 min at room temperature. Then 2mL of metal salt solution was added to it dropwise. For tungsten catalyst, 0.2238 g Ammonium Metatungstate hydrate; for molybdenum catalyst, 0.2065 g Ammonium heptamolybdate was dissolved in 2 mL distilled water to prepare metal salt solution. Then the mixture was stirred for 16 h at room temperature. The water was then removed by rotary evaporator. Solid material was then dried at 60 ˚C followed by heating at 150 ˚C for 6 h in high vacuum. Then the tungsten materials are calcined at 550 ˚C for 2 h

and molybdenum catalysts are calcined at 550 ˚C for 5 h in the presence of air at heating rate 2 ˚C/min.

3.2.2.2. Characterization of catalysts

Catalysts were characterized with XRD, Ammonia-TPD, N_2-Sorption techniques. The catalysts were well characterized in previous articles from our research group.[28, 30]

3.2.2.2.1. X-ray Powder Diffraction (XRD)

X-ray diffraction (XRD) analysis was done to understand the phase purity in the material. For analysis, sample was prepared as a thin layer on a glass plate and analysed by Rigaku Miniflex diffractometer (Netherland) using a Ni-filtered monochromatic Cu K_α radiation (λ = 1.5406 Å). The samples were scanned between a 2θ range of 5-80˚ at a scan rate of 2˚/min.

3.2.2.2.2. Temperature Programmed Desorption (TPD)

TPD analysis was carried out in Micrometrics AutoChem-2920 instrument equipped with TCD detector. At first, catalyst was activated at 600 ˚C at a heating rate of 4 ˚C/min in helium flow of 30 mL/min for 1h. Then the temperature was decreased to 50 ˚C and NH_3 was absorbed by exposing the samples to 10% NH_3 in helium for 1h. It was then flushed with helium for 1h at 100 ˚C to remove all the physisorbed NH_3. Desorption of NH_3 was carried out in helium flow (30 mL/min) by increasing the temperature from 100 to 600 ˚C at the rate of 10 ˚C/min.

3.2.2.2.3. N_2- Sorption Study

The surface areas of catalysts were calculated using a Nova 1200 and Autosorb 1C Quanta Chrome Instruments, USA. At first, samples are pelletized and activated at 250 ˚C for 2h under high vacuum (10^{-6} bar). The surface area was determined using BET method, pore size was obtained using BJH method.

3.2.2.2.4. ICP-OES analysis

Samples were analysed in Spectro Arcos Germany, FHS 12 instrument. In a polypropylene bottle *ca.* 20 mg catalyst is weighed and few drops of HF is added to this. Then the mixture is heated at 70 °C upto dryness. Upon addition of HF, it reacts with silica in the sample to form silicon tetrafluoride (SiF_4) which is volatile and removed from the sample. Silica is removed completely by this process and thereby prevents interference in ICP analysis. After that, 3 mL freshly prepared aquaregia (1:3 *v/v* concentrated HNO_3: concentrated HCl) is added to it and heated at 70 °C for 15-20 min. In this process, metals are digested in aquaregia by forming chloride or nitrate salts and became soluble in water. The resultant mixture is then diluted and made to 50 mL in a volumetric flask and filtered through 0.22 μm filter. The filtered solution is then analysed by ICP-OES instrument.

3.2.2.2.5. Elemental analysis

Analysis was done in Thermo Finnigan, Italy, model EA1112 series flash elemental analyser. The amount of C, H, N in samples was determined by rapid combustion of small amounts (1-2 mg) of the sample in pure oxygen (Dumas method or "flush combustion")

3.2.3. Catalytic runs

Catalytic runs were performed in a Parr make batch reactor (100 mL). In a typical reaction, 30 mL of 311 μmol solution of substrate was taken. To it, 0.4 g of silica-alumina (SA) was added. Reactions were performed under 0.5 MPa nitrogen pressure (charged at room temperature). Then the autoclave was heated to desired temperature at 100 rpm. Upon attaining the desired reaction temperature, stirring was increased to 700 rpm. After the completion of reaction, catalyst was separated from reaction mixture through centrifugation. The reaction mixture is then analysed by High Performance Liquid Chromatography (HPLC).

3.2.4. Extraction of products in organic solvents

To identify and understand the nature of unknown compounds, extraction of these compounds was done in organic solvents and then the organic layer was injected to Gas Chromatography (GC) and GC-Mass spectrometry (GC-MS). The scheme of extraction procedure is shown in Figure 3.2.

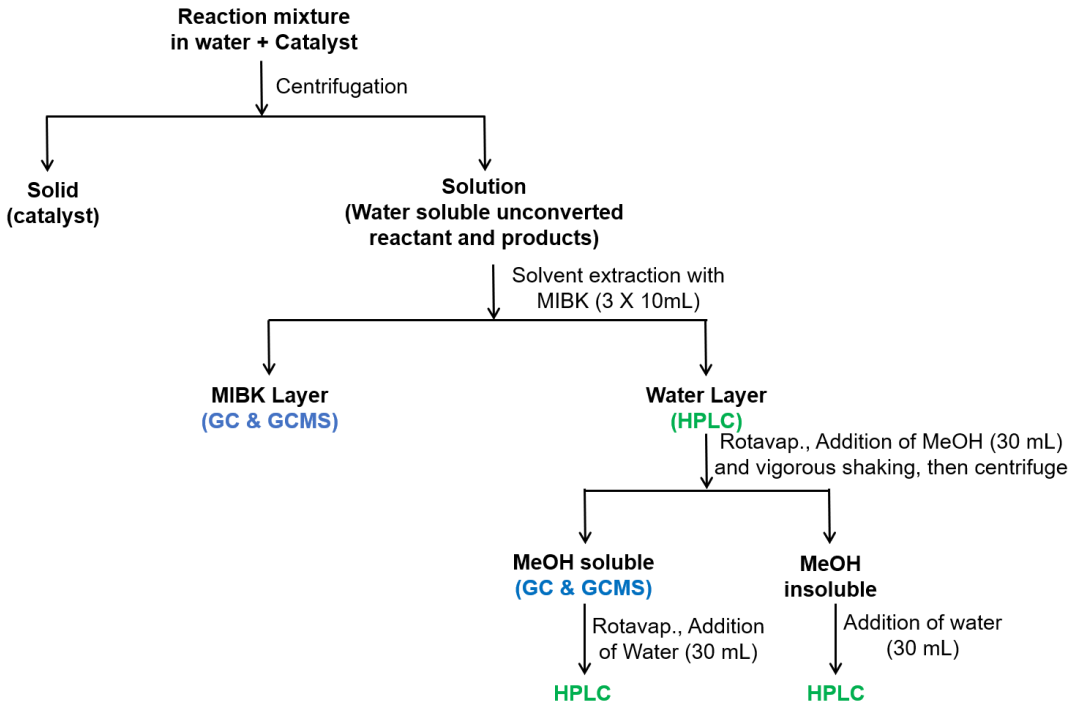


Figure 3.2. Scheme for the extraction of products

3.2.5. Analysis of reaction mixtures

Reaction mixtures were analysed using High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) and GC-Mass Spectroscopy (GC-MS).

3.2.5.1. High Performance Liquid Chromatography (HPLC)

Aqueous reaction mixture was filtered through 0.22 um PTFE filter and injected to Agilent 1200 HPLC equipped with Pb^{2+} column (300 mm x 7.8 mm I.D, 8 μm), variable wavelength detector (VWD, wavelength kept at 195 nm), refractive index detector (RID, cell temperature kept at 40 °C) and Millipore water as mobile phase.

Column temperature was kept at 80 °C and the flow rate of mobile phase was 0.5 mL/min.

3.2.5.2. Gas Chromatography (GC)

Extracted products soluble in organic solvents were injected to Agilent 7890B GC equipped with HP-1 column (30 m x 0.22 μm I.D) and flame ionization detector (FID).

3.2.5.3. Gas Chromatography- Mass Spectrometry (GC-MS)

Extracted products soluble in organic solvents were analysed using GC-MS system equipped with Agilent 7890B GC (DB-5 column-50 m x 0.22 μm I. D) and MS 5977A system.

3.2.6. Calculations

Weight of formed 5-HMF is calculated from area from HPLC using calibration curve. Standards were prepared with the known quantity of 5-HMF in water. All the yields mentioned in this chapter were calculated based on HPLC results. Details on the calculations are mentioned below,

Glucosamine ($GlcNH_2$) ⟶ 5-HMF

M.W = 179 M.W = 126

Molecular weight of 5-HMF is 126 and considering 100% yield and selectivity, 126 g 5-HMF yield is possible from 179 g $GlcNH_2$.

% Yield of 5-HMF = [weight of 5-HMF formed (HPLC)/weight of 5-HMF (theoretical)] x 100

3.3. Results & discussion

3.3.1. Conversion of Glucosamine to 5-HMF

Initially, evaluation of various solid acid catalysts for their activities on the dehydration of glucosamine ($GlcNH_2$) to 5-HMF was done. As seen from Figure

3.3, various catalysts showed varying activity and maximum activity was seen with m-SAPO-44 catalyst to achieve 5-HMF with highest yield (26%).

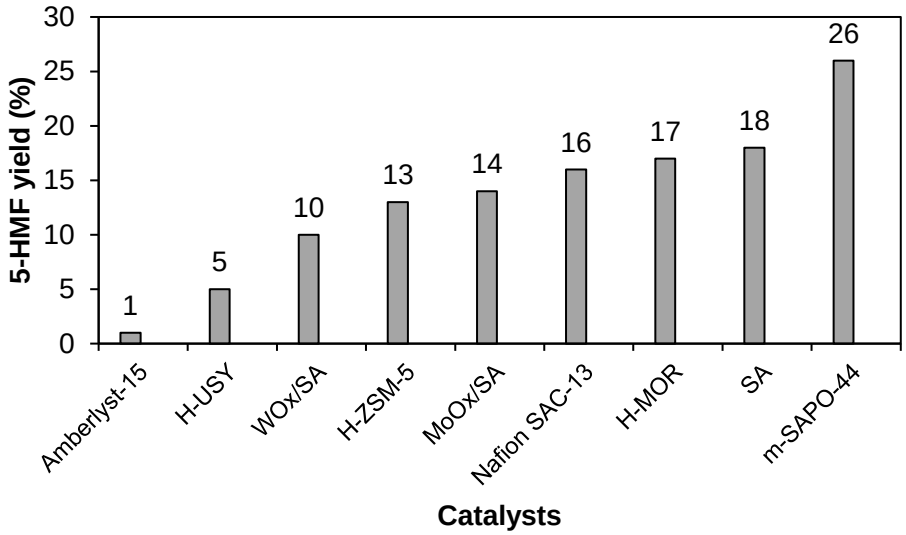


Figure 3.3. Dehydration of $GlcNH_2$ using various solid acid catalysts. Reaction conditions: $GlcNH_2$ (311 μmol), catalyst (0.4 g), water (30 mL), 170 °C, 0.5 MPa N_2 pressure at RT, 4 h

As seen from the Figure 3.3, different types of catalysts were evaluated for e.g., catalysts having defined structure with crystallinity (porosity/channel) or catalysts without defined structure such as having amorphous structure. It was expected that catalysts having defined structure would show higher activity than the amorphous catalysts due to difference between the accessibility of active sites by the substrates to undergo dehydration reactions. Nevertheless, results are contradictory to this belief as catalysts having defined structure (H-USY, H-ZSM-5) showed poor results than amorphous catalysts (silica-alumina (SA), MoOx/SA, WOx/SA, Nafion SAC-13). To explain this further, size of $GlcNH_2$ was calculated based on ChemDraw 3D after energy minimization and it was observed to be 0.50 nm x 0.71 nm (W x L) (Figure 3.4 and Table 3.2). Kinetic diameter, width and length of $GlcNH_2$, GlcNAc and 5-HMF can be calculated by using following formula. [31]

Kinetic diameter (σ) = 1.234 X $(M.W)^{1/3}$

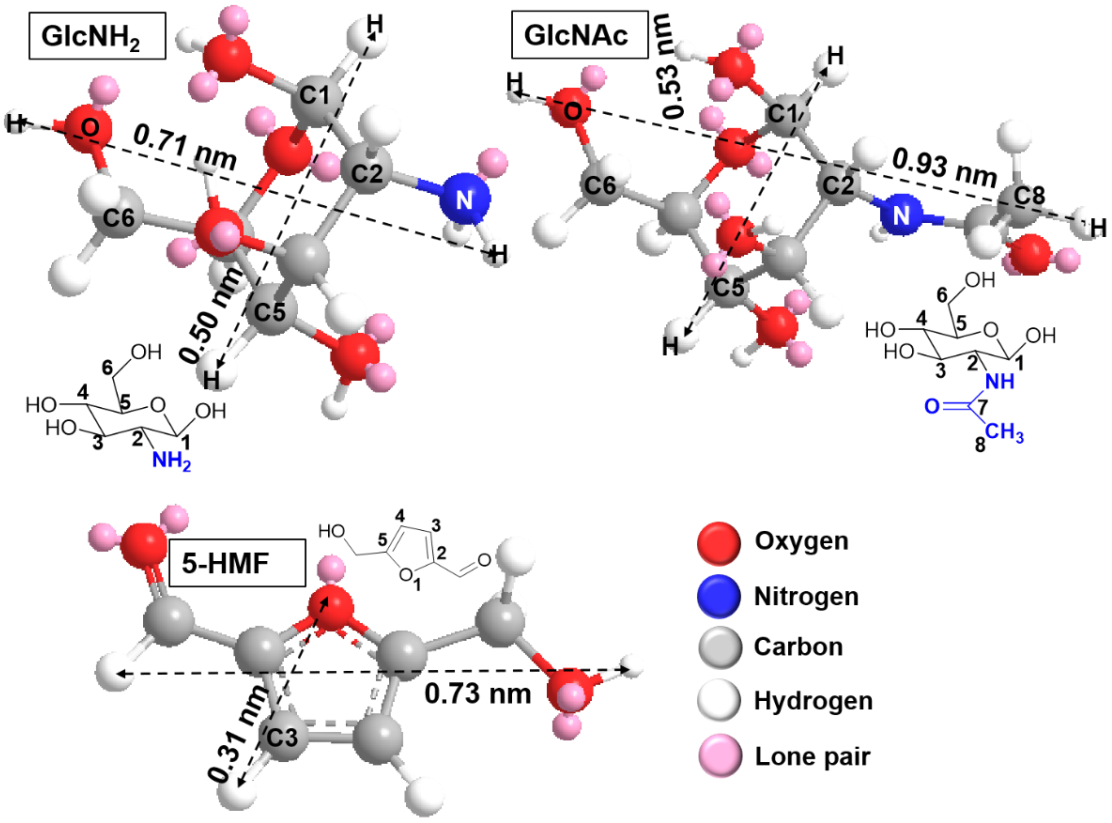


Figure 3.4. Energy minimized structures of $GlcNH_2$, GlcNAc and 5-HMF and their dimensions using ChemDraw 3D software

Table 3.2. Size of $GlcNH_2$, GlcNAc and 5-HMF

Properties	$GlcNH_2$	GlcNAc	5-HMF
Width (nm) (from Chem 3D)	0.50 (H of C1 to H of C5)	0.53 (H of C1 to H of C5)	0.31 (O of furan ring to H of C3)
Length (nm) (from Chem 3D)	0.71 (H of OH-C6 to H of NH_2-C2)	0.93 (H of OH-C6 to H of CH_3-C8)	0.73 (H of -CHO to H of -CH_2OH)
Kinetic diameter (nm) (from M.W)	0.69	0.75	0.62

Pore size of structured catalysts i.e., H-USY (0.74 nm x 0.74 nm), H-ZSM-5 (0.55 nm x 0.51 nm and 0.56 nm x 0.53 nm) and H-MOR (0.70 nm x 0.65 nm and 0.57 nm x 0.26 nm) are well bigger (Figure 3.5, Table 3.3) than the size of substrate but

still, those observed to be showing poor activity than amorphous catalysts having varying pore size. Another reason for discrepancy in the activity based on structure of catalyst would be number of active sites present on the catalyst. Structural properties of the structured catalysts and their structures are given below (Figure 3.5 and Table 3.3)

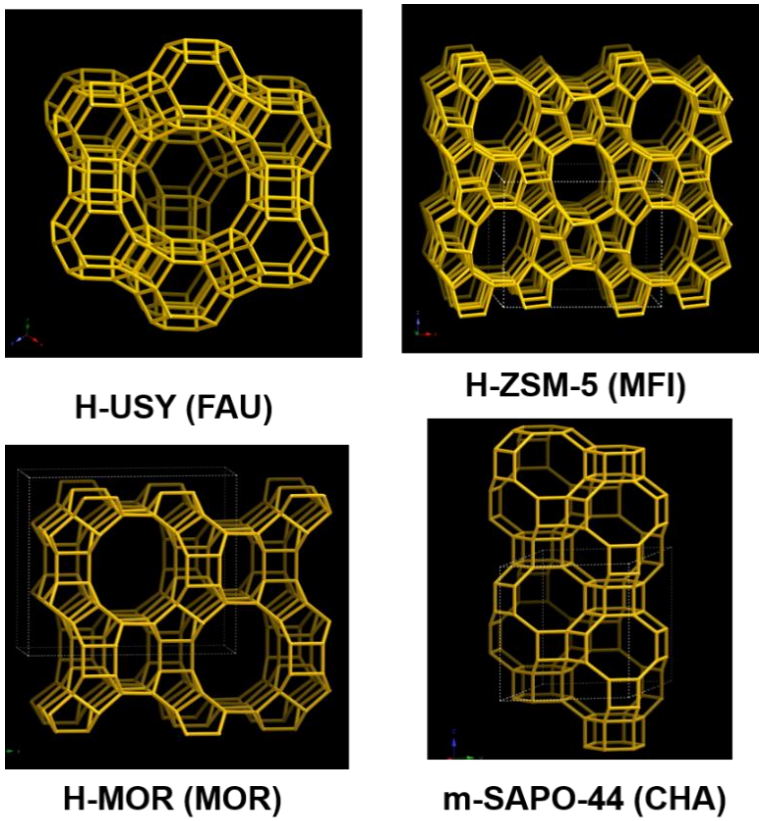


Figure 3.5a. Structures of zeolite frameworks adapted from IZA website

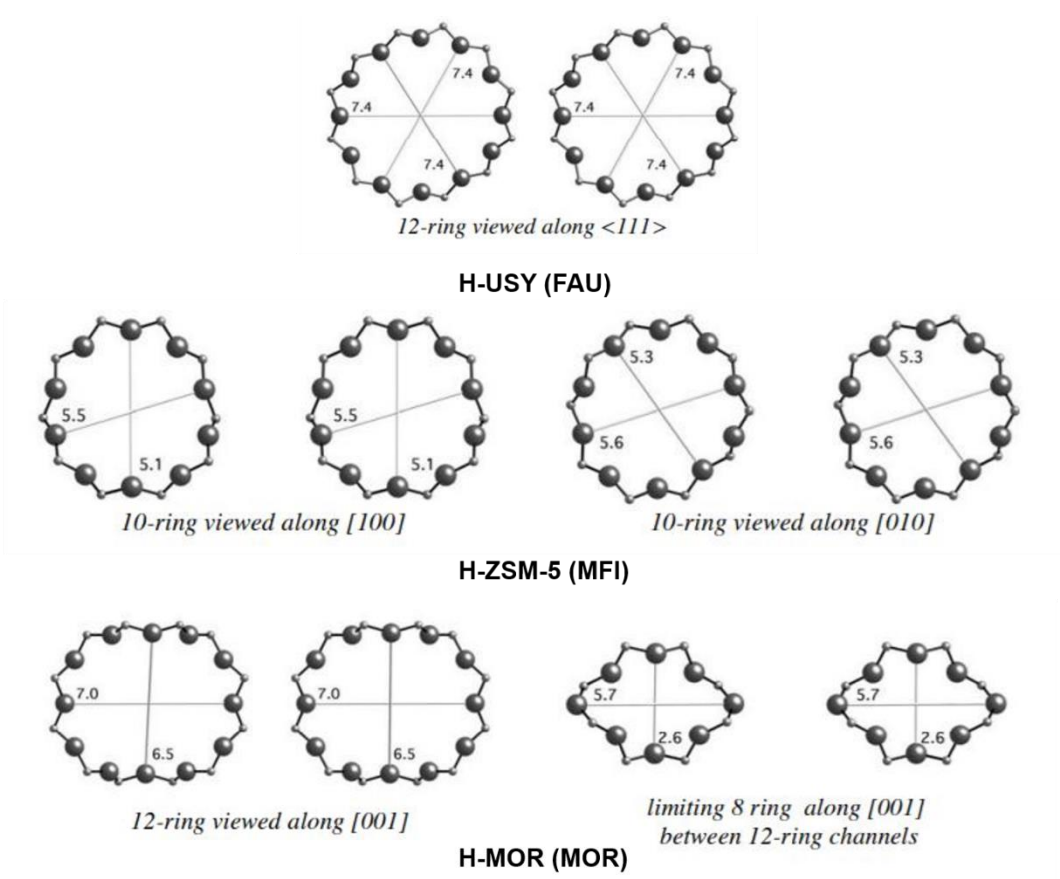


Figure 3.5b. Pore diameters of zeolite frameworks adapted from IZA website

Table 3.3. Structural properties of structured catalysts used for the reaction

Zeolite	Framework	Ring sizes	Channel system	BET surface area (m^2/g)	Pore diameter (nm)	Total acidity (mmol/g)
H-USY (Si/Al=15)	FAU	12,6,4	3D	873	(0.74 x 0.74)[32]	0.55
H-ZSM-5 (Si/Al=11.5)	MFI	10,6,5,4	3D	423	(0.55 x 0.51), (0.56 x 0.53)[32]	0.98

H-MOR (Si/Al=10)	MOR	12,8,5, 4	2D	528	(0.70 x 0.65), (0.57 x 0.26)[32]	1.15
m-SAPO-44	CHA	8,6,4	3D	337	0.41	1.02
SA	-	-	-	380	3.92	0.67
MoOx/SA	-	-	-	88	1.55	0.69
WOx/SA	-	-	-	195	1.91	0.72
Amberlyst-15	-	-	-	53[33]	-	4.7[33]
Nafion SAC-13	-	-	-	200[34]	>10[34]	0.17[34]

As seen from Table 3.3, acid sites on each catalyst are different and also their strength is different. This may contribute to the change in activity of these catalysts. However, to arrive at any logical explanations on this, reactions with similar concentration of acid sites were performed. It was observed that when reactions with H-MOR were conducted with substrate to catalyst (S/C) ratio 1.16 mole/mole (0.268 mmol of acid sites), 29% yield of 5-HMF were observed. As the increase in yield is observed by decreasing H-MOR quantity, the catalyst quantity was decreased further and slight decrease in yield (27%) was observed with S/C ratio 4.64 mole/mole (0.067 mmol of acid sites) and further decrease in yield (24%) is observed when substrate to catalyst ratio is decreased further to 9.28 mole/mole (0.0335 mmol of acid sites). Effect of decreasing catalyst quantity for m-SAPO-44 was also checked as it has given best 5-HMF yield. Decrease in 5-

HMF yield was observed with decrease in catalyst quantity (S/C ratio- 4.64 mole/mole and 0.067 mmol of acid sites, 24%; S/C ratio- 9.28 mole/mole and 0.0335 mmol of acid sites, 23%). Effect of S/C ratio for SA catalyst is explained in next section.

It was also essential to compare the activity of well-known solid acid catalysts like ion-exchanged resins (Amberlyst-15 and Nafion SAC-13). Same reaction conditions were employed for the reactions with these two catalysts. Amberlyst-15 showed poor yield of 5-HMF (1%) instead of having very high acid amount. This is obvious due to its very low hydrothermal stability (<120 °C).[21] 16% 5-HMF yield was obtained from Nafion SAC-13 but sulfonic acid groups present in the catalyst got solvated by reaction medium[35], i.e., water. Therefore, Nafion SAC-13 will not show good recyclability with consistent 5-HMF yield. Activity of metal oxides on silica alumina (WO_x/SA, MoO_x/SA) were also checked as amorphous SA was already found to give better yield of 5-HMF. 10% and 14% 5-HMF yield were achieved from WO_x/SA and MoO_x/SA respectively, but when the ICP-OES analysis of reaction mixture (after reaction) was performed, very high amount of W and Mo leaching were observed. Hence, it can be concluded that metal oxides are not stable under reaction condition.

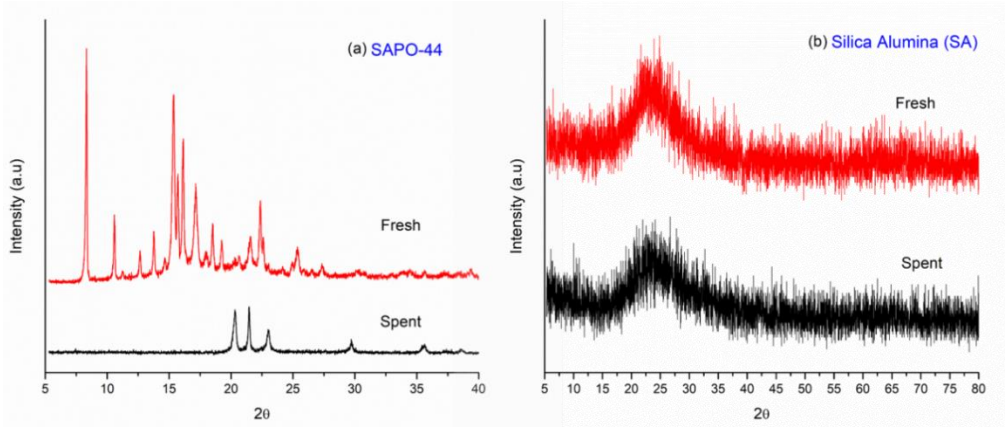


Figure 3.6. (a) XRD patterns of fresh and spent m-SAPO-44, (b) XRD patterns of fresh and spent silica alumina

Although, it was seen from Figure 3.3 that m-SAPO-44 catalyst showed highest yield of 5-HMF (26%), from the spent m-SAPO-44 catalyst characterization (Figure 3.6a) it was seen that it undergoes structural changes during reaction. This observation was unexpected because in our earlier work on m-SAPO-44 catalysts, we have shown it to be stable under reaction conditions used for biomass conversion.[9] However, fundamental difference between earlier work and this work is that in earlier work bi-phasic solvent system (water:MIBK) was used while in this work only water is used as a solvent. As observed from Figure 3.3, under similar reaction conditions, H-MOR and SA showed better yield (18%) compared to any other catalysts. The analysis of SA catalyst (Figure 3.6b) indicates that the catalysts are stable under the reaction condition but SA gives best activity in lower catalyst quantity while decrease in yield is observed when H-MOR quantity is decreased. SA is found to be better catalyst as it can give highest yield in lowest catalyst quantity and it is stable under reaction condition. Detailed study on the effect of S/C ratio of SA catalyst is discussed in next section. Detailed characterization of fresh and spent m-SAPO-44 and SA studies were performed to understand any change in the accessibility of acid sites due to deformation of the catalyst structure which is discussed on Section 3.3.6. Moreover, ICP-OES data (Table 3.6) also reveals that no substantial leaching of Si or Al is seen. This quantitatively and substantially proves that amorphous SA catalyst is more stable under reaction conditions and thus further studies were performed with this catalyst.

3.3.2. Effect of reaction parameters in Glucosamine reactions

Our earlier experience and literature educate us that humin formation and other degradation/side reactions are predominant if reaction temperatures are too harsh for a reaction. To understand effect of temperature on glucosamine dehydration reactions, those were carried out in the temperature range of 120-190 °C under 0.5 MPa N_2 pressure at RT for 4 h. On the expected lines, it was seen that with increase in temperature, yield of 5-HMF enhanced (120 °C, 10%; 130 °C,

12%; 140 ˚C, 15%; 160 ˚C, 17%; 170 ˚C, 18%; 180 ˚C, 16% and 190 ˚C, 13%) except until 170 ˚C. Total pressure of the reaction system is increased when the temperature was elevated (140 ˚C, 0.7 MPa; 160 ˚C, 0.8 MPa; 170 ˚C, 0.9 MPa; 180 ˚C, 1 MPa; 190 ˚C, 1.2 MPa). Above 180 ˚C, yield of 5-HMF started decreasing due to either thermal or catalytic side reactions started becoming predominant.

As like effect of temperature was studied, effect of nitrogen pressure too was studied on the reaction as it is known fact that with increase in overall pressure, diffusivity of substrate on the catalytically active sites increases, which in turn enhances the reaction rate. When reaction was carried out at 170 ˚C for 4 h without nitrogen pressure the yield of HMF is 15% but with 0.5 MPa (18%), 1 MPa (18%) and 2 MPa (18%) nitrogen pressure (initial), yield of 5-HMF increased initially but later was stabilized. 0.9 MPa total pressure was observed at reaction temperature (170 ˚C) when 0.5 MPa nitrogen was charged at room temperature. Not much dependency of activity on nitrogen pressure was seen as amorphous catalyst, SA was used in this study, which is devoid of any channel structure wherein diffusivity parameter shows higher impact.

Effect of time was recorded at 170 ˚C under 0.5 MPa N_2 pressure at RT and it was observed that 5-HMF yield was maximum within 2 and 4 h (1 h, 15%; 2 h, 18%; 3 h, 17%; 4 h, 18%; 5 h, 16%, 6 h, 14%). Logically it is apparent that with change in reaction time, conversions will vary but as reaction is carried out for longer times, side reactions and formation of degradation products are to be expected to decrease the yield of 5-HMF.

Effect of substrate to catalyst ratio (S/C ratio) was studied with SA catalyst at 170 ˚C for 4h under 0.5 N_2 pressure at RT. Figure 3.7 shows the effect of S/C ratio on 5-HMF yield. It was observed that the yield of 5-HMF is increasing with decreasing catalyst quantity. Higher catalyst quantity might be the reason for more side reaction, hence decrease in 5-HMF yield. Best 5-HMF yield was found with 9.28 mole/mole substrate to catalyst ratio.

As observed in chapter 2 (Section 2.3.1. and Table 2.6a), $GlcNH_2$, GlcNAc and 5-HMF are completely soluble in Water, that's why, water was preferably chosen as reaction medium. Moreover, water is known as green solvent and also environmentally favourable.[36] Furthermore, water forms hydronium ions with labile protons of solid acid catalysts which is very helpful in case of solid acids with smaller pore size than substrate.[37-38] That's why, all the reactions were carried out in water medium.

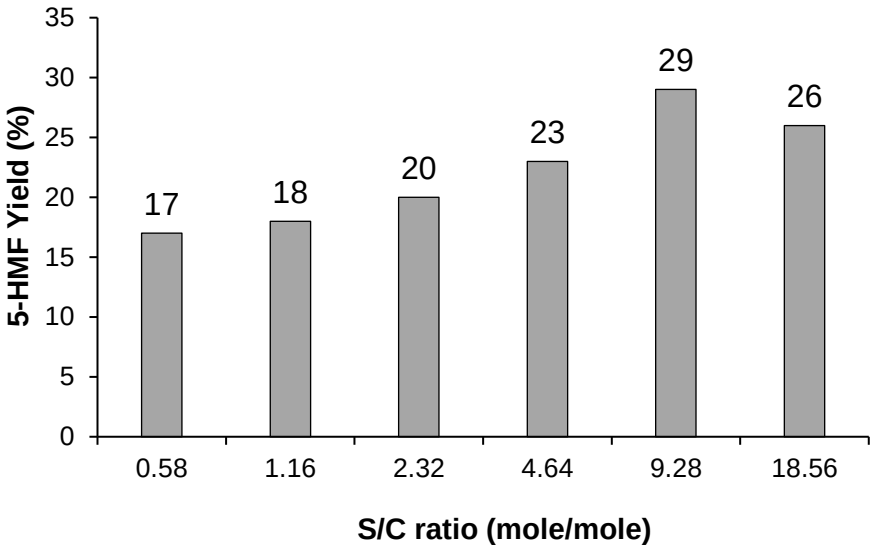


Figure 3.7. Effect of substrate to catalyst ratio (S/C ratio). Reaction conditions: substrate ($GlcNH_2$), catalyst (SA) water (30 mL), 170 °C, 0.5 MPa N_2 pressure at RT, 4 h

3.3.3. Extraction of products in organic solvents

As observed from the HPLC chromatograms (Figure 3.8), only one peak of substrate ($GlcNH_2$) was observed when the initial (before reaction) sample was injected. After the reaction, extra peaks for products were observed which reveals that the substrate is converted to some other products in presence of solid acid catalysts. 5-HMF was identified by injecting the standard of the same. Sugar compounds and organic compounds formed in the reaction are completely

soluble in reaction medium, i.e., water. Therefore, the aim of this study was to extract organic compounds in organic solvent to isolate/separate those from sugar compounds and also identify those using GC and GC-MS methods. To achieve this, from a reaction mixture, organic compounds were extracted in MIBK and methanol subsequently as given in Figure. 3.2. As observed from the HPLC chromatograms (Figure 3.8), 5-HMF was extracted in MIBK and it was confirmed by HPLC, GC and GC-MS analysis too. GC profiles of the extracted samples (Figure 3.9) also confirms the formation of 5-HMF which was not clearly visible in Figure 3.8 because peak of MIBK and 5-HMF have merged with each other. It is observed that three compounds are extracted in methanol however at this juncture only $GlcNH_2$ (unconverted) could be identified. Further work on this aspect is ongoing in our group and will be reported in our future communications.

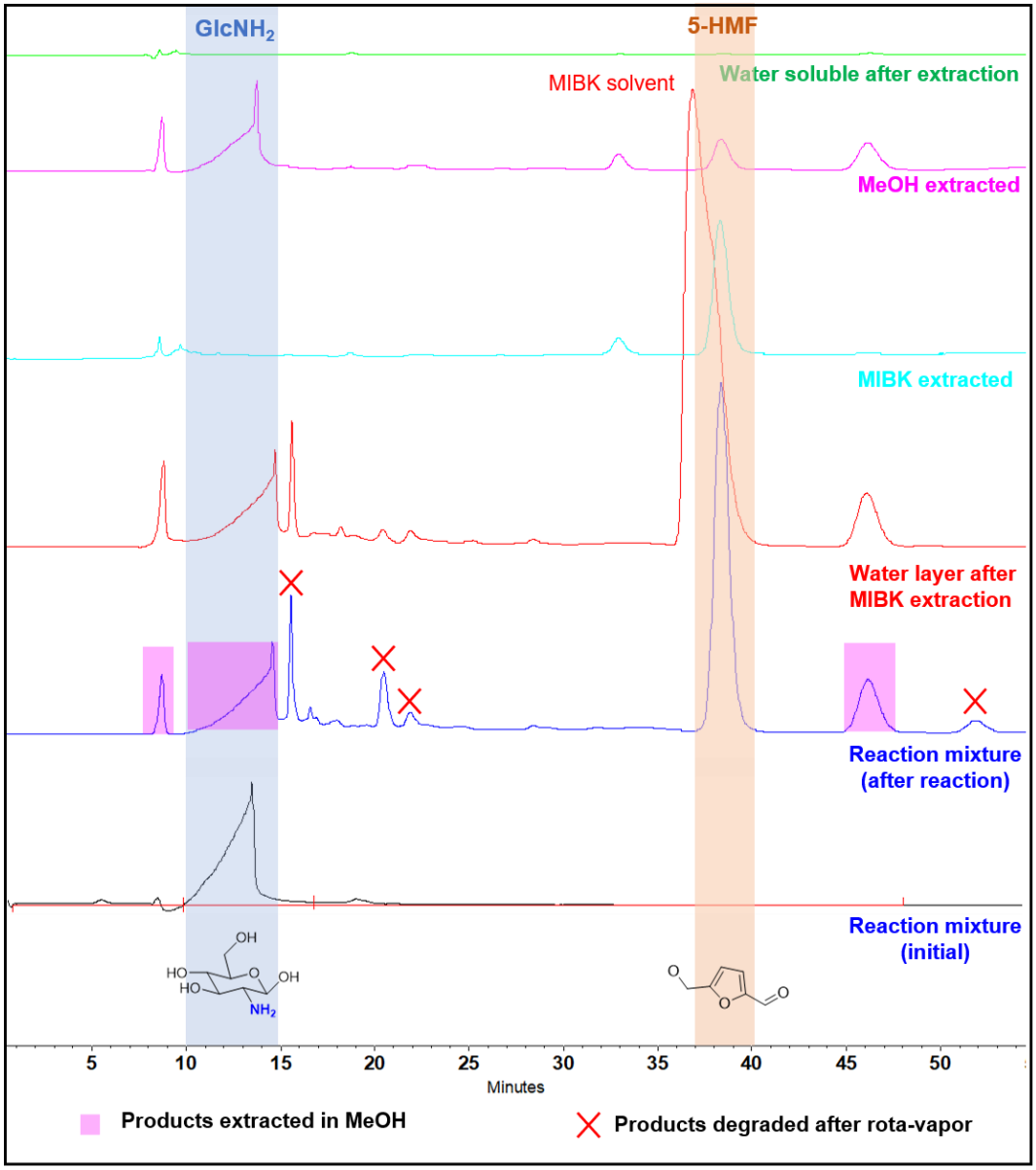


Figure 3.8. HPLC chromatograms for reaction mixture extracted in organic solvents. Reaction conditions: $GlcNH_2$ (311 μmol), SA (0.4 g), water (30 mL), 170 °C, 0.5 MPa N_2 pressure, 4 h

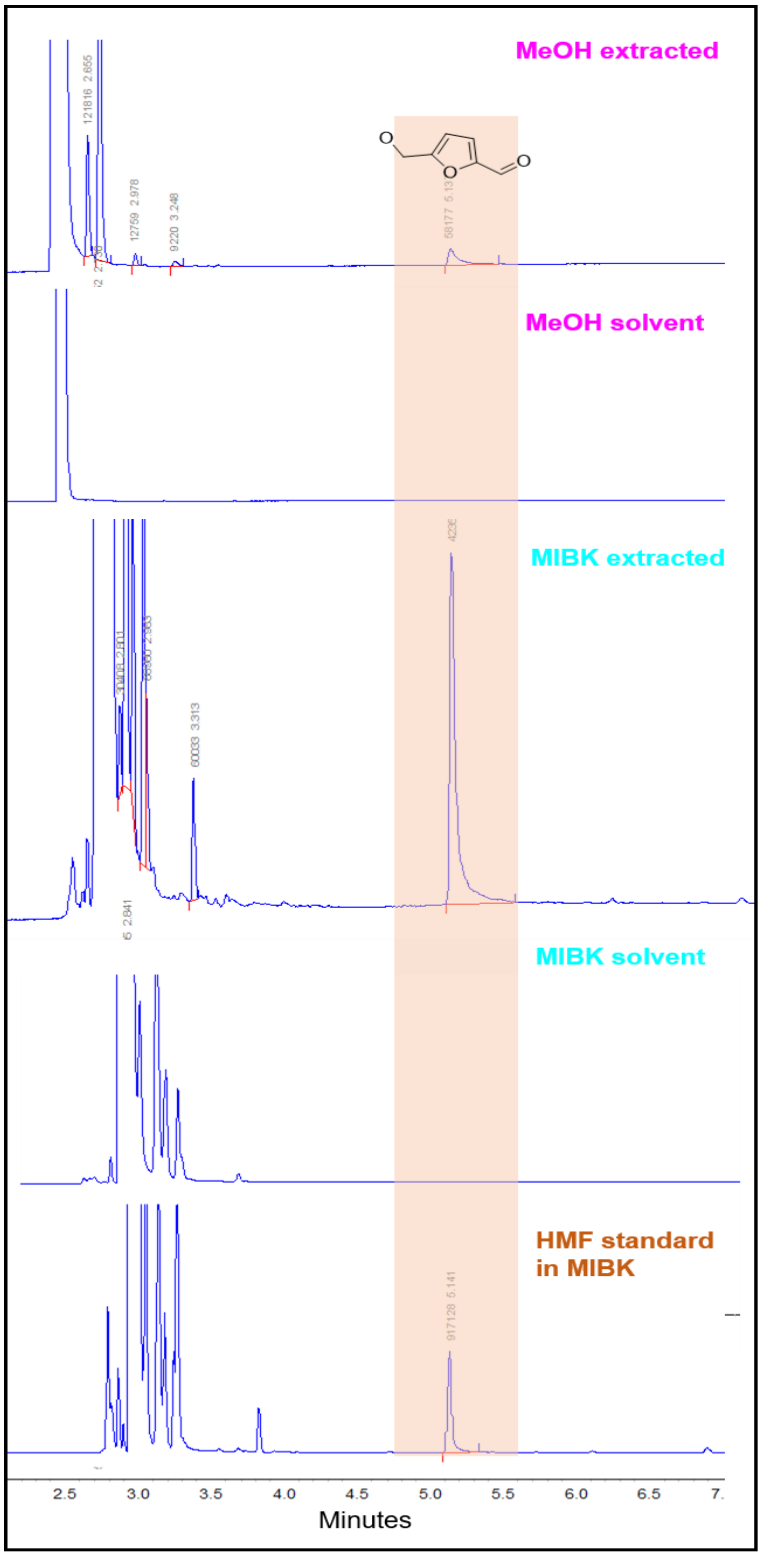


Figure 3.9. GC chromatograms for reaction mixture extracted in organic solvents. Reaction conditions: $GlcNH_2$ (311 μmol), SA (0.4 g), water (30 mL), 170 °C, 0.5 MPa N_2 pressure, 4 h

After rota-vaporisation of water from reaction mixture, few peaks were missing (marked by red cross in Figure. 3.8). To understand the observation, the water from the reaction mixture was evaporated using rota-evaporator and then again 30 mL water was added to it and stirred vigorously. Then the water-soluble part is again injected to HPLC. It is observed from HPLC chromatographs (Figure 3.10) that some peaks are vanished (marked with red cross mark) and intensity of 5-HMF is also decreased. 5-HMF might be reacting with the other products during rota-vapor and drying process. The vanished products might be consisting of such functional groups which can react with 5-HMF, as 5-HMF is well known for condensation reactions and humin formation.

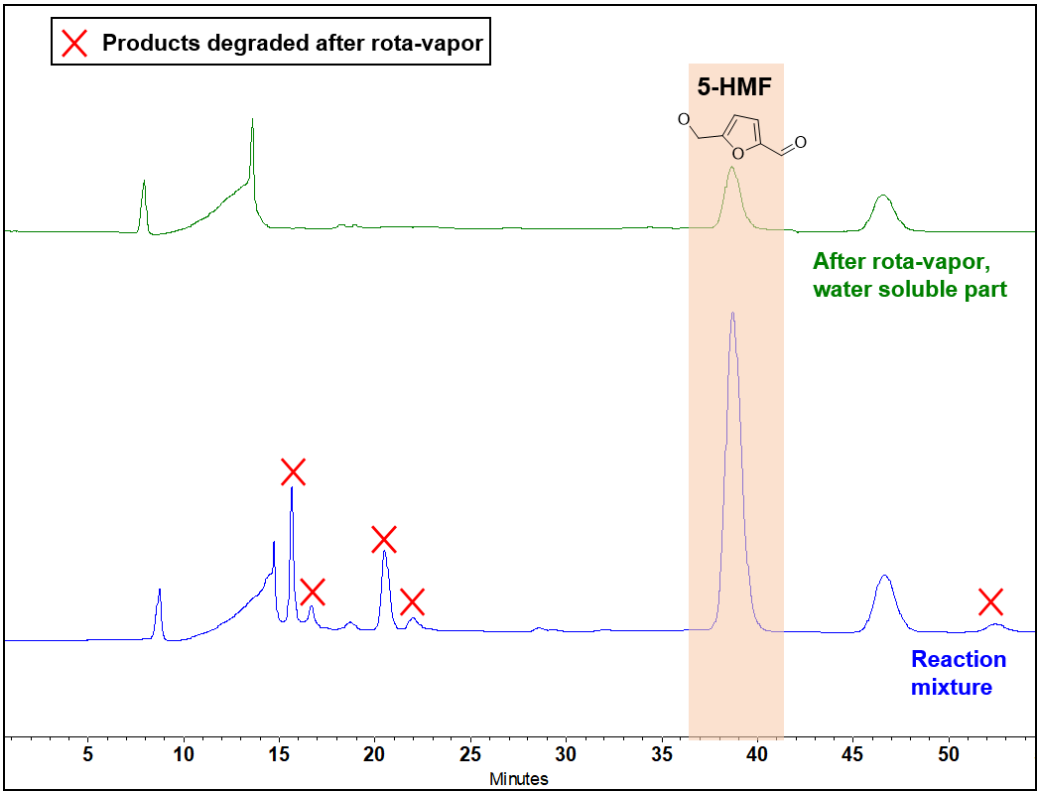


Figure 3.10. HPLC chromatographs of reaction mixture of $GlcNH_2$ and water-soluble part after rota. Reaction conditions: $GlcNH_2$ (311 µmol), SA (0.4 g), water (30 mL), 170 ˚C, 0.5 Mpa N_2 pressure at RT, 4 h

3.3.4. N-Acetyl-D-glucosamine (GlcNAc) as a substrate

Reaction with another chito-monomer, N-Acetyl-D-glucosamine was also done under same conditions (170 ˚C, 0.5 MPa N_2 pressure, 4 h) using m-SAPO-44 as a catalyst. The 5-HMF yield in the reaction with GlcNAc was only 1%, which was very surprising considering that difference between two substrates is only change at C2 position. Besides this, products formed in this reaction were different from the reactions done with $GlcNH_2$ as a substrate except peak for 5-HMF (Figure 3.11). This also suggests that although both monomers are almost similar (Except at C2 position), mechanisms followed by both these substrates are different. From these observations, it can be assumed that chitosan (hetero polymer of $GlcNH_2$ and GlcNAc) will give better 5-HMF yields than chitin (homo polymer of GlcNAc) as chitosan is composed of more than 60% deacetylated units ($GlcNH_2$).

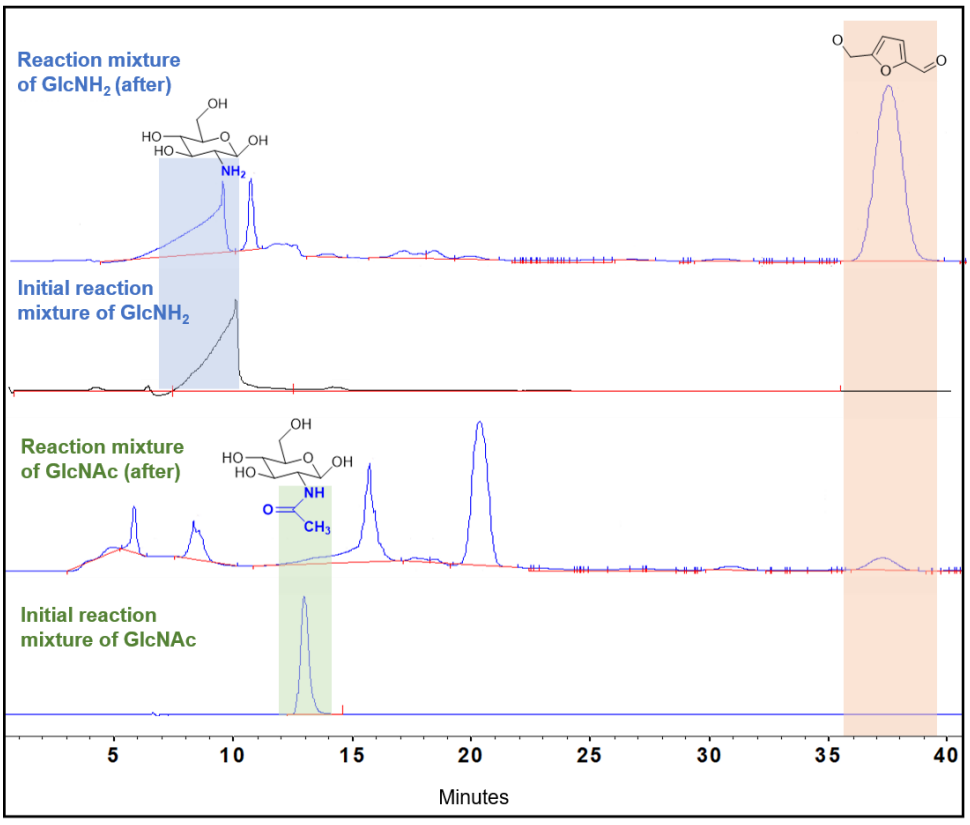


Figure 3.11. HPLC chromatograms of $GlcNH_2$ and GlcNAc catalytic reactions (Before and after reaction) Reaction conditions: $GlcNH_2$ (311 µmol)/GlcNAc (226 µmol), m-SAPO-44 (0.4 g), water (30 mL), 170 ˚C, 0.5 MPa N_2 pressure, 4 h

Possible mechanistic reason for this difference in the results is shown in Figure 3.12a. In case of $GlcNH_2$, at first, H^+ ion (reactions are done using acidic catalyst) attacks basic NH_2 and NH_3 is liberated immediately as NH_3 is known as a good leaving group. After that, stable five membered furan ring is formed which undergoes dehydration to yield 5-HMF. In case of GlcNAc, Acetamide (-$NHCOCH_3$) group is bulky and due to steric hindrance, attack of H^+ and liberation of acetamide group is not favourable. This might be the possible reason for poor yield of 5-HMF in GlcNAc reaction.

Protonation of -NH may occur better than the protonation of amides (N of amide). This is because in case of amides due to increased resonance stabilization of the O-protonated species, protonation of oxygen occurs predominantly rather than nitrogen (Figure 3.12b).[39-40] Also, it is known that amines are better leaving group than amides.

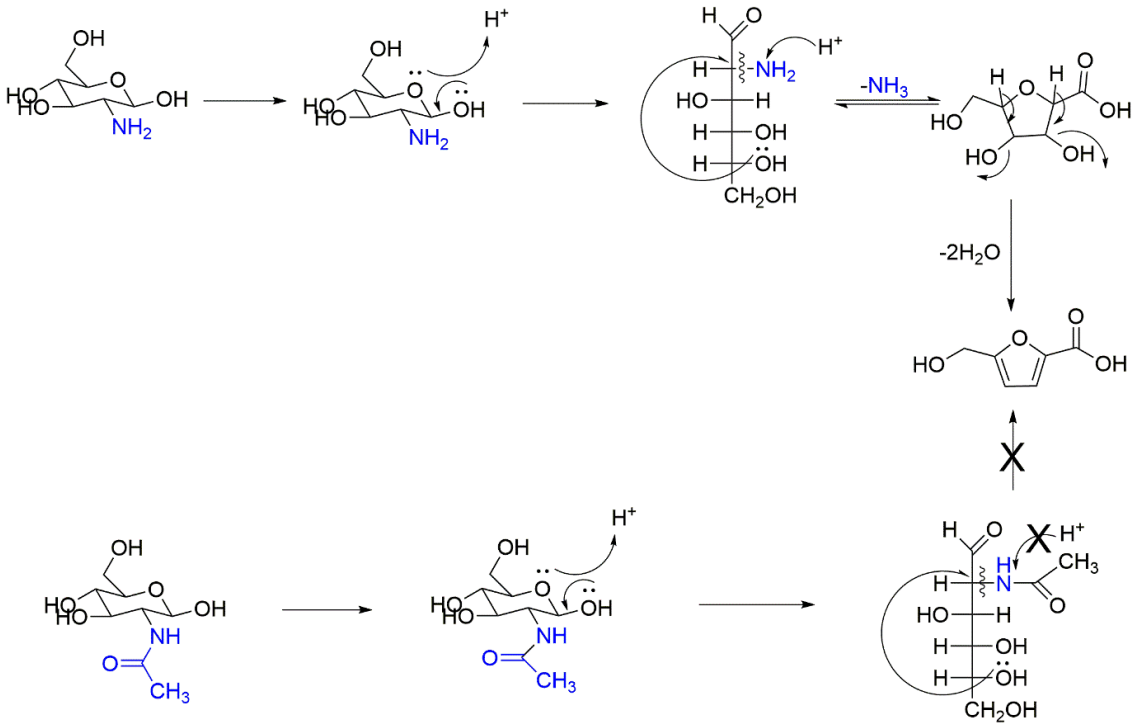


Figure 3.12a. Possible reaction mechanism for reactions with $GlcNH_2$ and GlcNAc

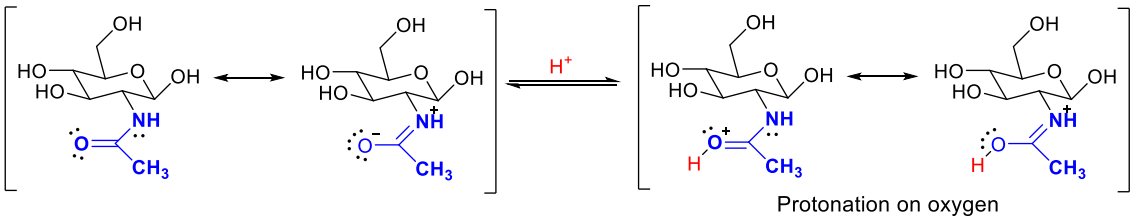


Figure 3.12b. Protonation of amide group in presence of H^+

3.3.5. Catalyst recycles study

From the spent catalyst characterization data (Figure 3.6) it is revealed that, SA catalyst is stable under reaction conditions and thus recycle study with this catalyst was carried out. After the reaction, catalyst was recovered by centrifugation, oven dried and used in the next reaction. As seen from Figure 3.13, slight increase in the yield of 5-HMF was observed. To understand this fact, adsorption study of $GlcNH_2$ and 5-HMF was performed on SA catalyst surface. In the study, 30 mL of 311 μmol $GlcNH_2$ and 310 μmol 5-HMF (taken according to 100% 5-HMF yield) were taken in two separate RBs. To it, 0.4 g SA catalyst was added and stirred for 4 h at room temperature. The mixture was filtered and injected to HPLC. It is found that 5% $GlcNH_2$ and 2% 5-HMF were being adsorbed on SA catalyst surface in comparison to initial sample (before addition of SA). This adsorption of substrate and product could be a probable reason for higher yield in 2^{nd} to 6^{th} catalytic runs. This is because, equilibrium for adsorption of substrate and product on catalyst surface is already achieved in first run and the same is still present when catalyst is used in the next run. Since, in the next runs already adsorbed substrate and product are present their contribution from that run will not reflect in the results. The elemental analysis (Table 3.5) of spent SA catalyst also revealed that ca. 4% carbon and 2% nitrogen was present on the spent catalyst. Nonetheless, most important aspect of this work is that catalyst is recyclable and showed consistent results until 6th catalytic run.

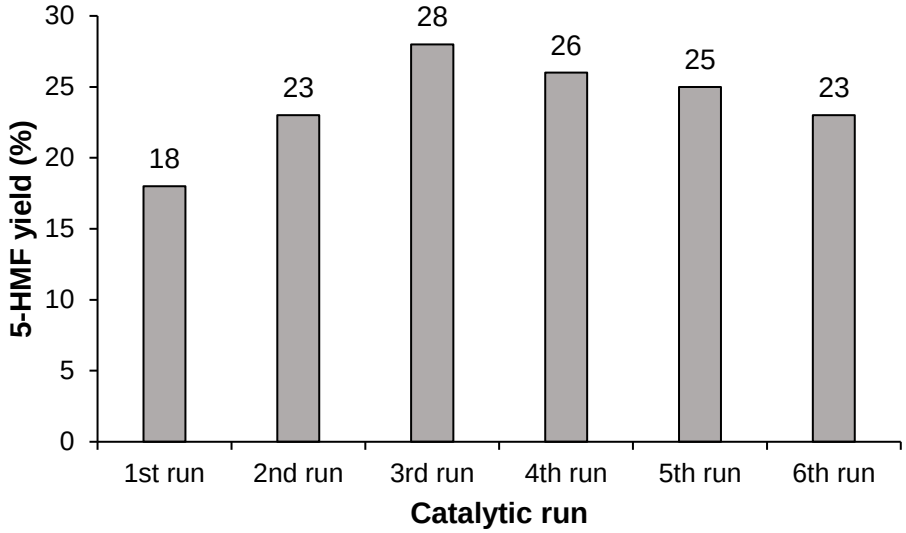


Figure 3.13. Catalyst recycle study. Reaction conditions: $GlcNH_2$, SA, S/C ratio (1.16 mole/mole), Water (30 mL), 170 °C, 0.5 MPa N_2 pressure at RT, 4 h

3.3.6. Catalyst characterization study

The XRD patterns for fresh and spent m-SAPO-44 are shown in Figure 3.6a. The peak pattern in fresh m-SAPO-44 matches well with CHA morphology. XRD pattern of spent m-SAPO-44 revealed the breaking of structure and CHA morphology is completely disturbed after reaction. Figure 3.6b shows the XRD patterns of SA. Amorphous SA shows no change in XRD pattern after reaction and hence, silica alumina is stable under reaction condition. XRD analysis of other fresh catalysts were also done (Figure 3.14) and in case of zeolites sharp peaks were observed in the XRD pattern which confirms that the materials are crystalline in nature. Nafion SAC-13 shows amorphous phase as no sharp peak is observed. The tungsten oxide on silica alumina also shows amorphous phase but some sharp peaks were observed in molybdenum oxide on silica alumina which confirms the crystalline phase in MoO_x/SA. Presence of orthorhombic α-MoO_3 crystals (JCPDS File no. 76-1003) was confirmed with its crystalline phase.

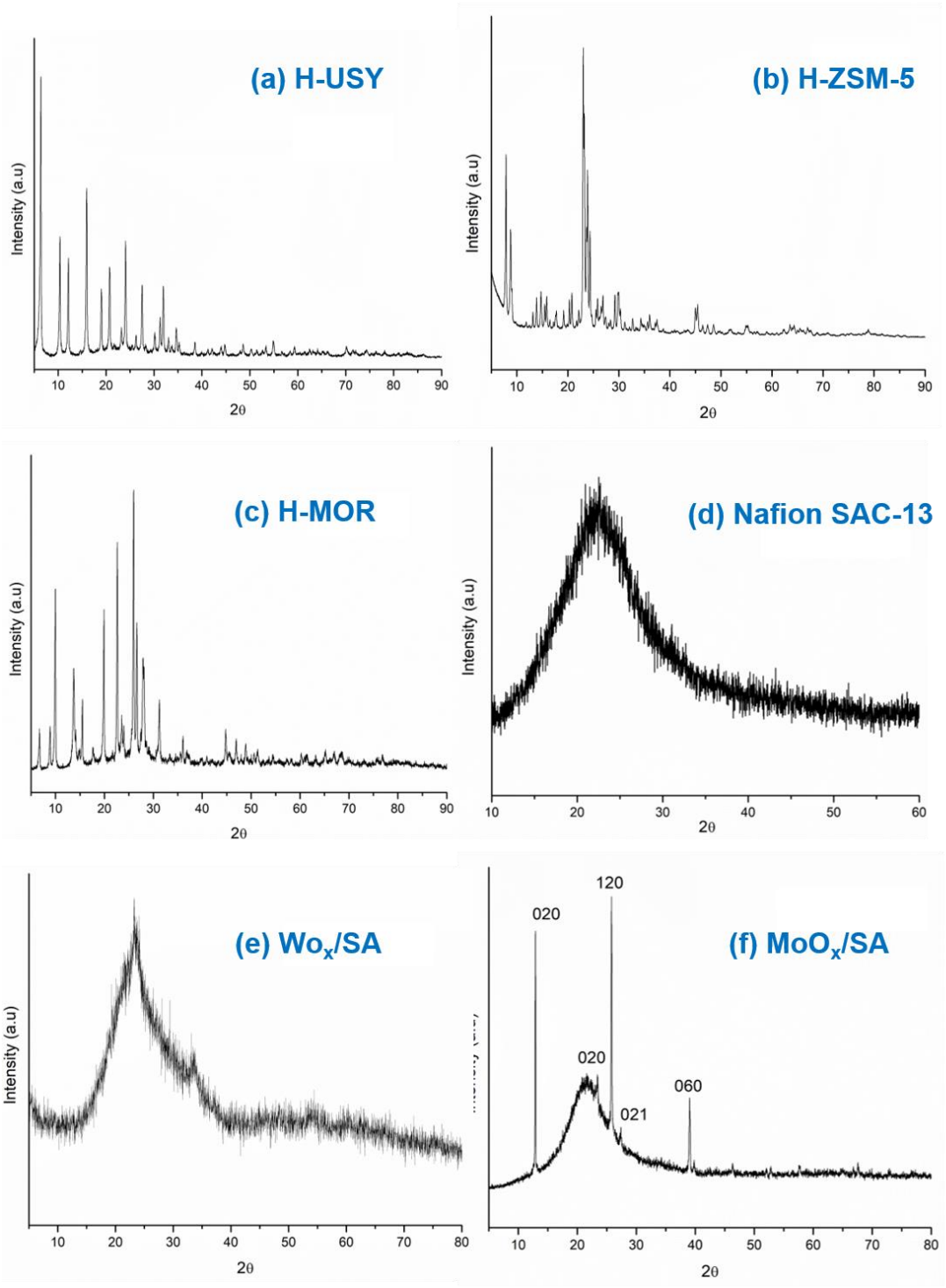


Figure 3.14. XRD patterns of structured solid acid catalysts (H-USY, H-ZSM-5, H-MOR), Nafion SAC-13, wet impregnated metal oxides (WO_x/SA, MoO_x/SA)

Results on NH_3-TPD study of fresh and spent SA and m-SAPO-44 are shown in Table 3.4. Fresh m-SAPO-44 showed best activity due to its higher acid amount than fresh SA in the 1st run. But, reduction in total acidity is observed in spent m-

SAPO-44 after 1st run due to breaking of structure and CHA morphology. On the other hand, the acidity of spent SA shows marginal decrease after 1st run which may be the reason to get better recyclability with SA.

Table 3.4. NH_3-TPD study of fresh and spent catalyst

Catalyst	Total acidity (mmol/g) of fresh catalyst	Total acidity (mmol/g) of spent catalyst
SA (Si/Al=5.3)	0.67	0.60
m-SAPO-44	1.02	0.45

Elemental analysis of fresh and spent m-SAPO-44 and SA is shown in Table 3.5. Elemental analysis of fresh m-SAPO-44 and SA shows absent of C and N but spent catalysts shown presence of C and N, which proves the adsorption of substrate and products on the catalyst surface which also supports the adsorption study data in Section 3.3.5. Absence of N in spent m-SAPO-44 revealed that substrate was not adsorbed on the m-SAPO-44 catalyst as it has smaller pore diameter than the size of $GlcNH_2$ (kinetic diameter, length, width of the substrates and product are shown in Table 3.2.)

Table 3.5. Elemental analysis of fresh and spent catalysts

Catalyst	Fresh		Spent	
	C (%)	N (%)	C (%)	N (%)
SA (Si/Al=5.3)	absent	absent	4.1	1.9
m-SAPO-44	absent	absent	1.9	absent

ICP-OES analysis data (shown in Table 3.6) of SA shows very less amount of Al, i.e, 0.25% is leached in the solution which proves the rigidity of the catalyst under reaction condition. But, in case of m-SAPO-44 comparatively more amount of Al (10.93%) is leached in the reaction mixture which also proved that the structure of m-SAPO-44 was getting disturbed during reaction.

Table 3.6. ICP-OES analysis of fresh, spent catalyst and reaction mixture (calculated w.r.t catalyst amount taken for reaction, i.e., 0.4 g)

Sample	Al content in mg (per 0.4 g)
Fresh SA (Si/Al=5.3)	36.73
Spent SA	35.24
Reaction mixture (when SA is used as catalyst	0.09
Fresh m-SAPO-44	66.98
Spent m-SAPO-44	58.68
Reaction mixture (when m-SAPO-44 is used as catalyst	7.32

Nitrogen sorption data for fresh and spent m-SAPO-44 and SA catalysts is shown in Table 3.7. Surface area of both catalysts are comparatively in similar range, however, there are differences in pore size and pore volume between these two catalysts. Higher pore radius of SA allows substrate molecules to enter and access the acid sites. On the other hand, the substrate molecule cannot enter the pore and access the acid sites as pore radius of m-SAPO-44 is lower than molecular size of $GlcNH_2$. But, the reaction with m-SAPO-44 still showed better activity which suggests that some of the protons present on m-SAPO-44 are labile and those are in the water in the form of hydronium ions. The similar phenomenon of partial proton transfer to water from Brønsted acid sites of zeolite to form hydronium ion is also proven earlier with the support of infra-red spectroscopy and quantum chemical ab initio studies.[37-38] However, due to very rapid transfer of H^+ from one water molecule to another may ultimately remove the labile protons from catalyst surface into the bulk water where bigger substrate molecules are present. These protons will then help in dehydration reaction.

Table 3.7. Summary on nitrogen sorption data of fresh and spent catalysts

Catalyst		BET surface area (m^2/g)	Pore volume (V) (cm^3/g)	Pore radius (nm)
SA (Si/Al=5.3)	Fresh	380	0.34	3.92
	Spent	144	0.29	1.93
m-SAPO-44	Fresh	337	0.10	0.41
	Spent	nd	nd	nd

3.4. Conclusion

In this work, structured catalyst m-SAPO-44 as well as amorphous catalyst SA showed good yield of 5-HMF from $GlcNH_2$. Due to morphological changes in m-SAPO-44 after 1st catalytic run, the characteristic peak of CHA morphology vanished and total acidity was also reduced. SA shows better stability under reaction condition and could be recycled up to 6th run. We have developed a solid acid based catalytic system in water as medium without the addition of any mineral aid, organic acid, high boiler solvents, metal chlorides etc. In future, more work is needed for analysis of other products formed. The complete identification of all the products requires extensive studies and those are not beyond the capabilities of our research facilities. Moreover, efforts should be put for identification of other products, which can also help to understand each step in reaction mechanism. Nevertheless, most important aspect of this work is that catalyst is recyclable and showed consistent results until 6th catalytic run.

3.5. References

1. Binder, J. B.; Raines, R. T., Simple chemical transformation of lignocellulosic biomass into furans for fuels and chemicals. *Journal of the American Chemical Society* **2009,** *131* (5), 1979-1985.

2. Gallezot, P., Conversion of biomass to selected chemical products. *Chemical Society Reviews* **2012,** *41* (4), 1538-1558.

3. Deepa, A. K.; Dhepe, P. L., Lignin depolymerization into aromatic monomers over solid acid catalysts. *ACS Catalysis* **2015,** *5* (1), 365-379.

4. Fukuoka, A.; Dhepe, P. L., Catalytic conversion of cellulose into sugar alcohols. *Angewandte Chemie International Edition* **2006,** *45* (31), 5161-5163.

5. Sahu, R.; Dhepe, P. L., A one-pot method for the selective conversion of hemicellulose from crop waste into C5 sugars and furfural by using solid acid catalysts. *ChemSusChem* **2012,** *5* (4), 751-761.

6. Yan, N.; Yuan, Y.; Dykeman, R.; Kou, Y.; Dyson, P. J., Hydrodeoxygenation of lignin-derived phenols into alkanes by using nanoparticle catalysts combined with brønsted acidic ionic liquids. *Angewandte Chemie* **2010,** *122* (32), 5681-5685.

7. Yan, N.; Zhao, C.; Luo, C.; Dyson, P. J.; Liu, H.; Kou, Y., One-step conversion of cellobiose to C6-alcohols using a ruthenium nanocluster catalyst. *Journal of the American Chemical Society* **2006,** *128* (27), 8714-8715.

8. Omari, K. W.; Besaw, J. E.; Kerton, F. M., Hydrolysis of chitosan to yield levulinic acid and 5-hydroxymethylfurfural in water under microwave irradiation. *Green Chemistry* **2012,** *14* (5), 1480.

9. Bhaumik, P.; Dhepe, P. L., Influence of properties of SAPO's on the one-pot conversion of mono-, di-and poly-saccharides into 5-hydroxymethylfurfural. *RSC advances* **2013,** *3* (38), 17156-17165.

10. Peleteiro, S.; Rivas, S.; Alonso, J. L.; Santos, V.; Parajó, J. C., Furfural production using ionic liquids: A review. *Bioresource technology* **2016,** *202*, 181-191.

11. Bhaumik, P.; Dhepe, P. L., Solid acid catalyzed synthesis of furans from carbohydrates. *Catalysis Reviews* **2016,** *58* (1), 36-112.

12. Agarwal, B.; Kailasam, K.; Sangwan, R. S.; Elumalai, S., Traversing the history of solid catalysts for heterogeneous synthesis of 5-hydroxymethylfurfural from carbohydrate sugars: A review. *Renewable and Sustainable Energy Reviews* **2018,** *82*, 2408-2425.

13. Delbecq, F.; Wang, Y.; Muralidhara, A.; El Ouardi, K.; Marlair, G.; Len, C., Hydrolysis of hemicellulose and derivatives—A review of recent advances in the production of furfural. *Frontiers in chemistry* **2018,** *6*, 146.

14. Holladay, J.; Bozell, J.; White, J.; Johnson, D., Top value-added chemicals from biomass. *DOE Report PNNL* **2007,** *16983*.

15. Zhou, D.; Shen, D.; Lu, W.; Song, T.; Wang, M.; Feng, H.; Shentu, J.; Long, Y., Production of 5-hydroxymethylfurfural from chitin biomass: A review. *Molecules* **2020,** *25* (3), 541.

16. Chen, X.; Chew, S. L.; Kerton, F. M.; Yan, N., Direct conversion of chitin into a N-containing furan derivative. *Green Chem.* **2014,** *16* (4), 2204-2212.

17. Mascal, M.; Nikitin, E. B., Dramatic advancements in the saccharide to 5-(chloromethyl)furfural conversion reaction. *ChemSusChem* **2009,** *2* (9), 859-61.

18. Lee, S. B.; Jeong, G. T., Catalytic Conversion of Chitosan to 5-Hydroxymethylfurfural Under Low Temperature Hydrothermal Process. *Appl Biochem Biotechnol* **2015,** *176* (4), 1151-61.

19. Park, M.-R.; Kim, H. S.; Kim, S.-K.; Jeong, G.-T., Thermo-chemical conversion for production of levulinic and formic acids from glucosamine. *Fuel Processing Technology* **2018,** *172*, 115-124.

20. Wang, Y.; Pedersen, C. M.; Deng, T.; Qiao, Y.; Hou, X., Direct conversion of chitin biomass to 5-hydroxymethylfurfural in concentrated ZnCl2 aqueous solution. *Bioresour Technol* **2013,** *143*, 384-90.

21. Yu, S.; Zang, H.; Chen, S.; Jiang, Y.; Yan, B.; Cheng, B., Efficient conversion of chitin biomass into 5-hydroxymethylfurfural over metal salts catalysts in dimethyl sulfoxide-water mixture under hydrothermal conditions. *Polymer Degradation and Stability* **2016,** *134*, 105-114.

22. Kim, H. S.; Kim, S.-K.; Jeong, G.-T., Efficient conversion of glucosamine to levulinic acid in a sulfamic acid-catalyzed hydrothermal reaction. *RSC advances* **2018,** *8* (6), 3198-3205.

23. Feng, J. X.; Zang, H. J.; Yan, Q.; Li, M. G.; Cheng, B. W. In *Conversion of chitosan into 5-hydroxymethylfurfural via hydrothermal synthesis*, Advanced Materials Research, Trans Tech Publ: 2015; pp 411-414.

24. Li, M.; Zang, H.; Feng, J.; Yan, Q.; Yu, N.; Shi, X.; Cheng, B., Efficient conversion of chitosan into 5-hydroxymethylfurfural via hydrothermal synthesis in ionic liquids aqueous solution. *Polymer Degradation and Stability* **2015,** *121*, 331-339.

25. Zang, H.; Yu, S.; Yu, P.; Ding, H.; Du, Y.; Yang, Y.; Zhang, Y., Hydrothermal conversion of N-acetyl-d-glucosamine to 5-hydroxymethylfurfural using ionic liquid as a recycled catalyst in a water-dimethyl sulfoxide mixture. *Carbohydrate research* **2017,** *442*, 1-8.

26. Kalane, N. D.; Krishnan, R. A.; Yadav, V. D.; Jain, R.; Dandekar, P., Synergistic effect of hetero- and homo-catalysts on the 'green' synthesis of 5-hydroxymethylfurfural from chitosan biomass. *Cellulose* **2019,** *26* (4), 2805-2819.

27. Tzeng, T.-W.; Bhaumik, P.; Chung, P.-W., Understanding the production of 5-hydroxymethylfurfural (HMF) from chitosan using solid acids. *Molecular Catalysis* **2019,** *479*, 110627.

28. Bhaumik, P.; Dhepe, P. L., Efficient, stable, and reusable silicoaluminophosphate for the one-pot production of furfural from hemicellulose. *ACS Catalysis* **2013,** *3* (10), 2299-2303.

29. Prakash, A.; Unnikrishnan, S.; Rao, K., Synthesis and characterization of silicon-rich SAPO-44 molecular sieves. *Applied Catalysis A: General* **1994,** *110* (1), 1-10.

30. Bhaumik, P.; Kane, T.; Dhepe, P. L., Silica and zirconia supported tungsten, molybdenum and gallium oxide catalysts for the synthesis of furfural. *Catalysis Science & Technology* **2014,** *4* (9), 2904-2907.

31. Wang, H.; Frenklach, M., Transport properties of polycyclic aromatic hydrocarbons for flame modeling. *Combustion and flame* **1994,** *96* (1-2), 163-170.

32. commission, I. s. Database of zeolite structures. https://asia.iza-structure.org/IZA-SC/ftc_table.php.

33. Catalyst, D. A. D. P. https://www.dupont.com/content/dam/dupont/amer/us/en/water-solutions/public/documents/en/45-D00927-en.pdf.

34. information, M. p. https://www.sigmaaldrich.com/IN/en/product/aldrich/474541.

35. Liu, Y.; Lotero, E.; Goodwin Jr, J. G., A comparison of the esterification of acetic acid with methanol using heterogeneous versus homogeneous acid catalysis. *Journal of Catalysis* **2006,** *242* (2), 278-286.

36. Capello, C.; Fischer, U.; Hungerbühler, K., What is a green solvent? A comprehensive framework for the environmental assessment of solvents. *Green Chemistry* **2007,** *9* (9), 927-934.

37. Nascimento, M., *Theoretical aspects of heterogeneous catalysis*. Springer Science & Business Media: 2006; Vol. 8.

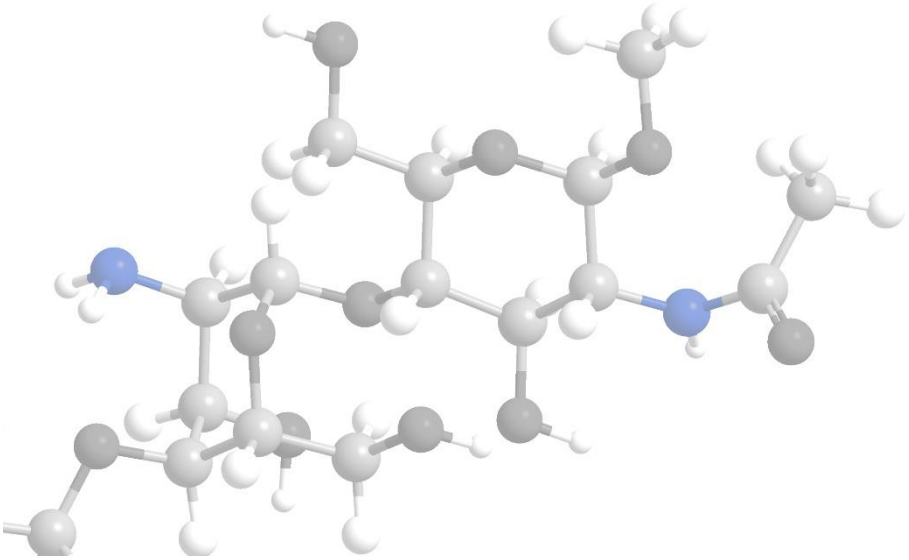
38. Sauer, J.; Horn, H.; Häser, M.; Ahlrichs, R., Formation of hydronium ions on Brønsted sites in zeolitic catalysts: a quantum-chemical ab initio study. *Chemical physics letters* **1990,** *173* (1), 26-32.

39. Morgan, J.; Greenberg, A.; Liebman, J. F., Paradigms and paradoxes: O-and N-protonated amides, stabilization energy, and resonance energy. *Structural Chemistry* **2012,** *23* (1), 197-199.

40. Cho, S. J.; Cui, C.; Lee, J. Y.; Park, J. K.; Suh, S. B.; Park, J.; Kim, B. H.; Kim, K. S., N-Protonation vs O-Protonation in Strained Amides: Ab Initio Study. *The Journal of Organic Chemistry* **1997,** *62* (12), 4068-4071.

Chapter 04

Understanding commercial chitin and chitosan samples through physico-chemical characterizations



4.1. Introduction

Chitin is a homo-polymer made up of β-1,4 linked N-acetyl-D-glucosamine (GlcNAc) units. It is 2nd abundant natural polysaccharide which is discovered in 1811 by a French professor Henri Braconnot.[1] Chitin occurs naturally in the exoskeleton of arthropods or cell walls of fungi and yeast.[2] Commercially, chitin is extracted from crustaceans by demineralization upon acid treatment (removal of calcium carbonate) followed by deproteinization with base treatment.[3] Chitin occurs as two polymorphs in nature, namely α-chitin and β-chitin.[3-4] α-chitin is most abundant and found in fungal and yeast cells as well as crab, lobster, shrimp shells. α-chitin has an antiparallel arrangement of chains which leads to intensive inter and intra-hydrogen bonding between -OH and H groups of several units. β-chitin is mainly found in squid or loligo pens and exhibits weaker hydrogen bonding network due to parallel arrangement of chains.[5-6] The structural differences between α-chitin and β-chitin can be studied using different techniques like infrared spectroscopy and ^{13}C NMR spectroscopy.[7] Due to structural rigidity of chitin, it is insoluble in most solvents which limits its utilization. Chitin is soluble only in some environment unfriendly solvents like hexafluoro isopropanol, hexafluoroacetone sesquihydrate, methanesulfonic acid, inorganic salts such as LiCNS, Ca $(CNS)_2$, CaI_2, $CaCl_2$, concentrated mineral acids like trichloroacetic acid, dichloroacetic acid, mineral bases like NaOH. Moreover, Lithium Chloride (LiCl) can form a complex with chitin which is soluble in dimethylacetamide (DMAc), N,N-dimethylpropionamide (NMP) and 1,3-dimethyl-2-imidazolldione. Hence chitin can be solubilised in above mentioned binary mixtures through complex formation with LiCl.[8] Chitosan is derived from chitin by deacetylation using base, with a minimum deacetylation of 70%. Thus, chitosan is a heteropolymer made up of β-1,4 linked D-Glucosamine ($GlcNH_2$) and N-acetyl-D-glucosamine (GlcNAc) units.[9] Chemical modification to chitin is done to produce chitosan which is more utilizable derivative.[9] Utilization and valorisation of chitin and chitosan has drawn attention in past decades due to its

availability and unique structural properties as it contains 7% biologically fixed nitrogen. Presence of nitrogen makes it different from other biomass sources. The conversion of chitin and chitosan to value added chemicals seems to be important as the area is not much explored earlier.[10] Structural difference of chitin and chitosan can be studied using different analytical techniques. As the source and the extraction methods can be different for the chitosan and chitin samples, structural differences can be found in the different chitin and chitosan samples. Literature also suggests that the molecular weight, structure, crystallinity, ash content etc. can be varied from source to source.[11-12] Therefore, it is very important to characterize the various chitosan and chitin samples systematically before starting the depolymerisation study.

4.2. Experimental section

4.2.1. Materials

Eight chitosan and two chitin samples which have different physico-chemical properties were procured from different suppliers and used for the depolymerisation study. The details of these substrates are summarized in Table 4.1. All the substrates were used for as received without any pre-treatment.

Table 4.1. Summary on substrate used for depolymerisation study

S. No.	Chemicals	Sample code	Supplier	Product Code	Viscosity (cP) given by suppliers
1.	Aldrich low molecular weight chitosan	ALMWC	Sigma-Aldrich	448869	20-300* [13]
2.	Aldrich medium molecular weight chitosan	AMMWC	Sigma-Aldrich	448877	200-800* [14]

3.	Aldrich high molecular weight chitosan	AHMWC	Sigma-Aldrich	419419	800-2000* [15]
4.	TCI chemicals low molecular weight chitosan	TCLMWC	TCI Chemicals	C2395	5-20** [16]
5.	TCI chemicals medium molecular weight chitosan	TCMMWC	TCI Chemicals	C2396	20-100** [17]
6.	TCI chemicals high molecular weight chitosan	TCHMWC	TCI Chemicals	C0831	200-600** [18]
7.	Aldrich shrimp shell chitosan	ASSC	Sigma-Aldrich	417963	>200* [19]
8.	Aldrich crab shell chitosan	ACSC	Sigma-Aldrich	48165	-
9.	TCI chemicals chitin	TCC	TCI Chemicals	C0072	-
10.	Loba Chemie chitin	LCC	Loba Chemie	02695	-

*Measured for 1 *wt.*% substrate in 1% acetic acid at 25°C.

**Measured for 0.5 *wt*.% substrate in 0.5% acetic acid at 20°C.

It was believed that there may be difference in source and extraction procedure of chitosan and chitin samples as they are procured from different suppliers and they also have different viscosity ranges. Therefore, all the procured chitosan and chitin samples were thoroughly characterized using various physico-chemical techniques.

4.2.2. Characterization techniques

Understanding the properties of substrate samples is necessary before starting the depolymerization experiments. Therefore, all the chitosan and chitin samples were thoroughly characterized using various techniques like XRD, SEM-EDAX analysis, TG-DTA, CHNS elemental analysis, ash content measurement, ICP-OES analysis, ATR, 13C NMR, viscosity measurement, etc.

4.3. Results and discussions

4.3.1. X-Ray Diffraction (XRD) analysis

It is well known that XRD, based on wide-angle elastic scattering of X-rays, has been the most important analytical technique to determine the structure of any material. The XRD patterns are obtained by the measurements of the angles at which X-ray beam is diffracted by the sample. From the Bragg's equation, distance between two *hkl* planes (*d*) and and angle of diffraction (2θ) can be related as, $n\lambda = 2dsin\theta$, where λ= wavelength of X-rays, n = an integer known as the order of the reflection (*h, k, l* represent Miller indices of the respective plane). The uniqueness of the structures, phase, degree of crystallinity, and unit cell parameters can be determined from the diffraction patterns. XRD analysis was performed for different chitosan and chitin samples to know their morphology (Figure 4.1). The powder XRD of chitosan and chitin samples were performed in PAN analytical X'pert Pro, Netherlands, with a dual goniometer diffractor. The

source of X-rays was Cu Kα (1.5418 Å) radiation with Ni filter. Scanning was done from 2θ value 5° to 90° at the scanning rate of 4° /min.

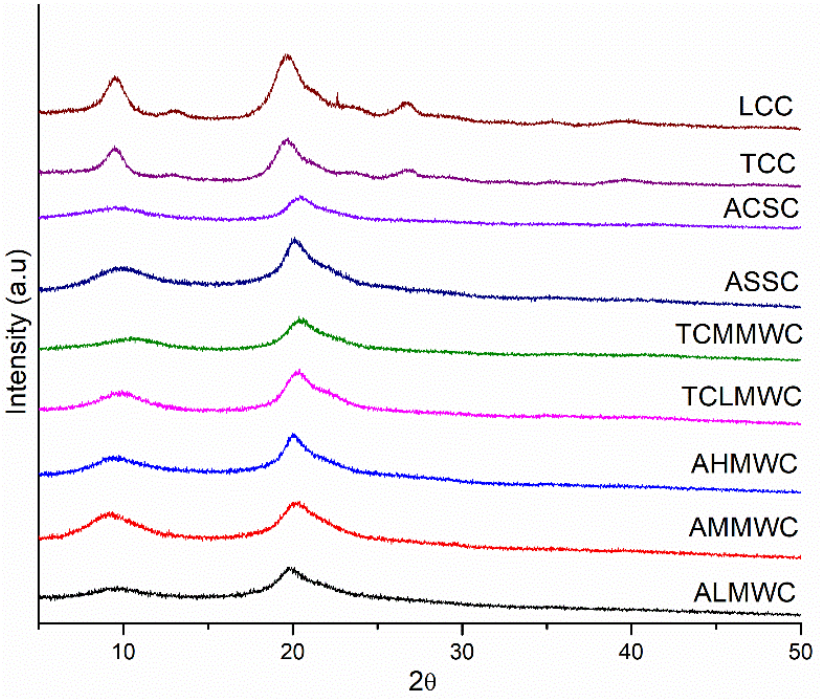


Figure 4.1. XRD pattern of chitin and chitosan samples

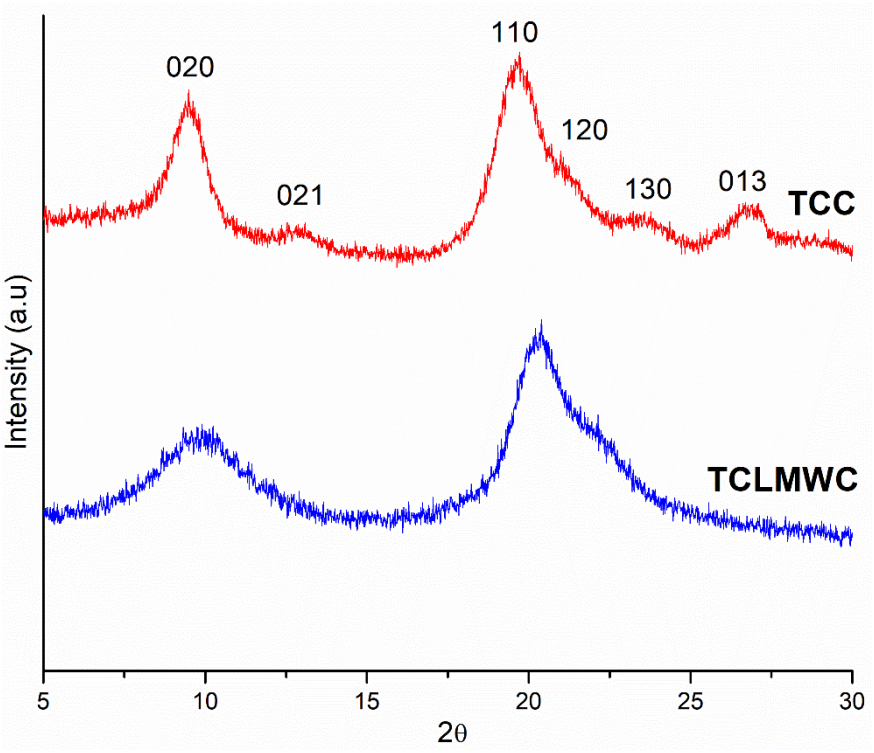


Figure 4.2. Comparative XRD pattern of TCC (chitin) and TCLMWC (chitosan)

A careful look at the XRD pattern of chitin (Figure 4.2) depicts six diffraction peaks. at 9.5°, 12.9°, 19.7°, 21.1°, 23.5°, 26.8° are present. These peaks indexed as (020), (021), (110), (120), (130) and (013) respectively. The presence of these intense diffraction peaks in both TCC and LCC proves the α-chitin structure, which is confirmed from previous literature reports.[20-22] α-chitin is more crystalline polymorph because of its antiparallel arrangement.[12] Figure. 4.2 also reveals a decrease in intensity of diffraction peaks of chitosan. Some of the peaks disappeared completely. Literature also suggests the decrease in crystallinity of chitosan.[23] The decline in crystallinity is due to NaOH treatment during deacetylation of chitin to chitosan which leads to breakage of interchain H-bonds.[24]

4.3.2. Scanning Electron Microscopy-Energy Dispersive X-Ray Analysis (SEM-EDAX)

Crude shell waste contains calcium carbonate ($CaCO_3$) and protein in its structure along with chitin. $CaCO_3$ is removed through demineralization process with the treatment of hydrochloric acid (HCl) and then sodium hydroxide (NaOH) is used in deproteinization process. Furthermore, chitin is deacetylated to produce chitosan by NaOH treatment. So, there is a possibility that traces of Ca, Na may be present in the chitosan and chitin samples. To confirm the elements present in chitosan and chitin samples, SEM-EDAX technique is used. In this technique, sample is irradiated with a source of X-ray, which ejects an electron present in the inner shell and creates a hole. When an electron from a higher energy shell fills this hole, energy is released in the form of X-rays. The generated X-rays are recorded, which is characteristic for particular element. The SEM micrographs of the samples were recorded on a Leo Leica Cambridge UK Model Stereoscan 440 scanning electron microscope, with an electron beam of 5-50 eV. Chitosan and chitin samples were mounted on carbon tapes and were analysed. SEM Images (Figure 4.3a and 4.3b) shows non-homogeneous, non-smooth surface of chitosan. There is difference in appearance between two low molecular weight chitosan

(ALMWC and TCLMWC) though the magnification is same. Same trend is also observed in medium and high molecular weight chitosan from two different suppliers.

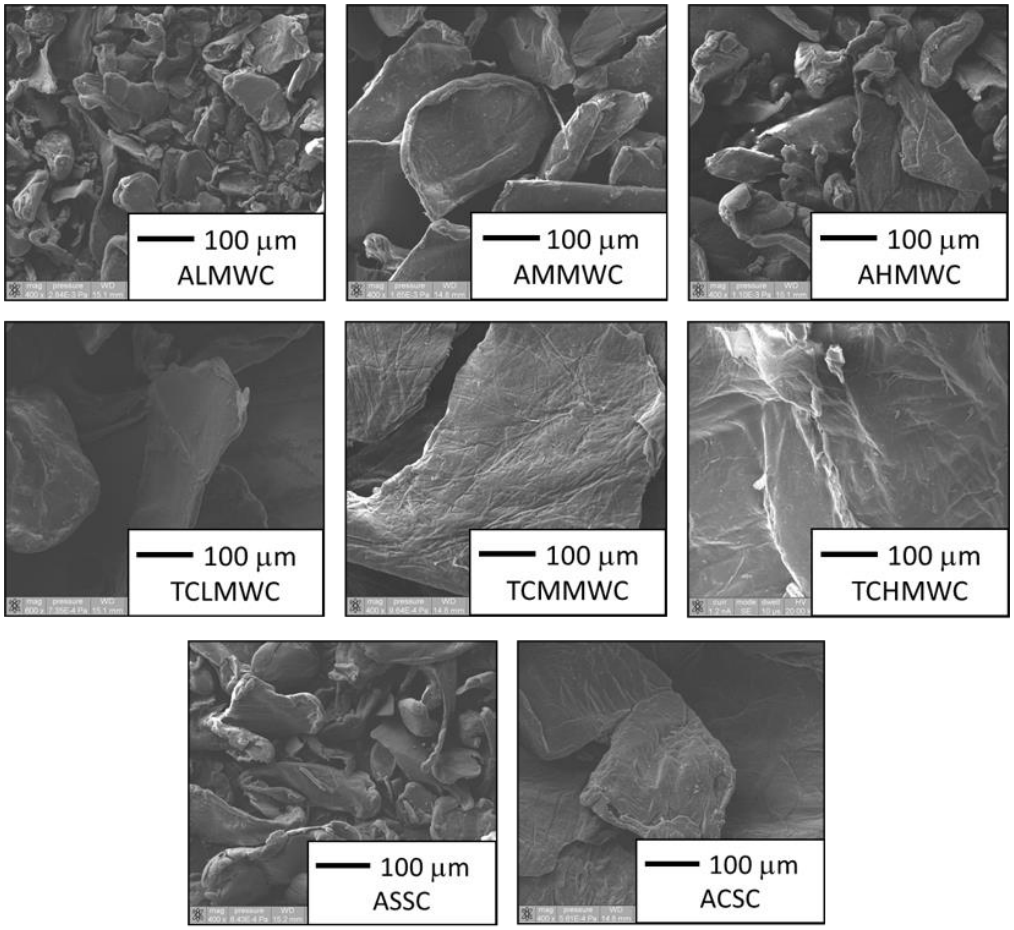


Figure 4.3a. SEM Images of different chitosan samples

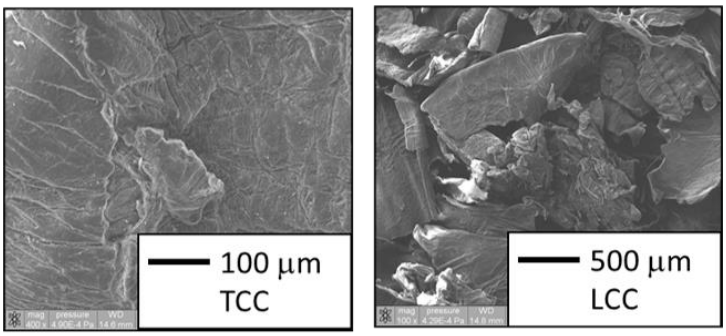


Figure 4.3b. SEM images of chitin samples

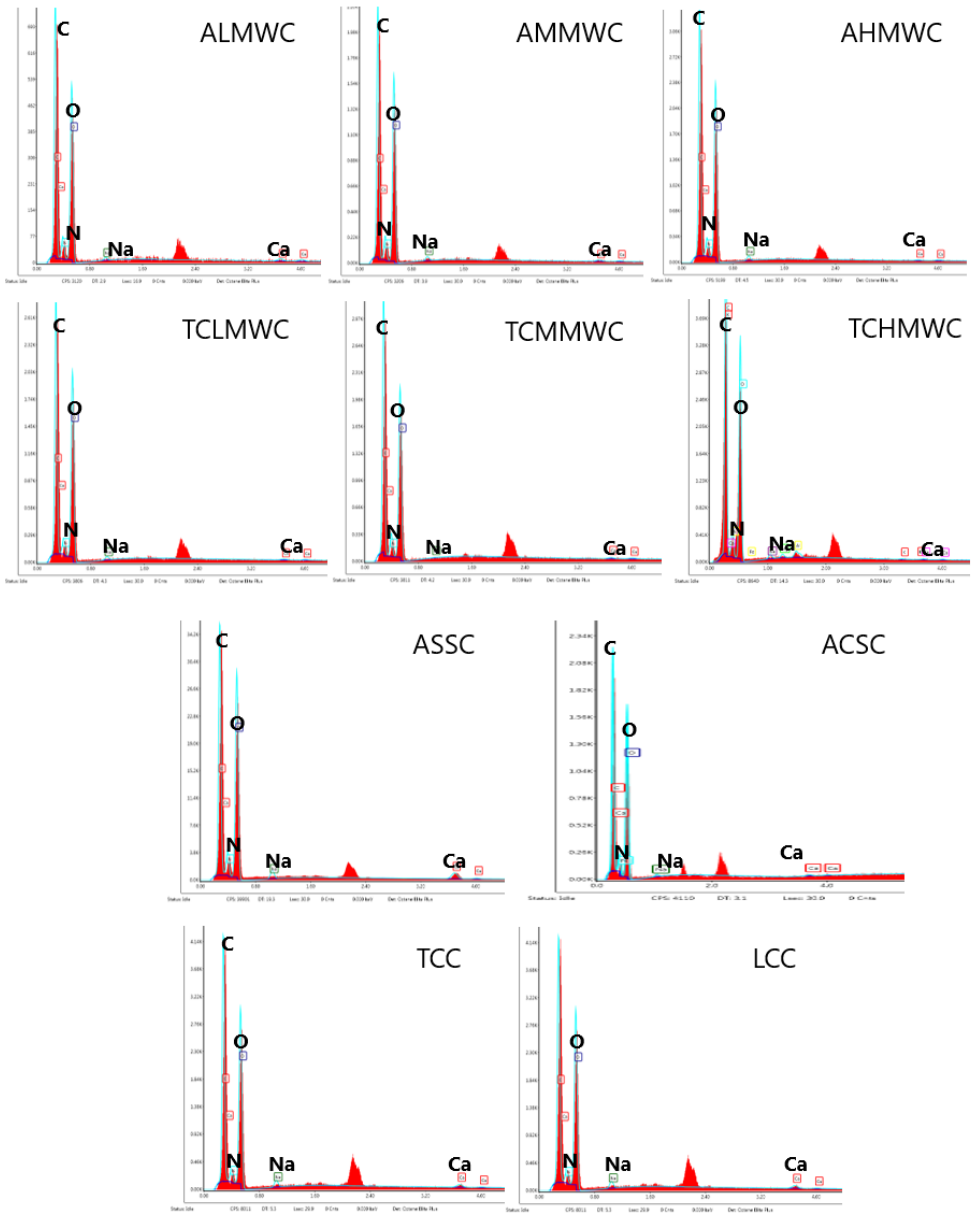


Figure 4.4. EDAX of different chitin and chitosan

EDAX analysis (Figure 4.4) shows presence of Ca and Na along with C, N, O. This proves that Ca is remaining after demineralization step and presence of Na can be attributed to the use of NaOH at deproteinization and deacetylation step.

4.3.3. Thermo Gravimetric Analysis – Differential Thermal Analysis (TGA-DTA)

The change in weight of the sample is measured as a function of temperature or time in TGA-DTA analysis. Loss of water, solvent loss, pyrolysis, decarboxylation, decomposition happening at these temperatures, and the weight % of ash

(unburnt residue) can be analysed by TGA-DTA analysis. TGA instrument consists of a sample pan that is supported by a precision balance. The pan is kept inside a furnace which is heated and cooled during the experiment. The mass of the sample is monitored during the experiment. Purge gas (nitrogen or air) is used to control the sample environment. TGA-DTA analysis was done using METTLER TOLEDO TGA/SDTA85a series, USA instrument. To study the thermal decomposition of chitosan and chitin TGA-DTA was performed with a temperature range from 25 ˚C to 1000 ˚C with a heating rate of 10 ˚C/min. Thermal degradation of chitosan and chitin samples was studied in N_2 and air atmosphere. TGA-DTA analysis for all the chitosan and chitin samples were shown in Figure. 4.5a to Figure. 4.5j.

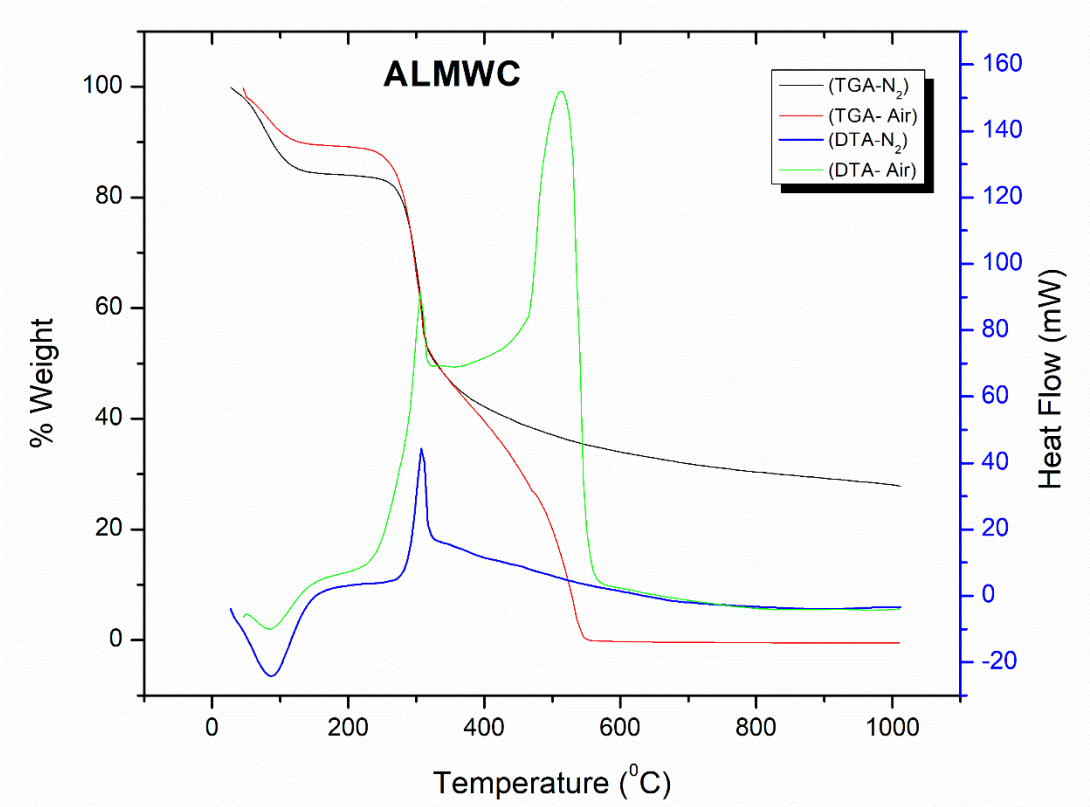


Figure 4.5a. TGA-DTA analysis of ALMWC

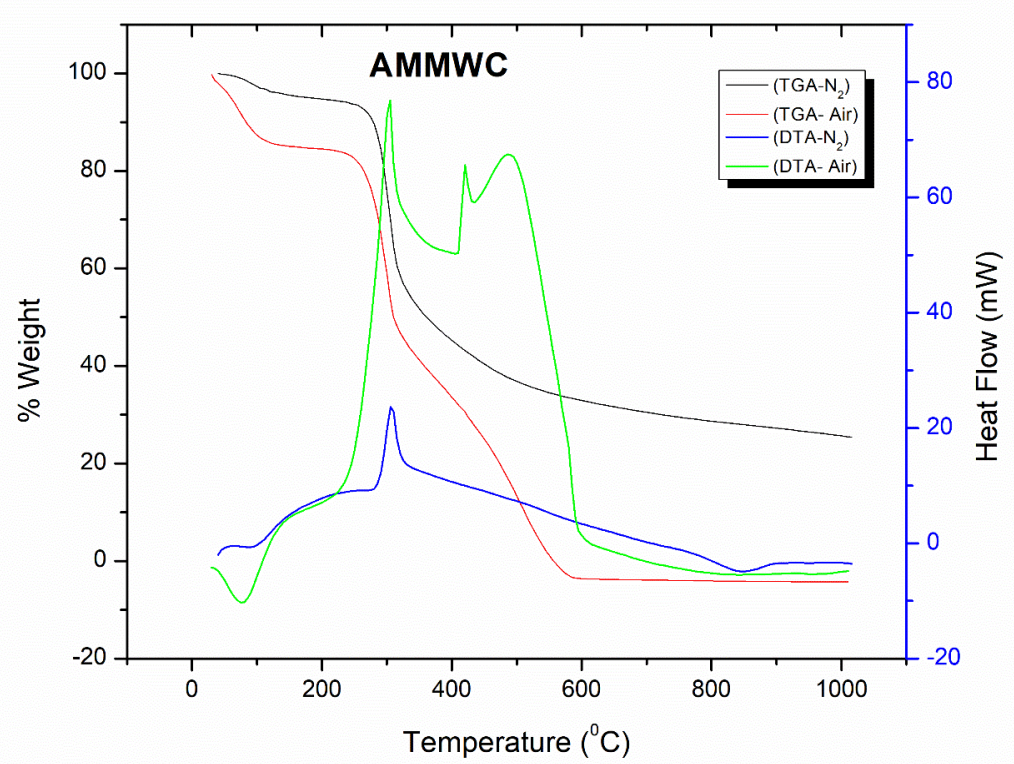


Figure 4.5b. TGA-DTA analysis of AMMWC

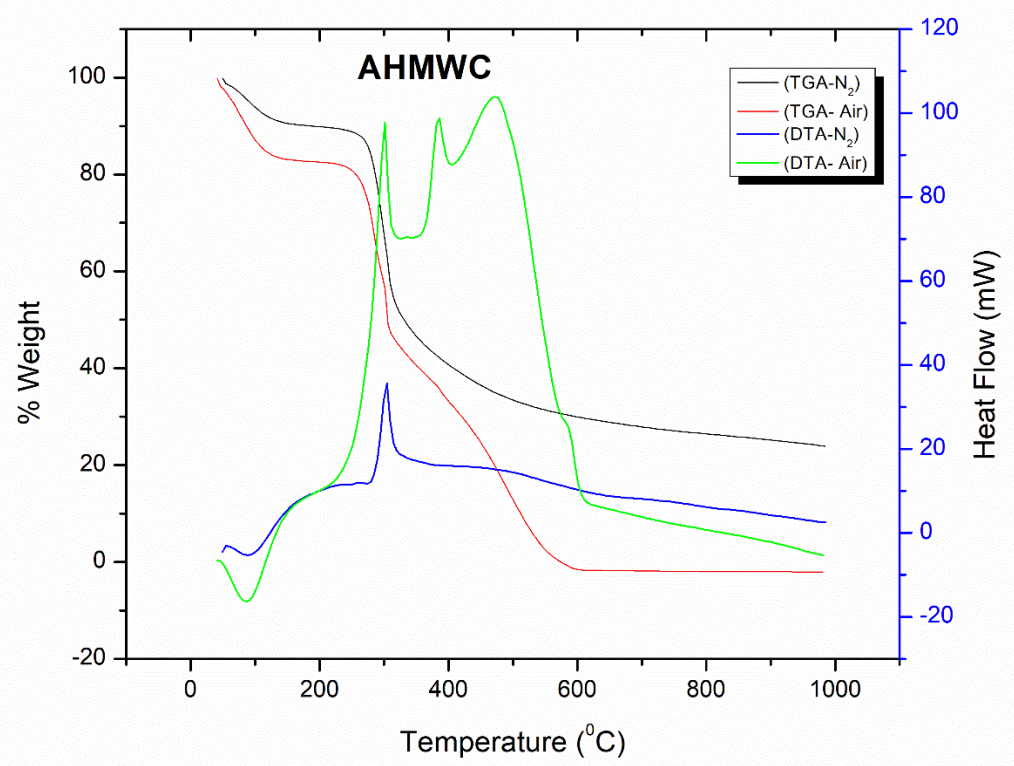


Figure 4.5c. TGA-DTA analysis of AHMWC

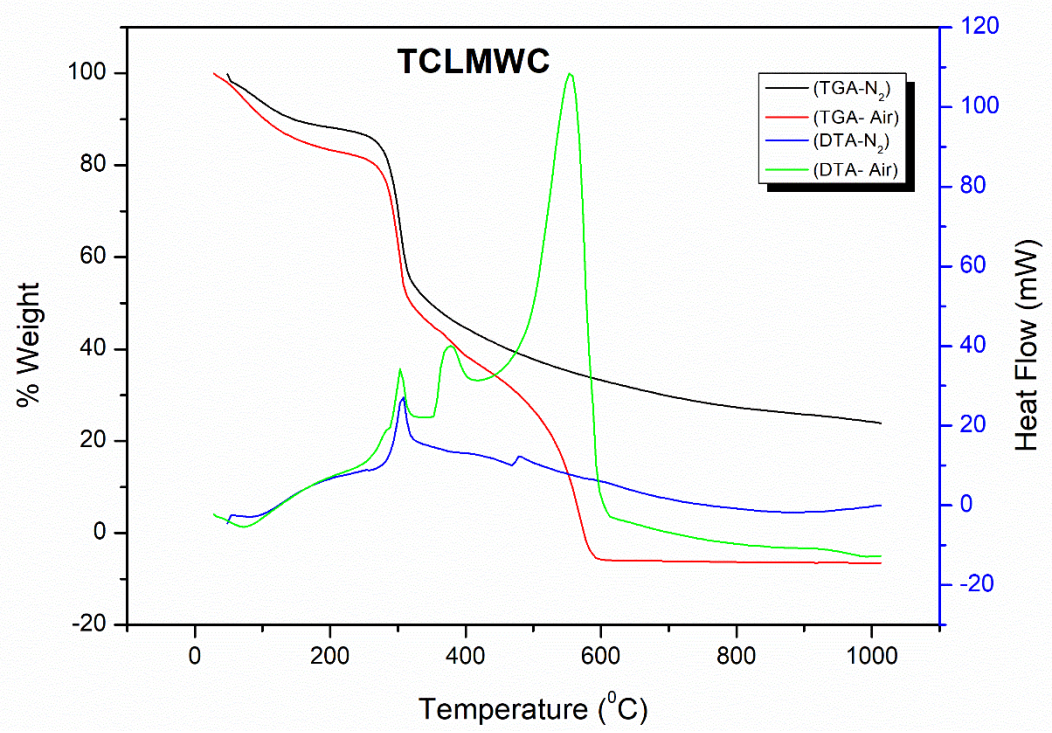


Figure 4.5d. TGA-DTA analysis of TCLMWC

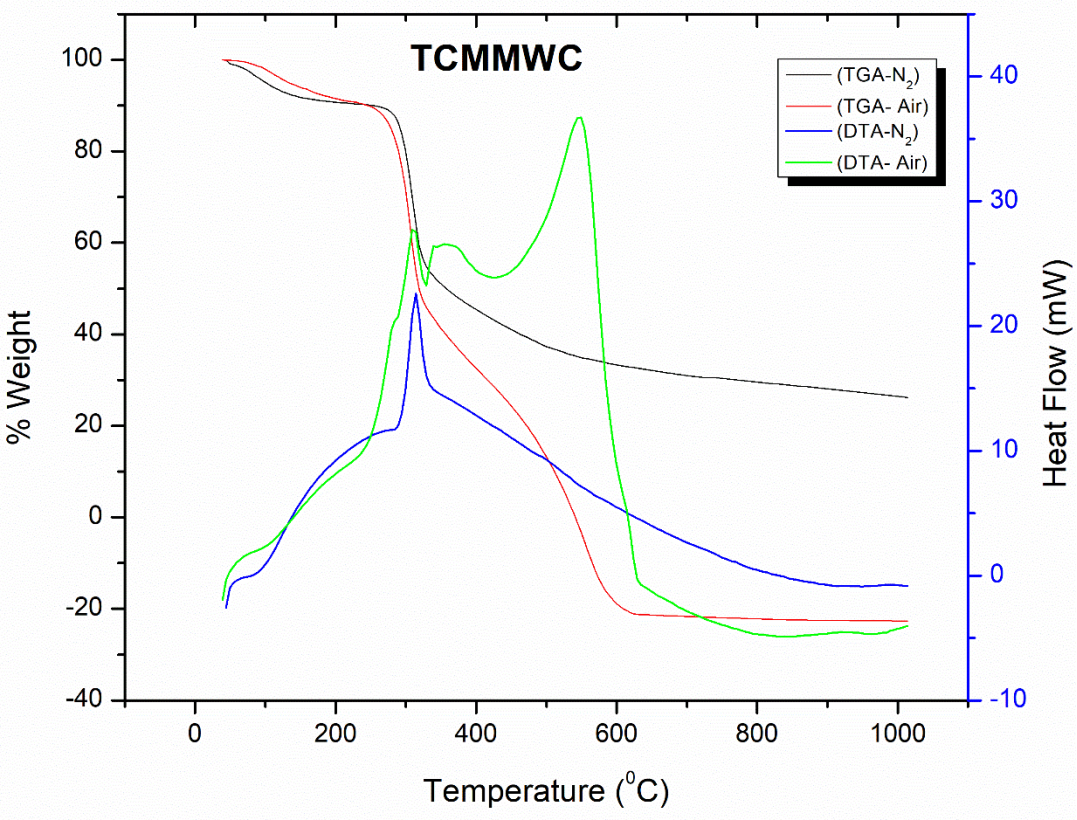


Figure 4.5e. TGA-DTA analysis of TCMMWC

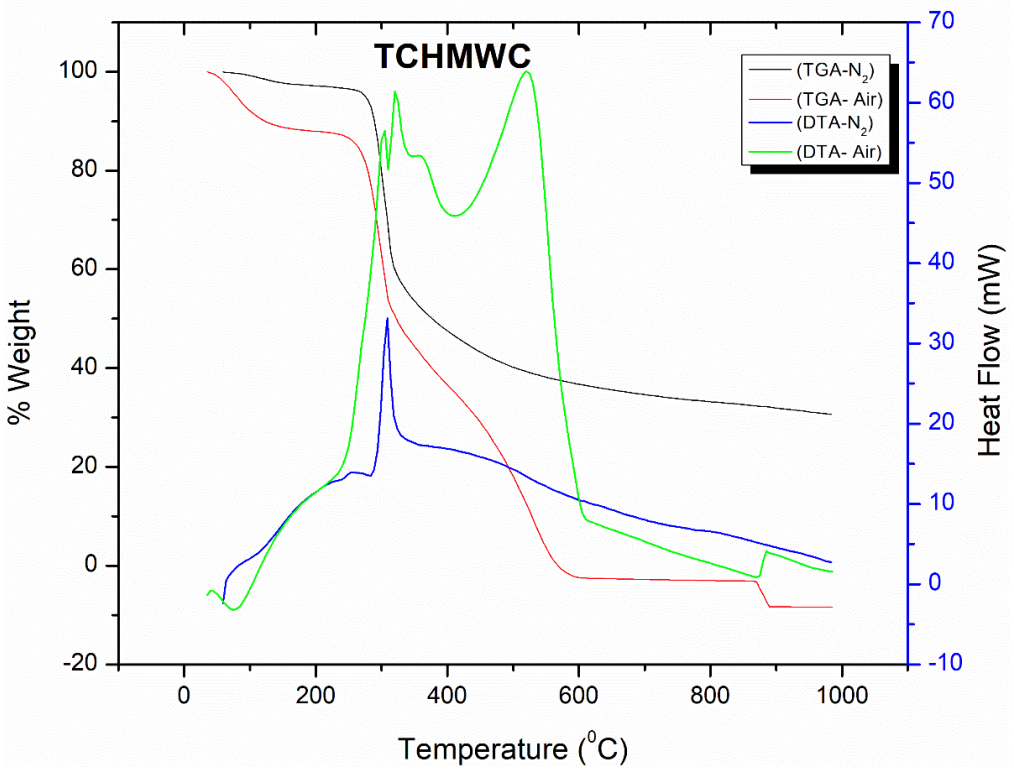


Figure 4.5f. TGA-DTA analysis of TCHMWC

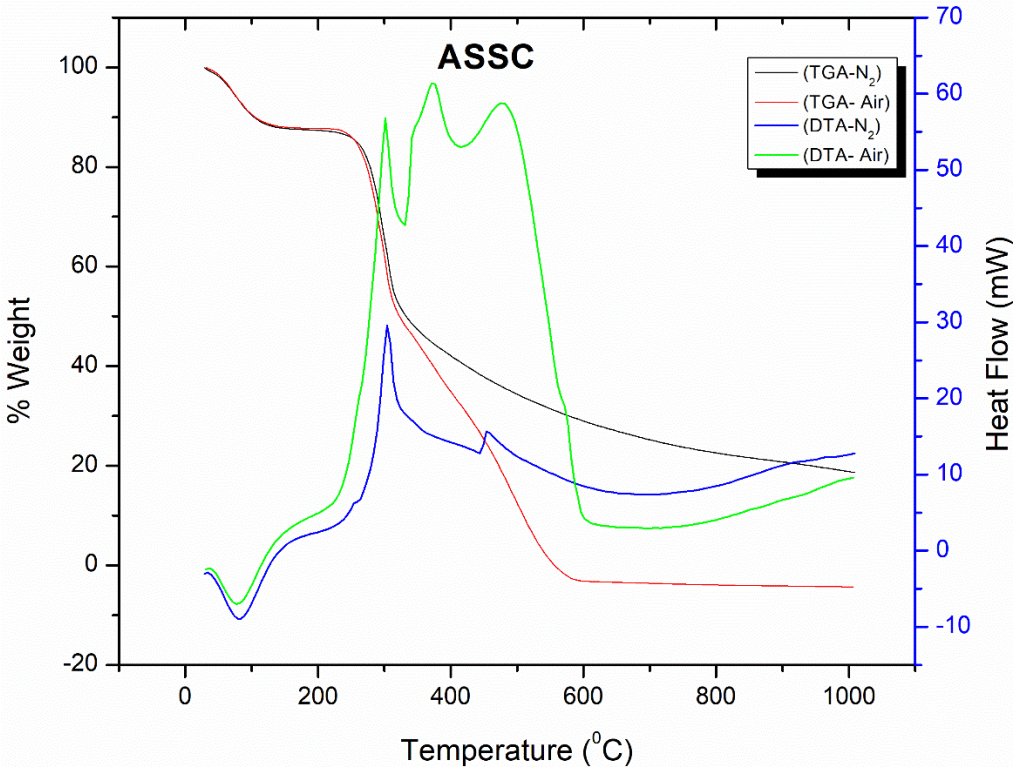


Figure 4.5g. TGA-DTA analysis of ASSC

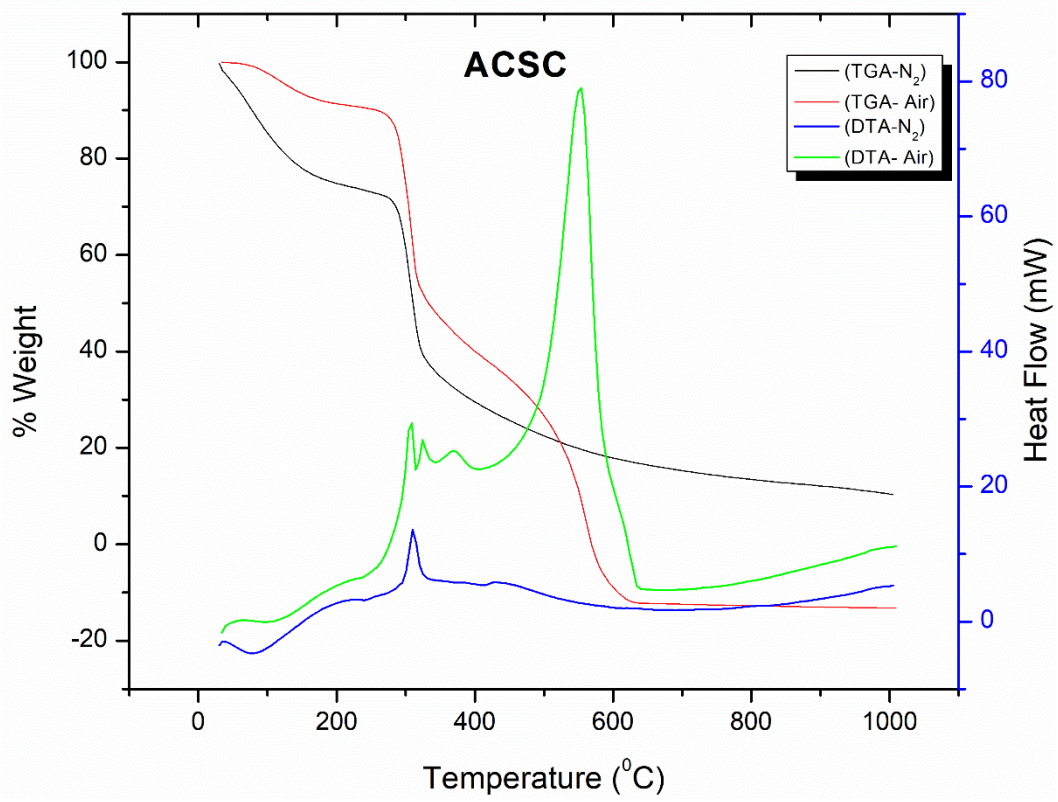


Figure 4.5h. TGA-DTA analysis of ACSC

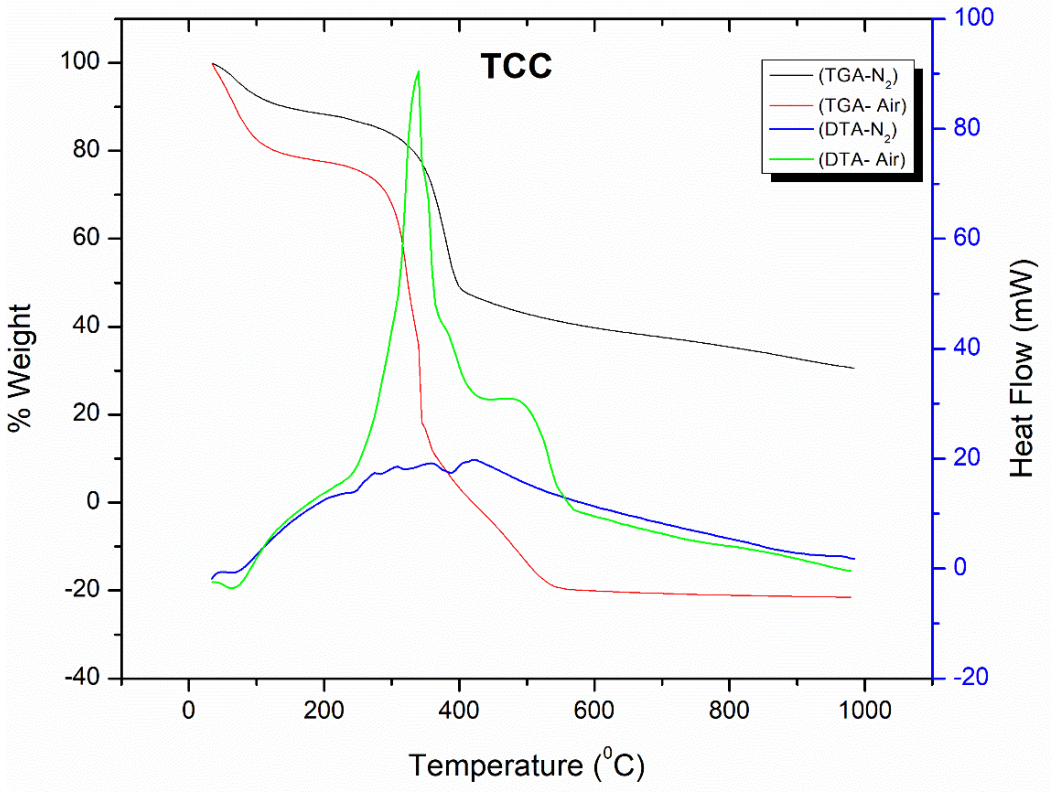


Figure 4.5i. TGA-DTA analysis of TCC

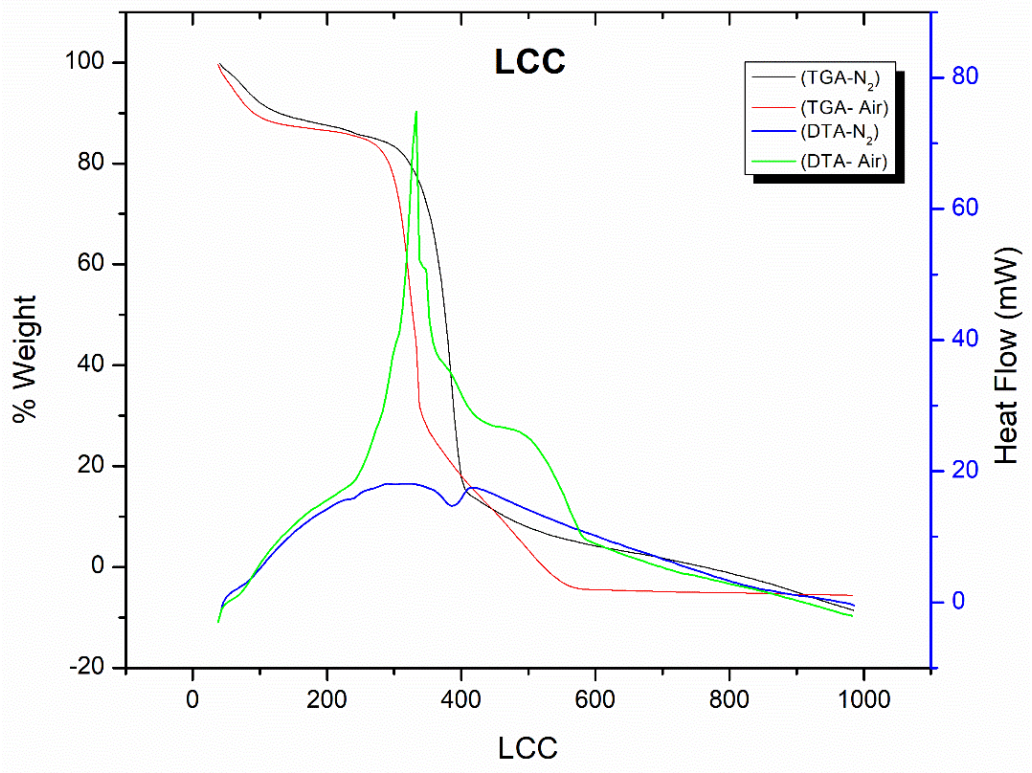


Figure 4.5j. TGA-DTA analysis of LCC

It was observed that all the chitosan samples show weight loss in the range of 50-120 ˚C, which corresponds to the removal of moisture. Under nitrogen atmosphere, the thermogram shows two step decomposition. The first step is due to loss of moisture, as discussed earlier. Second step weight loss in the range of 300-600 ˚C is attributed to degradation of saccharide structure of the molecule including the dehydration of saccharide rings, deacetylation (loss of -CH_3CO) from acetylated part of chitosan, elimination of NH_3 from deacetylated part of chitosan, and elimination of CO, CH_4. Under nitrogen atmosphere, some unburnt residue (18-34%) has remained which could be explained based on carbon, hydrogen, nitrogen and oxygen present in the chitosan. In the inert nitrogen atmosphere, first water molecules will be eliminated due to dehydration of polysaccharide ring and then carbon will be eliminated in the form of CO and CH_4 as per the remaining oxygen and hydrogen present in the chitosan structure. Hence, some carbon will still remain unburned with some inorganic material. Under air environment, 3 step degradation and weight loss were observed. The

first two steps are similar as happened in the nitrogen medium. At the third step, weight loss occurred due to the removal of remaining carbon and hydrogen as CO, CO_2, H_2O in the presence of air environment. This step can be called as oxidative elimination. DTA curve shows that the chitosan samples are thermally stable up to 300 °C. The TGA-DTA results are summarised in Table. 4.2. Thermal analysis of chitin shows same pattern of weight loss steps. In nitrogen atmosphere, 2 step weight loss was found. First step of weight loss happened in the range of 50-140 °C due to removal of moisture present in the sample. 2nd step of weight loss happened in the range of 300-600 °C due to dehydration of saccharide ring, deacetylation and elimination of remaining carbon in the form CO, CH_4 with remaining oxygen and hydrogen. Under air environment, 3 step degradation was found. The first two steps are similar with nitrogen atmosphere. Third step weight loss happened due to oxidative elimination of remaining carbons. DTA shows that chitin is stable up to 330 °C. The stability of chitin is higher than chitosan is due to H-bonding network in chitin which leads to structural rigidity in chitin. This proves that H-bonding network got disturbed during deacetylation of chitin as strong base is used during this process. The observed thermal degradation steps of chitosan and chitin are confirmed with the available literature reports.[25-26]

Table 4.2. Summary on thermal analysis of chitosan and chitin

Sample	Thermal stability up to (◦C)
ALMWC	309
AMMWC	305
AHMWC	301
TCLMWC	305
TCMMWC	312
TCHMWC	309
ASSC	305

ACSC	309
TCC	336
LCC	330

4.3.4. CHNS elemental analysis

CHNS elemental analysers provides the rapid and simultaneous determination of carbon, hydrogen, nitrogen and sulphur in organic samples, based on the principle of "Dumas method" which involves the complete and instantaneous oxidation of the sample by "flash combustion". The combustion products (NO_2, CO_2, H_2O, SO_2) are then separated by chromatographic column and detected by thermal conductivity detector. A wide variety of samples such as, solids, liquids, viscous samples can be analysed by using this technique.[27]

Chitosan and chitin both are polymers of amino sugars and it was expected to have elements like carbon (C), hydrogen (H), nitrogen (N) and oxygen (O). CHNS elemental analysis was performed to know elemental composition. Elemental analysis was done using Thermo Finnigan, Italy; model EA1112 series Flash Elemental Analyzer. 2-3 mg of oven dried sample was used for the analysis and the amount of elements (C, H and N) present in the samples were analysed by rapid combustion of the sample in pure oxygen (Dumas or flash combustion method). CHNS elemental analysis shows that chitosan contains 39.4-40.5% C, 6.5-7.3% H and 6.7-7.5% N. Elemental analysis of chitin shows that 44.2-44.5% C, 5.9-6.3% H and 6.2-6.5% N are present. Based on these results, a general monomer molecular formula for all chitosan and chitin samples are derived and summarized in Table no. 4.3. The general formula for all the chitosan and chitin samples can be expressed as $C_aH_bN_cO_d$ (a = 6.2-6.9, b = 12.9-13.7, c = 1, d = 5.2-6.1) and $C_wH_xN_yO_z$ (w = 7.9-8.4, x = 12.5-14.2, y = 1, z = 5.8-6.1) respectively which correlates well with the literature.[28] Degree of acetylation (DA) can be calculated from the carbon/nitrogen (C/N) ratio. In the completely deacetylated polymer

with $C_6H_{11}O_4N$ repeating monomer unit C/N ratio will be 5.145 and in fully N-acetylated polymer with $C_8H_{13}O_5N$ repeating monomer unit C/N ratio will be 6.861. C/N ratio can be varied from 6.861 to 5.145. Therefore, the degree of acetylation (DA) and degree of deacetylation (DD) can be calculated by using formula (1) and formula (2) respectively.[29] DA (%) and DD (%) values are tabulated in Table no. 4.3.

$$\boldsymbol{DA}\ (\%) = \frac{(\boldsymbol{C/N - 5.145})}{(\boldsymbol{6.861 - 5.145})} \times \boldsymbol{100} \qquad \text{..... (1)}$$

$$\boldsymbol{DD}\ (\%) = \boldsymbol{100} - \boldsymbol{DA}(\%) \qquad \text{..... (2)}$$

Table 4.3. Elemental analysis of various chitin and chitosan

Sample	C (%)	N (%)	H (%)	O (%)	Experimental Molecular formula	DA (%)	DD (%)
ALMWC	39.4	6.9	6.8	46.9	$C_{6.6}H_{13.7}N_1O_{5.9}$	31.8	68.2
AMMWC	39.8	6.7	6.6	46.9	$C_{6.9}H_{13.7}N_1O_{6.1}$	46.0	54.0
AHMWC	39.9	6.8	6.6	46.7	$C_{6.8}H_{13.6}N_1O_{6.0}$	43.0	57.0
TCLMWC	40.3	6.9	6.6	46.2	$C_{6.2}H_{13.6}N_1O_{5.3}$	41.8	58.2
TCMMWC	40.5	7.5	7.0	45.0	$C_{6.4}H_{13.6}N_1O_{5.6}$	14.9	85.1
TCHMWC	40.5	7.1	6.5	45.9	$C_{6.8}H_{13.5}N_1O_{5.8}$	33.9	66.1
ASSC	39.8	7.2	6.9	46.1	$C_{6.3}H_{13.1}N_1O_{5.2}$	23.9	76.1
ACSC	40.1	7.5	7.3	45.1	$C_{6.6}H_{12.9}N_1O_{5.6}$	13.3	86.7
TCC	44.2	6.5	5.9	43.4	$C_{7.9}H_{12.5}N_1O_{5.8}$	-	-
LCC	44.5	6.2	6.3	43.0	$C_{8.4}H_{14.2}N_1O_{6.1}$	-	-

4.3.5. Measurement of ash content and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES)

ICP-OES is one of the well-known techniques for the elemental analysis. It is a highly specific technique capable of analyzing all the metals with good detection limits. A plasma source is used to dissociate the sample into its constituent atoms or ions and exciting them to a higher energy level. They return to their ground state by emitting photons of a characteristic wavelength depending on the element present. The light is recorded by an optical spectrometer. The amount of elements can be quantified by calibrating against standards of different concentrations. For ICP-OES analysis, SPECTRO ARCOS Germany, FHS 12 instrument was used. For the preparation of the samples for ICP-OES analysis, 3-4 g of chitosan and chitin sample was weighed in a crucible and then kept in the muffle furnace. Under air, the sample was heated to 650 °C for 6h with a ramp rate of 5 °C/min. During this process, C, H, N and O will burn off in the form of CO_2, CO, CH_4, NH_3, NO_2 etc. and in the crucible unburned inorganic residue will remain. This unburned inorganic residue is called as ash. The amount of ash in chitosan and chitin samples are calculated in mg per 1g of chitosan/chitin. This residue was then dissolved in aqua regia and diluted with water.

SEM-EDAX analysis was shown the presence of Ca and Na. SEM-EDAX is a surface technique, therefore, the exact quantity of metals cannot be analysed. ICP-OES can measure the amount of metals quantitatively as this is a bulk analysis. ICP-OES data (Table 4.4) also shows the presence of magnesium (Mg) and Iron (Fe) metals. Among all the chitosan samples, AMMWC sample has shown highest ash content. ICP-OES data shows that high amount of Na is present in AMMWC and this is due to use of NaOH at deproteinization and deacetylation step. TCC also shows high ash and Na content which can be attributed to use of NaOH in deproteinization step.

Table 4.4. Ash content and ICP-OES analysis

Sample	Ash content (mg/g)	Ca (mg/g)	Na (mg/g)	Mg (mg/g)	Fe (mg/g)	Al (mg/g)
ALMWC	2.2	0.4	0.7	0.06	0.08	0.02
AMMWC	9.1	1.1	7.4	0.4	0.3	0.10
AHMWC	1.3	0.08	1.0	0.01	0.05	0.01
TCLMWC	1.7	0.3	0.07	0.2	0.08	0.0005
TCMMWC	0.8	0.2	0.02	0.07	0.02	0.0006
TCHMWC	0.7	0.3	0.01	0.04	0.01	0.0002
ASSC	3.1	0.2	2.3	0.04	0.1	0.06
ACSC	0.7	0.2	0.04	0.07	0.01	0.0001
TCC	6.7	1.4	2.3	0.1	0.06	0.05
LCC	3.9	0.4	0.8	0.2	0.3	0.17

4.3.6. Attenuated Total Reflection (ATR) spectroscopy

Functional groups present in chitosan and chitin samples were analysed using ATR technique. Analysis was done on Perkin Elmer Spectrum Two FT-IR Spectrometer; in the range of 500-4000 cm^{-1}. ATR is a sampling technique that is used in combination with infrared spectroscopy which allows samples to be examined directly in the solid state without any further sample preparation. Before the analysis, all the samples were dried in vacuum oven (10^{-4} MPa) for 3h. ATR for all the chitosan samples are shown in Figure 4.6a. to 4.6h.

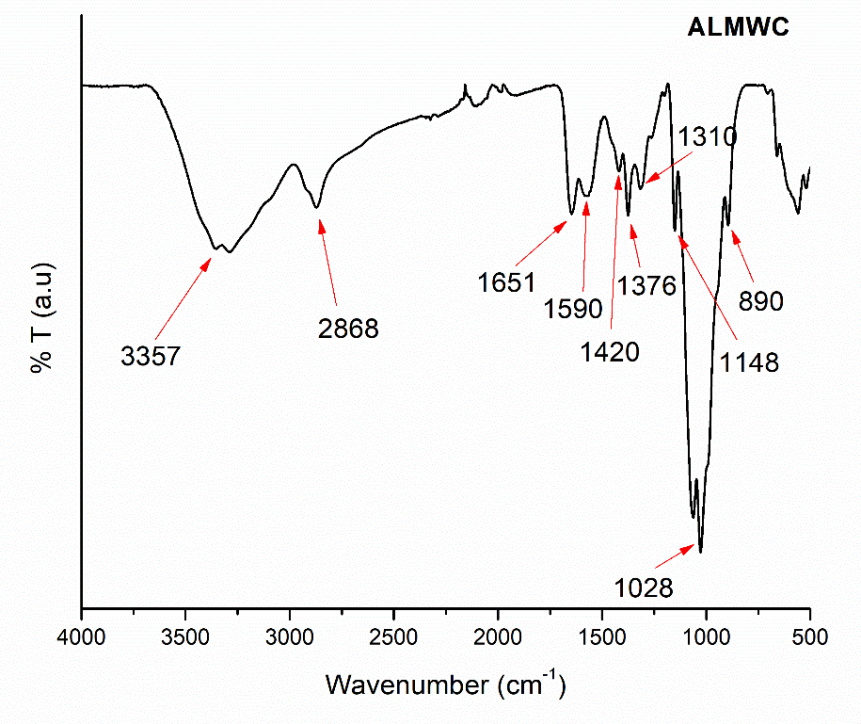


Figure 4.6a. ATR spectra of ALMWC

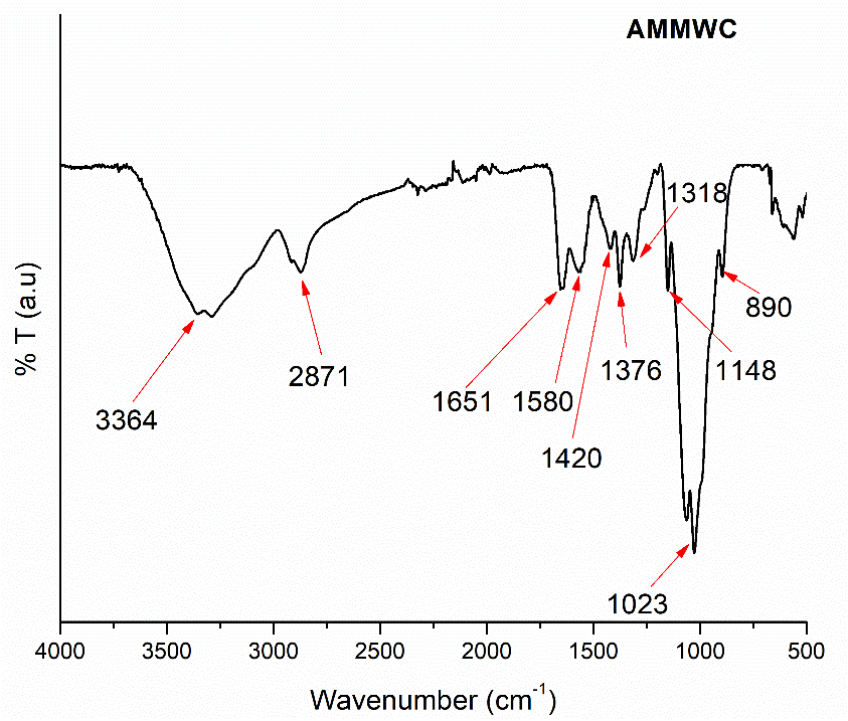


Figure 4.6b. ATR spectra of AMMWC

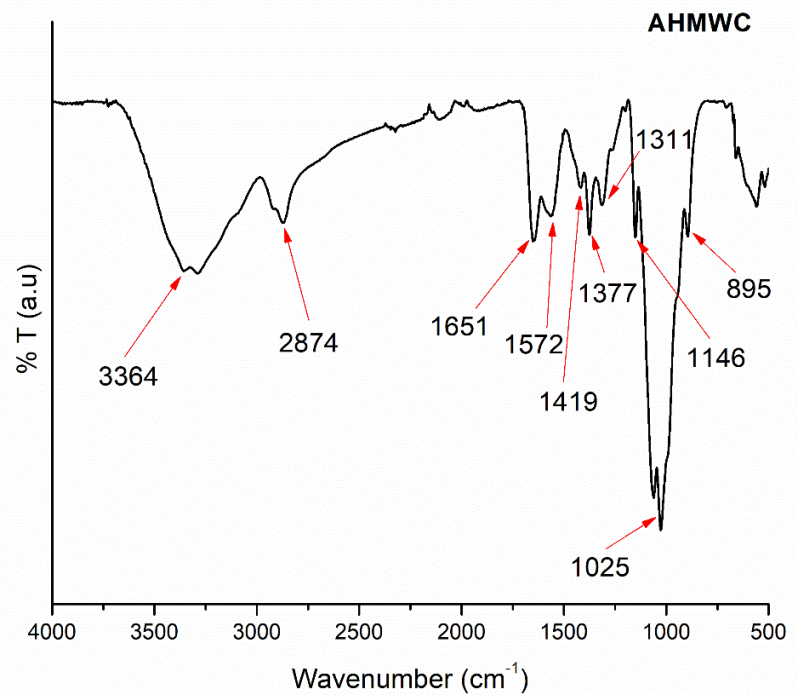


Figure 4.6c. ATR spectra of AHMWC

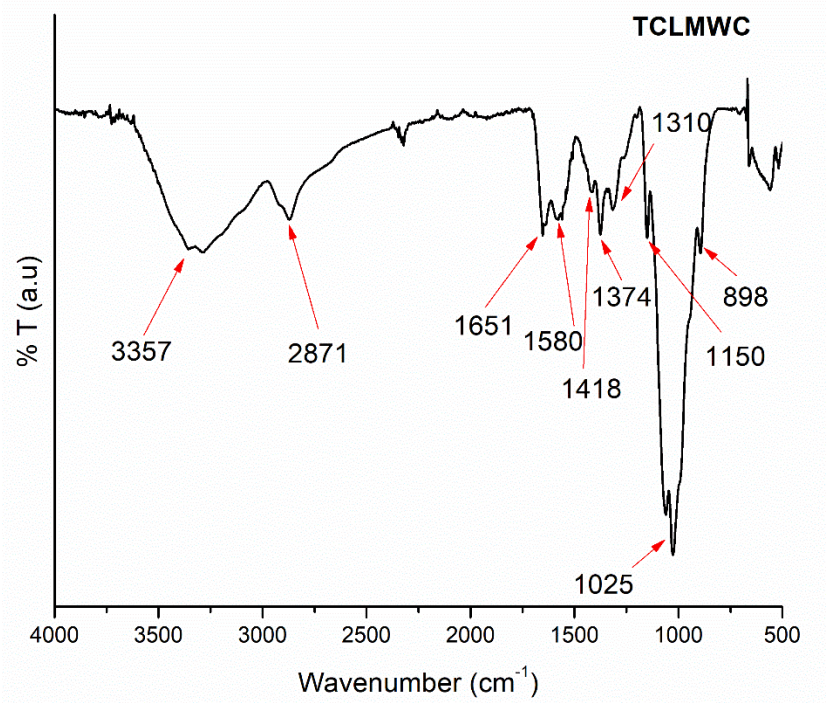


Figure 4.6d. ATR spectra of TCLMWC

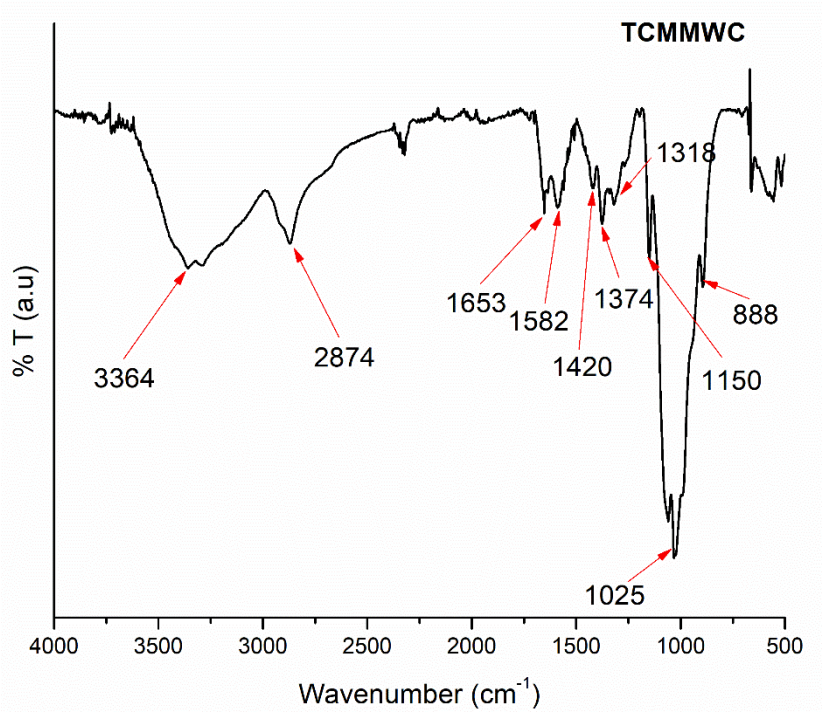


Figure 4.6e. ATR spectra of TCMMWC

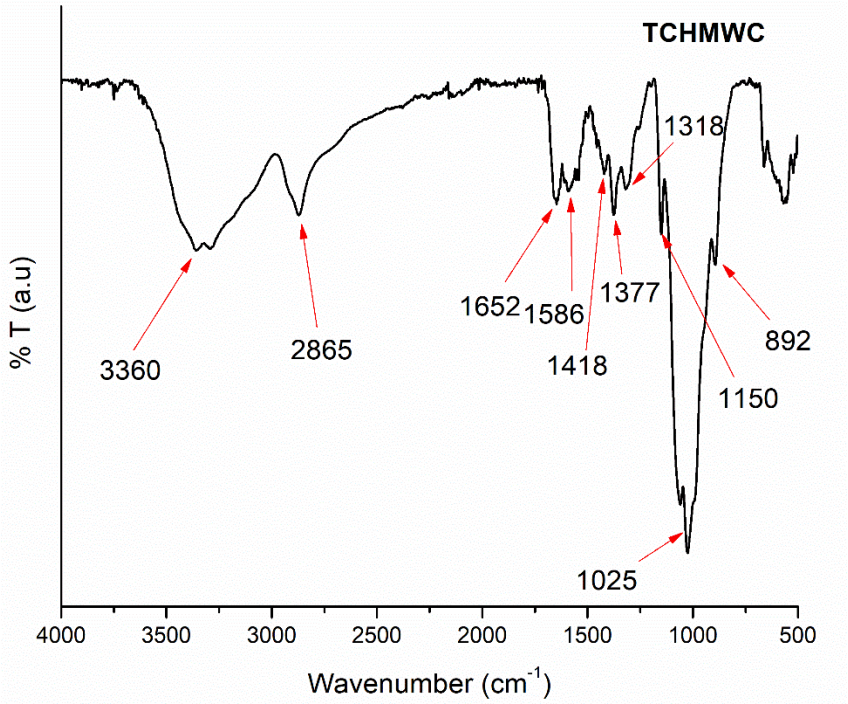


Figure 4.6f. ATR spectra of TCHMWC

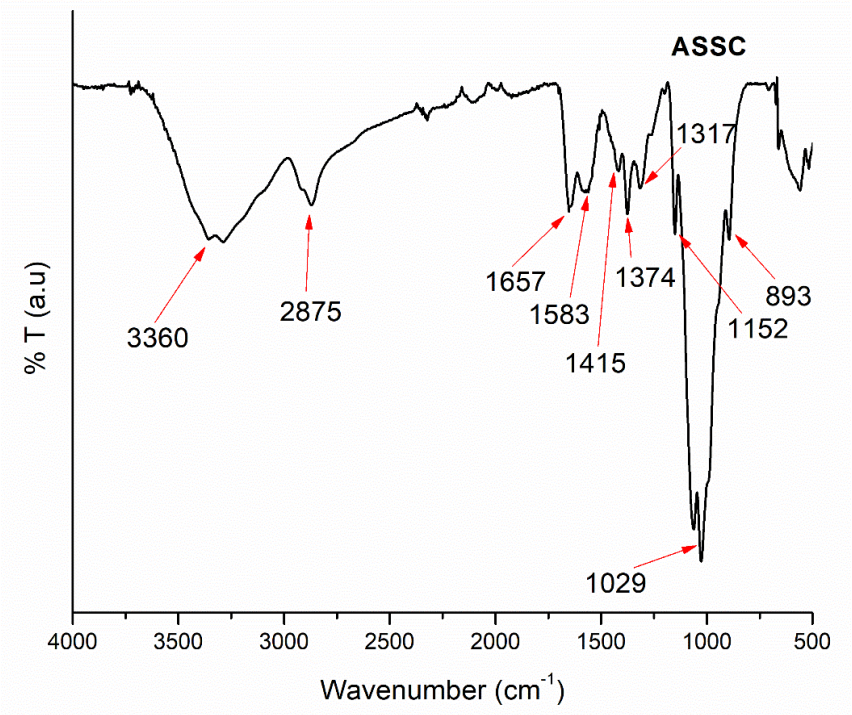


Figure 4.6g. ATR spectra of ASSC

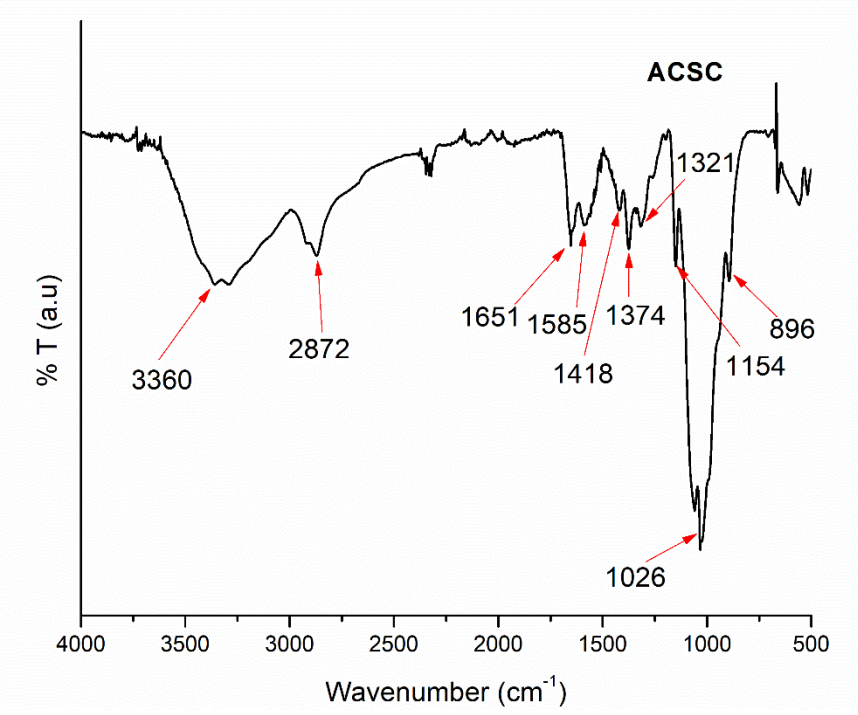


Figure 4.6h. ATR spectra of ACSC

In order to determine different functional groups present in the chitosan samples, ATR analysis was performed. It is known that band appearing between 3500-3100

cm^{-1} is due to stretching vibrations of hydroxyl groups. Stretching of N-H bond can also be found in that region. C-H asymmetric stretching vibration of methyl and methylene groups can be found in 2880-2860 cm^{-1} region. The band observed at 1660-1650 cm^{-1} can be attributed to the presence of amide I C=O which proves that the chitosan samples are not fully deacetylated. The band at 1590-1570 cm^{-1} can be due to amide II C-N stretching or bending of N-H bonds. The band at 1420-1410 cm^{-1} can be the characteristic of bend vibration of C-H bond. C-H symmetric stretching vibration was found at 1380-1370 cm^{-1}. Band at 1325-1310 cm^{-1} can be attributed to the presence of wagging vibration of CH_2. C-O-C asymmetric stretching vibration was found at 1155-1145 cm^{-1}. Sharp and intense absorption band at 1030-1020 cm^{-1} was found due to presence of C-O asymmetric stretching of ring. Band at 885-896 cm^{-1} can be attributed to CH ring stretching of saccharide ring.

All the chitosan samples show the presence of similar functional group with similar intensities. Below, the summary of various bands observed in chitosan samples are presented in Table 4.5.

Table 4.5. Summary on ATR bands present in various chitosan samples

Band (cm^{-1}) and type of vibration	**Wavenumber (cm^{-1})**							
	ALMWC	**AMMWC**	**AHMWC**	**TCLMWC**	**TCMMWC**	**TCHMWC**	**ASSC**	**ACSC**
3500-3100 (O-H stretching and N-H str-etching)	3357	3364	3364	3357	3364	3360	3360	3360
2880-2860 (C-H asymmetric	2868	2871	2874	2871	2874	2865	2875	2872

stretching in methyl/methylene groups)								
1660-1650 (Amide I C=O stretching of acetylated monomers)	1651	1651	1651	1651	1653	1652	1657	1651
1590-1570 (Amide II C-N stretching/N-H bending)	1590	1580	1572	1580	1582	1586	1583	1585
1420-1410 (CH2 bending)	1420	1420	1419	1418	1420	1418	1415	1418
1380-1370 (CH3 symmetrical deformation/ C-H bending)	1376	1376	1377	1374	1374	1377	1374	1374
1325-1310 (CH2 wagging)	1310	1318	1311	1310	1318	1318	1317	1321
1155-1145 (C-O-C asymmetric stretching	1148	1148	1146	1150	1150	1150	1152	1154
1030-1020 (C-O asymmetric stretching)	1028	1023	1025	1025	1025	1025	1029	1026
885-896 (CH ring stretching of saccharide ring)	890	890	895	898	888	892	890	896

Furthermore, ATR analysis of chitin samples was also performed to determine the different functional groups are present in chitin. Band at 3450-3440 cm^{-1} was observed which can be due to stretching vibration of hydroxyl groups. Band at 3265-3255 cm^{-1} and 3105-3100 cm^{-1} can be attributed to vibrational modes of N-H (intermolecular hydrogen bonding between C=O and NH & H-bond between NH groups). C-H stretching vibrations of methyl and methylene groups were present at 2880-2870 cm^{-1}. C=O stretching of amide I was found at 1620-1610 cm^{-1}. Band at 1560-1540 cm^{-1} can be attributed to stretching vibration of amide II C-N bond. Bending vibration of N-H bond also can come at same place. Band for $-CH_2$ bending was found at 1430-1420 cm^{-1}. Band at 1375-1370 cm^{-1} can be due to C-CH_3 deformation and also for C-H bending. Wagging vibration of $-CH_2$ was found at 1310-1300 cm^{-1}. Band appearing at 1165-1160 cm^{-1} was found due to C-O-C asymmetric stretching for bridge oxygen. Again, C-O-C asymmetric stretching vibration was seen at 1070-1060 cm^{-1}. C-O asymmetric stretching in ring was found at 1015-1011 cm^{-1}. Wagging vibration of CH_3 was found at 950-945 cm^{-1}. All chitin samples show the presence of similar functional groups. ATR spectra of chitin samples are shown in Figure. 4.7a. to Figure. 4.7b. and the summary of bonds observed in chitins are presented in Table 4.6.

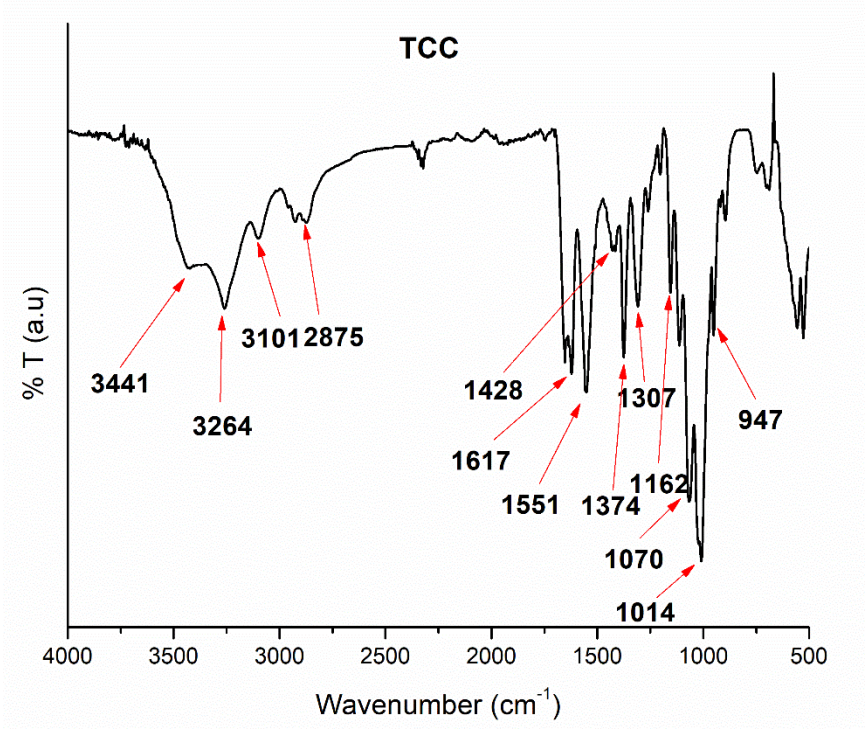


Figure 4.7a. ATR spectra of TCC

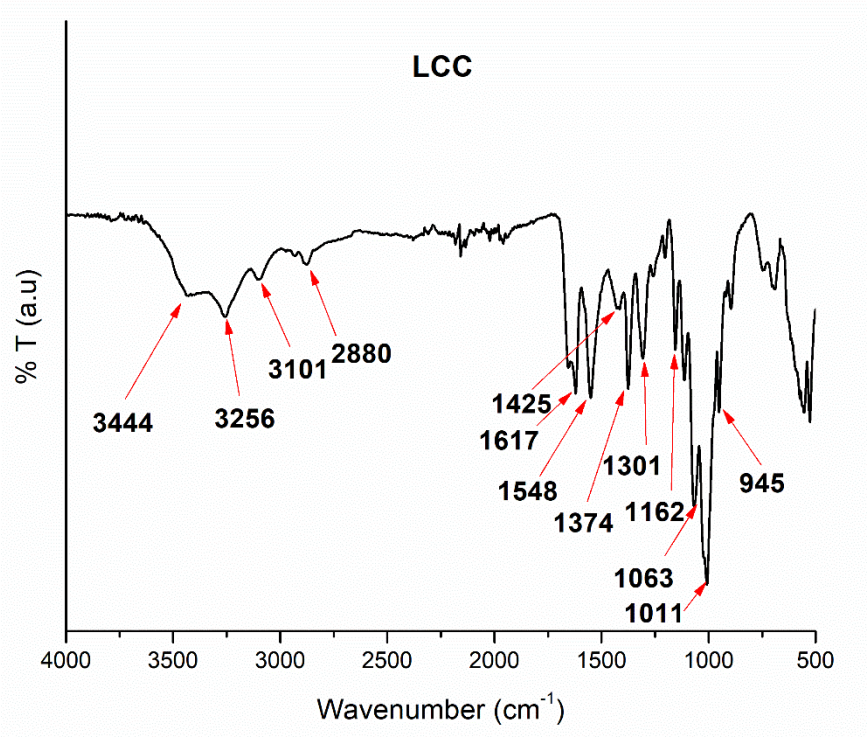


Figure 4.7b. ATR spectra of LCC

Table. 4.6. Summary on ATR bands present in various chitin samples

Band (cm^{-1}) and type of vibration	Wavenumber (cm^{-1})	
	TCC	**LCC**
3450-3440 (Stretching vibration of O-H groups)	3441	3444
3265-3255 and 3105-3100 (N-H stretching)	3264	3256
2880-2870 (C-H stretching of methyl and methylene group)	2875	2880
1620-1610 (Amide I C=O stretching)	1617	1617
1560-1540 (Amide II C-N stretching/N-H bending)	1551	1548
1430-1420 (CH_2 bending)	1428	1423
1375-1370 (CH3 symmetrical deformation/C-H bending)	1374	1374
1310-1300 (CH_2 wagging)	1307	1301
1165-1160 (C-O-C asymmetric stretching)	1162	1162
1070-1060 (C-O-C asymmetric stretching in saccharide rings)	1070	1063
1015-1010 (C-O asymmetric stretch in ring	1014	1011
950-945 (CH_3 wagging)	947	945

A careful look on comparative spectra (Figure. 4.8) of chitin (TCC) and chitosan (TCLMWC) reveals that the shoulder peak at 3479 cm^{-1} in the spectrum of TCC (chitin) is due to intermolecular hydrogen bonding between OH of C6 carbon and C=O which completely disappears in the ATR spectra of TCLMWC (chitosan). This observation proves that the initial structure of chitin was broken during deacetylation process.[30] Again, the band at 3264 cm^{-1} and 3101 cm^{-1} in TCC (chitin) is attributed to vibrational modes of N-H bonds. Presence of these vibrations also proves the presence of intermolecular hydrogen bonding between C=O and NH & also H bonded NH groups. This bands also disappears in ATR spectra of TCLMWC (chitosan). The bands at 1653 cm^{-1} and 1617 cm^{-1} are due to amide Ⅰ C=O bond. According to literature, the splitting of IR bands in polymers happened due to interchain interactions in the crystals.[23, 30] In case of TCC (chitin), peak at 1653 cm^{-1} attributed to stretching of C=O which are bonded only with NH groups in $-NHCOCH_3$ part. The band at 1617 cm^{-1} is specifically due to C=O group bonded with both NH and OH of C6 carbon (H-bonding). It is observed from the IR spectra of TCLMWC (chitosan) that intensity of this region is very less with respect to TCC (chitin) which suggests deacetylation of N-acetylated group. Furthermore, the splitting of these bands is found to be very less in case of TCLMWC (chitosan) which proves breaking of interchain interactions during deacetylation process. Again, decrease in intensity of amide Ⅱ C-N stretching or N-H bending is also observed in TCLMWC (chitosan) which also proves the deacetylation of acetamide group. Changes in shape and intensity of CH_2 bending band at 1428 cm^{-1} is also observed which suggests the change in arrangement and environment of primary alcohol ($-CH_2OH$) group at C6 carbon. These structural changes also found in previous literature reports.[23, 31]

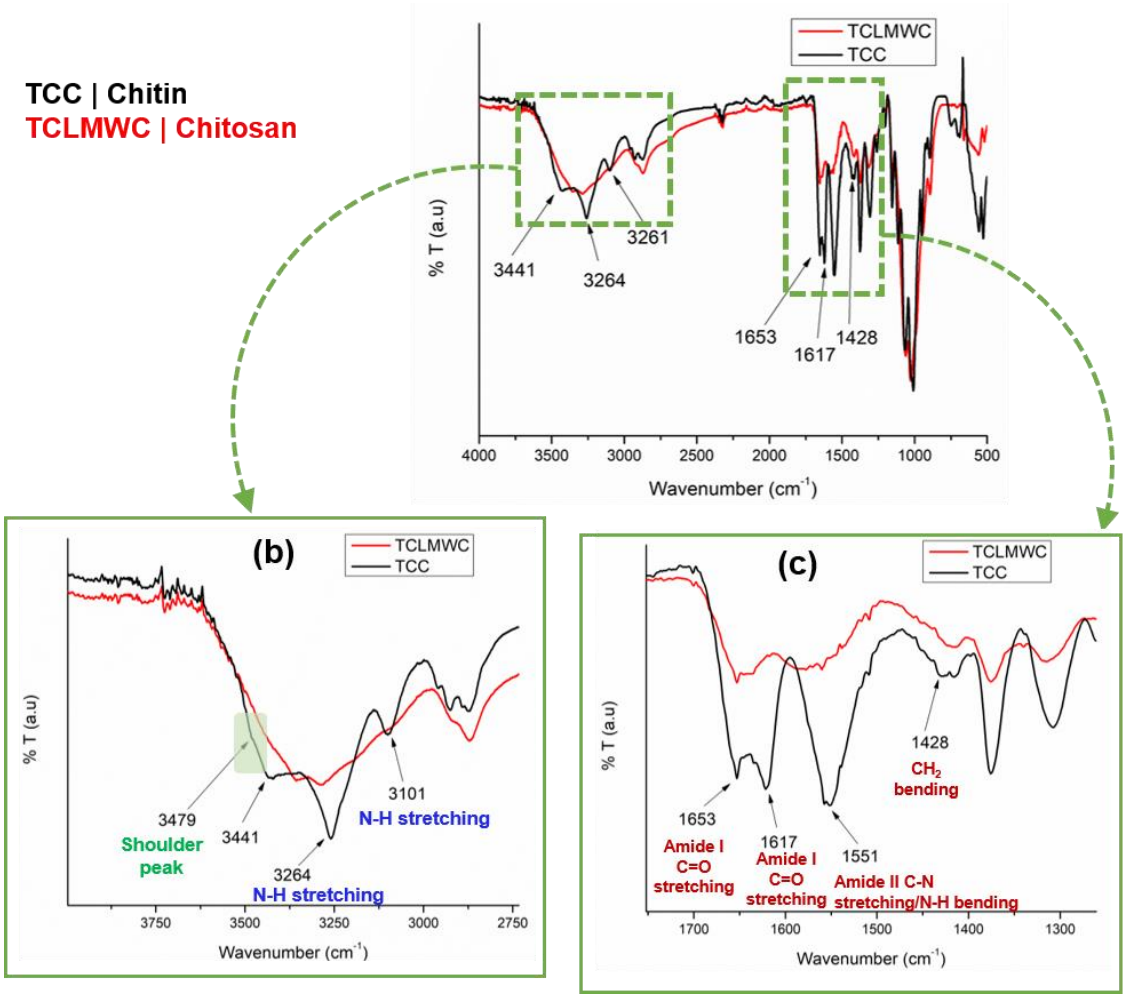


Figure 4.8. (a) Comparison of ATR spectra of TCC and TCLMWC, (b) zoomed ATR spectra between 3880-2750 cm^{-1} range, (c) zoomed ATR spectra between 1800-1200 cm^{-1} range

4.3.7. Solid state ^{13}C NMR spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is one of the most important and powerful tools to understand the structure of a molecule. Atomic nuclei consisting of odd number of protons and/or neutrons possessing a nuclear spin $I \neq 0$ and consequently a magnetic moment $\mu = \gamma\hbar I$ (γ = gyromagnetic ratio), when placed in a magnetic field of strength B_0, Zeeman interaction results in quantized orientations of the nuclear magnetic moments. The nucleus can adopt $2I + 1$ Eigen states with energies $E(m) = -m\gamma\hbar B_0$, where m = (I, I–1,...., –I). Transitions between neighbouring energy states ($\Delta m = \pm 1$) can be induced by

electromagnetic radiation (energy $E = h\nu$) of frequency $\nu_0 = \gamma B_0/2\pi$. The chemical shift interaction arises from secondary local magnetic fields induced by the interaction of the electrons surrounding the nucleus. The induced local field opposes B_0 and hence shields the nucleus under observation. The shielding is spatially anisotropic due to the non-spherical electron distribution around the nucleus.[32]

The skeleton of chitosan and chitin were explored using ^{13}C NMR spectroscopy. ^{13}C NMR analysis was done in Bruker AV300, Germany; at 10 kHz with a pulse program Cp, av and no. of scans was 8192. All the chitosan and chitin samples showed similar type of functional groups in ATR analysis. Figure. 4.9 shows the representative structure of chitosan in which two monomer units $GlcNH_2$ and GlcNAc are attached together as chitosan is the heteropolymer of these two units. It is known that chitosan and chitin have polysaccharide backbone (C1-C6) and in addition to the polysaccharide backbone, one methyl carbon (C8) and one carbonyl carbon (C8) are also present which are characteristic peak for GlcNAc moiety. As chitin is homopolymer of GlcNAc, it contains 8 carbons [6 carbons from polysaccharide backbone (C1-C6), one methyl carbon (C7) and one carbonyl carbon(C8)]. Therefore, it is obvious that peaks for C7 and C8 carbons are less intense in case of chitosan than chitin as chitosan is partially deacetylated derivative of chitin.

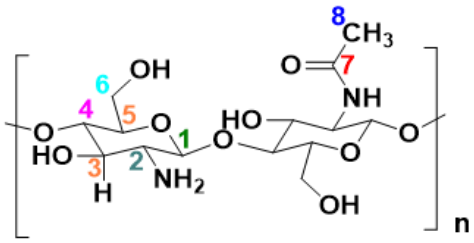


Figure 4.9. Representative structure of chitosan with carbon labelling

To confirm the presence of polysaccharide backbone and other functional groups in chitosan and chitin samples, ^{13}C NMR was performed and spectra are presented

in Figures 4.10a. to 4.10i. Chemical shift of C1, C2, C4, C6 were observed at 104-105 ppm, 55-58 ppm, 82-84 ppm and 60-62 ppm respectively. Peaks of C3 and C5 are merged together and appears as a single peak at 74-77 ppm. ^{13}C NMR spectra of chitin samples showed 8 well defined peaks for polysaccharide backbone, methyl group and carbonyl group. Chemical shifts for all the peaks are tabulated in Table. 4.7. Two distinct peaks for C3 (76-77 ppm) and C5 (74 ppm) proves that this chitin samples are α-chitin which is suggested by previous literature also.[33]

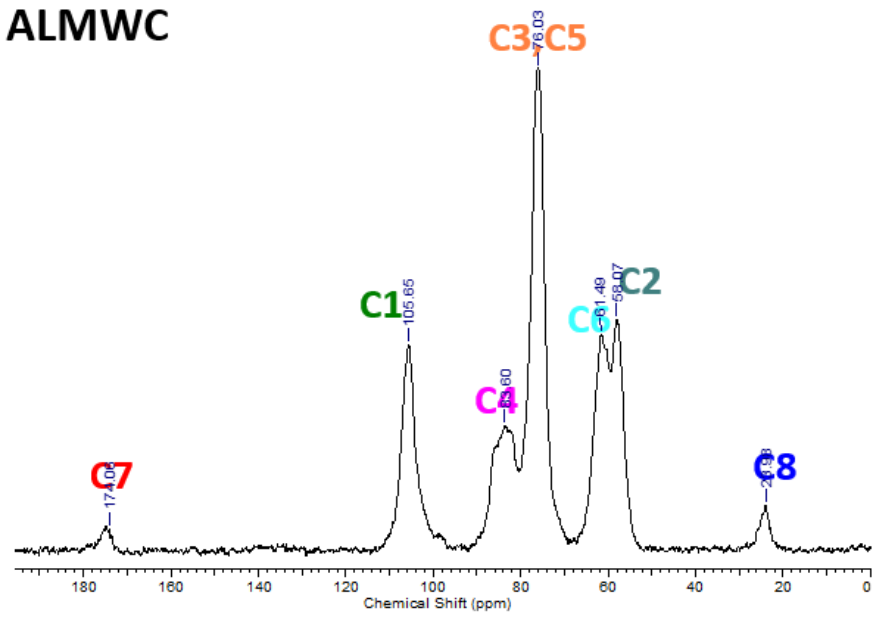


Figure 4.10a. Solid state ^{13}C NMR spectra of ALMWC

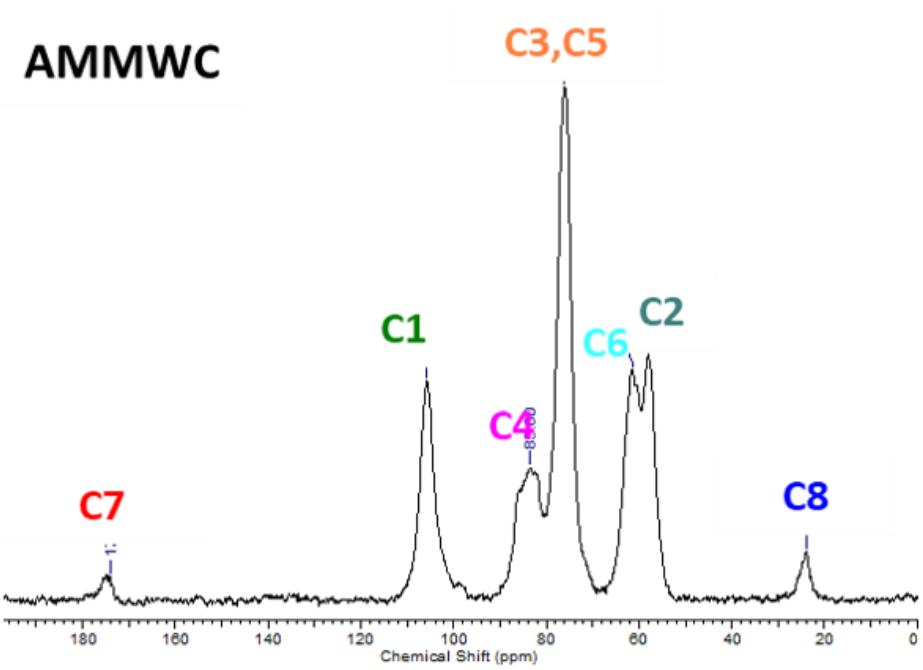


Figure 4.10b. Solid state ^{13}C NMR spectra of AMMWC

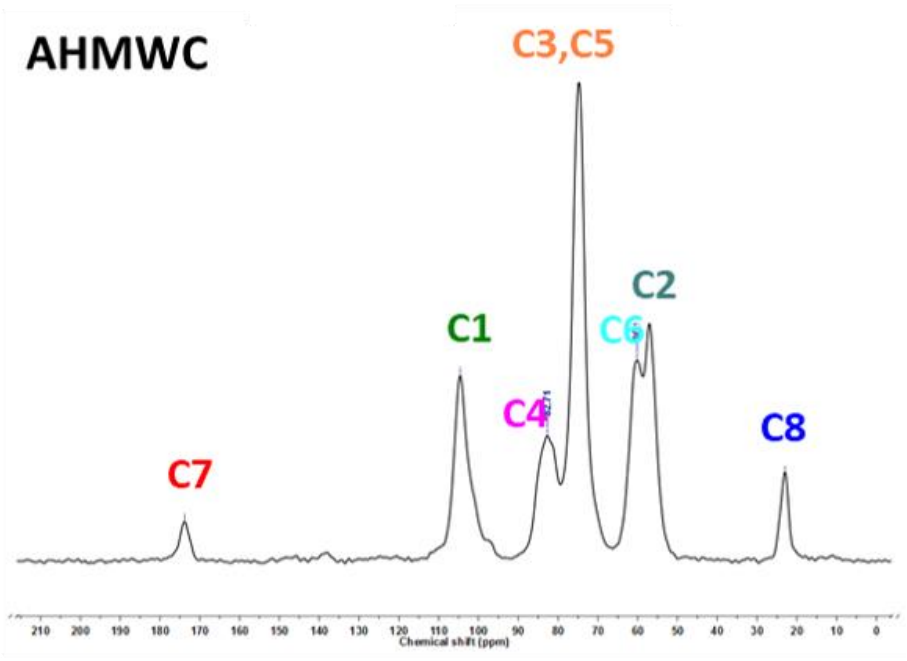


Figure 4.10c. Solid state ^{13}C NMR spectra of AHMWC

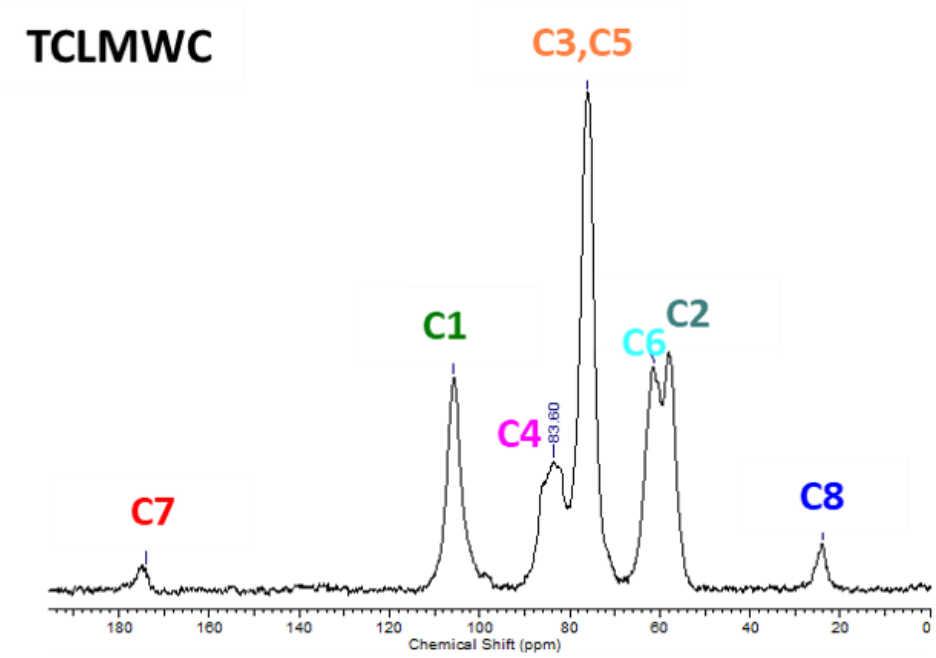


Figure 4.10d. Solid state ^{13}C NMR spectra of TCLMWC

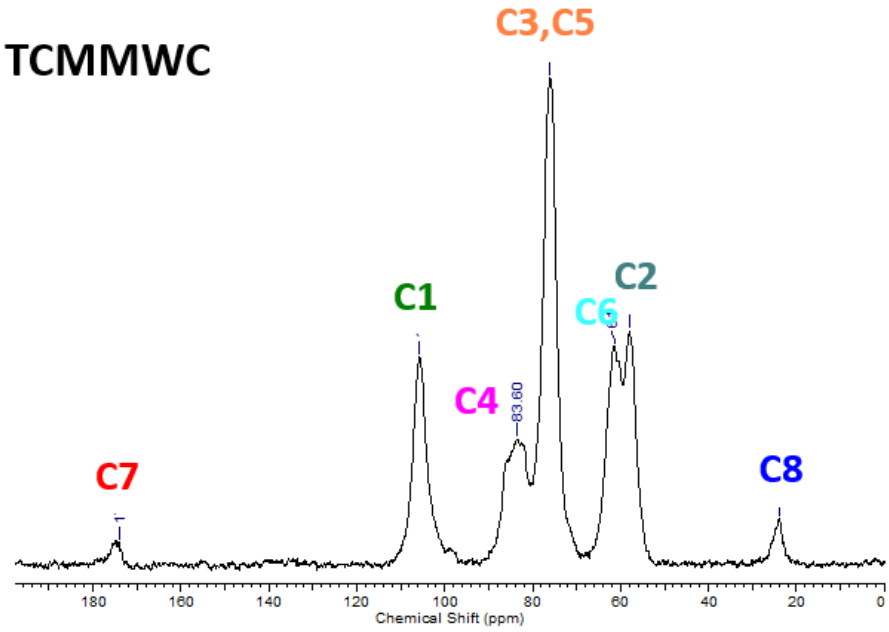


Figure 4.10e. Solid state ^{13}C NMR spectra of TCMMWC

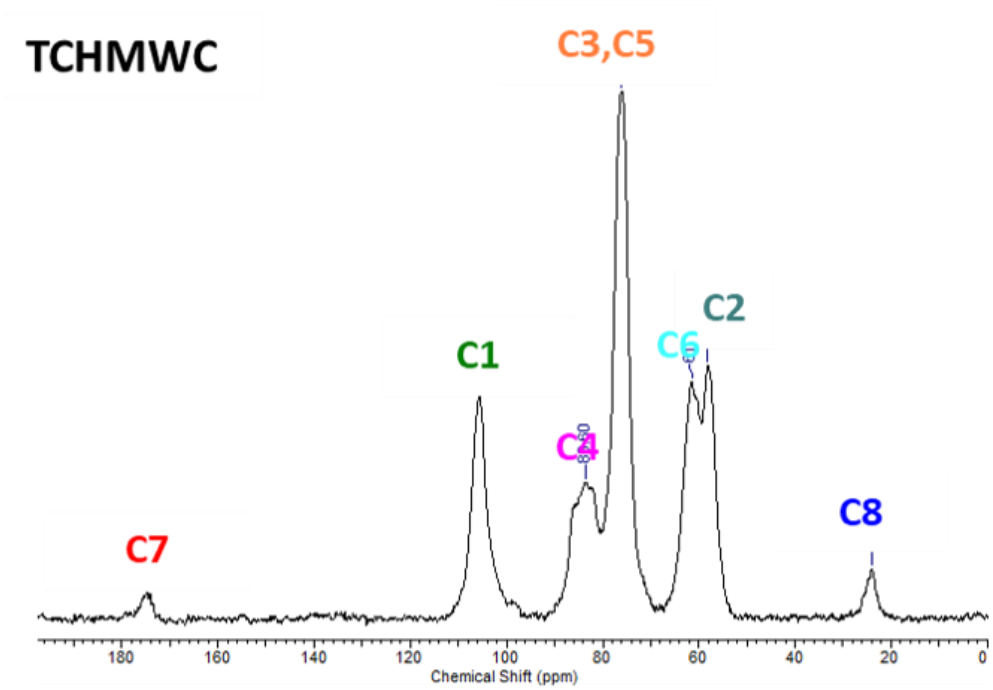


Figure 4.10f. Solid state ^{13}C NMR spectra of TCHMWC

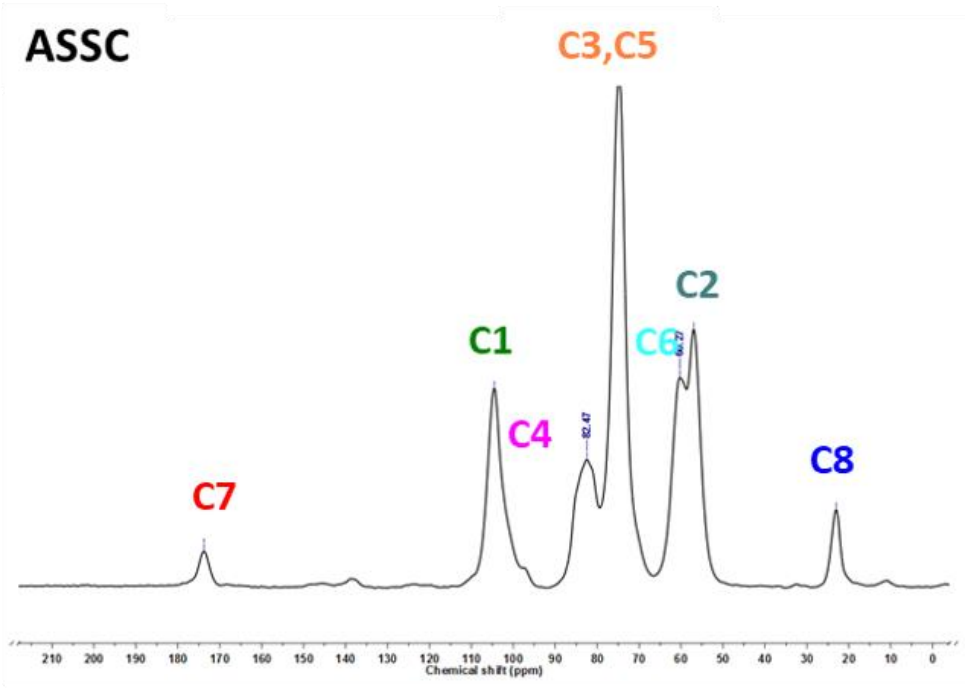


Figure 4.10g. Solid state ^{13}C NMR spectra of ASSC

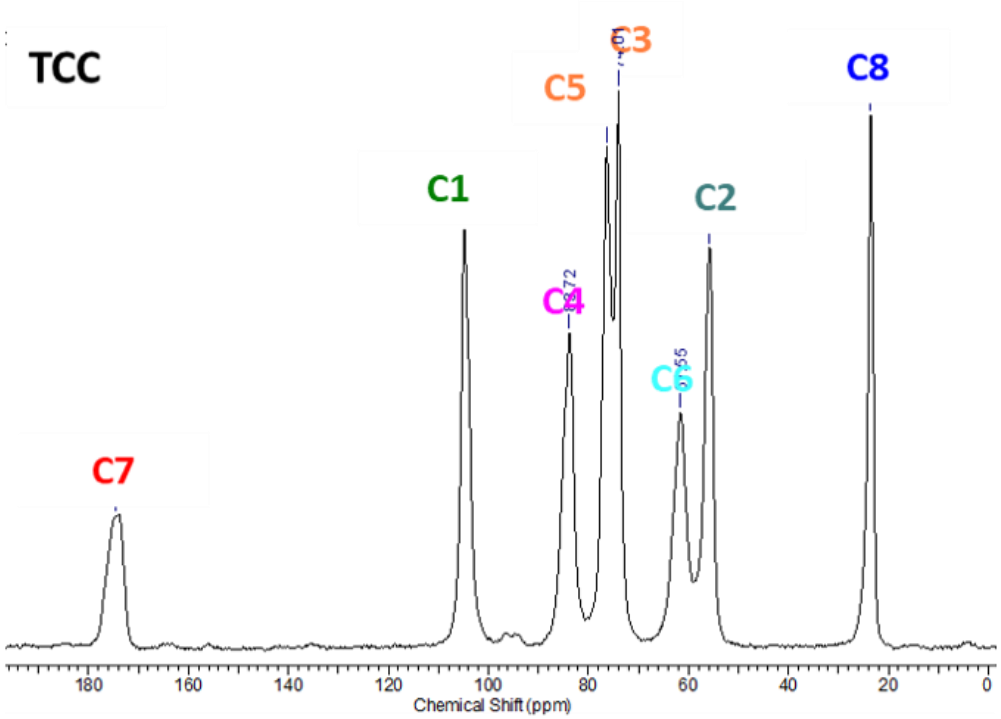


Figure 4.10h. Solid state ^{13}C NMR spectra of TCC

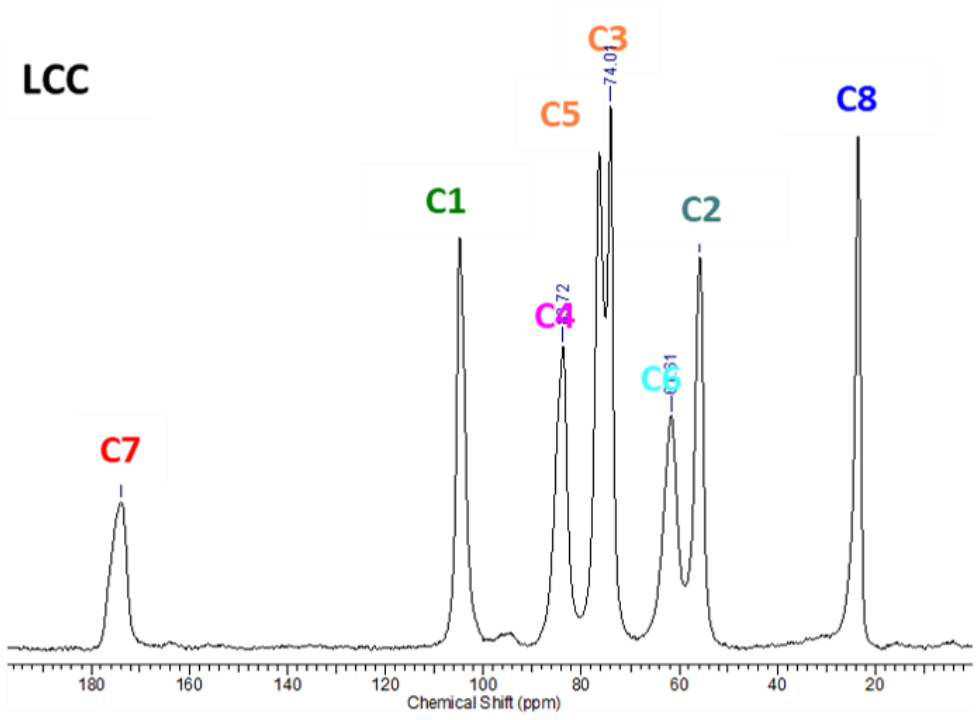


Figure 4.10i. Solid state ^{13}C NMR spectra of LCC

Table 4.7. Summary on chemical shifts (ppm) of various chitosan and chitin from ^{13}C NMR

Sample code	Chemical shift (ppm)							
	Polysaccharide Backbone						-C=O	-CH_3
	C1	C2	C3	C4	C5	C6	C7	C8
ALMWC	105.65	58.07	76.03	83.60	76.03	61.49	174.08	23.98
AMMWC	105.71	58.13	76.09	83.72	76.09	61.73	174.98	24.17
AHMWC	104.59	57.05	74.76	82.71	74.76	60.16	173.83	23.01
TCLMWC	105.77	58.25	76.27	83.42	76.27	61.73	174.61	24.41
TCMMWC	105.66	58.25	75.98	83.90	75.98	61.49	175.04	24.29
TCHMWC	105.65	58.07	76.03	83.70	76.03	61.49	174.06	23.98
ASSC	104.53	56.96	74.76	82.47	74.76	60.27	173.79	23.01
TCC	104.79	55.75	76.33	83.72	74.01	61.55	173.88	23.49
LCC	104.79	55.81	76.33	83.72	74.01	61.61	173.82	23.49

4.3.8. Absolute viscosity measurement

Absolute viscosity is a measure of the internal friction of a fluid. Absolute viscosity can be defined as the tangential force per unit area of two parallel planes at a unit distance apart when the space between them is filled with fluid, and one plane

moves with unit velocity in its own plane relative to the other. The greater amount of force required to cause the movement if the friction is greater. As per Newton's law, the force required to maintain the speed is proportional to the difference in speed through the liquid. This is called as "shear". According to Newton's law, $F/A = \eta . dv/dx$, η is a constant for a given material and is called as "viscosity". dv/dx is a measure of the change in speed which can be called as shear rate (. The term /A is the force per unit area required to produce the shearing action and it can be called as shear stress and symbolized as τ. Therefore, viscosity = shear stress/shear rate. The unit of viscosity is poise (P) / Pascal-seconds (Pa.s). One Pascal-second is equal to ten poise. Therefore, one milli-pascal-second is equal to one centipoise.

Viscosity was measured by Brookfield-ultra rheometer with a cone-plate arrangement. As viscosity is temperature dependent, the temperature of the samples was controlled by using Julabo thermostat. The calibration of the instrument was done against the reported viscosity data of water. 10 mg chitosan samples were taken and solubilized in 1 mL of 1 *wt%* acetic acid solution. ACSC, TCC and LCC could not be solubilized in 1 *wt%* acetic acid solution; that's why measurements of these samples could not be done. The measured viscosity data for chitosan samples (Table. 4.8) falls in the range which is provided by suppliers.

Table 4.8. Absolute viscosity measurement

Sample	Measured viscosity, η (cP)	Viscosity, η (cP) given by suppliers
ALMWC	42	20-300
AMMWC	257	200-800
AHMWC	1019	800-2000
TCLMWC	6	5-20
TCMMWC	82	20-100
ASSC	485	>200

As viscosity has a proportional relationship with molecular weight, sample with less viscosity will have lower molecular weight. Viscosity measurement data matches well with the range of viscosity provided by suppliers. Data suggests that TCLMWC has lowest molecular weight among all chitosan samples.

4.4. Conclusions

Various physico-chemical characterizations were done for all the commercially procured chitosan and chitin samples to know their physical and chemical properties. Structural and functional properties can be different for chitin and chitosan samples depending on their source and extraction method. Hence, the complete characterization of all chitosan and chitin samples were performed before starting the depolymerisation experiments using various techniques like CHNS, ICP-OES elemental analysis, SEM-EDAX, ATR, ^{13}C NMR, XRD analysis. CHNS elemental analysis revealed that chitosan contains 39.4-40.5% C, 6.5-7.3% H and 6.7-7.5% N. Elemental analysis of chitin shows that 44.2-44.5% C, 5.9-6.3% H and 6.2-6.5% N are present. Furthermore, degree of deacetylation (DD %) was calculated from C/N ratio and it is found that chitosan samples are 54-86% deacetylated. Morphology of chitosan and chitin samples were found in SEM analysis and EDAX analysis revealed that Ca and Na are present in the samples as impurity. Moreover, bulk level ICP-OES analysis was performed to quantify the metal content. ICP analysis shows the presence of Ca which proves that demineralization was not complete and presence of Na can be attributed to the use of NaOH in the deproteinization and deacetylation step. ATR and ^{13}C NMR spectroscopic measurements were performed to know the backbone and functional groups present in the samples. All the chitosan samples have similar type of functional groups. Moreover, a comparative study on structure of chitosan and chitin was done and it is found that major structural changes happened during deacetylation process. Interchain hydrogen bonding network was disturbed during deacetylation. DTA analysis revealed that chitosan samples are

thermally stable upto 300 °C and chitin has greater thermal stability that chitosan, i.e., 330 °C. The stability of chitin is higher than chitosan is due to H-bonding network in chitin which leads to structural rigidity in chitin. TG-DTA data also proves that H-bonding network got disturbed during deacetylation of chitin as strong base is used during this process. Morphology of chitosan and chitin samples were studied using XRD analysis. XRD patterns of chitin samples shows intense diffraction peaks which are characteristic peaks for α-chitin. In case of chitosan, decrease in intensity of diffraction peaks was observed and few peaks were disappeared. The decrease in crystallinity is due to NaOH treatment during deacetylation of chitin to chitosan. XRD data also suggests that inter-chain H-bonds may be broken during base treatment. Viscosity measurement was done to get an idea about the molecular weight of chitosan samples as viscosity have proportional relation with molecular weight. Measured viscosity data falls in the range which is provided by the suppliers. It is found that TCLMWC has the lowest viscosity among all chitosan samples. Characterization data of all chitin and chitosan samples revealed that chitosan will be easier to depolymerize than chitin due to its lower crystallinity, lesser interchain hydrogen bonding network, lower structural rigidity and lower molecular weight.

4.5. References

1. Khoushab, F.; Yamabhai, M., Chitin research revisited. *Mar Drugs* **2010,** *8* (7), 1988-2012.

2. Austin, P. R.; Brine, C. J.; Castle, J. E.; Zikakis, J. P., Chitin: New Facets of Research. *Science* **1981,** *212* (4496), 749-753.

3. Rinaudo, M., Chitin and chitosan: Properties and applications. *Progress in polymer science* **2006,** *31* (7), 603-632.

4. Rudall, K.; Kenchington, W., The chitin system. *Biological Reviews* **1973,** *48* (4), 597-633.

5. Kim, S.-K., *Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications*. CRC Press: 2010.

6. Kim, S. S.; Kim, S. H.; Lee, Y. M., Preparation, characterization and properties of β-chitin and N-acetylated β-chitin. *Journal of Polymer Science Part B: Polymer Physics* **1996,** *34* (14), 2367-2374.

7. Focher, B.; Naggi, A.; Torri, G.; Cosani, A.; Terbojevich, M., Structural differences between chitin polymorphs and their precipitates from solutions—evidence from CP-MAS 13C-NMR, FT-IR and FT-Raman spectroscopy. *Carbohydrate Polymers* **1992,** *17* (2), 97-102.

8. Pillai, C. K. S.; Paul, W.; Sharma, C. P., Chitin and chitosan polymers: Chemistry, solubility and fiber formation. *Progress in Polymer Science* **2009,** *34* (7), 641-678.

9. Ibitoye, E.; Lokman, I.; Hezmee, M.; Goh, Y.; Zuki, A.; Jimoh, A., Extraction and physicochemical characterization of chitin and chitosan isolated from house cricket. *Biomedical Materials* **2018,** *13* (2), 025009.

10. Chen, X.; Chew, S. L.; Kerton, F. M.; Yan, N., Direct conversion of chitin into a N-containing furan derivative. *Green Chemistry* **2014,** *16* (4), 2204-2212.

11. Hajji, S.; Younes, I.; Ghorbel-Bellaaj, O.; Hajji, R.; Rinaudo, M.; Nasri, M.; Jellouli, K., Structural differences between chitin and chitosan extracted from three different marine sources. *International Journal of Biological Macromolecules* **2014,** *65*, 298-306.

12. Sagheer, F. A. A.; Al-Sughayer, M. A.; Muslim, S.; Elsabee, M. Z., Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. *Carbohydrate Polymers* **2009,** *77* (2), 410-419.

13. Sigma-Aldrich 448869, Product information. https://www.sigmaaldrich.com/IN/en/product/aldrich/448869.

14. Sigma-Aldrich 448877, Product information. https://www.sigmaaldrich.com/IN/en/product/aldrich/448877.

15. Sigma-Aldrich 419419, Product information. https://www.sigmaaldrich.com/IN/en/product/aldrich/419419.

16. TCI C2395, Product information. https://www.tcichemicals.com/IN/en/p/C2395.

17. TCI C2396, Product information. https://www.tcichemicals.com/OP/en/p/C2396.

18. TCI C0831, Product information. https://www.tcichemicals.com/OP/en/p/C0831.

19. Sigma-Aldrich 417963, Product information. https://www.sigmaaldrich.com/IN/en/product/aldrich/448877.

20. Noishiki, Y.; Takami, H.; Nishiyama, Y.; Wada, M.; Okada, S.; Kuga, S., Alkali-Induced Conversion of β-Chitin to α-Chitin. *Biomacromolecules* **2003,** *4* (4), 896-899.

21. Wada, M.; Saito, Y., Lateral thermal expansion of chitin crystals. *Journal of Polymer Science Part B: Polymer Physics* **2001,** *39* (1), 168-174.

22. Duan, B.; Chang, C.; Ding, B.; Cai, J.; Xu, M.; Feng, S.; Ren, J.; Shi, X.; Du, Y.; Zhang, L., High strength films with gas-barrier fabricated from chitin solution dissolved at low temperature. *Journal of Materials Chemistry A* **2013,** *1* (5), 1867-1874.

23. Focher, B.; Naggi, A.; Torri, G.; Cosani, A.; Terbojevich, M., Chitosans from Euphausia superba. 2: Characterization of solid state structure. *Carbohydrate Polymers* **1992,** *18* (1), 43-49.

24. Burkhanova, N. D.; Yugai, S. M.; Pulatova, K. P.; Nikononvich, G. V.; Milusheva, R. Y.; Voropaeva, N. L.; Rashidova, S. S., Structural Investigations of Chitin and Its Deacetylation Products. *Chemistry of Natural Compounds* **2000,** *36* (4), 352-355.

25. de Britto, D.; Campana-Filho, S. P., Kinetics of the thermal degradation of chitosan. *Thermochimica acta* **2007,** *465* (1-2), 73-82.

26. Paulino, A. T.; Simionato, J. I.; Garcia, J. C.; Nozaki, J., Characterization of chitosan and chitin produced from silkworm crysalides. *Carbohydrate Polymers* **2006,** *64* (1), 98-103.

27. Thompson, M., CHNS elemental analysers. *The Royal Society of Chemistry* **2008**.

28. Yen, M.-T.; Yang, J.-H.; Mau, J.-L., Physicochemical characterization of chitin and chitosan from crab shells. *Carbohydrate polymers* **2009,** *75* (1), 15-21.

29. Kasaai, M. R.; Arul, J.; Charlet, G., Intrinsic viscosity–molecular weight relationship for chitosan. *Journal of Polymer Science Part B: Polymer Physics* **2000,** *38* (19), 2591-2598.

30. Pearson, F.; Marchessault, R.; Liang, C., Infrared spectra of crystalline polysaccharides. V. Chitin. *Journal of Polymer Science* **1960,** *43* (141), 101-116.

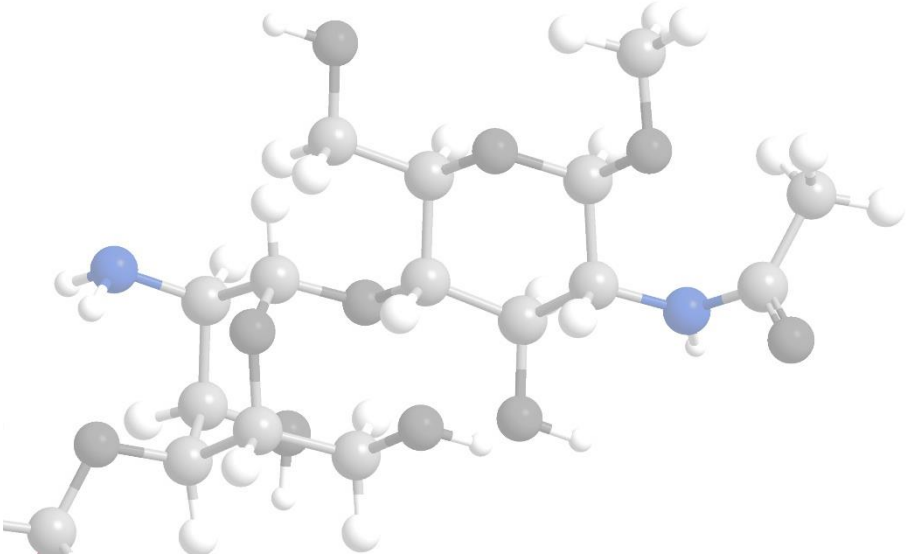
31. No, H. K.; Meyers, S. P., Preparation and characterization of chitin and chitosan—a review. *Journal of aquatic food product technology* **1995,** *4* (2), 27-52.

32. Duer, M. J., Solid state NMR spectroscopy: principles and applications. *John Wiley & Sons* **2008**.

33. Sila, A.; Mlaik, N.; Sayari, N.; Balti, R.; Bougatef, A., Chitin and chitosan extracted from shrimp waste using fish proteases aided process: efficiency of chitosan in the treatment of unhairing effluents. *Journal of Polymers and the Environment* **2014,** *22* (1), 78-87.

Chapter 05

Extraction of chitin from shell waste: Understanding through detailed characterization



5.1. Introduction

Global fisheries (including fish, crustaceans, molluscs) production is increasing day by day as sea-food demand grows and reached about 179 million tonnes in 2018.[1] Crustaceans are diverse arthropod taxon which have a hard exoskeleton to protect their body. Crabs, lobsters, crayfish, shrimps, krill, prawns are the examples of crustaceans.[2] According to FAO 2020 report, 9.4 million tonnes crustaceans comprising of prawns, shrimps, lobsters and crabs were produced annually in 2018.[1] Furthermore, production of freshwater crustacean farming has significantly increased in Asia in 2018.[1] The total marine coastal and inland crustacean annual production was 8.4 million tonnes in Asian countries which is 89.6% of the total world production of crustaceans.[1, 3] India also has long coastline of about 8118 km which includes West Bengal (WB), Orissa (OD), Andhra Pradesh (AP), Tamil Nadu (TN), Pondicherry (PD), Andaman and Nicobar Islands (AN) as east coast and Gujarat (GJ), Maharashtra (MH), Goa (GA), Karnataka (KA), Kerala (KL), Lakshadweep (LD) as west coast.[4] In 2018, total annual fisheries production is 9.7 million tonnes in India.[5] Table 5.1 shows species wise marine crustaceans production in coastal states of India.

Table 5.1. Species-wise marine crustaceans production in coastal states of India[5]

Production in coastal regions in India	**Species wise marine shells production in 2019-20 (in Lakh Tonnes)[5]**			
	Penaeid prawns (Penaeidae)	**Non-penaeid prawns (Sergestidae)**	**Lobsters (Nephropidae)**	**Crabs (Brachyura)**
East coast (AP, OD, TN, WB, AN, PD)	1.6	0.63	0.04	0.13
West coast (GA, GJ, KA, KL, MH)	1.22	0.93	0.03	0.19

Based on the production and consumption demand of crustaceans, generous amount of shell wastes is generated by farming and processing industries.[1] Shell (exoskeleton) and head of shrimps, prawns and crabs were discarded as waste after using the edible part.[6] Some part of shell wastes is used as food in aquaculture or for animals but they were openly disposed in landfills or sea in majority.[7] The shell waste disposal landfills produce bad odour, gases, dust and moreover, infectious diseases can be caused and transmitted by rodents, flies, mosquitoes and other pests.[3] Due to this, soil, air and water pollution can happen and economy and livelihood of people can be affected. Furthermore, sea water also gets polluted and hardness of water increases due to calcium carbonate content in shell waste which can harm aquatic animals. However, it is necessary to utilize the shell wastes which can bring both economic and environmental benefits.[8] As discussed in introduction chapter, valorisation of shell waste has gained immense interest in research community because it contains chitin which can be valorised to nitrogen containing value added chemicals.[8] The concept of shell biorefinery was recently proposed where crustacean shells were separated into different fractions and each fraction is upgraded into value added products.[9] Crustacean shell waste is mainly composed of chitin (15-40%), protein (20-40%), calcium carbonate (20-50%) and other minor components like lipid, astaxanthin and minerals.[9] All components of shell wastes have lots of applications (figure 5.1).[9]

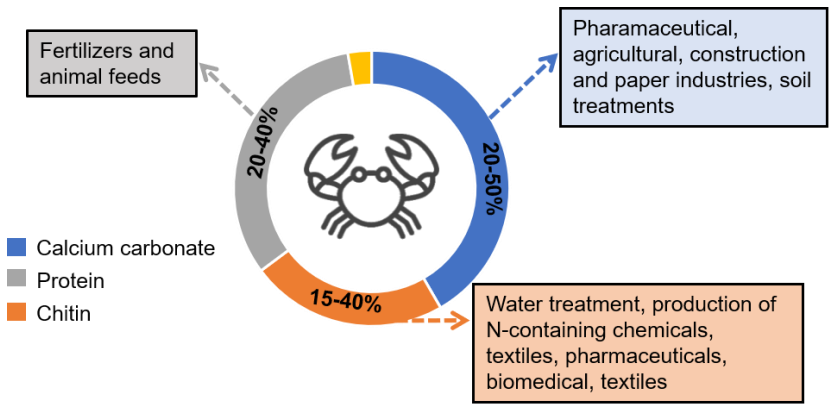


Figure 5.1. Components of shell wastes and their applications

Due to presence of these important components, finding innovative and industrially favourable methods for fractionation of shell waste is a very important topic in future. Different fractionation methods like industrial method (demineralization, deproteinization and decoloration)[10-12], solvent extraction method (using ionic liquids)[13-15] and bioprocessing methods (enzymatic method, lactic acid fermentation method)[16-17] were discussed in detail in introduction chapter. Most of these processes possess drawbacks like use of mineral acids/bases, use of harsh conditions, contamination of metals (Na), use of costly ionic liquids as solvent, use of costly enzymes and requirement of longer time, incomplete deproteinization etc.[3] After considering the drawbacks of all methods, industrial method was used in this work as it is an easy and effective method for extraction and a well-established protocol for chitin extraction is available in literature.

Literature suggests that the composition of shell wastes varies depending on the species, type of water and geographical location.[6] Chitin quantity can be varied seasonally in case of shrimps as they undergo molting (the shedding of their exoskeletons). Moreover, variation in the hardness of shells happened due to different habitat which showed change in chemical composition of shells from different geographical location.[3]

Chitin and its derivatives have many applications in wastewater treatment, cosmetics, textile, paper, agriculture industry and they are very much important materials for biomedical applications.[18] As shrimp shell is an important source of calcium, it is used as construction material. Shrimp shells were used as biosorbents for the removal of anionic dyes like Acid Blue 25.[3] Shell wastes were also used as fertilizers after removing chitin. In addition to this, chitin contains 7% biologically fixed nitrogen which makes it unique from other biomasses like cellulose, hemicellulose and lignin. Therefore, it holds potential to produce N-containing chemicals.[8] From the knowledge of ammonia synthesis, it is also

known that incorporation of nitrogen is a very tedious and highly energy-consuming process. In contrast to this tedious process, N-containing chemicals can be produced from a waste material which can enhance economic and ecological benefits.[8-9]

In the 4th chapter, physico-chemical properties of commercially available chitin and chitosan samples were discussed in detail. By considering the very low monetary value of shell waste and the environmental impact due to their disposal, it is preferable to use abundant and inexpensive shell waste material directly for the extraction of high value chitin which is a potential source of nitrogen containing value added chemicals. The purpose behind this work was to extract pure chitin from collected shell waste samples and understand the properties of extracted chitin using various characterization techniques. Moreover, comparison between lab-extracted chitin and commercially available chitin was also carried out to know the similarities or difference between them. Further, I have decided to collect different shell waste samples from different places to know how the properties changes with the geographical location. It is known that fresh and sea water contains different types of elements with different quantity. It was also decided to collect both fresh and sea water samples to understand if there is any effect of type of water on extracted chitin samples. Moreover, it was also understood from the literature that shell waste composition depends on the source or species. It was also decided to collect two different types of species (shrimp or crab) to understand the differences or similarities between the chitin extracted from the two different species. Furthermore, in industrial method acid and base were used subsequently to demineralize and deproteinize shell waste to extract chitin. Use of acid may depolymerize the chitin chain and base may deacetylate the chitin to chitosan. I have decided to use the well-known protocol for chitin extraction, i.e., industrial method to know that if acid and base can alter the structure of chitin or it remains unchanged while extraction procedure.

5.2. Experimental section

5.2.1. Chemicals and materials

Edible parts of crustacean shells e.g., shrimp, prawn, crab etc. are generally sold in local markets and the non-edible part i.e., shell waste is generally dumped in landfill and thrown away to waterbodies. Shell wastes were collected from local market of two different places of Maharashtra and one from Kerala. All the samples were first cleaned thoroughly with water to remove remaining edible parts, dried in sunlight for two days and further oven dried at 60 ◦C for 16 h. The dried shell waste samples were crushed to powder form and kept in oven at 60 ◦C for 16 h followed by vacuum drying at 150 ◦C for 4 h and stored at air-tight lid container. HCl (37%, LOBA Chemie), NaOH (98%, LOBA Chemie), Sodium hypochlorite solution (LOBA Chemie), HF (40%, LOBA Chemie) were purchased and used as received. Milli Q water has been used for the experiments.

5.2.2. Extraction of chitin from shell waste

5.2.2.1. Sample collection and preparation

Three different types of shell wastes were collected from local markets in India. Details of collected shell wastes were given below in Table 5.2.

Table 5.2. Details of collected shell wastes

S. No.	Place of collection	Type of shell	Water type	Isolated chitin sample name with code
1.	Pashan, Pune, Maharashtra	Shrimp	Fresh water	Pashan fresh water shrimp shell waste (PFWSC)
2.	Dapoli, Ratnagiri, Maharashtra	Shrimp	Sea water	Dapoli sea water shrimp chitin (DSWSC)
3.	Tirur, Malappuram, Kerala	Crab	Sea water	Kerala sea water crab chitin (KSWCC)

All the samples were washed thoroughly with water to remove any remaining edible part. Then the samples were dried in sunlight for 2 days. After that, the samples were dried in oven at 60 °C for 16 h. The dried shell waste samples were crushed to powder form and kept in oven at 60 °C for 16 h followed by vacuum drying at 150 °C for 4 h and stored at air-tight lid container.

5.2.2.2. Isolation of chitin from shell waste

Chitin was isolated from all the shell waste samples using well-established conventional and commercial protocol.[12] Isolation of chitin involves three basic steps, i.e., demineralization, deproteinization and decoloration.

5.2.2.2.1. Demineralization

Shell waste powder (3-5 g) was taken in a round bottom flask and 1N HCl solution was added dropwise with a solid to solvent ratio of 1:15 (*w/v*) and continuous vigorous stirring at ambient temperature. Shell waste samples generally contains 20-50% Calcium carbonate ($CaCO_3$). $CaCO_3$ reacted with HCl and formed CO_2 gas and $CaCl_2$. Just after addition of HCl solution, bubbles were seen and CO_2 gas was coming out as the reaction proceeded. $CaCl_2$ remained in the solution as it is soluble in water. After the complete addition of 1N HCl (taken according to weight of shell waste powder), the reaction mixture was kept on stirring for 2 hours at room temperature. After 2 hrs, few drops of 1N HCl waste added to see the bubble formation. No bubble formation proves the removal of $CaCO_3$ from the shell waste powder. Then the reaction mixture was filtered using Whatman 41 and the washed thoroughly to neutrality with milli-Q water. The demineralized mass was then dried at 60 °C for 16 h, followed by vacuum drying at 150 °C for 4h. Then, the dried demineralized mass was used for next step.

5.2.2.2.2. Deproteinization

Demineralized mass was weighed and taken in a round bottom flask. 3.5 *wt%* NaOH solution was added gradually with a solid to solvent ratio of 1:10 (*w/v*) and

constant stirring. The reaction mixture was kept in oil bath at 65 °C with continuous stirring for 2 h. Shell wastes generally contains 20-40% protein which in presence of NaOH gets solubilized after breakage of peptide bonds. After 2 h, the reaction mixture was filtered and washed thoroughly to neutrality with milli-Q water. The deproteinized solid was then dried at 60 °C for 16 h, followed by vacuum drying at 150 °C for 4h.

5.2.2.2.3. Decoloration

The dried deproteinized mass was weighed and taken in a round bottom flask and 0.3% sodium hypochlorite solution was added to it with solid to solvent ratio of 1:10 (*w/v*) and stirred continuously for 5 min at room temperature. Active chlorine in sodium hypochlorite solution helps to decolorised the sample. The decolorised product was then collected through filtration, washed and dried at 60 °C for 16 h, followed by vacuum drying at 150 °C for 4h.

The schematic representation of the chitin isolation method is shown in Figure 5.2.

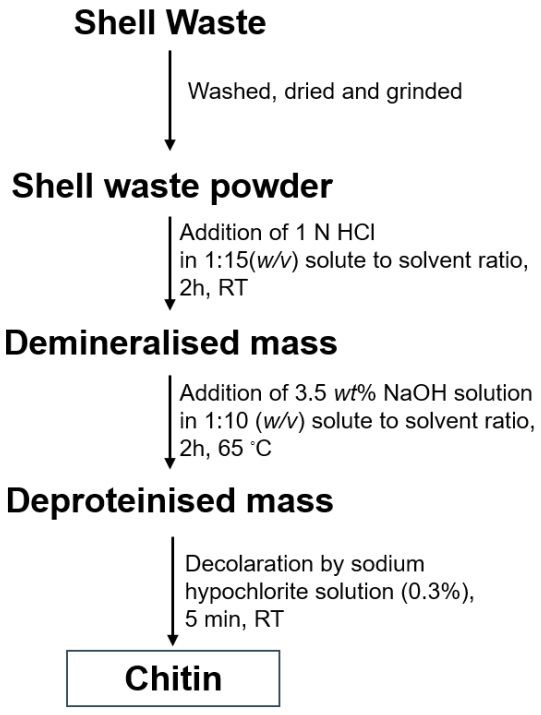


Figure 5.2. Schematic representation of chitin isolation method

After completion of all the above steps, extracted samples were stored in airtight lid container and used for further study. Detailed characterization of all the extracted samples were done using various physicochemical techniques to confirm that the structure and properties of extracted mass matches with chitin. The effect of source and water type can also be understood by characterization techniques.

5.2.3. Characterization techniques

Understanding the properties of extracted samples is necessary to confirm the structure of the extracted mass. Therefore, all the extracted samples were thoroughly characterized using various techniques like CHNS elemental analysis, ICP-OES analysis, SEM-EDAX analysis, ATR, ^{13}C NMR, TG-DTA, XRD, ash content measurement etc.

5.3. Results and discussions

5.3.1. X-Ray Diffraction (XRD) analysis

As discussed in chapter 4 (Section 4.3.1), XRD was done to know the morphology of the extracted samples can be understood from powder wide angle XRD analysis data (Figure 5.3). The instrument and procedure details were given in 4th chapter, Section 4.3.1.

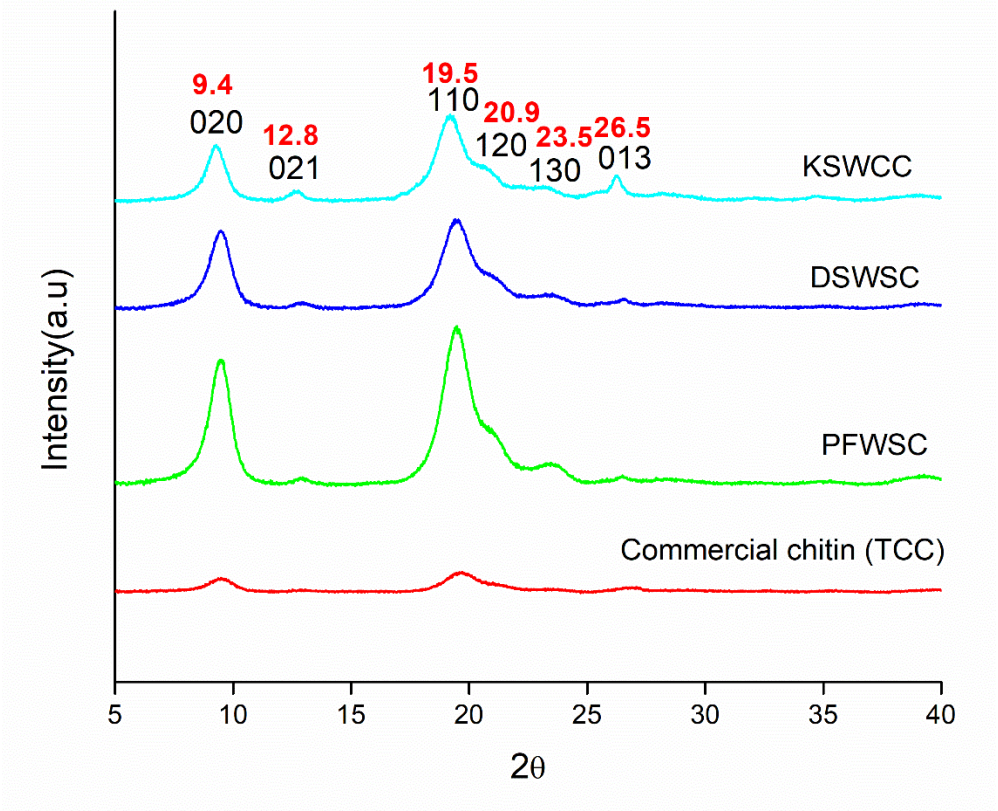


Figure 5.3. XRD patterns for extracted samples and commercial chitin (TCC)

Six diffraction peaks were observed in all extracted samples which matches well with the diffraction peaks of commercial chitin. Six diffraction peaks at 9.4°, 12.8°, 19.5°, 20.9°, 23.5°, 26.5° are present which can be indexed as (020), (021), (110), (120), (130) and (013) respectively. Diffraction peaks of extracted samples matches with diffraction peaks of α-chitin structure which is more crystalline polymorph because of its antiparallel arrangements.[19-22] Therefore, previous literature reports confirm that the structure of extracted samples matches with α-chitin. XRD data also shows that extracted samples are more crystalline than commercial chitin (TCC) sample. Moreover, PFWSC is more crystalline among all the extracted samples as it shows higher intensity peaks than the other two samples. Hence, fresh water chitin sample showed higher crystallinity.

5.3.2. Scanning Electron Microscopy-Energy Dispersive X-Ray Analysis (SEM-EDAX)

Crude shell waste contains calcium carbonate ($CaCO_3$) in its structure which is removed through demineralization with the HCl treatment. Furthermore, NaOH is used in deproteinization step. Hence, there is a possibility that trace amount of Ca and Na may be present in the extracted mass. To confirm the elements present in extracted mass, SEM-EDAX was done by following same instrument and sample preparation method discussed in 4th chapter (Section 4.3.2). Non-homogeneous, non-smooth surfaces of extracted masses were observed in SEM images (Figure 5.4). Same observations were also observed in commercial chitin sample (TCC)

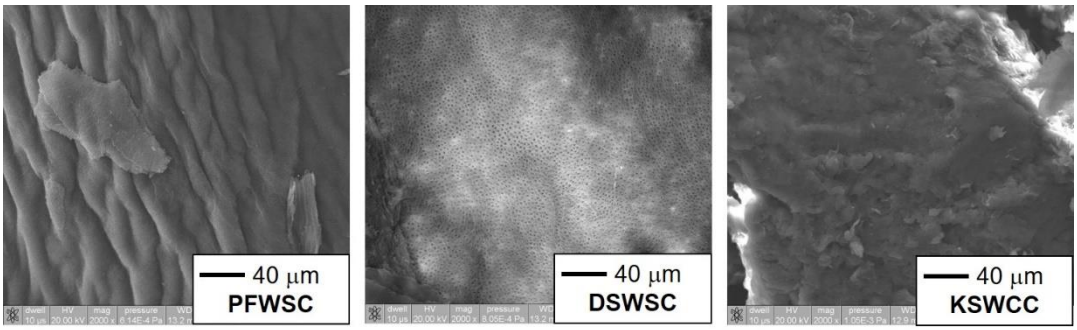


Figure 5.4. SEM images of extracted samples from shell wastes

EDAX analysis shows presence of Ca, Na, Al, Mg, Fe, K along with C, N, O. Presence of Ca depicts that traces of Ca are remaining after demineralization. Presence of Na can be attributed to the use of NaOH at deproteinization step. EDAX images are shown in Figure 5.5. Moreover, Ca & Na were also present in commercial chitin sample (TCC) along with C, N, O. Other metals (Al, Mg, Fe) were not found in EDAX data but they were observed when I performed bulk level ICP-OES analysis, presence of Al, Mg and Fe was seen and they were quantified. Those metals were not found in surface analysis technique, EDAX.

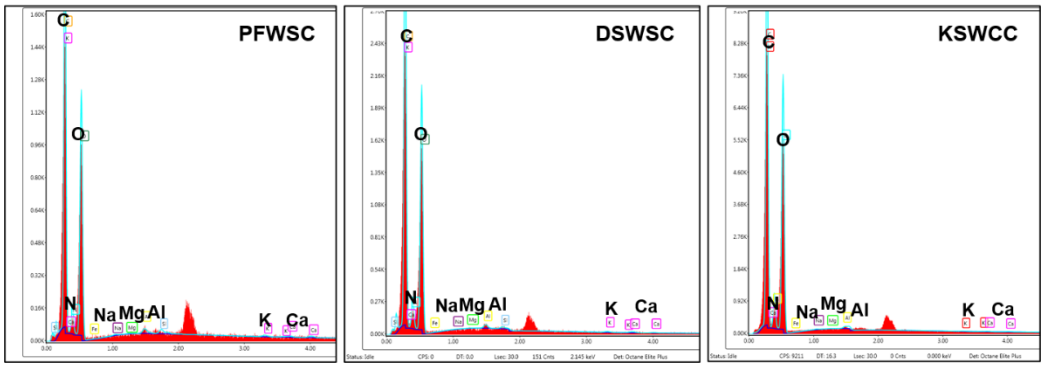


Figure 5.5. EDAX of extracted samples from shell wastes

5.3.3. Thermo Gravimetric Analysis- Differential Thermal Analysis (TGA-DTA)

TGA-DTA analysis is a useful technique to know the thermal stability of any material. As discussed in 4th chapter (Section 4.3.3), thermal degradation of extracted samples was studied in both N_2 and air atmosphere.

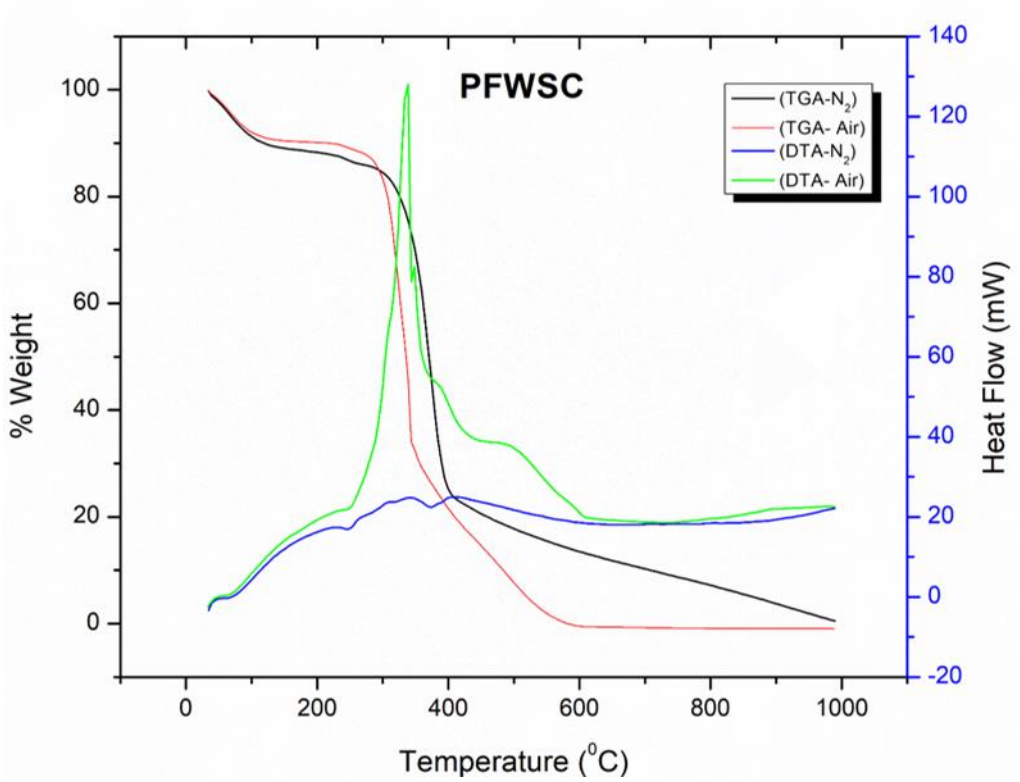


Figure 5.6a. TGA-DTA analysis of PFWSC

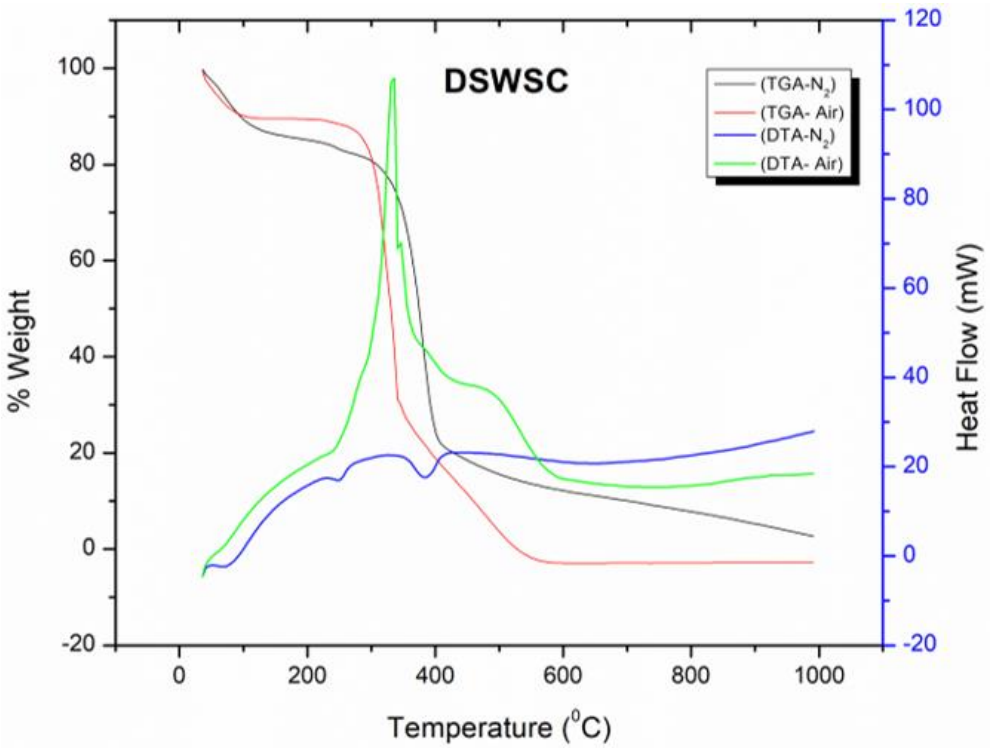


Figure 5.6b. TGA-DTA analysis of DSWSC

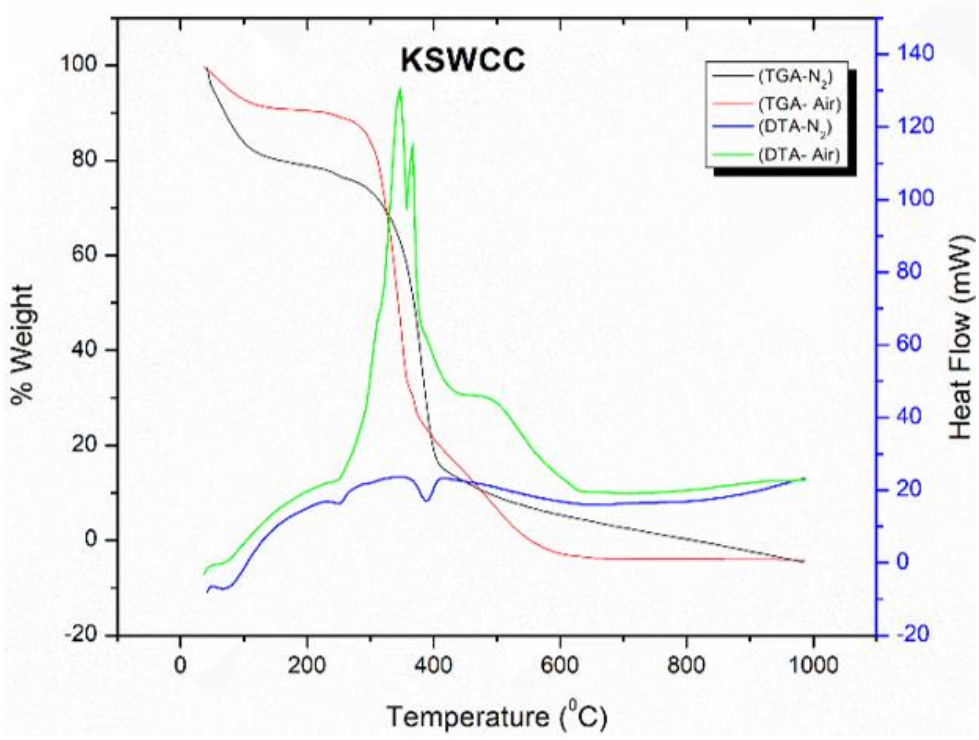


Figure 5.6c. TGA-DTA analysis of KSWCC

Thermal analysis of extracted samples (Figure 5.6a- 5.6c) shows two step weight loss in nitrogen atmosphere. First step of weight loss happened in the range of 50- 120 °C due to removal of moisture present in the sample. Then 2nd step of weight loss happened in the range of 330-400 °C due to dehydration of saccharide ring, deacetylation, elimination of carbons in the form of CO, CH_4 with remaining oxygen and hydrogen. Under air environment, 3 step degradation was found. The first two steps are similar with nitrogen atmosphere. Third step weight loss happened due to oxidative elimination of remaining carbons. The pattern of weight loss matches well with α-chitin commercial sample (TCC) which is discussed in previous 4th chapter (Section 4.3.3). Commercial chitin sample (TCC) also showed two step weight loss in nitrogen atmosphere (first step for removal of moisture and 2nd step due to dehydration of saccharide ring, deacetylation and elimination of remaining carbon in the form CO, CH_4 with remaining oxygen and hydrogen). On the other hand, three step weight loss was observed in air atmosphere (first two steps are same as nitrogen atmosphere and 3rd step is due to oxidative elimination of remaining carbons). Furthermore, DTA of extracted samples shows that the samples are stable up to 330 °C (Table 5.3) which is also comparable with thermal stability of commercial chitin sample TCC (336 °C). Hence, weight loss patterns and thermal stability matches well with commercial chitin TCC which confirms the α-chitin structure of extracted chitin samples. α-chitin shows good thermal stability due to presence of H-bonding network which leads to structural rigidity. The observed thermal degradation steps of extracted samples are also confirmed with the available literature reports.[23-24]

Table 5.3. Summary on the thermal stability of extracted samples and commercial chitin

Sample	Thermal stability up to (°C)
PFWSC	338
DSWSC	333

KSWCC	342
Commercial chitin (TCC)	336

5.3.4. CHNS elemental analysis

As discussed in 4th chapter (Section 4.3.4), rapid and simultaneous determination of carbon, hydrogen, nitrogen and sulphur in organic samples can be done by CHNS elemental analysers. CHNS elemental analysis was performed to know elemental composition. Extracted mass from shell waste must contain C, H and N. A general monomer molecular formula can be derived from the results of CHNS analysis which can confirm the structure of chitin. Complete extraction of protein can also be confirmed by the amount of carbon and nitrogen present in the sample. Elemental analysis of chitin shows that 42.3-44% C, 5.6-6.4% H and 6.2-6.3% N are present. Based on these results, a general monomer molecular formula for all extracted chitin samples and commercial chitin (TCC) are derived and summarized in Table 5.4. The general formula for all the extracted samples can be expressed as $C_aH_bN_cO_d$ (a = 7.9-8.3, b = 12.6-14.3, c = 1, d = 6.3) which matches well with theoretical monomer molecular formula of chitin.[25] Moreover, elemental composition matches well with commercial chitin sample TCC (44.2% C, 6.5% N, 5.9% H and 43.4% O) and derived monomeric formula is also comparable with TCC. Excess carbon and nitrogen are not present in the extracted sample which proves that protein has been completely eliminated during deproteinization step and only chitin was extracted from shell waste samples. Hence, CHNS analysis data also suggests the extracted sample is pure chitin which is free from protein and similar like commercial chitin sample.

Table 5.4. Elemental analysis of extracted samples from different shell wastes and commercial chitin

Sample	C (%)	N (%)	H (%)	O (%)	Experimental Molecular formula
PFWSC	43.6	6.2	5.6	44.6	$C_{8.2}H_{12.6}N_1O_{6.3}$
DSWSC	44.0	6.2	5.7	44.1	$C_{8.3}H_{13}N_1O_{6.3}$
KSWCC	42.3	6.3	6.4	45.0	$C_{7.9}H_{14.3}N_1O_{6.3}$
TCC	44.2	6.5	5.9	43.4	$C_{7.9}H_{12.5}N_1O_{5.8}$

5.3.5. Measurement of ash content and Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES)

ICP-OES analysis is a well known and highly specific technique to analyse all the metals with good detection limits. The elements can be quantified by calibrating against standards of different concentrations. Details of instrument and sample preparation method was discussed in 4th chapter (Section 4.3.5)

Presence of metals has been observed from SEM-EDAX analysis. As SEM-EDAX analysis is a surface technique, amount of metals can not be quantified exactly using SEM-EDAX. On the other hand, ICP-OES can measure the amount of metal quantitatively as this is a bulk analysis. ICP-OES data (Table 5.5) shows the presence of Ca, Na, Mg Al and Fe which matches with SEM-EDAX data (Figure 5.5). Higher amount of ash is found in sea water samples (DSWSC, KSWCC) than fresh water sample (PFWSC). Higher amount of Ca and Mg are present in sea water samples which is natural as sea water contains Ca, Mg and Na in large quantities. Moreover, extracted sample from crab shell waste has higher ash content than shrimp one. Ca content is higher in KSWCC than the other two samples which can be due to presence of remaining $CaCO_3$ after demineralization. Furthermore, presence of Na can be attributed to the use of NaOH during

deproteinization or presence of Na in seawater/freshwater. Furthermore, ash and elements content in commercial chitin sample (TCC) is compared with extracted chitin samples and the data shows that high amount of ash (6.7 mg/1g of chitin) is also present in commercial sample (TCC) along with high amount of Ca and Na. Other metals e.g., Mg, Al and Fe are also present in TCC. Calcium and sodium content in commercial sample is higher than lab-extracted chitin (PFWSC, DSWSC). Hence, it can be assumed that complete demineralisation was also not done while preparing chitin samples commercially and Na may come from deproteinization step. From all the extracted samples, fresh water shrimp chitin (PFWSC) is purer than the other extracted chitin samples and commercial chitin sample as it contains less amount of minerals.

Table 5.5. Measurement of ash and ICP-OES analysis

Sample name	Ash Content (mg/g)	Ca (mg/g)	Na (mg/g)	Mg (mg/g)	Al (mg/g)	Fe (mg/g)
PFWSC	2.38	0.11	0.29	0.07	0.31	0.18
DSWSC	4.40	0.85	0.21	0.26	0.27	0.42
KSWCC	8.29	2.73	0.17	0.13	0.16	0.16
TCC	6.7	1.4	2.3	0.1	0.05	0.06

5.3.6. Attenuated Total Reflection (ATR) spectroscopy

ATR spectroscopy is a powerful technique to analyse the functional groups present in the extracted samples which can help to understand the structural features of the samples. Analysis is done in the same instrument as discussed in 4th chapter (Section 4.3.6)

ATR analysis of extracted samples was performed to determine the different functional groups present in extracted samples (Figure 5.7). Band at 3450-3440

cm^{-1} was observed which can be due to stretching vibration of hydroxyl groups. Band at 3265-3255 cm^{-1} and 3105-3100 cm^{-1} can be attributed to vibrational modes of N-H (intermolecular hydrogen bonding between C=O and NH & H-bond between NH groups). C-H stretching vibrations of methyl and methylene groups were present at 2880-2870 cm^{-1}. C=O stretching of amide Ⅰ was found at 1620-1610 cm^{-1}. Band at 1560-1540 cm^{-1} can be attributed to stretching vibration of amide Ⅱ C-N bond. Bending vibration of N-H bond also can come at same place. Band for $-CH_2$ bending was found at 1430-1420 cm^{-1}. Band at 1375-1370 cm^{-1} can be due to $C-CH_3$ deformation and also for C-H bending. Wagging vibration of $-CH_2$ was found at 1310-1300 cm^{-1}. Band appearing at 1165-1160 cm^{-1} was found due to C-O-C asymmetric stretching for bridge oxygen. Again, C-O-C asymmetric stretching vibration was seen at 1070-1060 cm^{-1}. C-O asymmetric stretching in ring was found at 1015-1011 cm^{-1}. Wagging vibration of CH_3 was found at 950-945 cm^{-1}. The summary of bonds observed in extracted samples and commercial chitin are presented in Table 5.6. Peak positions of extracted samples match well with commercial chitin which confirms that the extracted samples have same functional groups like chitin. The ATR data also matches well with previous literature reports for IR of chitin.[26-27]

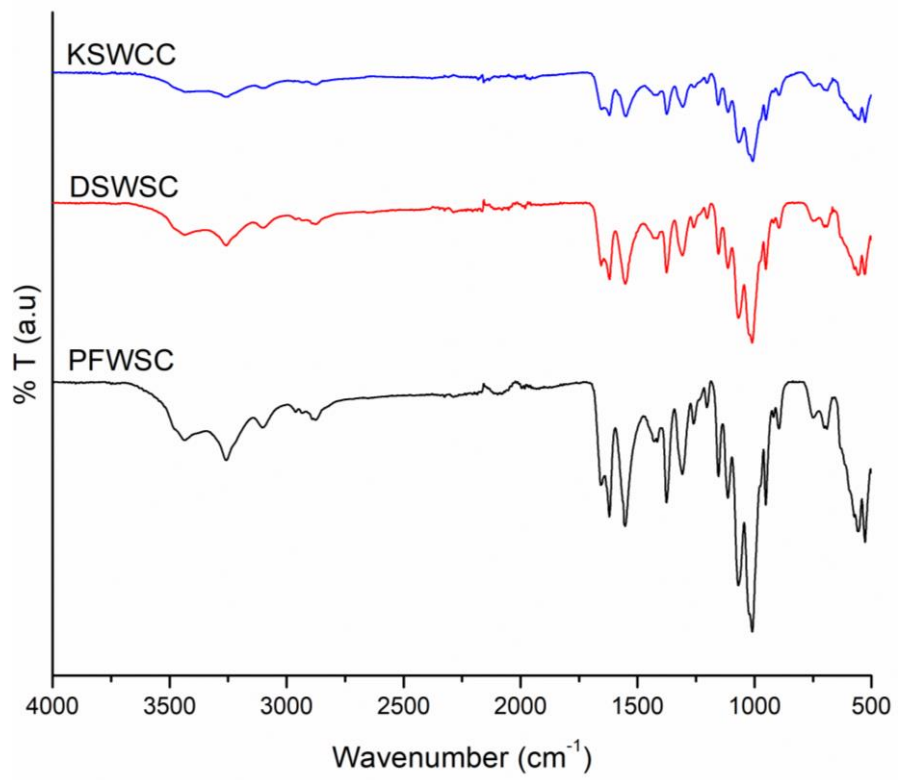


Figure 5.7. ATR spectra of extracted samples

Table. 5.6. Summary on ATR bands present in various chitin samples

Band (cm^{-1}) and type of vibration	Wavenumber (cm^{-1})			
	PFSWC	**DSWSC**	**KSWCC**	**Commercial chitin (TCC)**
3450-3440 (Stretching vibration of O-H groups)	3437	3435	3441	3441
3265-3255 and 3105-3100 (N-H stretching)	3263	3262	3258	3264
2880-2870 (C-H stretching of methyl and methylene group)	2880	2879	2882	2875

1620-1610 (Amide I C=O stretching)	1617	1618	1621	1617
1560-1540 (Amide II C-N stretching/N-H bending)	1552	1550	1556	1551
1430-1420 (CH_2 bending)	1422	1430	1428	1428
1375-1370 (CH_3 symmetrical deformation/C-H bending)	1376	1381	1374	1374
1310-1300 (CH_2 wagging)	1306	1306	1308	1307
1165-1160 (C-O-C asymmetric stretching)	1162	1157	1165	1162
1070-1060 (C-O-C asymmetric stretching in saccharide rings)	1069	1064	1066	1070
1015-1010 (C-O asymmetric stretch in ring	1008	1013	1011	1014
950-945 (CH_3 wagging)	953	948	945	945

5.3.7. Solid state ^{13}C NMR spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy helps to understand the structure of a molecule. The skeleton of extracted samples can be explored using ^{13}C NMR spectroscopy. The details of instrument are given in chapter 4 (Section 4.3.7). ^{13}C NMR spectra (Figure 5.8a to 5.8c) shows eight distinct peaks which suggests that the extracted samples contain 8 different carbons. Peaks at 174.20-174.39 ppm, 104.17-104.31 ppm, 83.39-83.54 ppm, 73.54-73.64 ppm, 75.77-75.87 ppm, 60.97-61.07 ppm, 55.34-55.44 ppm and 22.97-23.02 ppm were observed in all samples which matches well with the ^{13}C NMR peaks for commercial chitin sample. Peak at 174.20-174.39 ppm can be assigned to carbon for carbonyl group (-C=O). NMR peak at 22.97-23.02 ppm can be attributed to presence of methyl group.

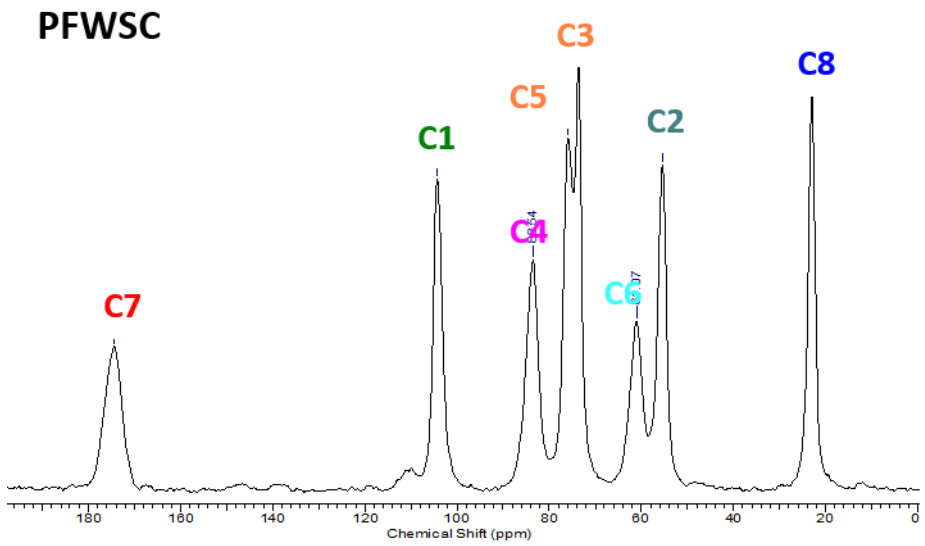


Figure 5.8a. Solid state ^{13}C NMR spectra of PFWSC

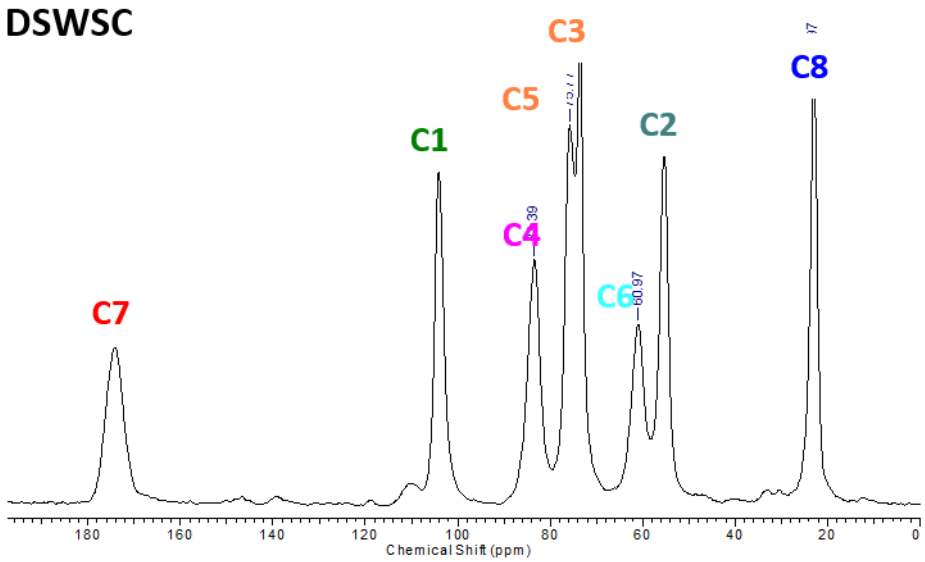


Figure 5.8b. Solid state ^{13}C NMR spectra of DSWSC

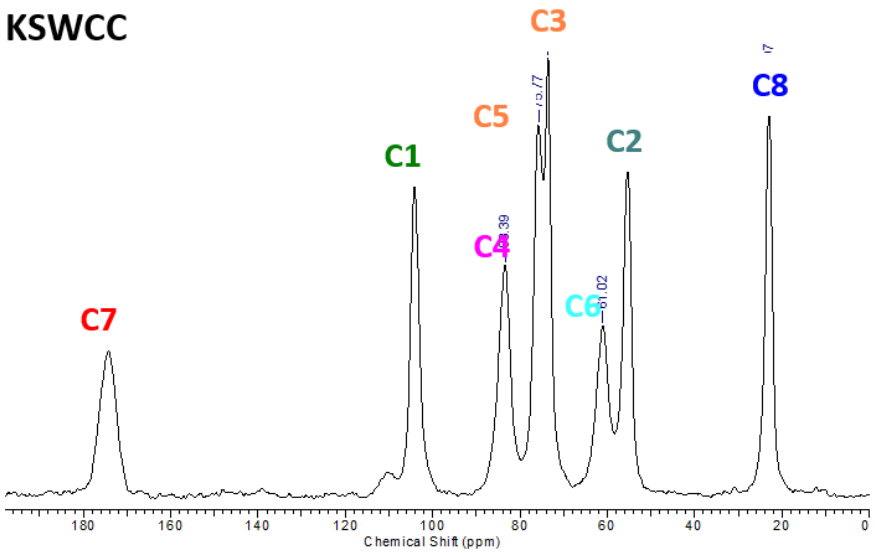


Figure 5.8c. Solid state ^{13}C NMR spectra of KSWCC

Table 5.7. Summary on chemical shifts (ppm) of extracted samples and commercial chitin from ^{13}C NMR

Sample code	Chemical shift (ppm)							
	Polysaccharide Backbone						-C=O	-CH_3
	C1	C2	C3	C4	C5	C6	C7	C8
PFWSC	104.31	55.44	73.64	83.54	75.87	61.07	174.39	23.02
DSWSC	104.17	55.34	73.54	83.39	75.77	60.97	174.20	22.97
KSWCC	104.20	55.34	73.54	83.39	75.77	61.02	173.21	22.98
Commercial chitin (TCC)	104.79	55.75	76.33	83.72	74.01	61.55	173.88	23.49

Table 5.7 shows that the peaks for extracted samples matches well with peaks of commercial chitin. From the previous 4th chapter (section 4.3.7), it was confirmed that chitin has a polysaccharide backbone which contains six different carbons (C1-C6), one carbonyl carbon (C7) and one methyl carbon (C8).

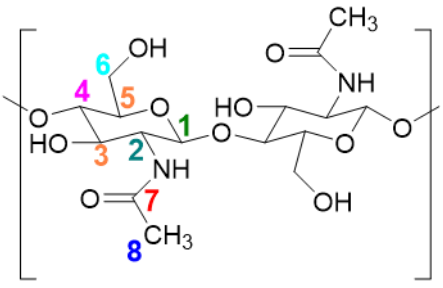


Figure 5.9. Representative structure of α-chitin with carbon labelling

Figure 5.9 shows representative structure of chitin with all carbon marking. Therefore, from the observations from previous chapter, it can be concluded that

chemical shifts of C1, C2, C3, C4, C5, C6, C7, C8 were observed at 104.17-104.31 ppm, 55.34-55.44 ppm, 75.77-75.87 ppm, 83.39-83.54 ppm, 73.54-73.64 ppm, 60.97-61.07 ppm, 174.20-174.39 ppm and 22.97-23.02 ppm respectively (Table 5.6). Previous literature reports suggest that the ^{13}C NMR peaks were exactly match with the α-chitin structure.[23] Moreover, ^{13}C NMR data supports the ATR data. No extra carbon peaks also prove the complete removal of proteins.

5.4. Conclusions

Shell waste samples were fractionated using well known industrial method and extracted samples were thoroughly characterized using different physico-chemical techniques. First of all, XRD analysis shows that the planes which are present in extracted mass matches well with the planes reported for α-chitin structure. In addition to this, XRD data also shows that extracted samples are more crystalline than commercial chitin sample (TCC). Comparing XRD data of extracted and commercial chitin samples, it can be assumed that either vendors are using more concentrated acid and base solutions for demineralization and deproteinization step respectively which also breaks the inter chain hydrogen bonding network in chitin or they are extracting chitin in more harsh reaction conditions (high temperature, longer time, use of mechanical energy) which decrystallize chitin along with demineralization and deproteinization. Moreover, fresh water chitin sample (PFWSC) is highly crystalline than sea water chitin samples (DSWSC, KSWSC) as it shows higher intensity peaks than the other two samples. Hence, highly crystalline pure α-chitin was extracted in lab from different shell waste samples. Furthermore, another surface analysis SEM-EDAX shows non-homogeneous, non-smooth surface of the extracted sample and presence of Ca, Na, Al, Mg, Fe, K along with C, N, O. The SEM-EDAX data of commercial samples showed only Ca, Na along with C, N, O. But SEM-EDAX is surface technique and can not detect the elements which is present in bulk, that's why bulk analysis was carried out. To quantify the elements, bulk level CHNS analysis

and ICP-OES analysis were done. Monomeric formula was derived from CHNS data and the experimental monomeric formula of extracted samples matches with theoretical monomeric formula of chitin. Moreover, the elemental composition and monomeric formula matched well with commercial chitin sample, TCC. Excess carbon and nitrogen were not present in the extracted sample which proves that protein has been completely eliminated during deproteinization step and pure chitin was extracted from shell waste. ICP-OES data shows the presence of Ca, Na, Mg, Al and Fe which matches with SEM-EDAX data. Higher amount of ash is found in sea water samples (DSWSC, KSWCC) than fresh water sample (PFWSC). Higher amount of Ca and Mg are present in sea water samples which is obvious as high amount of Ca and Mg are present in sea water. Moreover, extracted sample from crab shell waste has higher ash content than shrimp one. Ca content is higher in sea water crab chitin (KSWCC) than the other two samples which can be due to presence of remaining $CaCO_3$ after demineralization. Furthermore, presence of Na can be attributed to the use of NaOH during deproteinization. Further, ash and elements content in commercial chitin sample (TCC) is compared with extracted chitin samples and the data shows that high amount of ash (6.7 mg/1g of chitin) is also present in commercial sample (TCC) along with high amount of Ca and Na. Other metals e.g., Mg, Al and Fe are also present in TCC. Calcium and sodium content in commercial sample is higher than lab extracted chitin (PFWSC, DSWSC). Hence, it can be assumed that complete demineralisation was also not done while preparing chitin samples commercially and Na may come from deproteinization step. From all the extracted samples, fresh water shrimp chitin (PFWSC) is purer than the other extracted chitin samples and commercial chitin sample as it contains less amount of minerals. Furthermore, DTA of extracted samples shows that the samples are stable up to 330 °C which is comparable with thermal stability of commercial chitin sample. The pattern of weight loss matches well with α-chitin. Hence, α-chitin shows good thermal stability due to presence of H-bonding network which leads to structural rigidity. Weight loss patterns are

similar in all the extracted samples and commercial chitin sample. ATR analysis shows that functional groups present in extracted samples matches well with functional groups of chitin. Moreover, ^{13}C NMR study confirms that the structure of extracted mass from shell waste matches with structure of α-chitin. No extra carbon peaks also prove the complete removal of proteins. The IR data and ^{13}C NMR data of extracted chitin samples showed similar and comparable results with commercial chitin (TCC). Extracted chitin samples showed differences in crystallinity and mineral content which was observed in XRD and ICP-OES analysis. Extracted chitin samples were found to be more crystalline than commercial chitin sample. Further, high amount of ash, Ca and Na are present in commercial chitin sample which is higher that extracted chitin samples (PFWSC, DSWSC). It was also observed that the minerals composition also varies species wise and depends on habitat (fresh water or sea water). Fresh water chitin sample contains less ash, Ca and Mg that sea water samples. Moreover, shrimp samples showed less ash and calcium content that crab chitin sample. Fresh water chitin sample showed more crystallinity than sea water chitin samples as well. However, the detailed characterization study proves that pure α-chitin has been extracted from shell waste samples which have similar structural properties like commercial chitin but the differences was found in their crystallinity and mineral contents.

5.5. References

1. FAO. The state of world fisheries and aquaculture 2020. https://www.fao.org/documents/card/en/c/ca9229en.

2. Crustacean. https://en.wikipedia.org/wiki/Crustacean.

3. Mathew, G. M.; Mathew, D. C.; Sukumaran, R. K.; Sindhu, R.; Huang, C.-C.; Binod, P.; Sirohi, R.; Kim, S.-H.; Pandey, A., Sustainable and eco-friendly strategies for shrimp shell valorization. *Environmental Pollution* **2020,** *267*, 115656.

4. Marine fisheries, Department of fisheries. https://dof.gov.in/marine-fisheries.

5. Department of fisheries, Ministry of fisheries, animal husbandry and dairying, Govt. of India, Handbook on fisheries statistics 2020. https://dof.gov.in/sites/default/files/2021-02/Final_Book.pdf.

6. Sachindra, N. M.; Bhaskar, N.; Mahendrakar, N. S., Carotenoids in different body components of Indian shrimps. *Journal of the Science of Food and Agriculture* **2005,** *85* (1), 167-172.

7. Xu, Y.; Bajaj, M.; Schneider, R.; Grage, S. L.; Ulrich, A. S.; Winter, J.; Gallert, C., Transformation of the matrix structure of shrimp shells during bacterial deproteination and demineralization. *Microbial Cell Factories* **2013,** *12* (1), 90.

8. Chen, X.; Yang, H.; Yan, N., Shell biorefinery: dream or reality? *Chemistry–A European Journal* **2016,** *22* (38), 13402-13421.

9. Yan, N.; Chen, X., Sustainability: Don't waste seafood waste. *Nature* **2015,** *524* (7564), 155-157.

10. Shahidi, F.; Synowiecki, J., Isolation and characterization of nutrients and value-added products from snow crab (Chionoecetes opilio) and shrimp (Pandalus borealis) processing discards. *Journal of agricultural and food chemistry* **1991,** *39* (8), 1527-1532.

11. Beaney, P.; Lizardi-Mendoza, J.; Healy, M., Comparison of chitins produced by chemical and bioprocessing methods. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology* **2005,** *80* (2), 145-150.

12. No, H. K.; Meyers, S. P.; Lee, K. S., Isolation and characterization of chitin from crawfish shell waste. *Journal of Agricultural and Food Chemistry* **1989,** *37* (3), 575-579.

13. Xie, H.; Zhang, S.; Li, S., Chitin and chitosan dissolved in ionic liquids as reversible sorbents of CO 2. *Green Chemistry* **2006,** *8* (7), 630-633.

14. Wu, Y.; Sasaki, T.; Irie, S.; Sakurai, K., A novel biomass-ionic liquid platform for the utilization of native chitin. *Polymer* **2008,** *49* (9), 2321-2327.

15. Shimo, M.; Abe, M.; Ohno, H., Functional comparison of polar ionic liquids and onium hydroxides for chitin dissolution and deacetylation to chitosan. *ACS Sustainable Chemistry & Engineering* **2016,** *4* (7), 3722-3727.

16. Sedaghat, F.; Yousefzadi, M.; Toiserkani, H.; Najafipour, S., Chitin from Penaeus merguiensis via microbial fermentation processing and antioxidant activity. *International journal of biological macromolecules* **2016,** *82*, 279-283.

17. Duan, S.; Li, L.; Zhuang, Z.; Wu, W.; Hong, S.; Zhou, J., Improved production of chitin from shrimp waste by fermentation with epiphytic lactic acid bacteria. *Carbohydrate Polymers* **2012,** *89* (4), 1283-1288.

18. Dutta, P. K.; Ravikumar, M.; Dutta, J., Chitin and chitosan for versatile applications. *Journal of Macromolecular Science, Part C: Polymer Reviews* **2002,** *42* (3), 307-354.

19. Noishiki, Y.; Takami, H.; Nishiyama, Y.; Wada, M.; Okada, S.; Kuga, S., Alkali-induced conversion of β-chitin to α-chitin. *Biomacromolecules* **2003,** *4* (4), 896-899.

20. Wada, M.; Saito, Y., Lateral thermal expansion of chitin crystals. *Journal of Polymer Science Part B: Polymer Physics* **2001,** *39* (1), 168-174.

21. Duan, B.; Chang, C.; Ding, B.; Cai, J.; Xu, M.; Feng, S.; Ren, J.; Shi, X.; Du, Y.; Zhang, L., High strength films with gas-barrier fabricated from chitin solution dissolved at low temperature. *Journal of Materials Chemistry A* **2013,** *1* (5), 1867-1874.

22. Al Sagheer, F.; Al-Sughayer, M.; Muslim, S.; Elsabee, M., Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. *Carbohydrate polymers* **2009,** *77* (2), 410-419.

23. Jang, M. K.; Kong, B. G.; Jeong, Y. I.; Lee, C. H.; Nah, J. W., Physicochemical characterization of α-chitin, β-chitin, and γ-chitin separated from natural resources. *Journal of Polymer Science Part A: Polymer Chemistry* **2004,** *42* (14), 3423-3432.

24. Paulino, A. T.; Simionato, J. I.; Garcia, J. C.; Nozaki, J., Characterization of chitosan and chitin produced from silkworm crysalides. *Carbohydrate Polymers* **2006,** *64* (1), 98-103.

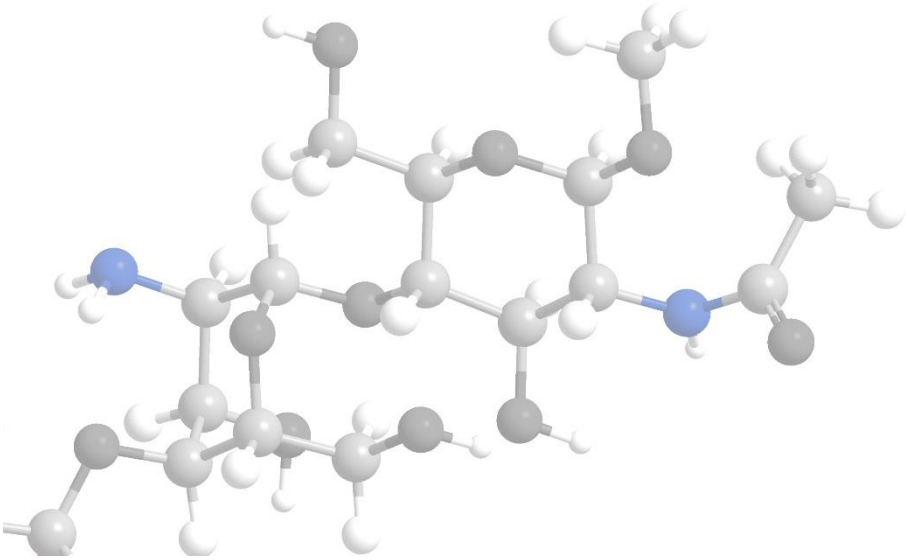
25. Yen, M.-T.; Yang, J.-H.; Mau, J.-L., Physicochemical characterization of chitin and chitosan from crab shells. *Carbohydrate polymers* **2009,** *75* (1), 15-21.

26. Ibitoye, E.; Lokman, I.; Hezmee, M.; Goh, Y.; Zuki, A.; Jimoh, A., Extraction and physicochemical characterization of chitin and chitosan isolated from house cricket. *Biomedical Materials* **2018,** *13* (2), 025009.

27. Van de Velde, K.; Kiekens, P., Structure analysis and degree of substitution of chitin, chitosan and dibutyrylchitin by FT-IR spectroscopy and solid state 13C NMR. *Carbohydrate polymers* **2004,** *58* (4), 409-416.

Chapter 06

Conversion of chitosan and chitin using solid acid catalysts



6.1. Introduction

Chitin is excellent feedstock for production of value-added chemicals as they contain naturally fixed nitrogen along with carbon, hydrogen and oxygen. Moreover, it is 2nd abundantly available bio-polymer on earth. As discussed in 1st chapter (Section 1.8), many nitrogen containing chemicals can be synthesized from chitin which is very beneficial because insertion of nitrogen in any molecule or synthesizing nitrogen containing molecule is an energy extensive process (e.g.- Haber's process of ammonia synthesis).[1-2] Chitosan is a derivative of chitin which is more suitable for the chemical transformation because of its enhanced solubility due to the weakening of crystallinity in the structure (refer characterisation data in 4th chapter, Section 4.3). Both chitin and chitosan are excellent feedstock for production of important chemicals.[1, 3] On this context, conversion of chitin and chitosan to value added chemicals has gained immense attention in the recent few years.[4]

Chitin and chitosan can be converted to various chemicals, e.g.- depolymerisation of chitin and chitosan gives N-acetyl-D-glucosamine (GlcNAc), D (+)-glucosamine ($GlcNH_2$) in acidic medium[5-6], acid catalysed further dehydration of chito-monomers to 5-hydroxymethylfurfural (5-HMF)[7-13], levullinic acid (LA)[14-15], formic acid (FA) [14], chromogen I [16-18], chromogen III [16-18] and 3-acetamido-5-acetylfuran (3A5AF)[1, 19-21], pyrolysis in basic condition to form fructosazine (FZ), deoxyfructosazine (DFZ)[22-23], hydrogenation of chitin to 2-acetamido-2-deoxysorbitol (ADS), N-acetylmonoethanolamine (NMEA), C4 & C6 polyols[24-25], oxidation of chitin and chitosan to glucosaminic acid (AGA)[26], acetic acid (AA)[27-28] etc. For these transformations, mineral acids, mineral bases, organic acids, metal chlorides, ionic liquids, supported metal catalysts were used as catalysts. In addition to this, mineral acids, organic acids, high boiling solvents, ionic liquids were used as solvents. In hydrogenation reactions, high hydrogen pressure (4 MPa) was used. As discussed in 1st chapter, solid acid catalysts were widely used

for the hydrolysis of cellulose to form D-glucose[29-30] and its further dehydration to form 5-HMF.[31] As cellulose has very similar structure like chitin and chitosan (only differs in C2 position, hydroxyl group is replaced by acetamido/amino group), I thought that solid acids can be effectively used for hydrolysis of chitin and chitosan to form $GlcNH_2$ and GlcNAc and their dehydration products (refer Figure 1.12, 1st chapter). In the 1st chapter, literature reports on acid catalysed transformations of chitin and chitosan were discussed in detail (refer Section 1.8). In those reports, mineral acids, organic acids, metal chlorides, ionic liquids were used as catalyst along with mineral acids, organic acids, high boiling solvents (DMSO, NMP), ionic liquids as solvents. As per best of my knowledge, only two reports on solid acid catalysed conversion of chitosan were reported which were also associated with few drawbacks like use of organic acids as solvent & co-catalyst, instability of catalysts, non-recyclability of the catalysts. [10, 32] However, solid acid catalysed system for conversion of chitosan and chitin to value added chemicals (both with and without nitrogen) under mild reaction conditions (T ≤ 170 °C and inert atmosphere) was developed for the first time where only water was used as solvent without the addition of any mineral and organic acids (Figure 6.1).

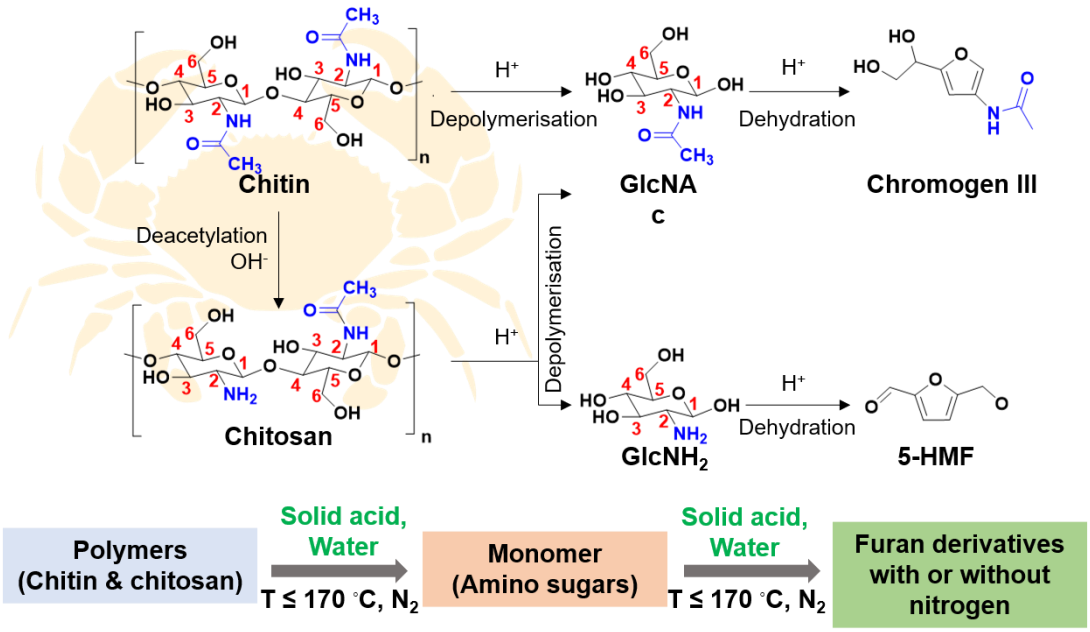


Figure 6.1. Scheme for the conversion of chitin and chitosan over solid acid catalysts

In this chapter, I have also discussed the study on the conversion of different chitosan and chitin substrates. Moreover, stable, reusable, recyclable water tolerant solid acid catalytic system is developed for the first time. Furthermore, detailed probable mechanistic routes for the conversion of chitosan and chitin are discussed in this chapter.

6.2. Experimental Section

6.2.1. Materials and reagents

Chitosan and chitin samples were procured from different suppliers and detail characterization study of these chitosan and chitin samples were discussed in 4th chapter. The details of the chitosan and chitin samples are summarized in Table 6.1. All the samples were used without pre-treatment.

Table 6.1. Summary on details of chitosan and chitin substrates

S. No.	Chemicals	Sample code	Supplier	Product Code	Viscosity (cP) given by suppliers
1.	Aldrich low molecular weight chitosan	ALMWC	Sigma-Aldrich	448869	20-300* [33]
2.	Aldrich medium molecular weight chitosan	AMMWC	Sigma-Aldrich	448877	200-800* [34]
3.	Aldrich high molecular weight chitosan	AHMWC	Sigma-Aldrich	419419	800-2000* [35]
4.	TCI chemicals low molecular weight chitosan	TCLMWC	TCI Chemicals	C2395	5-20** [36]
5.	TCI chemicals medium molecular weight chitosan	TCMMWC	TCI Chemicals	C2396	20-100** [37]
6.	TCI chemicals high molecular weight chitosan	TCHMWC	TCI Chemicals	C0831	200-600** [38]

7.	Aldrich shrimp shell chitosan	ASSC	Sigma-Aldrich	417963	>200* [39]
8.	Aldrich crab shell chitosan	ACSC	Sigma-Aldrich	48165	-
9.	TCI chemicals chitin	TCC	TCI Chemicals	C0072	-
10.	Loba Chemie chitin	LCC	Loba Chemie	02695	-

*Measured for 1 *wt.*% substrate in 1% acetic acid at 25 °C.

**Measured for 0.5 *wt.*% substrate in 0.5% acetic acid at 20 °C.

Zeolites, H-USY (CBV-720, Si/Al = 15), NH_4-ZSM-5 (CBV-2314, Si/Al= 11.5), NH_4-MOR (CBV21A, Si/Al = 10) were obtained from Zeolyst International. Prior to use, zeolites (except H-USY) were calcined at 550°C for 12 h in muffle furnace under air flow to achieve H^+ form of the same. SiO_2–Al_2O_3 (Aldrich, Si/Al=5.3), K10 clay (Sigma-Aldrich), Nafion SAC-13 (Sigma-Aldrich), Amberlyst-15 (Fluka) were also purchased and prior to the catalytic runs, all the catalysts were oven dried at 60 °C for 16 h followed by vacuum drying at 150 °C for 4 h (10^{-4} MPa). Pseudoboehmite (Marathwada Chemical Industries Pvt. Ltd., India, 65-78%), Fumed silica (Sigma-Aldrich, USA, 99%), Ortho phosphoric acid (Loba Chemie, 85%), Cyclohexyl amine (Sigma-Aldrich, 99%), Ammonium heptamolybdate (Loba chemie, 99%), Ammonium metatungstate hydrate (Sigma-Aldrich, USA, >66.5%), Phosphorus Acid (Loba Chemie, 98%) were procured and used for catalyst preparation. Monomers of chitin and chitosan, i.e., D- (+)-glucosamine ($GlcNH_2$, 99%), N-acetyl-D-glucosamine (GlcNAc, 99%) and expected dehydration product i.e., 5-hydroxymethylfurfural (5-HMF, 99%) were purchased from Sigma-Aldrich

and used for HPLC calibration. All the chemicals were used as received. Solvents like Methyl-iso-butyl ketone (MIBK, 99% AR), Methanol (MeOH, 99% AR) were purchased from Loba Chemie. Hydrochloric acid (HCl, 37%, Loba Chemie), Hydrofluoric acid (HF, 48%, Loba Chemie) were purchased and used for sample preparation for ICP-OES analysis. Millipore water was used for all the experiments.

6.2.2. Synthesis of catalysts

6.2.2.1. Synthesis of Modified SAPO-44 (m-SAPO-44)

Synthesis of m-SAPO-44 catalyst is discussed in 3rd chapter (refer Section 3.2.2.1.1). Details about synthesis conditions were summarized in Table 3.1 (Chapter 3).

6.2.2.2. Synthesis of wet-impregnated supported metal oxides

Four wet-impregnated supported metal oxides were prepared. Silica and silica-alumina (SA) support were used for the synthesis of supported metal oxide catalysts by wet-impregnation method. 10 *wt%* metal on support material was prepared using typical procedure.[40] 1.0 g of activated support i.e., silica (SiO_2) and silica alumina (SA) was dispersed in 8 mL distilled water by stirring it using magnetic stirred for 30 min at room temperature. Then 2 mL of metal salt solution was added to it dropwise. For tungsten catalyst, 0.2238 g Ammonium Metatungstate hydrate; for molybdenum catalyst, 0.2065 g Ammonium heptamolybdate was dissolved in 2 mL distilled water to prepare metal salt solution. Then the mixture was stirred for 16 h at room temperature. The water was then removed by rotary evaporator. Solid material was then dried at 60 °C followed by heating at 150 °C for 6 h in high vacuum. Then the tungsten materials are calcined at 550 °C for 2 h and molybdenum catalysts are calcined at 550 °C for 5 h in the presence of air at heating rate of 2 °C/min.

6.2.3. Characterization of catalysts

Catalysts were characterized with XRD, Ammonia-TPD, N_2-Sorption techniques, ICP-OES analysis, CHNS elemental analysis. Details on the instruments and methods used for these characterizations were discussed in details in Chapter 3, Section 3.2.2.2. The catalysts were well characterized in previous articles from our research group.[40-41]

6.2.4. Solid acid catalysed conversion of chitosan and chitin

Catalytic runs were performed in a Parr make batch reactor (100 mL). In a typical reaction, 0.31 mmol chitosan in 30 mL water was taken and 400 mg of catalyst was added to it. Reactions were performed under 2MPa nitrogen pressure (charged at room temperature). Then the autoclave was heated to desired temperature at 100 rpm. Upon attaining the desired reaction temperature, stirring was increased to 700 rpm. After the completion of reaction, catalyst was separated from reaction mixture through centrifugation. The reaction mixture is then analysed by High Performance Liquid Chromatography (HPLC).

6.2.5. Analysis of reaction mixtures

Reaction mixtures were analysed using High Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography (GC).

6.2.5.1. High Performance Liquid Chromatography (HPLC)

Aqueous reaction mixture was filtered through 0.22 μm PTFE filter and injected to two HPLC instrument simultaneously noted below.

i. Agilent 1200 HPLC equipped with Rezex Phenomenex Pb^{2+} column (300 mm x 7.8 mm I.D, 8 μm), G1314 variable wavelength detector (VWD, wavelength kept at 195 nm), G1362 refractive index detector (RID, cell temperature kept at 40 °C), Millipore water as mobile phase. Column

temperature was kept at 80 °C and the flow rate of the mobile phase was 0.5 mL/min.

ii. Shimadzu LC-9A HPLC system equipped with Agilent C18 column (250 mm x 4.6 mm x 5 μm), SPD-6A variable wavelength detector (VWD, wavelength kept at 195 nm), acetonitrile: water (1: 9 *v/v*) as mobile phase. Column temperature was kept at 25 °C and the flow rate of the mobile phase was 0.5 mL/min.

6.2.5.2. Liquid Chromatography- Mass Spectrometry (LC-MS)

Aqueous reaction mixture was analysed using Shimadzu LCMS-8040 triple quadrupole mass spectrometer equipped with electro spray ionization technique (ESI), both positive and negative polarity, 3.0 L/min nebulizing gas flow, 10 L/min drying gas flow, 250 °C DL temperature, 400 °C heat block temperature. LC analytical conditions were kept same as Section 6.2.6.1 (ii).

6.2.5.3. Gas Chromatography (GC)

Extracted products soluble in organic solvents were injected to Agilent 7890B GC equipped with HP-1 column (30 m x 0.22 μm I.D) and flame ionization detector (FID).

6.2.6. Calculations

Calculation for chitosan reaction was done using below formulae.

% Yield of D (+)- glucosamine ($GlcNH_2$) = {weight of $GlcNH_2$ (HPLC) / weight of $GlcNH_2$ (theoretical)} x 100

% Yield of N-acetyl-D-glucosamine (GlcNAc) = {weight of GlcNAc (HPLC) / weight of GlcNAc (theoretical)} x 100

% Yield of 5-HMF= {weight of 5-HMF (HPLC) / weight of 5-HMF (theoretical)} x 100

Weight of formed products are calculated from area from HPLC using calibration curve. Standards were prepared with the known quantity of $GlcNH_2$, GlcNAc and 5-HMF in water. All the yields mentioned in this chapter were calculated based on HPLC results.

Calculation of chitin reaction was carried out using below formulae.

% Yield of N-acetyl-D-glucosamine = {weight of GlcNAc (HPLC) / weight of GlcNAc (theoretical)} x 100

% Yield of 5-HMF= {weight of 5-HMF (HPLC) / weight of 5-HMF (theoretical)} x 100

6.3. Results & discussion

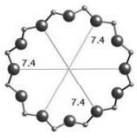
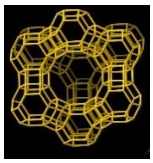
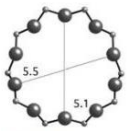
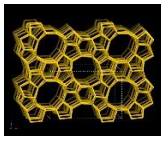
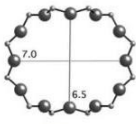
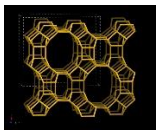
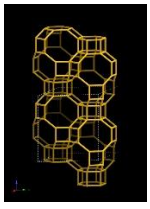
6.3.1. Evaluation of the effects of various structured solid acid catalysts for chitosan and conversion

6.3.1.1. Structural and physicochemical properties of the structured solid acid catalysts used for the reaction

Structured solid acid catalysts have definite pore structure with crystallinity, porosity and channel. It was expected that catalysts having defined structure would show higher activity than the amorphous catalysts due to their comparatively higher acidity and surface area. I have selected 3 zeolites (H-USY, H-ZSM-5, H-MOR) which have different pore size, acidity and surface area. Moreover, zeolites are also well known for the depolymerisation of cellulose, hemicellulose and lignin.[42-44] Silicoaluminophosphate SAPO-44 was also selected for the chitosan conversion reaction because of their higher hydrothermal stability and acidity. They have been also showed good results for depolymerisation of hemicellulose. Activity of SAPO-44 have studied in detail for hemicellulose conversion by our research group.[31, 45] Clay (Montmorillonite, K10) was used for the conversion of chitosan and chitin as they are very cheap, environmentally benign and they are also well known for the biomass

conversion.[46-47] Furthermore, it was also necessary to compare the activity of well-known solid acid catalysts like ion-exchanged resins (Amberlyst-15, Nafion SAC-13). Therefore, various structured catalysts were screened for the conversion of chitosan and chitin which have difference in physico-chemical properties (Table 6.2).

Table 6.2. Physico-chemical properties of structured solid acids used for chitosan and chitin conversion (figures of channel and structure of zeolites are adapted from IZA website)

Catalyst	Ring	Pore dimension (nm)	Channel	Structure	BET surface area (m^2/g)	Total acidity (mmol/g)
H-USY (FAU)	12, 6, 4	0.74 x 0.74[48]	3D 		873[49]	0.55[49]
H-ZSM-5 (MFI)	10, 6, 5, 4	0.55 x 0.51[48]	3D 		423[49]	0.98[49]
H-MOR (MOR)	12, 8, 5, 4	0.70 x 0.65[48]	2D 		528[49]	1.15[50]
m-SAPO-44 (CHA)	8, 6, 4	0.41	-		337	1.02
K10	-	-	-	-	250[50]	0.42[50]

Amberlyst -15	-	-	-	-	53[51]	4.7[51]
Nafion SAC-13	-	>10	-	-	200[52]	0.17[52]

6.3.1.2. Effect of different structured solid acid catalysts

At first, the activities of various structured solid acid catalysts were evaluated for the conversion of chitosan. From the detailed characterization study of chitosan and chitin, it was concluded that chitosan would be easier to depolymerise than chitin due to its lower crystallinity, lesser interchain hydrogen bonding network, lower structural rigidity and lower molecular weight. Moreover, characterization data reveals that TCLMWC has lowest viscosity and molecular weight among all the chitosan samples. Hence, TCLMWC will be easier to depolymerize and TCLMWC is selected for optimization study. As seen from Figure 6.2, various catalysts showed varying activity and maximum activity was seen with m-SAPO-44 catalyst to achieve highest 5-HMF yield (3.4%). Along with 5-HMF, other products were also formed as seen from HPLC chromatogram which will be discussed in Section 6.3.1.6. As, 5-HMF is identified and commercially available, amount of 5-HMF has been quantified on the basis of HPLC analysis by plotting calibration curve. As seen from Figure 6.2, no 5-HMF formation was observed when non-catalytic reaction was done in same reaction conditions. When solid acid catalyst was added to the reaction medium, 5-HMF yield was observed in the same reaction condition. Amberlyst-15 showed very poor 5-HMF yield (0.2%) instead of having high acid amount. This is obvious because Amberlyst-15 have very low hydrothermal stability (<120 °C).[53] At higher temperature, the catalyst had a tendency to lose the sulfonic groups, hence the activity of the catalyst was decreased at reaction temperature (170 °C). Nafion SAC-13 also showed poor 5-HMF yield (0.6%) because the sulfonic groups in the catalyst got solvated in the

reaction medium.[54] Hence, well known industrial catalysts are not stable and recyclable at reaction condition.

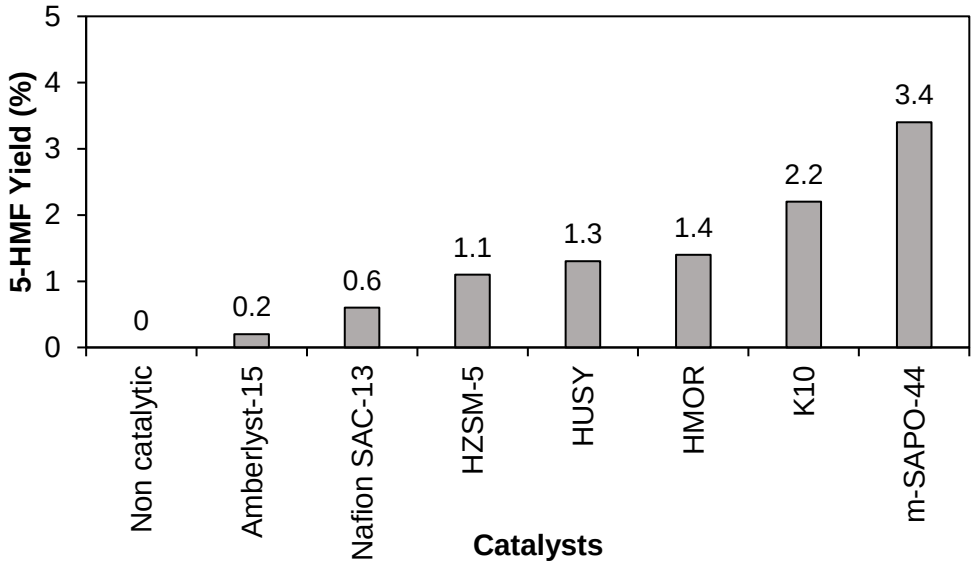


Figure 6.2. Conversion of chitosan into 5-HMF using various structured solid acid catalysts. Reaction condition: chitosan TCLMWC (0.31 mmol), catalyst (400 mg), water (30 mL), 170 °C, 2 MPa N_2 at RT, 24 h

Zeolites having different pore sizes were used for the reaction. The size of chitosan polymer is bigger than the size of the pores. Hence, bigger substrate molecule cannot enter the pores of the catalysts. In this case, labile protons on the catalyst surface for hydronium ion with water and help in the reaction. Partial proton transfer to water from Brönsted acid sites of zeolite to form hydronium ion is also proven earlier with the support of infra-red spectroscopy and quantum chemical ab initio studies.[55-56] Clay K10 and m-SAPO-44 showed better 5-HMF yield than the other catalysts. However, to arrive at any logical explanation, reaction with similar concentration of acid sites were performed with K10 and m-SAPO-44. When the reaction is carried out with 0.408 mmol of acid sites, the yield of 5-HMF is increased to 3% for K10 and almost similar 5-HMF yield was observed. This observation proves that total acid amount or amount of acid sites are playing a vital role in this reaction. M-SAPO-44 showed best results due to its higher acid

amount and m-SAPO-44 also followed same partial proton transfer phenomenon to water as zeolites because small pores of m-SAPO-44 could not be accessed by bigger substrate molecule.

6.3.1.3. Effect of reaction parameters

Degradation of side reactions can be predominant if the reaction conditions are too harsh for a reaction. To understand the effect of reaction condition like temperature, pressure, time and substrate to catalyst (S/C) ratio were studied. To understand the effect of temperature, the reactions were carried out in the temperature range of 130-190 °C under 2 MPa N_2 pressure at RT for 24 h. Expectedly, it was seen that yield of 5-HMF increased with the increasing temperature (130 °C, 2.1%; 150°C, 2.8%; 160 °C, 3%; 170 °C, 3.4%; 180 °C, 2.5% and 190 °C, 1.4%). Above 170 °C, yield of 5-HMF started decreasing probably due to side reactions. To understand the effect of total pressure at different temperature, total pressure was noted after reaching desired temperature. With the increase in temperature, total pressure of the reaction system also increased (130 °C, 2.4 MPa; 150°C, 2.7 MPa; 160 °C, 3.1 MPa; 170 °C, 3.4 MPa; 180 °C, 3.8 MPa and 190 °C, 4.1 MPa). It is also observed that the yield of 5-HMF increases with the increase in total pressure but after 170 °C the increase in total pressure did not help to increase the 5-HMF yield as side reaction becomes predominant at elevated temperature.

Similarly, effect of nitrogen pressure was also studied on the reaction as diffusivity of the substrate on the active sites of the catalysts increase with the increase in overall pressure and hence, reaction rate is increased. When the reaction was carried out at 170 °C for 24 h without nitrogen pressure the yield of 5-HMF is 2.3% but with 1 MPa (2.5%), 1.5 MPa (2.9%), 2 MPa (3.4%) and 3 MPa (3%) nitrogen pressure (initial), yield of 5-HMF increased initially and later was stabilized. 1.7 MPa (1 MPa initial), 2.8 MPa (1.5 MPa initial), 3.4 MPa (2 MPa initial). 5 MPa (3 MPa initial) total pressure was observed respectively at reaction temperature, 170

˚C. Less 5-HMF yield (2.5%) is observed at lower pressure, 1 MPa initial (1.7 MPa at 170 ˚C) and then the yield is increased with the increase in pressure. Temperature and pressure optimization data showed that the total reaction pressure helps to increase the reaction rate initially as comparative 5-HMF yield was found at similar reaction total pressure (150 ˚C, 2.7 MPa total pressure, 2.8%; 170 ˚C, 2.8 MPa total pressure, 1.5 MPa initial pressure at RT, 2.9%). Then, the yield of 5-HMF increases with elevated temperature and total pressure (160 ˚C, 3.1 MPa total pressure, 3%; 170 ˚C, 3.4 MPa total pressure, 3.4%). From co-relation between temperature and pressure optimization data, it can be concluded that the yield of 5-HMF depends on both reaction temperature and total pressure initially but at higher temperature (at or above 180 ˚C) degradation or side reactions are predominant which suppress the 5-HMF yield.

Effect of reaction time was checked at 170 ˚C under 2 MPa N_2 pressure at RT. It was observed that 5-HMF yield was maximum at 18 h (6 h, 2.8%; 12 h, 3.1%; 18 h, 3.6%; 24 h, 3.4%; 30 h, 2.7% and 36 h, 2.5%). The yield of 5-HMF increased with time but after 24 h it is decreased. It is expected that if the reaction is carried out for longer times, degradation products may be formed which suppress the 5-HMF yield.

Effect of substrate to catalyst ratio (S/C ratio) was studied with m-SAPO-44 catalyst at 170 ˚C under 2 MPa nitrogen pressure at RT for 24 h. It was observed that the yield of 5-HMF decreased with the decreasing catalyst quantity (S/C ratio-0.7 mole/mole, 3.4%; 1.4 mole/mole, 1.4%; 2.8 mole/mole, 0.8%). To understand the reason behind the decreasing catalytic activity with decreasing catalyst amount, a simple calculation and experiment was carried out.

Molecular weight of D (+)- Glucosamine ($GlcNH_2$) is 179 g

Therefore, molecular weight of chitosan will be 161 g after removal of 1 water molecule (18 g) during polymerization. (For the simplicity of the calculation, chitosan is considered as homo-polymer of $GlcNH_2$).

Hence, moles of chitosan taken in the reaction is 0.31 mmol.

Acidity of m-SAPO-44 is 1.02 mmol/g. Hence, acidity of m-SAPO-44 taken in the reaction is 0.41 mmol.

0.31 mmol of chitosan contains same amount of amino (NH_2) group which will be present in the reaction medium. Amino group is a basic functional group which has a tendency to react with H^+. Hence, there is a possibility of deactivation of some acid sites present in the catalyst. Therefore, when S/C ratio is 0.7 mole/mole, 0.10 mmol acidity is still remaining for the depolymerisation and dehydration reaction if 0.31 mmol acid sites are killed by 0.31 mmol NH_2 group. This can be the probable reason for decrease in 5-HMF yield with decreasing catalyst quantity (increasing S/C ratio).

To prove the removal of NH_2 group during reaction, a simple experiment was carried out. After the reaction, the gas from the outlet was bubbled very slowly in the Millipore water (pH 6.50) (Figure 6.3).

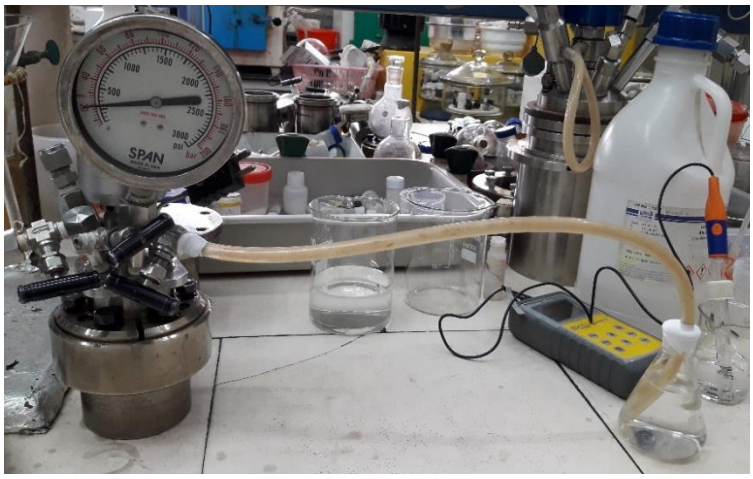


Figure 6.3. Set up for gas trap experiment

After the completion of gas release in water through bubbling, the final pH of the take Millipore water was checked and pH was increased to 7.24. The increase in pH proves that basic groups (NH_2) is getting liberated during reaction. From this trap experiment followed by pH study, it can also be concluded that $GlcNH_2$ was formed due to depolymerisation of chitosan during reaction which is further converted to 5-HMF after deamination (loss of NH_3) and dehydration (loss of water molecule).

pH of before and after the reaction were also checked (Table 6.3). At first, 0.31 mmol chitosan was added to the Millipore water (30 mL) and stirred vigorously for 30 minutes in a closed beaker. pH of Millipore water was also checked initially and it was 6.51. Then, pH is increased to 6.80 after the addition of chitosan which proves that chitosan has a basic character due to presence of amino groups. 400 mg of m-SAPO-44 catalyst was added to the previous solution and stirred again for 30 minutes and pH was checked. pH of the resulting solution was decreased to 4.55 due to the presence of H^+ ions which formed hydronium ions with water and gives acidity to the water. Then the reaction was done with solution at 170 °C under 2 MPa nitrogen pressure at RT for 24 h. After the reaction, reaction mixture was cooled down and the pH was again checked. After the reaction, pH was increased to 5.61. Therefore, increase in pH after the reaction also proves that 5-HMF is formed by deamination (loss of NH_3) of $GlcNH_2$ which is formed due to depolymerisation of chitosan.

Table 6.3. pH study of the reaction mixture

Sample	pH
Millipore water	6.51
Water after adding chitosan	6.80
Water + chitosan + solid acid (before reaction)	4.55
Reaction mixture after reaction	5.61

6.3.1.4. Conversion of different chitosan and chitin

After the optimization of the reaction conditions for the conversion of TCLMWC chitosan substrate (substrate, m-SAPO-44, S/C ratio (0.7 mole/mole), water; 30 mL, 170 °C, 2 MPa nitrogen pressure at RT, 24 h), various other chitosan and chitin substrates like ALMWC, AMMWC, AHMWC, TCMMWC, TCHMWC, ASSC, ACSC, TC, LC (refer to Table 6.1) for their full form and viscosity) were evaluated under this optimized condition. Figure 6.4 shows that all the chitosan samples gave 5-HMF with 1.5-3.9% yield. Moreover, chitin samples gave less 5-HMF yield (0.6-0.8%) which is due to different reaction pathway followed by its monomer, N-acetyl-D-glucosamine (GlcNAc) due to presence of bulky acetamido group. Detailed discussions were made in 3rd chapter, Section 3.3.4 on the probable mechanisms followed by $GlcNH_2$ and GlcNAc. It was also observed that 5-HMF yield does not only depend on viscosity or molecular weight because TCMMWC (η= 20-100 cP, 5-HMF yield= 2.1%) showed lower 5-HMF yield than AMMWC (η= 200-800 cP, 5-HMF yield= 3.2%). Moreover, the 5-HMF yield depends on the degree of crystallisation of chitosan samples because less crystalline ALMWC showed higher 5-HMF yield (3.9) than the more crystalline TCLMWC (3.4%) instead of having lower viscosity. Therefore, the hydrolysis of chitosan and dehydration of monomers to 5-HMF does not depend on only viscosity or molecular weight, it depends on many factors like degree of polymerisation, monomer present at the reducing end, degree of crystallisation and degree of deacetylation.

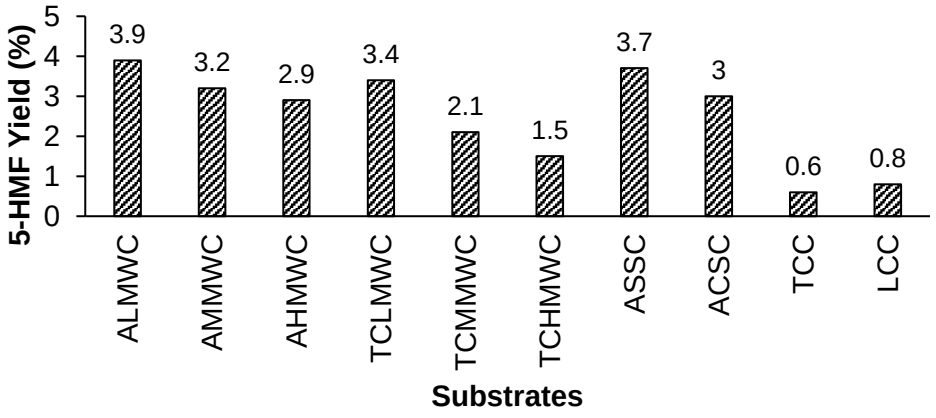


Figure 6.4. Conversion of different substrates into 5-HMF using structured solid acid catalyst, m-SAPO-44. Reaction condition: substrate, m-SAPO-44, S/C ratio (0.7 mole/mole), water (30 mL), 170 ˚C, 2 MPa N_2 at RT, 24 h

6.3.1.5. Reaction with monomers and probable mechanistic pathway

To understand the probable reaction pathways, reactions were carried out with monomers ($GlcNH_2$ and GlcNAc) at similar reaction condition (m-SAPO-44; 400 mg, water; 30 mL, 170 ˚C, 2 MPa nitrogen pressure at RT, 4 h). Time of the reaction was reduced from chitosan reaction as 4 h was found to be best optimized time for monomer reactions in 3rd chapter. When HPLC-VWD chromatograms for chitosan, $GlcNH_2$ and GlcNAc were compared (Figure 6.5a), it is found that, peak at retention time (RT) 20.47 min in chitosan reaction matches with the peak at RT 20.50 min in $GlcNH_2$ reaction which suggests that product eluting at RT 20.47 min came from $GlcNH_2$ units present in chitosan (no peak at same RT in GlcNAc reaction). Again, a sharp peak found at RT 22.29 min in chitosan reaction, matches well with peak at RT 22.30 min in GlcNAc reaction which proves that the product eluting at RT 22.29 min can be formed from GlcNAc units present in chitosan (as no peak at same RT observed in $GlcNH_2$ reaction). The peak at RT 22.29 min gave very intense response in UV detector which may suggest the presence of electron rich functional groups (chromophore) present in that product. Moreover, on an expected line and discussed in chapter 3, 5-HMF was formed from $GlcNH_2$ units

rather than GlcNAc units as attack of H^+ is easy in $GlcNH_2$ and NH_3 can be liberated immediately as it is a good leaving group. On the other hand, Acetamide ($-NHCOCH_3$) group is bulky and due to steric hindrance, attack of H^+ and liberation of acetamide group is not favourable and this might be the possible reason for poor yield of 5-HMF in GlcNAc and chitin reactions.

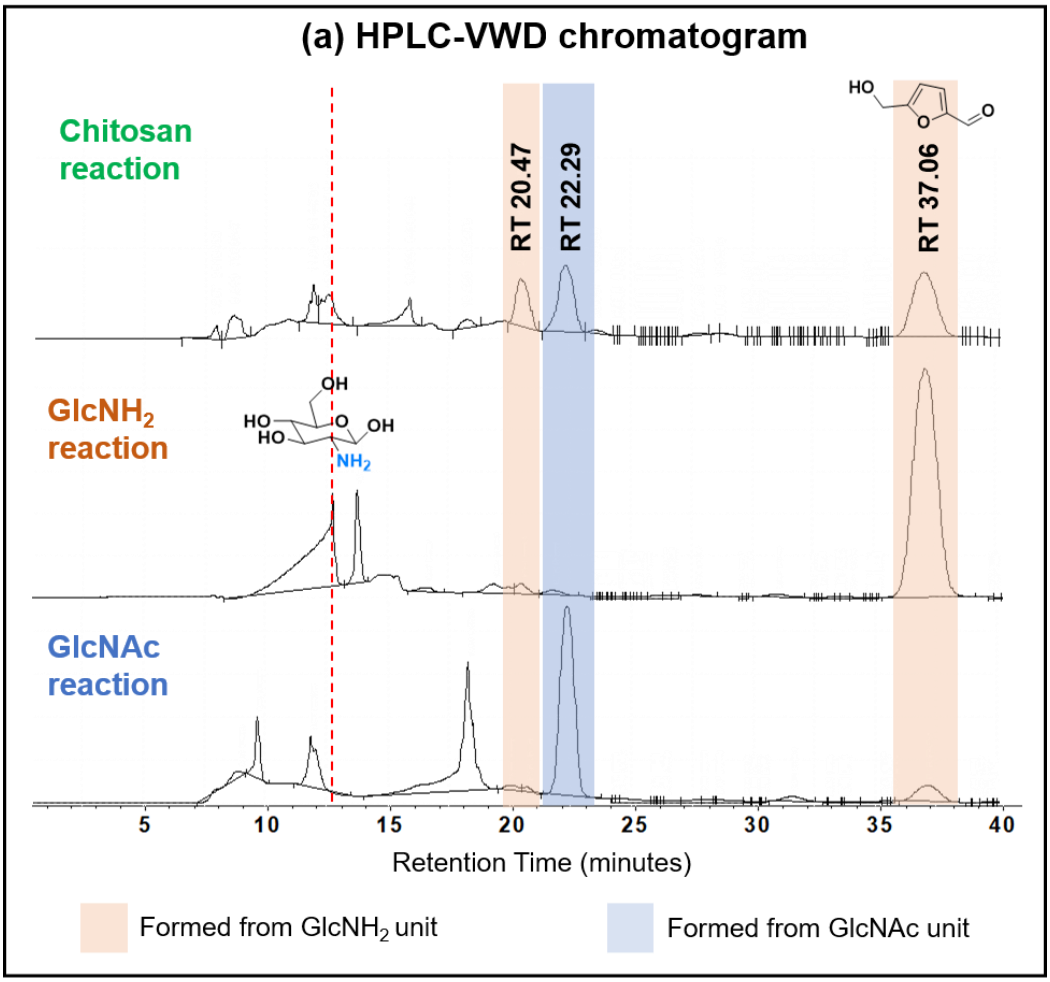


Figure 6.5a. HPLC-VWD chromatograms for chitosan, $GlcNH_2$ and GlcNAc reactions

Careful look on HPLC-RID chromatograms (Figure 6.5b) of chitosan, $GlcNH_2$ and GlcNAc reactions showed presence of one peak at similar RT (Chitosan- RT 12.87 min, $GlcNH_2$ – RT 12.90 min, GlcNAc – RT 12.87 min). This peak at RT 12.90 min in $GlcNH_2$ is due to presence of unconverted $GlcNH_2$ which was confirmed by injecting standard of $GlcNH_2$. This observation creates a confusion regarding the

formation of $GlcNH_2$ in the chitosan and GlcNAc reaction as this peak is visible in all these three reactions at similar position. The doubt can be clarified after observing HPLC-VWD chromatograms (Figure 6.5a) carefully. In VWD chromatogram of $GlcNH_2$ reaction, the peak for unconverted $GlcNH_2$ (RT 12.77 min) is present which was confirmed from VWD chromatogram of $GlcNH_2$ standard. On the other hand, no peak at same RT is observed in VWD chromatograms of chitosan and GlcNAc reactions and the small peaks observed nearby is in also different peak shape, not like $GlcNH_2$. From here, it can be concluded that the product eluting at RT 12.87 min in HPLC-RID chromatogram is not $GlcNH_2$ in chitosan reaction and this product is formed form GlcNAc units present in chitosan. The careful look on both VWD and RID chromatograms tells that this above discussed product eluting at RT 12.87 min in both chitosan and GlcNAc reactions is clearly detectable with only RID and no sharp peak is observable in VWD chromatograms which suggests that electron rich functional groups are not attached with the product which can enhance electronic transition for UV.

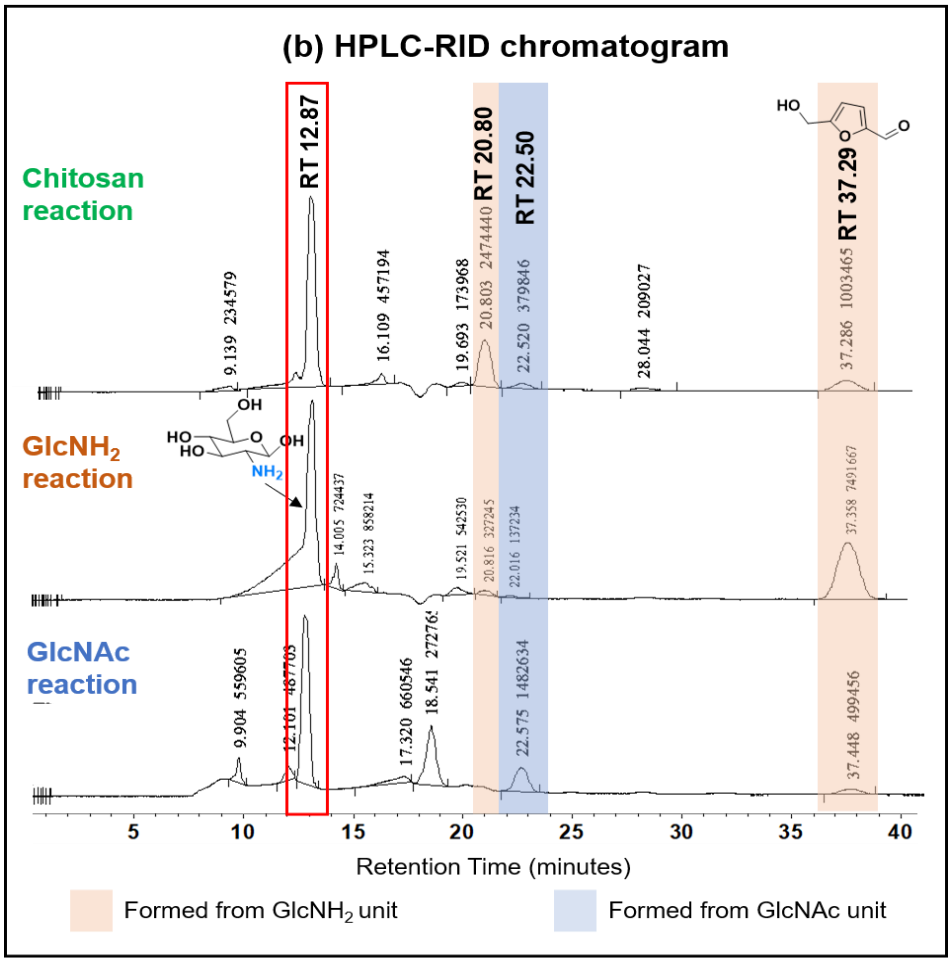


Figure 6.5b. HPLC-RID chromatograms for chitosan, $GlcNH_2$ and GlcNAc reactions

These observations become more confirmatory when I tried to extract products in methanol (MeOH). In the 2nd chapter (Section 2.3.1), it was discussed that the amino sugars are soluble in water (completely) and methanol (partially). That's why methanol was used for the extraction study. After the reaction, solid catalyst and unconverted substrate was separated through centrifugation and the water layer was filtered and injected in HPLC. After that, the water was evaporated using rota-vapour and 10 mL methanol was added to the solid remaining after rota-vapour and shaking vigorously. The soluble part was separated using centrifugation. To the solid part, again 10 mL methanol was added and above process is repeated twice. After separating methanol soluble part through centrifugation, 30 mL water was added to the solid part and injected it to HPLC

(water soluble products). GC of the Methanol soluble part was carried out (like 3rd Chapter, Section 3.3.3) but much information could be interpreted. Then the methanol was vaporised using rota-vaporisation and 30 mL of water was added when solvent is vaporised completely. This methanol extracted part was injected in HPLC after solubilizing in water. HPLC-VWD and HPLC-RID chromatograms of methanol soluble products of chitosan, $GlcNH_2$ and GlcNAc reactions are shown in Figure 6.6a and Figure 6.6b. From the chromatograms of $GlcNH_2$ reaction (both VWD and RID), it was observed that unconverted $GlcNH_2$ was extracted in methanol as $GlcNH_2$ is partially soluble in MeOH (2nd Chapter, Section 2.3.1) and the peak of $GlcNH_2$ was observed at RT 11.61 min in VWD & RT 12.04 min in RID. On the contrary, no peaks were observed in VWD & RID chromatograms of chitosan and GlcNAc reaction at the same position of $GlcNH_2$. Chromatograms for methanol extracted products confirmed that $GlcNH_2$ is not present in chitosan and GlcNAc reaction mixtures. Furthermore, it was found that product eluting at RT 22.29 min is extracted in methanol which suggests the product has organic nature.

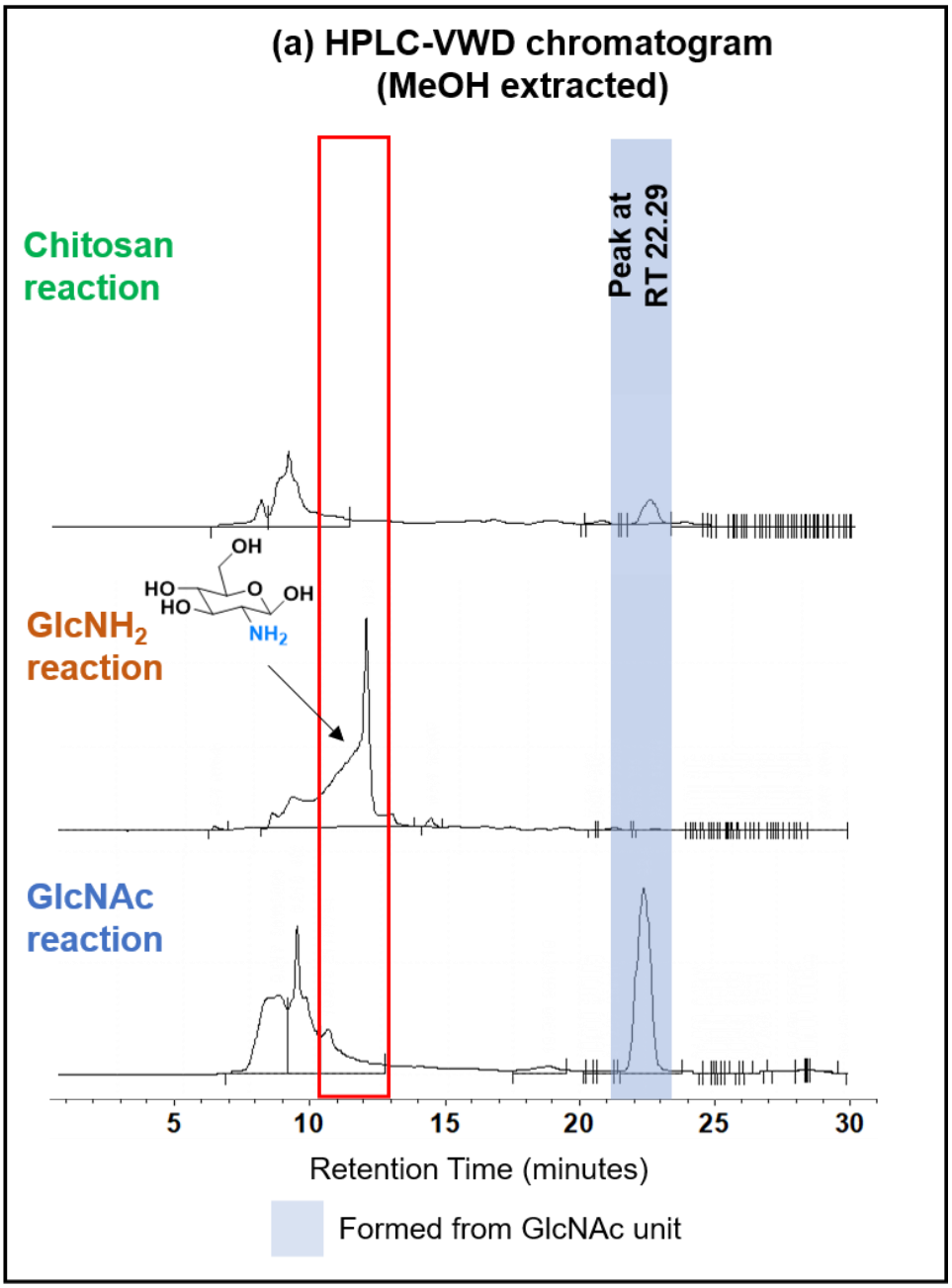


Figure 6.6a. HPLC-VWD chromatograms for methanol extracted products in chitosan, $GlcNH_2$ and GlcNAc reactions

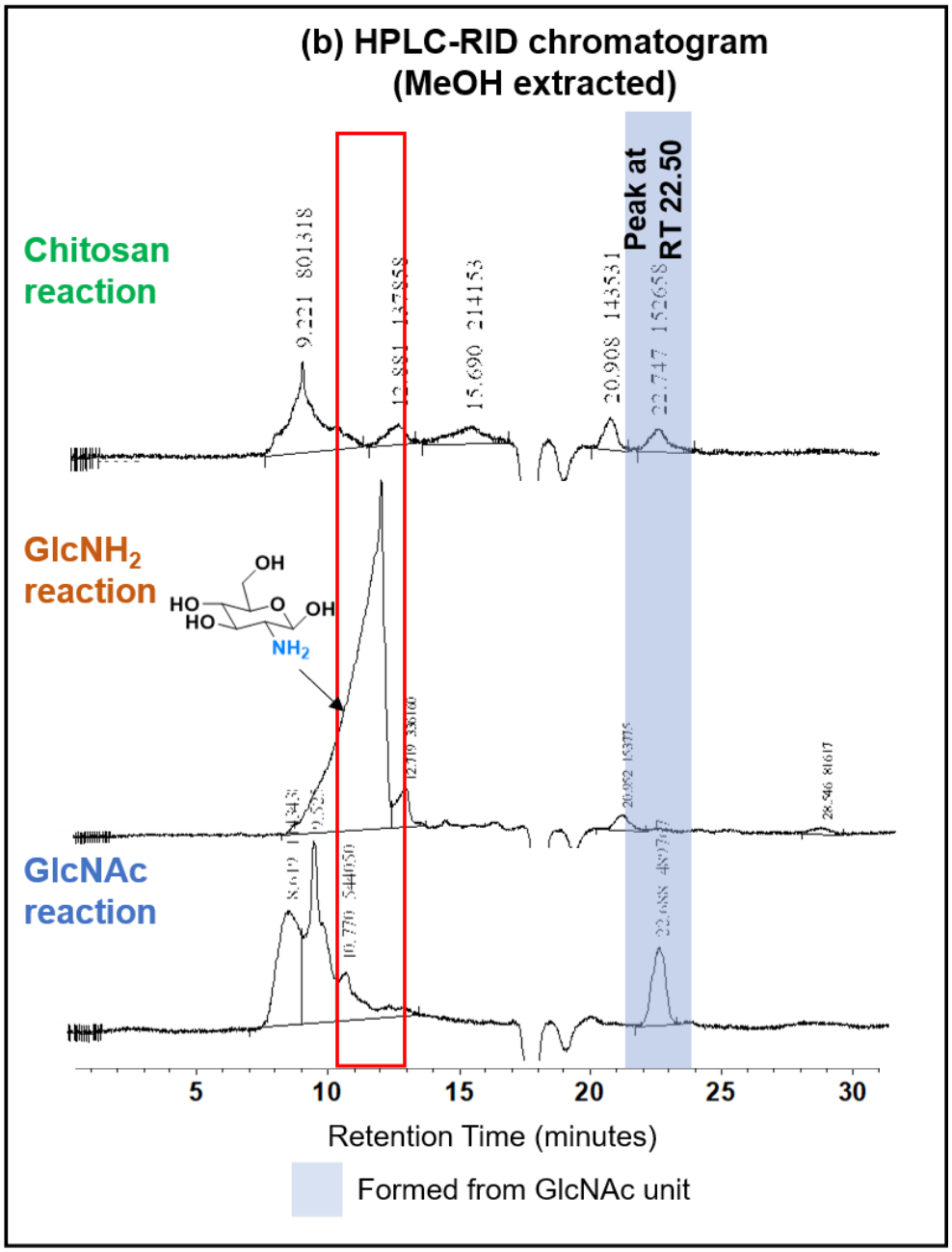


Figure 6.6b. HPLC-RID chromatograms for methanol extracted products in chitosan, $GlcNH_2$ and GlcNAc reactions

After separating methanol soluble part using centrifugation, the solid part was solubilised in water and injected in HPLC. HPLC-RID chromatograms (Figure 6.7) of water-soluble products in chitosan, $GlcNH_2$ and GlcNAc reaction showed presence of the product eluting at RT 12.92 min (same product eluted at RT 12.87 min before extraction procedure) in chitosan and GlcNAc reaction but not in $GlcNH_2$ reaction. This observation again confirms that the product at RT 12.87 min

is not $GlcNH_2$ and it forms from GlcNAc units. Here is the summary on details of the products forming in chitosan reaction based on extraction and HPLC study (Table 6.4). HPLC suggests both the detectors are very important for the complete detection of all the products in case of this type of complicated reactions and data from only one detector can miss some products due to detectability issues.

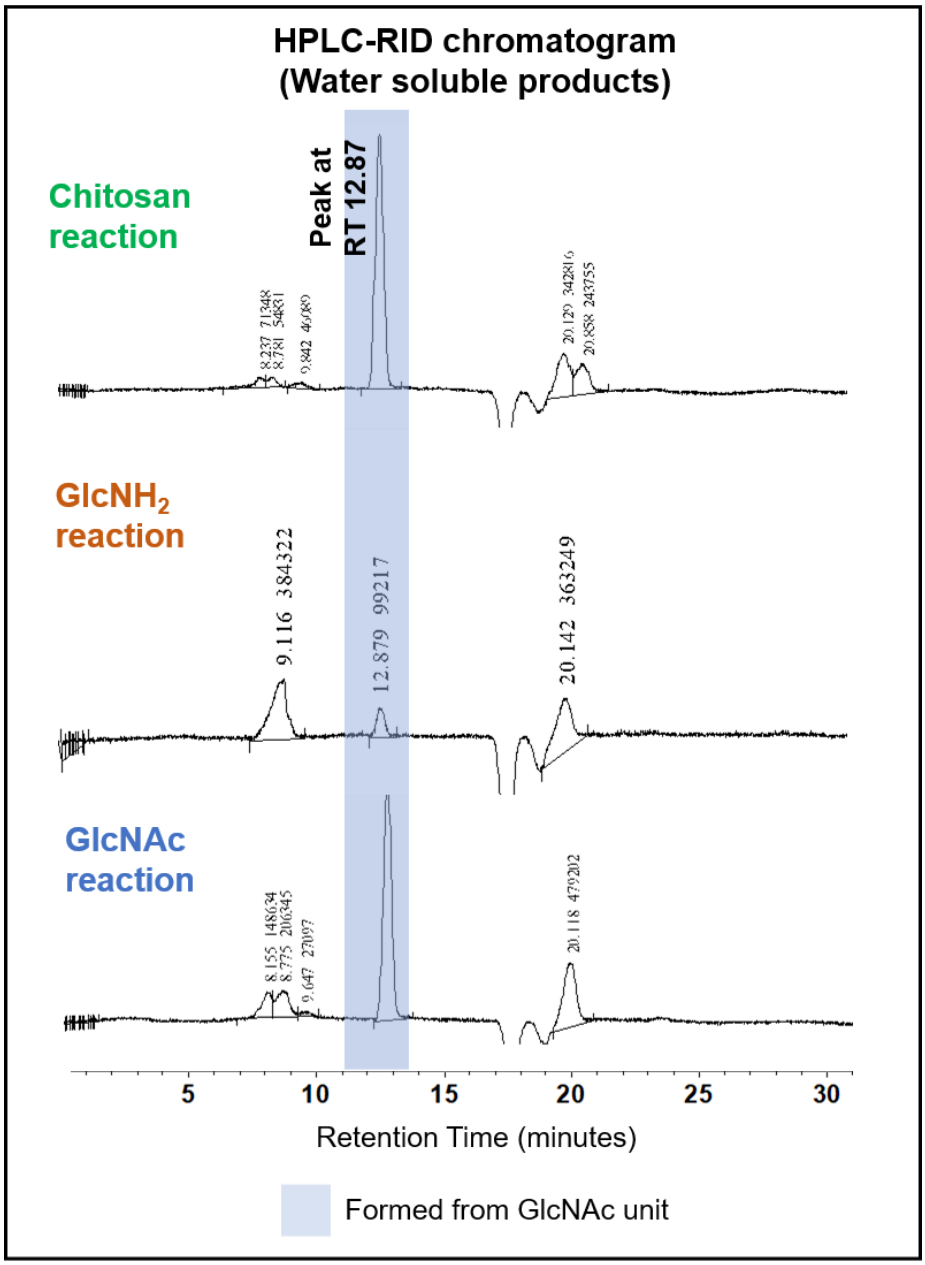


Figure 6.7. HPLC-RID chromatograms for water soluble products in chitosan, $GlcNH_2$ and GlcNAc reactions

All the reaction mixtures, extracted samples were simultaneously injected in two HPLC columns discussed in experimental section, Section 6.2.5.1 (C18 and Pb^{2+}) but here I have shown and discussed on the basis of Pb^{2+} column only because products are well separated in Pb^{2+} column due to its medium polarity and optimum adsorption-desorption of the products. In case of C18 column, some peaks are merging with each other or not separated well due to its non-polar stationary phase and due to this the comparative study can be misleading.

Table 6.4. Summary on details of the products formed in chitosan reaction (based on solvent extraction and HPLC study)

Peak position, RT (min)		Monomer unit from which it formed	Product name if identified
VWD	**RID**		
Not detectable clearly	12.87	GlcNAc	Not identified
20.47	20.80	$GlcNH_2$	Not identified
22.29	22.50	GlcNAc	Not identified
37.06	37.29	$GlcNH_2$	5-HMF

6.3.1.6. LCMS study and probable products

The probable and commercially available compounds which can be formed from chitosan were also injected in both HPLC systems along with reaction mixture (Figure 6.8 and Table 6.5) but the RT of the standard samples were not matching with the peak positions of the products from chitosan reaction except 5-HMF. HPLC chromatogram in C18 column is shown in Figure 6.8.

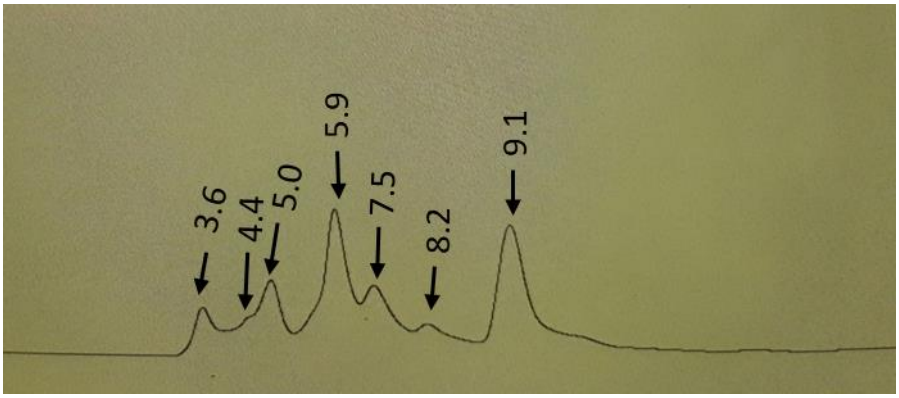


Figure 6.8. HPLC profile for chitosan reaction (C18 column)

Table 6.5. Summary of the peaks present in chitosan reaction and probable compounds which can be predicted to form in chitosan reaction

Reaction of standard	Peak ID	RT (min)	Peak identity	Molecular weight
Reaction of chitosan with m-SAPO-44	Peak-1	3.6	Unknown	-
	Peak-2	4.4	Unknown	-
	Peak-3	5.0	Unknown	-
	Peak-4	5.9	Unknown	-
	Peak-5	7.5	Unknown	-
	Peak-6	8.2	Unknown	-
	Peak-7	9.1	5-HMF	126
Standards	-	4.6	$GlcNH_2$	179
	-	5.4	GlcNAc	221
	-	9.1	5-HMF	126
	-	6.0	AcOH	60

To know the molecular weight of the products formed in the chitosan reaction, LCMS was done in both positive and negative mode. The LC profile showed all the seven peaks (Figure 6.9) and m/z observed was tabulated in Table 6.6. From the molecular weight, the presence of nitrogen can be predicted. It is known that if one nitrogen atom (atomic weight= 7) is present in any molecule, the molecular weight of that compound must be an odd number. From this theory, it is concluded that the product is nitrogen containing or not. It was observed that

three nitrogen containing compounds are formed in the reaction and other four are without nitrogen. I have searched the literature and found two probable compound which can be formed from chitosan under acid medium and those are matching with observed molecular weights. Figure 6.10 shows structure of probable compounds which can be formed in the acid catalysed conversion of chitosan.

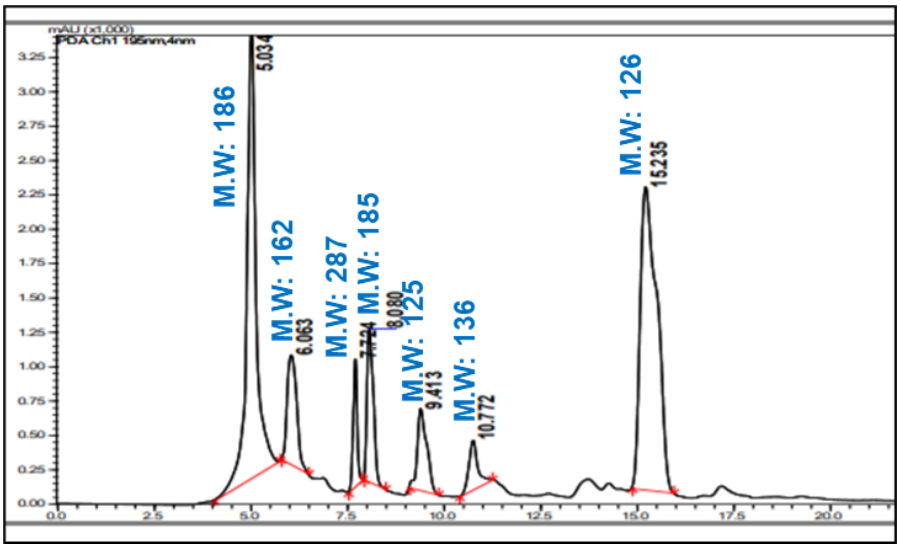


Figure 6.9. LC profile

Table 6.6. MS data with peaks observed

Peak ID	Mass observed (m/z)	Molecular weight	Type of compound
Peak-1	187 amu (M+H) +	186	Without nitrogen
Peak-2	161 amu (M-H)-	162	Without nitrogen
Peak-3	288 amu (M+H) +	287	With nitrogen
Peak-4	184 amu (M-H) -	185	With nitrogen
Peak-5	126 amu (M+H) +	125	With nitrogen
Peak-6	137 amu (M+H) +	136	Without nitrogen
Peak-7	127 amu (M+H) +	126	Without nitrogen (5-HMF)

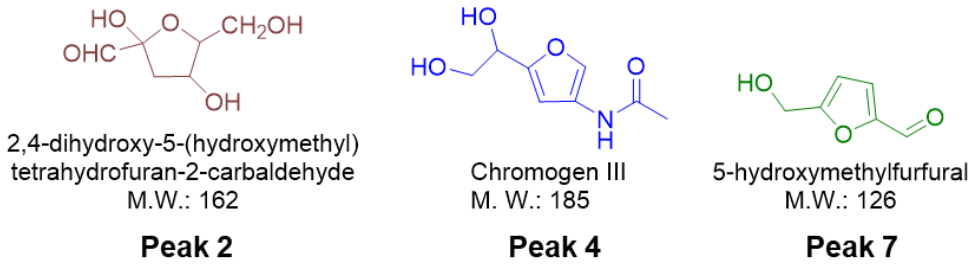


Figure 6.10. Probable products formed from chitosan

Probable mechanism for formation of 5-HMF from $GlcNH_2$ units is discussed in detail in 3rd chapter (Section 3.3.4, Figure 3.12). Probable mechanism for formation of Chromogen III (M.W – 185) from GlcNAc units are shown in Figure 6.11. In case of GlcNAc, acetamide group is bulky and due to steric hindrance, attack of H^+ and liberation of acetamide group is not favourable. Moreover, C1 is electropositive because oxygen is pulling electrons. To form stable five membered furan ring, OH at C4 position may attack and upon release of 2 water molecule chromogen III can be formed.

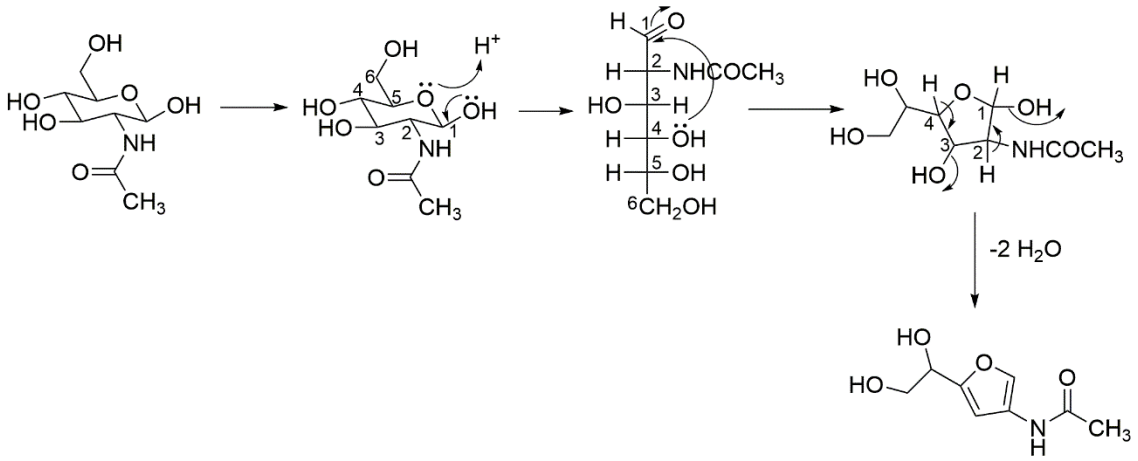


Figure 6.11. Probable mechanism for formation of Chromogen III from GlcNAc units

6.3.1.7. Characterization of spent structured solid acid catalysts

Characterizations of fresh and spent m-SAPO-44 was done to understand its stability after the reaction. The XRD patterns for fresh and spent m-SAPO-44 are shown in Figure 6.12. The peak pattern in fresh m-SAPO-44 matches well with

Chabazite (CHA) morphology. XRD pattern of spent m-SAPO-44 showed the breaking of the structure and CHA morphology is completely disturbed after the reaction which leads to decreased intensity of characteristic peak. Clay K10 also showed comparatively good 5-HMF yield after m-SAPO-44. That's why, stability of K10 was also checked and XRD of fresh and spent K10 was done. From fresh and spent XRD patterns of K10 (Figure 6.13), it was observed that structure of K10 was also altered after the reaction. Hence, m-SAPO-44 and K10 both are not stable at the reaction conditions as morphological changes were observed in both m-SAPO-44 and K10 catalysts.

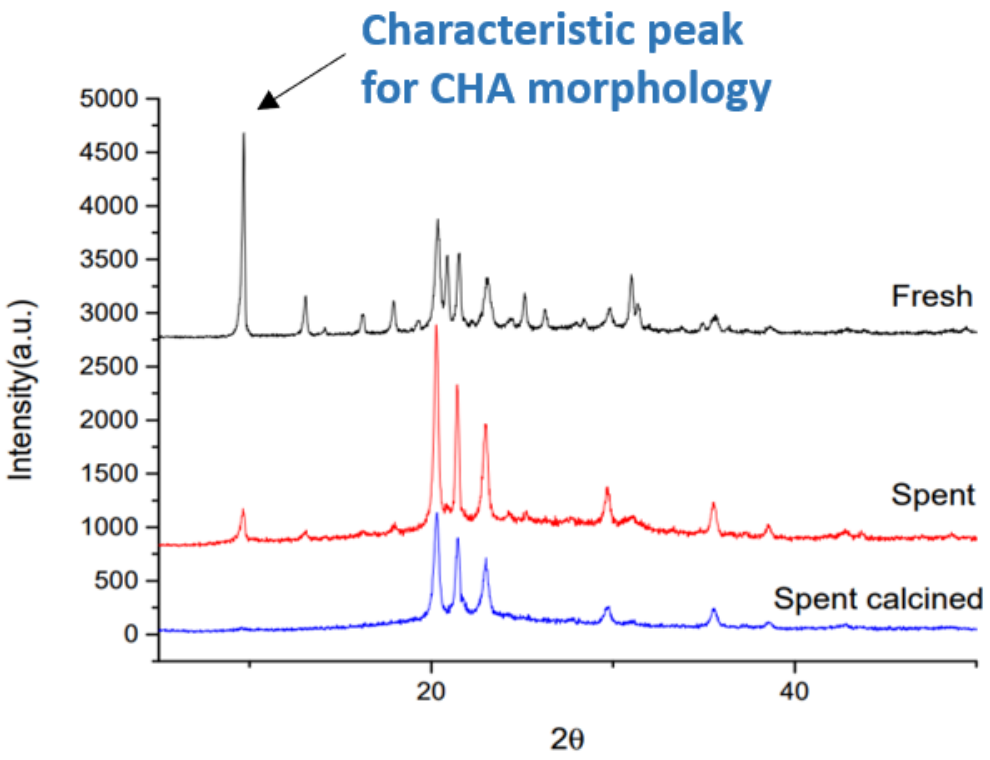


Figure 6.12. XRD of fresh and spent m-SAPO-44

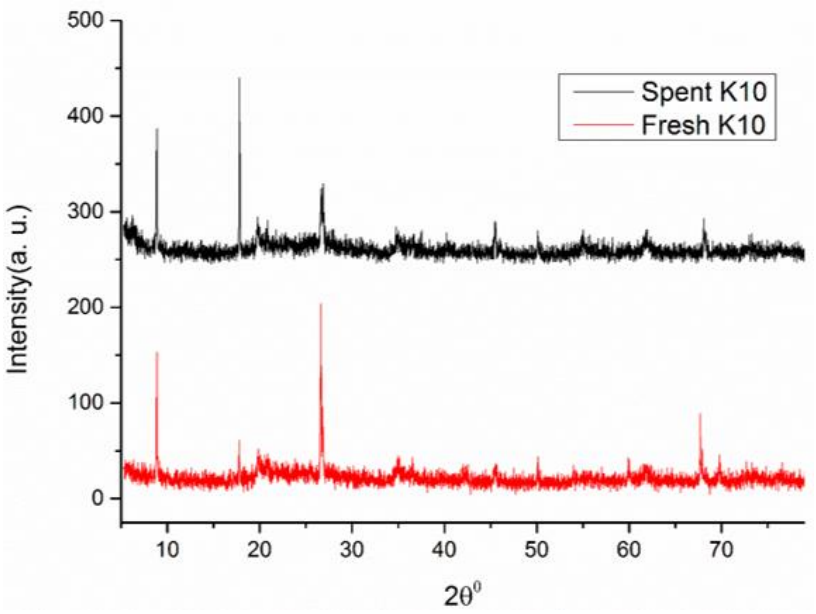


Figure 6.13. XRD of fresh and spent K10

NH3-TPD results of fresh and spent m-SAPO-44 are tabulated in Table 6.7. Fresh m-SAPO-44 showed best activity due to its higher acid amount than the other catalysts. Drastic reduction in total acidity is observed in spent m-SAPO-44 after 1st run which is due to breaking of pore structure and disturbed CHA morphology.

Table 6.7. NH_3-TPD study of fresh and spent m-SAPO-44

Catalyst	Total acidity (mmol/g) of fresh catalyst	Total acidity (mmol/g) of spent catalyst
m-SAPO-44	1.02	0.29

SEM images (Figure 6.14) of fresh m-SAPO-44 showed cubic morphology of the catalyst. SEM images of spent catalyst showed that the edges of the cubic crystals were broken after the reaction.

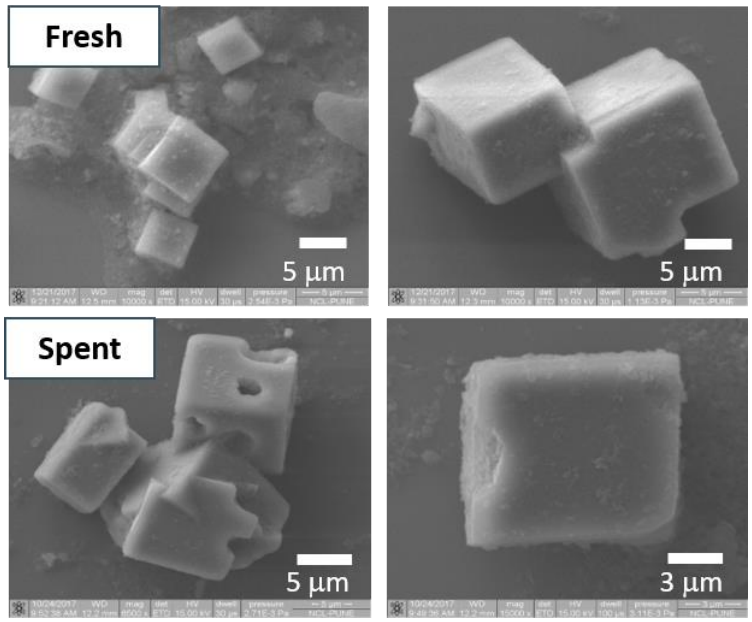


Figure 6.14. SEM images of fresh and spent m-SAPO-44

ICP-OES analysis data of fresh and spent m-SAPO-44 was shown in Table 6.8. Aluminium (Al) content of fresh m-SAPO-44 matched well with the theoretical Al content which proves that the m-SAPO-44 was synthesized properly. 12.72% Al was leached in the reaction mixture which supports the previous characterization data of spent m-SAPO-44 that the structure of catalyst was getting disturbed during reaction.

Table 6.8. ICP-OES analysis of fresh, spent m-SAPO-44 and reaction mixture (calculated w.r.t catalyst amount taken for the reaction, i.e., 400 mg)

Sample	Al content in mg (per 400 mg)
Fresh m-SAPO-44 (theoretical)	67.32
Fresh m-SAPO-44	66.98
Spent m-SAPO-44	54.72
Reaction mixture (when m-SAPO-44 is used as catalyst	8.52

Nitrogen sorption data for fresh and spent m-SAPO-44 catalyst is shown in Table 6.9. Substrate molecule cannot enter the pore and access the acid sites as pore

radius of m-SAPO-44 is lower than bigger polymer molecules. But, the reaction with m-SAPO-44 still showed better activity which suggests that some of the protons present on m-SAPO-44 are labile and those are in the water in the form of hydronium ions. The similar phenomenon of partial proton transfer to water from Brönsted acid sites of zeolite to form hydronium ion is also proven earlier with the support of infra-red spectroscopy and quantum chemical ab initio studies.[55-56] However, due to very rapid transfer of H^+ from one water molecule to another may ultimately remove the labile protons from catalyst surface into the bulk water where bigger substrate molecules are present.

Table 6.9. Summary on nitrogen sorption data of fresh and spent m-SAPO-44

Catalyst		BET surface area (m^2/g)	Pore volume (V) (cm^3/g)	Pore radius (nm)
m-SAPO-44	Fresh	337	0.10	0.41
	Spent	69	0.32	0.75

6.3.2. Evaluation of the effects of various amorphous solid acid catalysts for chitosan conversion

Based on above discussions on various characterization of fresh and spent catalysts like XRD, NH_3-TPD, SEM, ICP-OES, N_2 sorption analysis, it was confirmed that structured solid acids lost their catalytic activity due to the alteration in their structure and morphological changes. After considering the previous studies, it was decided that amorphous catalysts would be better choice than structured solid acids as they do not have definite pore structure. Hence, it was expected that amorphous solid acid catalysts can be more stable than the structured solid acid catalysts.

6.3.2.1. Structural and physicochemical properties of the amorphous solid acid catalysts used for the reaction

Amorphous solid acid catalysts do not have definite pore structure and channel structure. Due to this, it was expected that they will be stable under the reaction conditions. I have selected silica-alumina (SA) which have Brönsted acidity, comparatively high acid amount, surface area. SA is well known catalyst for industrial use and it is found to be very stable, active and recyclable catalyst in my previous work on D (+)- Glucosamine dehydration. Supported metal oxides are known for biomass conversions and have Brönsted and Lewis acidity. In supported metal oxide catalysts, -OH group attached to metal centre can give proton and acts as Brönsted acid sites and metal centre can act as Lewis acid site as vacant orbitals of metal can accept electron pair. Supported metal oxides were synthesized by wet impregnation method using two different metals like Tungsten (W), Molybdenum (Mo) on silica alumina (SA) and Silica (SiO_2) support. SA and SiO_2 both have high surface area (380 and 395 m^2/g respectively) which may help in effective dispersion of active centres on surface. Literature suggests that tungsten and molybdenum can form silicotungstic acid and silicomolybdic acid type species which can provide acid sites in catalysts additionally. The supported metal oxides were synthesized using wet impregnation method in which metals are adsorbed on the support via support-metal interaction. Therefore, catalysts synthesized in wet impregnation method can have active centres exposed on the surface which may help in catalytic activity. Physico-chemical properties of the amorphous solid acid catalysts used for chitosan and chitin conversion are tabulated in Table 6.10.

Table 6.10. Physico-chemical properties of amorphous solid acid catalysts used for chitosan and chitin conversion

Catalyst	Code	BET surface area (m^2/g)	Total acidity (mmol/g)
Silica alumina	SA	380	0.67
Niobium pentoxide	Nb_2O_5	155[49]	0.31[49]
WO_x on silica	WO_x/Silica	155[49]	0.09[49]
MoO_x on silica	MoO_x/Silica	63[49]	0.06[49]
WO_x on silica alumina	WO_x/SA	195	0.69
MoO_x on silica alumina	MoO_x/SA	88	0.72

6.3.2.2. Effect of different amorphous solid acid catalysts

Firstly, the activities of the amorphous solid acid catalysts were checked for the conversion of chitosan. As discussed earlier, TCLMWC is used for optimization of reaction conditions as it has lowest viscosity and molecular weight among all the chitosan samples. Optimized reaction condition for structured solid acid catalysts (substrate; 0.31 mmol, catalyst; 400 mg, water; 30 mL, 170 °C, 2 MPa nitrogen pressure at RT, 24 h) was employed for the evaluation of amorphous solid acids. As seen from Figure 6.15, various catalysts showed varying activity and maximum activity was shown with WO_x/SA catalyst to achieve highest 5-HMF yield (4.4%). No 5-HMF formation was observed when catalyst is not used for the reaction in the same reaction condition. Nb_2O_5 has shown very poor yield of 5-HMF (0.4%). WO_x/SiO_2 and MoO_x/SiO_2 showed better yield of 5-HMF (2% and 2.3%). As discussed earlier, catalyst active sites are exposed on the support surface which

helps in the reaction but metal leaching was observed for both the catalysts. High amount of W and Mo are leached in the reaction mixture (40.25 mg W from 400 mg WO_x/SiO_2, 22.98 mg Mo from the 400 mg MoO_x/SiO_2). Due to metal leaching, these catalysts are not stable and recyclable. Silica alumina catalyst also showed comparatively good activity (2.6% 5-HMF yield). Brönsted acid sites are helping in the hydrolysis of glycosidic linkages and further dehydration. Metal oxides supported on SA gave best activity due to their higher acidity and available acid sites on the surface of the support. WO_x/SA and MoO_x/SA showed best 5-HMF yield (4.4% and 3.4%). But same difficulties of metal leaching were observed like the previous case. High amount of W and Mo were leached out in the reaction mixture (38.16 mg W from 400 mg WO_x/SA, 18.65 mg Mo from the 400 mg MoO_x/SA). Leaching of metals is the main problem observed for the supported metal oxides even though they are showing better activity. By considering all the factors and mainly the stability of the catalyst, SA is showing considerably good yield of 5-HMF and stable under the reaction condition as very minimal aluminium leaching (4.03%) was observed in the reaction mixture.

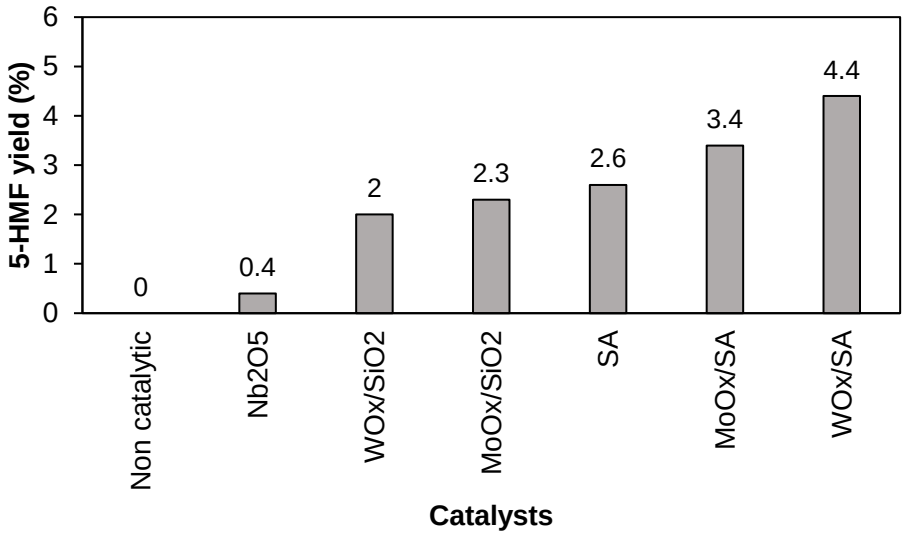


Figure 6.15. Conversion of chitosan into 5-HMF using various amorphous solid acid catalysts. Reaction condition: chitosan TCLMWC (0.31 mmol), catalyst (400 mg), water (30 mL), 170 °C, 2 MPa N_2 at RT, 24 h

6.3.2.3. Effect of reaction parameters

Optimized reaction condition for m-SAPO-44 (substrate TCLMWC; 0.31 mmol, catalyst; 400 mg, water; 30 mL, 170 ˚C, 2 MPa nitrogen pressure at RT, 24 h) was employed for the initial evaluation of different amorphous catalysts but it was expected that optimization is required to get comparatively good yield with SA. To understand the effect of reaction conditions like pressure, temperature, time, solvent was studied. To understand the effect of temperature, the reactions were carried out in the temperature range of 150- 190 ˚C under 2 MPa N_2 pressure at RT for 24h. As expected, it was seen that yield of 5-HMF was increased with the increasing temperature (150 ˚C, 0.7%; 160 ˚C, 1.1%; 170 ˚C, 2.6%; 180 ˚C, 2.1% and 190 ˚C, 1%) and above 180 ˚C, yield of 5-HMF is decreasing as at higher temperature thermal or catalytic side reactions or degradation reactions started becoming predominant.

Likewise, effect of nitrogen pressure was also studied as it is known that with increase in overall pressure, diffusivity of substrate on the catalytically active sites increases which helps to improve the reaction rate. When the reaction was carried out at 170 ˚C for 24 h without nitrogen pressure the yield of 5-HMF is 2.1% but with 1 MPa (2.6%), 2 MPa (2.6%) and 3 MPa (2.3%) nitrogen pressure (initial), yield of 5-HMF increased initially and later was stabilized. Total pressure at reaction temperature (170 ˚C) was 1.5 MPa when 1 MPa nitrogen was charged at room temperature. Not much dependency of activity on nitrogen pressure was observed as the amorphous catalyst, SA does not have any channel structure. Diffusivity parameter shows higher impact in case of structured catalysts where the catalysts have channel structure.

Effect of reaction time was checked at 170 ˚C under 1 MPa N_2 pressure at RT. It was observed that 5-HMF yield was maximum at 12 h (6 h, 2.3%; 12 h, 3.2%; 18 h, 3%; 24 h, 2.6% and 30 h, 1.9%). The yield of 5-HMF is increased with time but after

12 h it is decreased. It is expected that if the reaction is carried out for longer times, degradation products may be formed which can decrease the 5-HMF yield.

Initially water was preferably chosen as reaction medium as monomers ($GlcNH_2$, GlcNAc) and 5-HMF are completely soluble in water. From the literature reports it is understood that use of organic solvent along with water can decrease the chance of forming degradation products (humin). The purpose behind the use of organic solvent along with water in reaction system is to extract 5-HMF from water layer to organic layer and therefore, separating 5-HMF from acid catalysts to stop the further conversion of 5-HMF. 5-HMF has solubility in Methyl isobutyl ketone (MIBK) and MIBK/water biphasic system is also well-known solvent system for dehydration of glucose to 5-HMF. In this work, MIBK/water system (2/1 *v/v*) was used to check the effect of biphasic solvent system. Effect of biphasic solvent system on time was studied at 170 °C under 1 MPa N_2 pressure at RT in MIBK/water biphasic system (2/1 *v/v*). More 5-HMF yield was observed in less time when biphasic solvent system was used. It was observed that 5-HMF yield was maximum at 4 h (2 h, 2.6%; 4 h, 3.6%; 6 h, 3.1%; 8 h, 2.8%) and after that yield was decreased with increasing time. On the other hand, when only water was used as solvent, only 1.8% 5-HMF yield was observed in 4h.

6.3.2.4. Conversion of different chitosan and chitin

After the optimization of reaction conditions for the conversion of TCLMWC chitosan substrate (substrate to catalyst (S/C) ratio; 1.16 mole/mole, water; 30 mL, 170 °C, 1 MPa nitrogen pressure at RT, 24 h), various other chitosan and chitin substrates (ALMWC, AMMWC, AHMWC, TCMMWC, TCHMWC, ASSC, ACSC, TC, LC) were evaluated under this optimized reaction condition. All the chitosan samples showed 5-HMF yield with 1.5-3.1% (Figure 6.16). On an expected line, chitin samples gave less 5-HMF yield (0.4-0.5%) which can be attributed to the different reaction pathway followed by N-acetyl-D-glucosamine due o presence of bulky acetamido group. I have made a detailed discussions on probable

reaction pathways followed by $GlcNH_2$ and GlcNAc in 3rd chapter (Section 3.3.4). It was also found that the 5-HMF yield does not depend on only viscosity and molecular weight. This observation matches with the results found in the reaction with structured solid acids. ASSC ((η= 200 cP, 5-HMF yield= 2.8%) gave better yield TCMMWC ((η= 20-100 cP, 5-HMF yield= 2%). Therefore, the hydrolysis of chitosan does not depend on only viscosity or molecular weight, it depends on other factors like degree of polymerisation, degree of crystallisation, monomer present at reducing end and degree of acetylation.

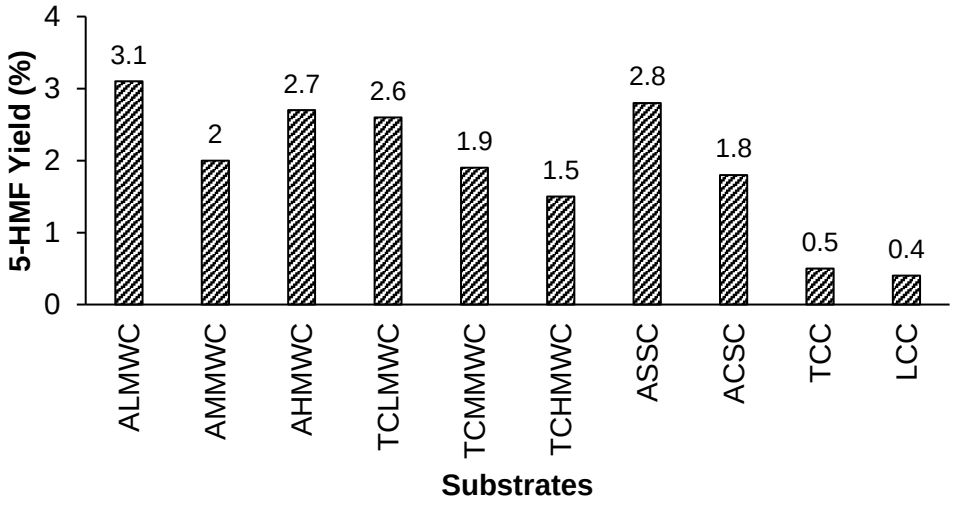


Figure 6.16. Conversion of different substrates into 5-HMF using amorphous solid acid catalyst, SA. Reaction conditions: Substrate, SA, S/C ratio (1.16 mole/mole), water (30 mL), 170 ˚C, 1 MPa N_2 pressure at RT, 24 h

6.3.2.5. Catalyst recycle study

Spent catalyst characterization data revealed that SA catalyst is stable under the reaction conditions and thus the recycle study was carried out with SA. After the reaction, catalyst was recovered by centrifugation, dried in oven and used in the next run. As seen from Figure 6.17, decrease in the 5-HMF yield was observed until 3rd run. The decrease in the 5-HMF yield is due to presence of unconverted chitosan from the previous cycle with the spent catalyst. After 3rd run, the catalyst was calcined at 600 ˚C in the muffle furnace under air stream to remove all the

unconverted chitosan remaining. The calcined catalyst was then used for the reaction and 2.5% 5-HMF yield was observed. Hence, the catalyst is stable and recyclable under the reaction condition and retains its catalytic activity. After each run, catalyst needs to be calcined to remove the unconverted substrate. Nevertheless, the most important aspect of this work is the catalyst is stable, recyclable and showed consistent results after removing unconverted chitosan through calcination. For the first time, stable and recyclable solid acid catalytic system is developed for the conversion of chitosan without the use of any mineral acids, organic acids, mineral bases, high boiling solvents and metal salts. The reaction conditions are also benign, green and only water is used as solvent.

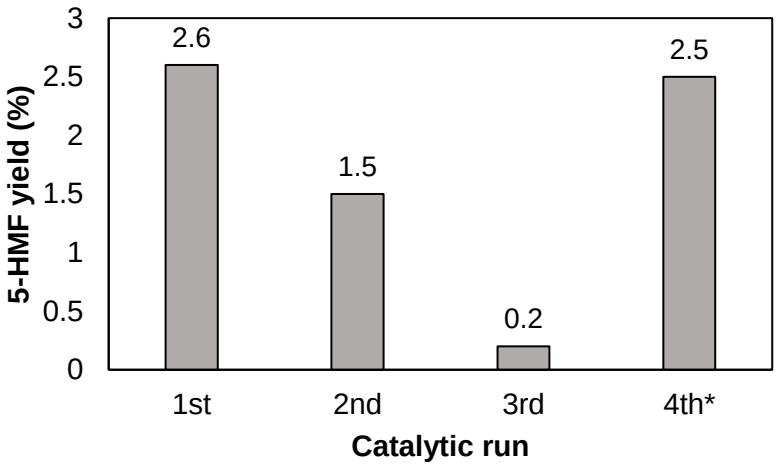


Figure 6.17. Catalyst recycle study. Reaction conditions: TCLMWC, SA, S/C ratio (1.16 mole/mole), water (30 mL), 170 °C, 1 MPa N_2 pressure at RT, 24 h

*Catalyst was calcined at 600 °C under air stream after 3rd run to remove unconverted chitosan

6.3.2.6. Characterization of spent amorphous solid acid catalysts

Characterizations of fresh and spent SA was done to understand its stability after the reaction. The XRD patterns for fresh, spent and calcined spent SA are shown in Figure 6.18. Amorphous silica alumina showed no change in XRD pattern after

the reaction and calcination. Hence, silica alumina is stable under reaction condition.

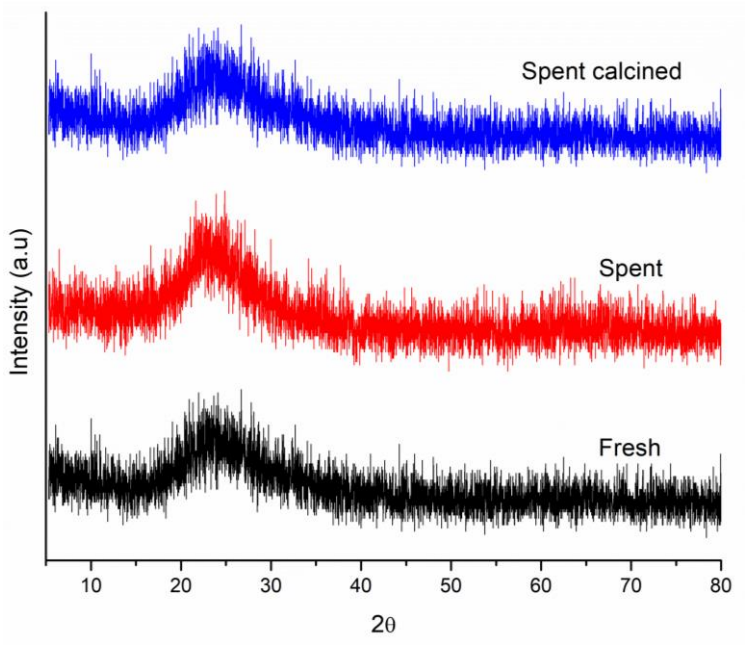


Figure 6.18. XRD patterns for fresh, spent and calcined spent silica alumina

NH_3-TPD study was done to know the total acid amount of the fresh catalyst and the changes in acid amount after the reaction. NH3-TPD results (Table 6.11) showed decreasing acid amount in spent SA because unconverted chitosan is mixed with spent catalyst. After calcination, the acid amount of SA increased and showed very marginal decrease in acid amount from fresh SA which may be the reason to get better recyclability with SA.

Table 6.11. NH_3-TPD study of fresh and spent catalyst

Sample	**Total acidity (mmol/g) of fresh catalyst**
Fresh SA	0.67
Spent SA	0.45
Calcined spent SA (After 3rd run)	0.61

ICP-OES analysis data (shown in Table 6.12) of SA shows very less amount of Al (4.03%) is leached in the reaction mixture which proves that the catalyst is rigid and stable under the reaction.

Table 6.12. ICP-OES analysis of fresh, spent SA and reaction mixture (calculated w.r.t catalyst amount taken for the reaction, i.e., 400 mg)

Sample	Al content in mg (per 400 mg)
Fresh m-SAPO-44	36.73
Spent m-SAPO-44	34.65
Reaction mixture (when m-SAPO-44 is used as catalyst	1.48

Nitrogen sorption data for fresh and spent SA catalyst is tabulated in Table 6.13. The difference in surface area of fresh and spent catalyst is due to presence of unconverted chitosan.

Table 6.13. Summary on nitrogen sorption data of fresh and spent SA

Catalyst		BET surface area (m^2/g)
SA	Fresh	380
	Spent	163

6.4. Conclusion

In this work, stable and recyclable solid acid catalysed system was developed for the first time where only water was as solvent instead of using any mineral acids, organic acids and high boiling solvents. Initially, structured solid acid m-SAPO-44 was used which gives good yield of 5-HMF. Mechanistic pathways for the chitosan reaction were investigated. Along with 5-HMF, other nitrogen containing chemicals were also achieved with the use of m-SAPO-44. M-SAPO-44 was also able to convert different chitosan and chitin substrates. Due to morphological

changes in m-SAPO-44 after 1st catalytic run, the characteristic peak of chabazite morphology vanished and total acidity was reduced drastically. Amorphous solid acid catalysts were then used for the conversion of chitosan and chitin. Amorphous solid acid, SA showed better stability under reaction condition and SA is also recyclable after calcining the spent catalyst to remove unconverted chitosan after every run. In future, more work is needed in this area for the analysis of other products formed. The complete identification of all the products formed from this complex reaction requires extensive studies and those are beyond the capabilities of our research facilities. Nevertheless, most important aspect of my work is that the catalyst is stable, recyclable and nitrogen containing chemicals can also be formed by using this solid acid catalytic system without the use of any mineral & organic acids as co-catalyst or solvent.

6.5. References

1. Chen, X.; Chew, S. L.; Kerton, F. M.; Yan, N., Direct conversion of chitin into a N-containing furan derivative. *Green Chemistry* **2014,** *16* (4), 2204-2212.

2. Ghosh, N.; Dhepe, P. L., HPLC method development for chitin and chitosan valorisation chemistry. *Carbohydrate Polymer Technologies and Applications* **2021,** *2*, 100139.

3. Chen, X.; Yang, H.; Yan, N., Shell biorefinery: dream or reality? *Chemistry–A European Journal* **2016,** *22* (38), 13402-13421.

4. Chen, X.; Yang, H.; Yan, N., Shell Biorefinery: Dream or Reality? *Chemistry* **2016,** *22* (38), 13402-21.

5. Yabushita, M.; Kobayashi, H.; Kuroki, K.; Ito, S.; Fukuoka, A., Catalytic Depolymerization of Chitin with Retention of N-Acetyl Group. *ChemSusChem* **2015,** *8* (22), 3760-3763.

6. Zhang, J.; Yan, N., Production of Glucosamine from Chitin by Co-solvent Promoted Hydrolysis and Deacetylation. *ChemCatChem* **2017,** *9* (14), 2790-2796.

7. Mascal, M.; Nikitin, E. B., Dramatic advancements in the saccharide to 5-(chloromethyl) furfural conversion reaction. *ChemSusChem: Chemistry & Sustainability Energy & Materials* **2009,** *2* (9), 859-861.

8. Wang, Y.; Pedersen, C. M.; Deng, T.; Qiao, Y.; Hou, X., Direct conversion of chitin biomass to 5-hydroxymethylfurfural in concentrated ZnCl2 aqueous solution. *Bioresource technology* **2013,** *143*, 384-390.

9. Kim, H. S.; Park, M. R.; Jeon, Y. J.; Kim, S. K.; Hong, Y. K.; Jeong, G. T., Valorization of Chitosan as Food Waste of Aquatic Organisms into 5-Hydroxymethylfurfural by Sulfamic Acid-Catalyzed Conversion Process. *Energy Technology* **2018,** *6* (9), 1747-1754.

10. Kalane, N. D.; Krishnan, R. A.; Yadav, V. D.; Jain, R.; Dandekar, P., Synergistic effect of hetero-and homo-catalysts on the 'green'synthesis of 5-hydroxymethylfurfural from chitosan biomass. *Cellulose* **2019,** *26* (4), 2805-2819.

11. Feng, J. X.; Zang, H. J.; Yan, Q.; Li, M. G.; Cheng, B. W. In *Conversion of chitosan into 5-hydroxymethylfurfural via hydrothermal synthesis*, Advanced Materials Research, Trans Tech Publ: 2015; pp 411-414.

12. Li, M.; Zang, H.; Feng, J.; Yan, Q.; Yu, N.; Shi, X.; Cheng, B., Efficient conversion of chitosan into 5-hydroxymethylfurfural via hydrothermal synthesis in ionic liquids aqueous solution. *Polymer Degradation and Stability* **2015,** *121*, 331-339.

13. Zhang, M.; Zang, H.; Ma, B.; Zhang, X.; Xie, R.; Cheng, B., Green Synthesis of 5-Hydroxymethylfurfural from Chitosan Biomass Catalyzed by Benzimidazole-Based Ionic Liquids. *ChemistrySelect* **2017,** *2* (32), 10323-10328.

14. Omari, K. W.; Besaw, J. E.; Kerton, F. M., Hydrolysis of chitosan to yield levulinic acid and 5-hydroxymethylfurfural in water under microwave irradiation. *Green Chemistry* **2012,** *14* (5), 1480-1487.

15. Kim, H. S.; Kim, S.-K.; Jeong, G.-T., Efficient conversion of glucosamine to levulinic acid in a sulfamic acid-catalyzed hydrothermal reaction. *RSC advances* **2018,** *8* (6), 3198-3205.

16. Osada, M.; Kikuta, K.; Yoshida, K.; Totani, K.; Ogata, M.; Usui, T., Non-catalytic synthesis of Chromogen I and III from N-acetyl-D-glucosamine in high-temperature water. *Green chemistry* **2013,** *15* (10), 2960-2966.

17. Zheng, X.-Y.; Peng, J.-B.; Livera, M. V. S.; Luo, Y.; Wang, Y.-Y.; Kong, X.-J.; Long, L.-S.; Zheng, Z.; Zheng, L.-S., Selective formation of chromogen I from N-acetyl-D-glucosamine upon lanthanide coordination. *Inorganic Chemistry* **2017,** *56* (1), 110-113.

18. Osada, M.; Kobayashi, H.; Miyazawa, T.; Suenaga, S.; Ogata, M., Non-catalytic conversion of chitin into Chromogen I in high-temperature water. *International journal of biological macromolecules* **2019,** *136*, 994-999.

19. Chen, X.; Liu, Y.; Kerton, F. M.; Yan, N., Conversion of chitin and N-acetyl-d-glucosamine into a N-containing furan derivative in ionic liquids. *Rsc Advances* **2015,** *5* (26), 20073-20080.

20. Omari, K. W.; Dodot, L.; Kerton, F. M., A simple one-pot dehydration process to convert N-acetyl-D-glucosamine into a nitrogen-containing compound, 3-acetamido-5-acetylfuran. *ChemSusChem* **2012,** *5* (9), 1767-1772.

21. Du, Y.; Zang, H.; Feng, Y.; Wang, K.; Lv, Y.; Liu, Z., Efficient catalytic system for converting N-acetyl-d-glucosamine into valuable chemical 3-acetylamino-5-acetylfuran. *Journal of Molecular Liquids* **2022,** *347*, 117970.

22. Jun, M.; Shao, Y.; Ho, C.-T.; Koetter, U.; Lech, S., Structural identification of nonvolatile dimerization products of glucosamine by gas chromatography− mass spectrometry, liquid chromatography− mass spectrometry, and nuclear magnetic resonance analysis. *Journal of agricultural and food chemistry* **2003,** *51* (21), 6340-6346.

23. Jia, L.; Wang, Y.; Qiao, Y.; Qi, Y.; Hou, X., Efficient one-pot synthesis of deoxyfructosazine and fructosazine from D-glucosamine hydrochloride using a basic ionic liquid as a dual solvent-catalyst. *RSC Advances* **2014,** *4* (83), 44253-44260.

24. Bobbink, F. D.; Zhang, J.; Pierson, Y.; Chen, X.; Yan, N., Conversion of chitin derived N-acetyl-D-glucosamine (NAG) into polyols over transition metal catalysts and hydrogen in water. *Green Chemistry* **2015,** *17* (2), 1024-1031.

25. Kobayashi, H.; Techikawara, K.; Fukuoka, A., Hydrolytic hydrogenation of chitin to amino sugar alcohol. *Green chemistry* **2017,** *19* (14), 3350-3356.

26. Dai, J.; Gözaydın, G. k.; Hu, C.; Yan, N., Catalytic conversion of chitosan to glucosaminic acid by tandem hydrolysis and oxidation. *ACS Sustainable Chemistry & Engineering* **2019,** *7* (14), 12399-12407.

27. Gao, X.; Chen, X.; Zhang, J.; Guo, W.; Jin, F.; Yan, N., Transformation of chitin and waste shrimp shells into acetic acid and pyrrole. *ACS Sustainable Chemistry & Engineering* **2016,** *4* (7), 3912-3920.

28. Savitri, E.; Juliastuti, S. R.; Handaratri, A.; Roesyadi, A., Degradation of chitosan by sonication in very-low-concentration acetic acid. *Polymer degradation and stability* **2014,** *110*, 344-352.

29. Huang, Y.-B.; Fu, Y., Hydrolysis of cellulose to glucose by solid acid catalysts. *Green Chemistry* **2013,** *15* (5), 1095-1111.

30. Shrotri, A.; Kobayashi, H.; Fukuoka, A., Cellulose depolymerization over heterogeneous catalysts. *Accounts of chemical research* **2018,** *51* (3), 761-768.

31. Bhaumik, P.; Dhepe, P. L., Solid acid catalyzed synthesis of furans from carbohydrates. *Catalysis Reviews* **2016,** *58* (1), 36-112.

32. Tzeng, T.-W.; Bhaumik, P.; Chung, P.-W., Understanding the production of 5-hydroxymethylfurfural (HMF) from chitosan using solid acids. *Molecular Catalysis* **2019,** *479*, 110627.

33. Sigma-Aldrich 448869, Product information. https://www.sigmaaldrich.com/IN/en/product/aldrich/448869.

34. Sigma-Aldrich 448877, Product information. https://www.sigmaaldrich.com/IN/en/product/aldrich/448877.

35. Sigma-Aldrich 419419, Product information. https://www.sigmaaldrich.com/IN/en/product/aldrich/419419.

36. TCI C2395, Product information. https://www.tcichemicals.com/IN/en/p/C2395.

37. TCI C2396, Product information. https://www.tcichemicals.com/OP/en/p/C2396.

38. TCI C0831, Product information. https://www.tcichemicals.com/OP/en/p/C0831.

39. Sigma-Aldrich 417963, Product information. https://www.sigmaaldrich.com/IN/en/product/aldrich/448877.

40. Bhaumik, P.; Kane, T.; Dhepe, P. L., Silica and zirconia supported tungsten, molybdenum and gallium oxide catalysts for the synthesis of furfural. *Catalysis Science & Technology* **2014,** *4* (9), 2904-2907.

41. Bhaumik, P.; Dhepe, P. L., Efficient, stable, and reusable silicoaluminophosphate for the one-pot production of furfural from hemicellulose. *ACS Catalysis* **2013,** *3* (10), 2299-2303.

42. Lanzafame, P.; Temi, D.; Perathoner, S.; Spadaro, A.; Centi, G., Direct conversion of cellulose to glucose and valuable intermediates in mild reaction conditions over solid acid catalysts. *Catalysis Today* **2012,** *179* (1), 178-184.

43. Dhepe, P. L.; Sahu, R., A solid-acid-based process for the conversion of hemicellulose. *Green Chemistry* **2010,** *12* (12), 2153-2156.

44. Bhaumik, P.; Deepa, A.; Kane, T.; Dhepe, P. L., Value addition to lignocellulosics and biomass-derived sugars: An insight into solid acid-based catalytic methods. *Journal of Chemical Sciences* **2014,** *126* (2), 373-385.

45. Bhaumik, P.; Dhepe, P. L., Influence of properties of SAPO's on the one-pot conversion of mono-, di-and poly-saccharides into 5-hydroxymethylfurfural. *RSC advances* **2013,** *3* (38), 17156-17165.

46. Deepa, A. K.; Dhepe, P. L., Lignin depolymerization into aromatic monomers over solid acid catalysts. *ACS Catalysis* **2015,** *5* (1), 365-379.

47. Rutkowski, P., Pyrolytic behavior of cellulose in presence of montmorillonite K10 as catalyst. *Journal of analytical and applied pyrolysis* **2012,** *98*, 115-122.

48. commission, I. s. Database of zeolite structures. https://asia.iza-structure.org/IZA-SC/ftc_table.php.

49. Bhaumik, P., Efficient ways to volorize biomass into sugars & furans: study on the properties of sapo's & metal oxide catalysts. **2014**.

50. Deepa, A., Depolymerization of lignin over heterogeneous catalyst having acidic functionality. **2014**.

51. Catalyst, D. A. D. P. https://www.dupont.com/content/dam/dupont/amer/us/en/water-solutions/public/documents/en/45-D00927-en.pdf.

52. information, M. p. https://www.sigmaaldrich.com/IN/en/product/aldrich/474541.

53. Yu, S.; Zang, H.; Chen, S.; Jiang, Y.; Yan, B.; Cheng, B., Efficient conversion of chitin biomass into 5-hydroxymethylfurfural over metal salts catalysts in dimethyl sulfoxide-water mixture under hydrothermal conditions. *Polymer Degradation and Stability* **2016,** *134*, 105-114.

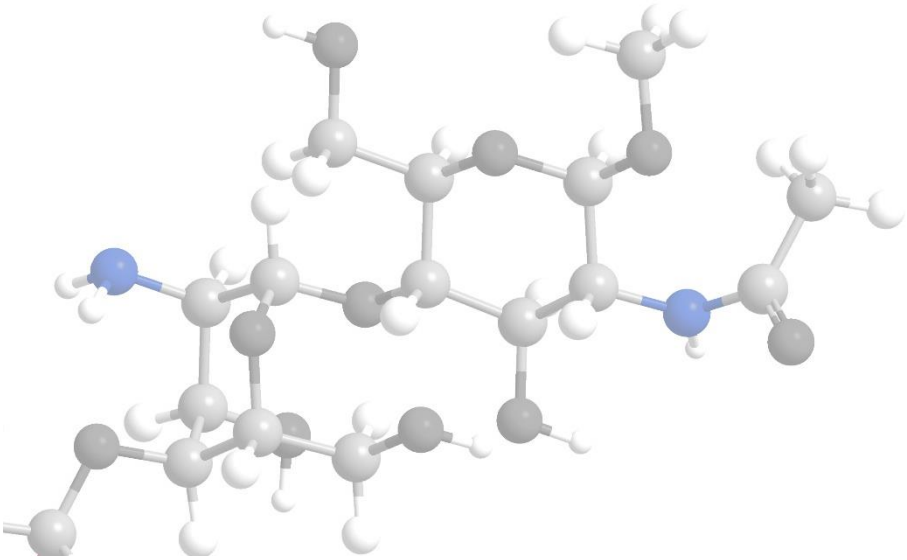
54. Liu, Y.; Lotero, E.; Goodwin Jr, J. G., A comparison of the esterification of acetic acid with methanol using heterogeneous versus homogeneous acid catalysis. *Journal of Catalysis* **2006,** *242* (2), 278-286.

55. Nascimento, M., *Theoretical aspects of heterogeneous catalysis*. Springer Science & Business Media: 2006; Vol. 8.

56. Sauer, J.; Horn, H.; Häser, M.; Ahlrichs, R., Formation of hydronium ions on Brønsted sites in zeolitic catalysts: a quantum-chemical ab initio study. *Chemical physics letters* **1990,** *173* (1), 26-32.

Chapter 07

Summary and Conclusions



Chitin and its derivatives (include chitosan, chito-monomers) have been recognized as a potential feedstock for the production of several value-added chemicals. Chitin is the second most plentiful bio-polymer on earth which contains carbon, hydrogen, nitrogen and oxygen. Presence of nitrogen makes it an important source of nitrogen containing chemicals which can not be synthesized from lignocellulosic biomass. Homogeneous catalytic processes have been mainly reported for the direct conversion of chitin/chitosan and chito-monomers into nitrogen containing chemicals and furan derivatives. However, there are several problems associated with the reported methods like difficulty in recovery of the catalyst, environmental issues, toxicity, corrosive to reactor parts, generation of neutralisation waste, use of high temperature and pressure, use of energy extensive processes (ball milling, microwave heating), etc. After considering these above-mentioned drawbacks, I have focused my work on the development of stable and recyclable solid acid catalysed system for the conversion of chitin biomass (included chitin, chitosan, N-acetyl-D-glucosamine and D (+)- glucosamine) into nitrogen containing chemicals and furan derivatives. Moreover, it was observed that there is a need of developing a proper analytical method to simultaneously separate and quantify the chitin derived compounds (e.g.- amino sugars, nitrogen containing chemicals and furan derivatives). So additionally, a HPLC method was developed to simultaneously separate and quantify amino sugars, nitrogen containing chemicals and furan derivatives in this current work. The Ph. D. thesis is divided into seven chapters in which the actual work carried out during my Ph.D. degree is discussed in 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} chapter and the results and conclusions from each chapter are summarized in this chapter.

Chapter 01

Chapter 1 starts with general introduction to the need of finding alternative for the fossil feedstocks & overview of biomass, its classification, structures of its components and its numerous advantanges over conventioanl fossil feedstocks. The details on the structure and source of animal derived biomass, chitin which is a potential source for chemicals (specially nitrogen containing ones) were discussed in detail. The statistics on fisheries and crustaceans production in the World and India was presented and it was found that a large amount of waste is genarated by farming and food processing industries and the wastes are just dumped in landfill or sea which creates several environmental issues. Components of crustacean shell waste and their potential uses were discussed in detail. Several processes are known for the fractionation of shell waste and extraction of chitin i.e., industrial/chemical method, solvent extraction method, bio-processing method. All these three processes were reviewed in this chapter. Also, the process of producing chitosan and its structural details were presented in the 1st chapter. Chitin biomass (includes chitin, chitosan and their monomers) were chosen as substrate in my work due to their potentiality for producing nitrogen containng chemiclas and furan derivatives. Earliar literature reports on the acid catalysed conversion of chitin biomass into amino sugar monomers, furan derivatives with or without nitrogen, organic acids (rehydration products) were discussed in detail and the gap between the known and scope of work was identified. After doing thorough literature survey, it was understood that there is a need to develop a catalytic system where catalyst is easily recoverable, stable and recyclable. Green solvent needs to be used as the reaction medium and the reactions should be done in ambient temperature and pressure.

Solid acids are well known to convert cellulose, hemicellulose by breaking β-1,4 glycosidic linkages and they are easily separable and recyclable. As chitosan and chitin has a very similar structure like cellulose, it is believed that they can also be

depolymerized using solid acid catalysts. Solid acid alone was not previously reported as catalyst along with water as solvent. From the literature study, it was also found that proper analytical techniques for separating amino sugars and furan derivatives are not available in previous literature reports. So, developing proper analytical technique for simultaneous separation and quantification of the amino sugars and furan derivatives is an important topic to work. Later detailed discussions on various structured and amorphous solid acid catalysts, their Brönsted and Lewis acid sites were discussed. At the end of this chapter statement of problem, objectives and scope of this thesis were summarized and the methodologies which were followed to achieve the objectives were also discussed. Roadmap and the outline of this thesis is shown in Figure 7.1.

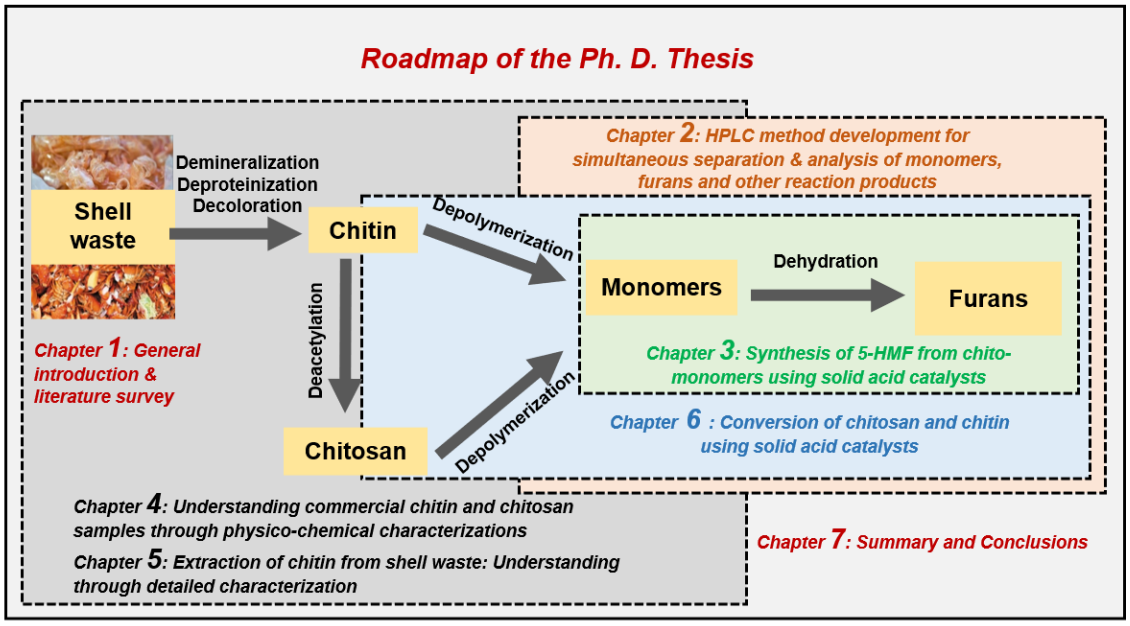


Figure 7.1. Roadmap and outline of this Ph. D thesis

Chapter 02

Chapter 2 deals with development of HPLC method for separating and quantifying amino sugar monomers (GlcNAc and $GlcNH_2$) and furan derivative (5-HMF) simultaneously. Moreover, other chitin derived nitrogen containing

chemicals and furan derivatives were also can be detected and separated using this developed method.

- ✓ First of all, the structural and chemical properties of $GlCNH_2$, GlcNAc and 5-HMF were checked. The size of the molecules (width and length) was calculated using Chem3D software after energy minimization and solubility of the compounds in different solvents was checked. The observation suggests that the compounds could not be separated through size exclusion chromatography (due to very minimal difference in size of these three molecules), column chromatography (completely soluble in only water, partially in methanol, polarity difference of $GlcNH_2$, GlcNAc is very less), gas chromatography ($GlcNH_2$ and GlcNAc will degrade at higher temperature, restricted solubility in organic solvents). In view of all these restrictions, HPLC seems to be the most suitable technique.
- ✓ At first, the non-polar C18 column was chosen for the analysis as researchers have previously used C18 column for the separation of chitin/chitosan derived products. UV-Visible spectroscopy study suggests that VWD wavelength should be set at 195 nm because $GlcNH_2$ and GlcNAc have λ_{max} at 195 nm. As lower wavelength corresponds to higher energy, more energy is required to excite the electrons in case of amino sugars, so, they cannot be detected at higher wavelength. Thus, it is essential to use correct wavelength to detect all the compounds in the sample.
- ✓ Choosing appropriate solvent as mobile phase is very important in case of HPLC analysis. From the literature, it was found that every solvent has their specific UV cut off. If measurement wavelength is less than this cut off value, then mobile phase also has some contribution in absorbance. Previously researchers have used methanol/water systems for the analysis of chitin/chitosan reaction mixtures and hence they required to keep the VWD above 205 nm wavelength since methanol has a UV cut-off at 205

nm. As detector wavelength must be kept at 195 nm to detect all the compounds mainly amino sugars, methanol has to be replaced by acetonitrile with lower UV cut off, i.e., <190 nm.

- ✓ Acetonitrile/water ratio was optimized to get better separation and acetonitrile/water (1/9 *v/v*) was found to be best for clear separation of these three compounds. Effect of column length was also checked and it was observed that longer columns are better for better separation. Moreover, separation of other sugars, N-containing chemicals and furan derivatives were checked using optimized HPLC conditions in C18 column and it was found that most of the compounds are eluting at same or nearby retention time and they can not be separated using this method.
- ✓ Then, effect of polar stationary phase, i.e., normal phase amino column was checked because it was thought that polar stationary phase can give better separation as polar compounds will have strong interactions with polar stationary phase. But it was observed that polar compounds have very strong interaction with the stationary phase of the column, leading to peak tailing. Moreover, amino column also fails to give separation for other chitin derived compounds.
- ✓ Ion-exchange column, monosaccharide Pb^{2+} column was then used which has medium polarity and with the use of this column, better separation of $GlcNH_2$, GlcNAc and 5-HMF was observed in comparison to other columns used (Figure 7.2). Moreover, this column could be able to separate all the chitin derived nitrogen containing chemicals, other sugars and furan derivatives. This developed HPLC method was demonstrated to be suitable to separate and quantify $GlcNH_2$, GlcNAc and 5-HMF simultaneously within 39 min. The regression equation showed very good linear relationship with R^2= 0.9904-0.9974. Therefore, the developed HPLC method is simple, rapid, accurate, precise, and specific and has the ability to separate and quantify amino sugars ($GlcNH_2$, GlcNAc) and 5-HMF simultaneously.

- ✓ Hence, non-polar C18 and mid-polar cation exchange Pb^{2+} columns are best suitable for separation of $GlcNH_2$, GlcNAc, other N-containing chemicals and furan derivatives. Moreover, Non-polar C18 column although showed better separation than NH_2 column but was unable to separate other sugars like glucose, fructose etc. from each other and other chemicals injected. Considering all the factors, mid-polar cation exchange Pb^{2+} column is best as it possesses optimum polarity to allow rapid adsorption-desorption of the compounds which helps to separate all the compounds.

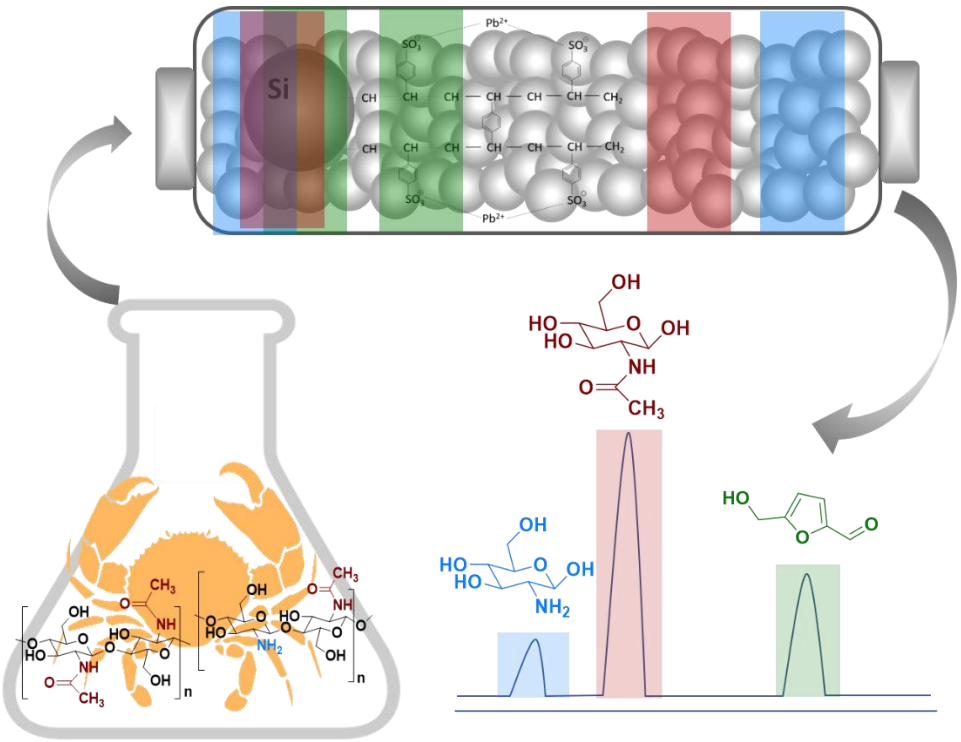


Figure 7.2. Separation of chitin derived $GlcNH_2$, GlcNAc and 5-HMF using ion-exchange Pb^{2+} column

Chapter 03

Chapter 3 discusses about synthesis of 5-HMF from chito-monomers ($GlcNH_2$ and GlcNAc) using different solid acid catalysts.

- ✓ Various solid acids were evaluated for the dehydration of $GlcNH_2$ to form 5-HMF. Reactions were carried out in a batch mode Parr reactor at 170 °C with 0.5 MPa N_2 for 4 h in water (30 mL) as solvent.
- ✓ Structured solid acid catalysts (m-SAPO-44, H-MOR, H-USY, H-ZSM-5), amorphous solid catalysts (silica alumina (SA), WO_x/SA, MoO_x/SA), commercial resins (Amberlyst-15, Nafion SAC-13) were evaluated for the dehydration of D-(+)-Glucosamine into 5-HMF. The reaction mixture was analysed using HPLC and yield of 5-HMF was quantified using calibration curve drawn by injecting standard samples of 5-HMF in different concentration.
- ✓ Among all the solid acid catalysts, structured solid acid catalyst m-SAPO-44 was observed to give maximum yield of 5-HMF (26%). Along with this, amorphous solid acid SA also gave good 5-HMF yield (18%).
- ✓ Catalyst characterization study showed that the due to morphological changes in m-SAPO-44 after 1st catalytic run, the characteristic peak of CHA morphology vanished and total acidity was also reduced. On the other hand, SA remains unchanged under the reaction condition and shows better stability.
- ✓ Optimization of reaction conditions like time, temperature, nitrogen pressure, substrate to catalyst ratio was performed and 170 °C, 0.5 MPa N_2, 4 h and S/C ratio 9.28 mole/mole were found to be best reaction condition to achieve 29% 5-HMF yield using SA catalyst.
- ✓ Reaction with another chito-monomer, N-Acetyl-D-glucosamine was also done under same conditions (170 °C, 0.5 MPa N_2 pressure, 4 h) using m-SAPO-44 as a catalyst. The 5-HMF yield in the reaction with GlcNAc was only 1%, which was very surprising considering that difference between two substrates is only change at C2 position. In case of $GlcNH_2$, at first, H^+ ion (reactions are done using acidic catalyst) attacks basic NH_2 and NH_3 is liberated immediately as NH_3 is known as a good leaving group. After that,

stable five membered furan ring is formed which undergoes dehydration to yield 5-HMF. In case of GlcNAc, Acetamide (-$NHCOCH_3$) group is bulky and due to steric hindrance, attack of H^+ and liberation of acetamide group is not favourable. This might be the possible reason for poor yield of 5-HMF in GlcNAc reaction.

- ✓ Catalyst recycles study shows that SA is stable and recyclable up to 6th catalytic run without much changes in the yield. The most important aspect of this work is that catalyst is recyclable and showed consistent results until 6th catalytic run (Figure 7.3).

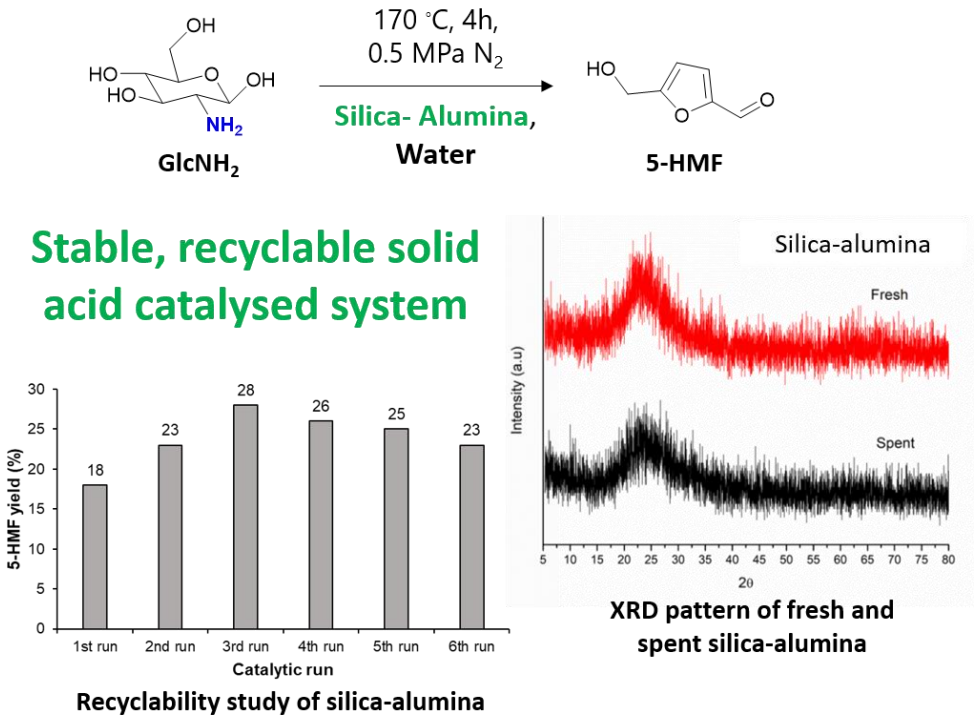


Figure 7.3. Stable, recyclable solid acid catalysed system for dehydration of $GlcNH_2$ to 5-HMF

- ✓ A solid acid based catalytic system is developed in water as medium without the addition of any mineral aid, organic acid, high boiler solvents, metal chlorides etc.

- ✓ In future, more work is needed for analysis of other products formed. The complete identification of all the products requires extensive studies and those are not beyond the capabilities of our research facilities.

Chapter 04

Chapter 4 includes the details on various commercially procured chitosan and chitin samples (ALMWC, AMMWC, AHMWC, TCLMWC, TCMMWC, TCHMWC, ASSC, ACSC, TCC, LCC) used in the solid acid catalysed conversion of chitosan and chitin. Physico-chemical properties of all these chitosan and chitin samples were performed using various analytical characterization techniques (Figure 7.4). Major findings are summarized below.

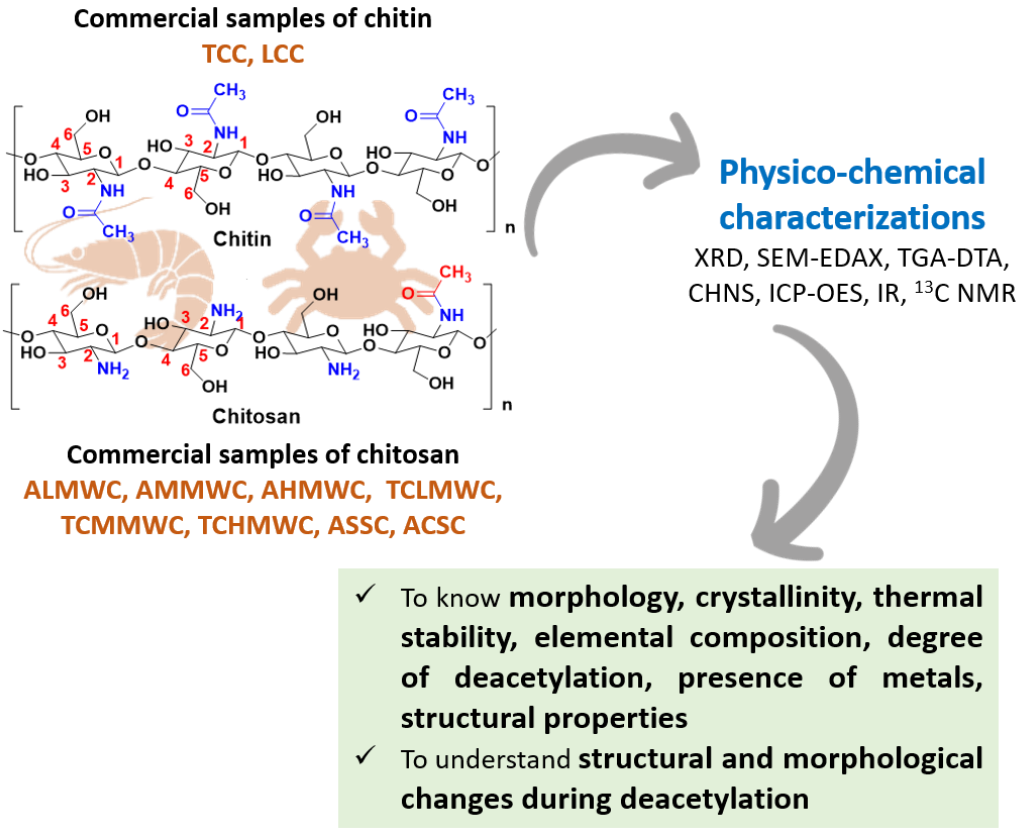


Figure 7.4. Detailed characterization to understand the physico-chemical properties of commercial chitosan and chitin samples

- ✓ Structural and functional properties can be different for chitin and chitosan samples depending on their source and extraction method. Hence, the complete characterization of all chitosan and chitin samples were performed before starting the depolymerisation experiments using various techniques like XRD analysis, SEM-EDAX, thermal analysis, CHNS, ICP-OES elemental analysis, ATR, ^{13}C NMR and absolute viscosity measurement.
- ✓ XRD patterns of chitin samples shows intense diffraction peaks which are characteristic peaks for α-chitin. In case of chitosan, decrease in intensity of diffraction peaks was observed and few peaks were disappeared. The decrease in crystallinity is due to NaOH treatment during deacetylation of chitin to chitosan. XRD data also suggests that inter-chain H-bonds may be broken during base treatment.
- ✓ DTA analysis revealed that chitosan samples are thermally stable up to 300 °C and chitin has greater thermal stability that chitosan, i.e., 330 °C. The stability of chitin is higher than chitosan is due to H-bonding network in chitin which leads to structural rigidity in chitin. TG-DTA data also proves that H-bonding network got disturbed during deacetylation of chitin as strong base is used during this process.
- ✓ CHNS elemental analysis revealed that chitosan contains 39.4-40.5% C, 6.5-7.3% H and 6.7-7.5% N. Elemental analysis of chitin shows that 44.2-44.5% C, 5.9-6.3% H and 6.2-6.5% N are present. The general formula for all the chitosan and chitin samples can be expressed as $C_aH_bN_cO_d$ (a = 6.2-6.9, b = 12.9-13.7, c = 1, d = 5.2-6.1) and $C_wH_xN_yO_z$ (w = 7.9-8.4, x = 12.5-14.2, y = 1, z = 5.8-6.1) respectively which correlates well with the literature. Furthermore, degree of deacetylation (DD %) was calculated from C/N ratio and it is found that chitosan samples are 54-86% deacetylated.

- ✓ Morphology of chitosan and chitin samples were found in SEM analysis and EDAX analysis revealed that Ca and Na are present in the samples as impurity. Moreover, bulk level ICP-OES analysis was performed to quantify the metal content. ICP analysis shows the presence of Ca which proves that demineralization was not complete and presence of Na can be attributed to the use of NaOH in the deproteinization and deacetylation step. Among all the chitosan samples, AMMWC sample has shown highest ash and Na content due to use of NaOH at deproteinization and deacetylation step. TCC also shows high ash and Na content which can be attributed to use of NaOH in deproteinization step.
- ✓ ATR and ^{13}C NMR spectroscopic measurements were performed to know the backbone and functional groups present in the samples. All the chitosan samples have similar type of functional groups. Moreover, a comparative study on structure of chitosan and chitin was done and it is found that major structural changes happened during deacetylation process which supports XRD and thermal analysis data.
- ✓ Viscosity measurement was done to get an idea about the molecular weight of chitosan samples as viscosity have proportional relation with molecular weight. Measured viscosity data falls in the range which is provided by the suppliers. It is found that TCLMWC has the lowest viscosity among all chitosan samples.
- ✓ Characterization data of all chitin and chitosan samples revealed that chitosan will be easier to depolymerize than chitin due to its lower crystallinity, lesser interchain hydrogen bonding network, lower structural rigidity and lower molecular weight.

Chapter 05

Chapter 5 deals with the extraction of chitin from different shell waste samples collected from different states of India and understanding the structural and functional properties of extracted chitin using various physico-chemical characterization techniques.

- ✓ Different shell waste samples were collected from different parts of India (Maharashtra and Kerala). Both shrimp and crab samples were collected to understand the difference between the extracted chitin from different crustacean source. Fresh and sea water both samples were collected to find out any difference between them.
- ✓ Pashan fresh water shrimp shell chitin (PFWSC), Dapoli sea water shrimp chitin (DSWSC) and Kerala sea water crab chitin (KSWCC) were extracted by following industrial/chemical method of extraction which involves three steps, i.e., demineralization using 1N HCl, deproteinization using 3.5 *wt%* NaOH and decoloration using 0.3% sodium hypochlorite solution.
- ✓ Understanding the properties of extracted samples is necessary to confirm the structure of the extracted mass. Therefore, all the extracted samples were thoroughly characterized using various techniques like CHNS elemental analysis, ICP-OES analysis, SEM-EDAX analysis, ATR, ^{13}C NMR, TG-DTA, XRD, ash content measurement etc.
- ✓ First of all, XRD analysis shows that the planes which are present in extracted mass matches well with the planes reported for α-chitin structure. In addition to this, XRD data also shows that extracted samples are more crystalline than commercial chitin sample (TCC). Comparing XRD data of extracted and commercial chitin samples, it can be assumed that either vendors are using more concentrated acid and base solutions for demineralization and deproteinization step respectively which also breaks

the inter chain hydrogen bonding network in chitin or they are extracting chitin in more harsh reaction conditions (high temperature, longer time, use of mechanical energy) which decrystallize chitin along with demineralization and deproteinization. Moreover, fresh water chitin sample (PFWSC) is highly crystalline than sea water chitin samples (DSWSC, KSWSC) as it shows higher intensity peaks than the other two samples. Hence, highly crystalline pure α-chitin was extracted in lab from different shell waste samples. Furthermore, another surface analysis SEM-EDAX shows non-homogeneous, non-smooth surface of the extracted sample and presence of Ca, Na, Al, Mg, Fe, K along with C, N, O.

- ✓ Furthermore, another surface analysis SEM-EDAX shows non-homogeneous, non-smooth surface of the extracted sample and presence of Ca, Na, Al, Mg, Fe, K along with C, N, O. The SEM-EDAX data of commercial samples showed only Ca, Na along with C, N, O. But SEM-EDAX is surface technique and cannot detect the elements which is present in bulk, that's why bulk analysis was carried out.
- ✓ Monomeric formula was derived from CHNS data and the experimental monomeric formula of extracted samples matches with theoretical monomeric formula of chitin. Moreover, the elemental composition and monomeric formula matched well with commercial chitin sample, TCC. Excess carbon and nitrogen were not present in the extracted sample which proves that protein has been completely eliminated during deproteinization step and pure chitin was extracted from shell waste.
- ✓ ICP-OES data shows the presence of Ca, Na, Mg, Al and Fe which matches with SEM-EDAX data. Higher amount of ash is found in sea water samples (DSWSC, KSWCC) than fresh water sample (PFWSC). Higher amount of Ca and Mg are present in sea water samples which is obvious as high amount of Ca and Mg are present in sea water. Moreover, extracted sample from crab shell waste has higher ash content than shrimp one. Ca content is

higher in sea water crab chitin (KSWCC) than the other two samples which can be due to presence of remaining $CaCO_3$ after demineralization. Furthermore, presence of Na can be attributed to the use of NaOH during deproteinization. Further, ash and elements content in commercial chitin sample (TCC) is compared with extracted chitin samples and the data shows that high amount of ash (6.7 mg/1g of chitin) is also present in commercial sample (TCC) along with high amount of Ca and Na. Other metals e.g., Mg, Al and Fe are also present in TCC. Calcium and sodium content in commercial sample is higher than lab extracted chitin (PFWSC, DSWSC). Hence, it can be assumed that complete demineralisation was also not done while preparing chitin samples commercially and Na may come from deproteinization step. From all the extracted samples, fresh water shrimp chitin (PFWSC) is purer than the other extracted chitin samples and commercial chitin sample as it contains less amount of minerals.

- ✓ Furthermore, DTA of extracted samples shows that the samples are stable up to 330 °C which matches well with commercial chitin samples (TCC, LCC). The pattern of weight loss matches well with α-chitin. Hence, α-chitin shows good thermal stability due to presence of H-bonding network which leads to structural rigidity. Weight loss patterns are similar in all the extracted samples and commercial chitin sample.
- ✓ ATR analysis shows that functional groups present in extracted samples matches well with functional groups of chitin. Moreover, ^{13}C NMR study confirms that the structure of extracted mass from shell waste matches with structure of α-chitin. No extra carbon peaks also prove the complete removal of proteins. The IR data and ^{13}C NMR data of extracted chitin samples showed similar and comparable results with commercial chitin (TCC).

- ✓ It was also observed that the minerals composition also varies species wise and depends on habitat (fresh water or sea water). Fresh water chitin sample contains less ash, Ca and Mg that sea water samples. Moreover, shrimp samples showed less ash and calcium content that crab chitin sample. Fresh water chitin sample showed more crystallinity than sea water chitin samples as well.
- ✓ However, the detailed characterization study proves that pure α-chitin has been extracted from shell waste samples which have similar structural properties like commercial chitin but the differences was found in their crystallinity and mineral contents. Summary of the 5th chapter is shown in Figure 7.5.

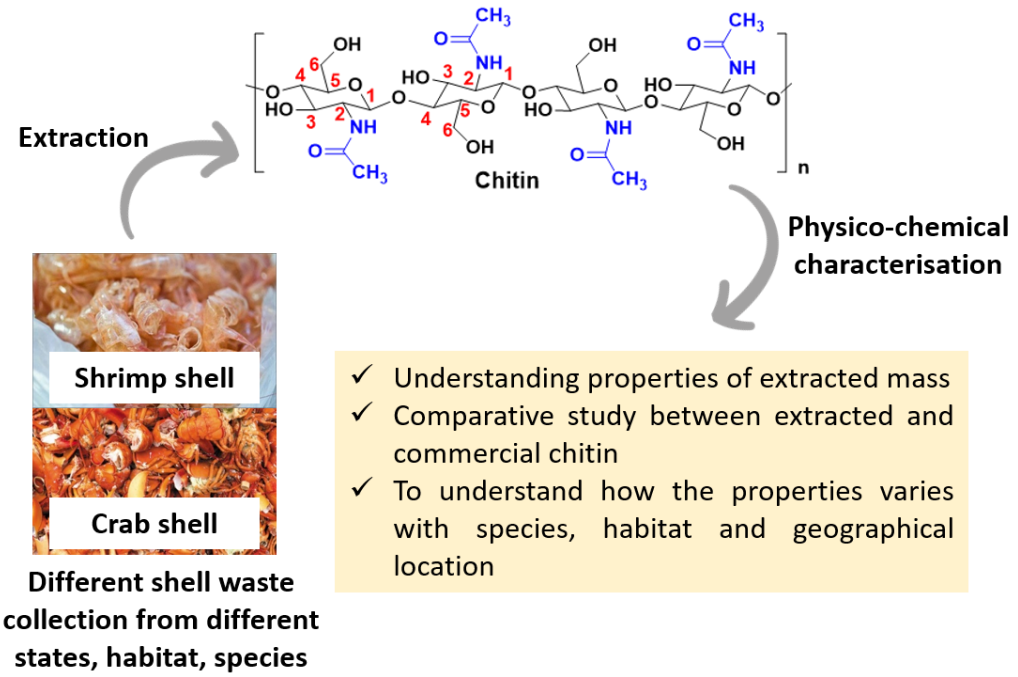


Figure 7.5. Extraction of pure chitin from different shell wastes and their detailed characterization to understand their properties

Chapter 06

Chapter 6 discusses the conversion of TCLMWC chitosan substrate to N-containing chemicals and furan derivatives using solid acid catalysts.

- ✓ Various types of structured solid acid catalysts were evaluated for the conversion of chitosan. Reactions were done in a batch mode Parr reactor at 170 °C with 2 MPa N_2 for 24 h in water (30 mL) as solvent (Figure 7.6).

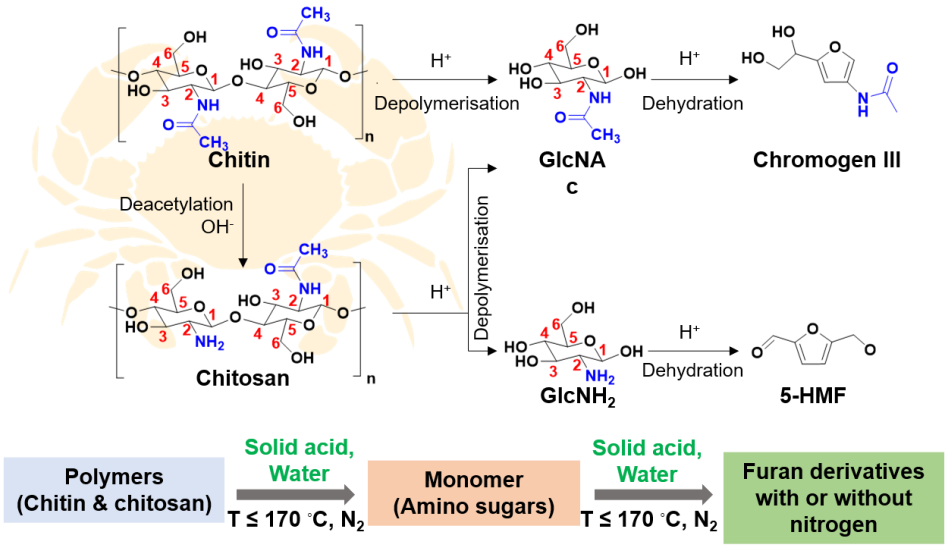


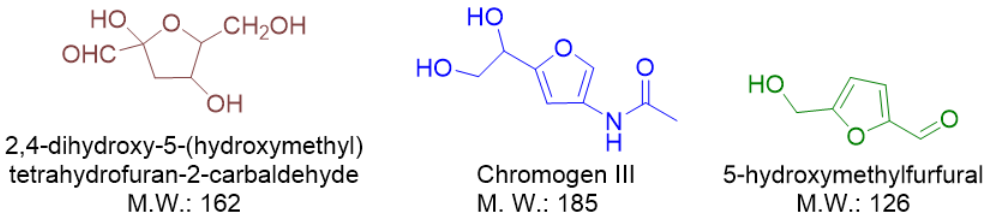
Figure 7.6. Scheme for the conversion of chitin and chitosan over solid acid catalysts

- ✓ Structured solid acid catalysts (H-USY, H-ZSM-5, H-MOR, m-SAPO-44, Montmorillonite K10 clay, Amberlyst-15, Nafion SAC-13) were evaluated for the conversion of chitosan substrate. Moreover, characterization data in 4th chapter reveals that TCLMWC has lowest viscosity and molecular weight among all the chitosan samples. Hence, TCLMWC will be easier to depolymerize and TCLMWC is selected for optimization study.
- ✓ Reaction mixture was analysed using HPLC. Among the structured solid acid catalysts m-SAPO-44 gave highest 5-HMF yield (3.4%). As, 5-HMF is identified and commercially available, amount of 5-HMF has been quantified on the basis of HPLC analysis by plotting calibration curve.
- ✓ Reaction conditions like temperature, time, nitrogen pressure, S/C ratio were optimized and it was observed that 170 °C, 2 MPa N_2, 18 h time, 0.7 mole/mole S/C ratio gave best 5-HMF yield. It was observed that the yield of 5-HMF decreased with the decreasing catalyst quantity due to presence

of NH_2 group which can kill acid sites. Moreover, pH after the reaction increased due to liberation of NH_3 while depolymerisation followed by dehydration process.

- ✓ M-SAPO-44 was able to give 5-HMF with 1.5-3.9% yield from different chitosan samples (ALMWC, AMMWC, AHMWC, TCMMWC, TCHMWC, ASSC, ACSC) and 0.6-0.8% from chitin substrates (TCC, LCC). Lower yield of 5-HMF was observed in case of chitin substrates which is due to different reaction pathway followed by its monomer, N-acetyl-D-glucosamine due to presence of bulky acetamido group. Moreover, it was also observed that 5-HMF yield does not only depends on viscosity or molecular weight because TCMMWC (η= 20-100 cP, 5-HMF yield= 2.1%) showed lower 5-HMF yield than AMMWC (η= 200-800 cP, 5-HMF yield= 3.2%). Therefore, the hydrolysis of chitosan and dehydration of monomers to 5-HMF does not depend on only viscosity or molecular weight, it depends on many factors like degree of polymerisation, monomer present at the reducing end, degree of crystallisation and degree of deacetylation.
- ✓ Mechanistic pathways for the chitosan reaction were investigated. To understand the probable reaction pathways, reactions were carried out with monomers ($GlcNH_2$ and GlcNAc) at similar reaction condition (m-SAPO-44; 0.4 g, water; 30 mL, 170 °C, 2 MPa nitrogen pressure at RT, 4 h). Careful look on HPLC chromatograms suggests that two compounds formed from $GlcNH_2$ unit (5-HMF and peak at 20.47 min RT in VWD) and another two compounds formed from GlcNAc unit (compounds eluted at 12.87 min RT in RID, 22.29 min RT in VWD).
- ✓ LCMS study was performed to know the molecular weight of the products formed in the chitosan reaction. It was concluded that three nitrogen containing compounds (M. W.-287, 185, 125) and four compounds without nitrogen (M. W.- 186, 162, 136, 5-HMF) was formed. I have searched the literature and found two probable compound which can be formed from

chitosan under acid medium and those are matching with observed molecular weights. Figure 7.7 shows structure of probable compounds which can be formed in the acid catalysed conversion of chitosan.



2,4-dihydroxy-5-(hydroxymethyl) tetrahydrofuran-2-carbaldehyde M.W.: 162

Chromogen III M. W.: 185

5-hydroxymethylfurfural M.W.: 126

Figure 7.7. Probable products formed from chitosan

Possible mechanism for formation of Chromogen III (M.W.-185) from GlcNAc units were discussed in detail.

- ✓ Characterization of fresh and spent catalyst shows that m-SAPO-44 undergoes morphological changes under the reaction condition and the structure of the catalyst was disturbed. Due to morphological changes in m-SAPO-44 after 1st catalytic run, the characteristic peak of chabazite morphology vanished and total acidity was reduced drastically.
- ✓ Amorphous solid acid catalysts were then used for the conversion of chitosan and chitin. Silica alumina (SA), niobium pentoxide, WO_x/Silica, MoO_x/Silica, WO_x/SA, MoO_x/SA were used for the conversion of chitosan and chitin. Optimized reaction condition for structured solid acid catalysts (substrate; 0.05g, catalyst; 0.4 g, water; 30 mL, 170 °C, 2 MPa nitrogen pressure at RT, 24 h) was employed for the evaluation of amorphous solid acids. Various catalysts showed varying activity (Figure 7.8) and maximum activity was shown with WO_x/SA catalyst to achieve highest 5-HMF yield (4.4%). High amount of W and Mo were leached out in the reaction mixture (38.16 mg W from 400 mg WO_x/SA, 18.65 mg Mo from the 400 mg MoO_x/SA). Due to metal leaching, these catalysts are not stable and recyclable. Silica alumina catalyst also showed comparatively good activity (2.6% 5-HMF yield). Brönsted acid sites are helping in the hydrolysis of

glycosidic linkages and further dehydration. Leaching of metals is the main problem observed for the supported metal oxides even though they are showing better activity. By considering all the factors and mainly the stability of the catalyst, SA is showing considerably good yield of 5-HMF and stable under the reaction condition as very minimal aluminium leaching (4.03%) was observed in the reaction mixture.

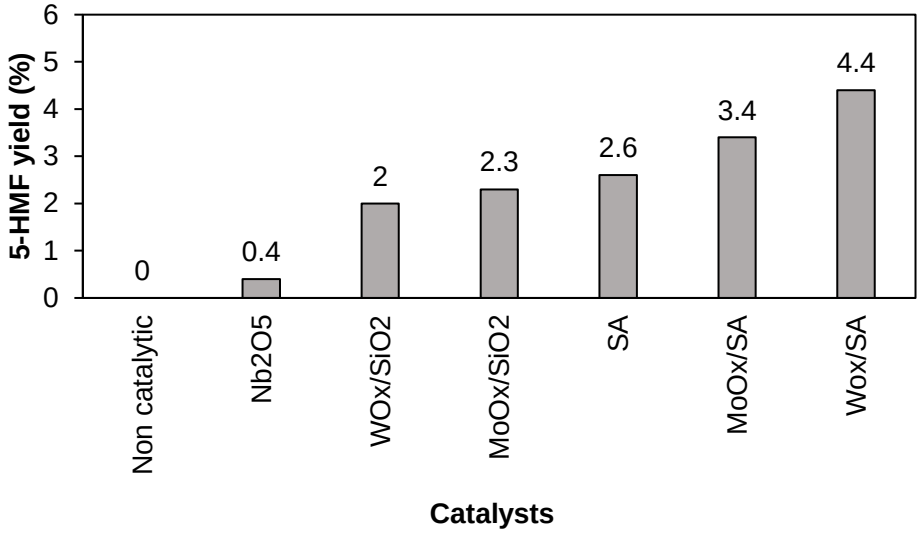


Figure 7.8. Conversion of chitosan into 5-HMF using various amorphous solid acid catalysts. Reaction condition: chitosan TCLMWC (0.05 g), catalyst (0.4 g), water (30 mL), 170 °C, 2 MPa N_2 at RT, 24 h

- ✓ Effect of reaction parameters were checked. It was found that 170 °C, 1 MPa nitrogen pressure, 12 h reaction time can give 3.2% 5-HMF yield. Effect of solvent system was carried out by using biphasic (MIBK/Water 2/1 *v/v*) solvent system. It was observed that 5-HMF yield was maximum at 4 h (2 h, 2.6%; 4 h, 3.6%; 6 h, 3.1%; 8 h, 2.8%) and after that yield was decreased with increasing time. On the other hand, when only water was used as solvent, only 1.8% 5-HMF yield was observed in 4h.
- ✓ SA was able to give 5-HMF with 1.5-3.1% yield from different chitosan samples (ALMWC, AMMWC, AHMWC, TCMMWC, TCHMWC, ASSC, ACSC) and 0.4-0.5% from chitin substrates (TCC, LCC).

- ✓ Spent catalyst characterization data revealed that SA catalyst is stable under the reaction conditions and thus the recycle study was carried out with SA. Consistent 5-HMF yield was observed when the spent catalyst is calcined to remove unconverted chitosan substrate. For the first time, stable and recyclable solid acid catalytic system is developed for the conversion of chitosan and chitin without the use of any mineral acids, organic acids, mineral bases, high boiling solvents and metal salts. The reaction conditions are also benign, green and only water is used as solvent.
- ✓ In future, more work is needed in this area for the analysis of other products formed. The complete identification of all the products formed from this complex reaction requires extensive studies and those are beyond the capabilities of our research facilities.

Chapter 07

All the major outcome and important results of my work were summarized in the 7th chapter. Furthermore, novelty of my Ph. D. work and future perspectives are discussed below.

Novelty of my work:

- A rapid, simple, accurate and precise HPLC method was developed for the simultaneous separation and quantification of chitin derived compounds
- A stable and recyclable solid acid catalytic system was developed for the dehydration of D (+)-Glucosamine into 5-HMF in water as medium and ambient reaction conditions (170 °C, 0.5 MPa N_2, 4h)
- All the commercially available chitosan and chitin samples were thoroughly characterized to understand their physico-chemical properties.

- Pure chitin was extracted from shrimp and crab shell waste using industrial/chemical method and their physico-chemical characterization was carried out to understand their properties. Comparative analysis between extracted and commercial chitin was done to understand similarities and differences.
- For the first time, stable and recyclable solid acid catalytic system is developed for the conversion of different types of chitosan and chitin without the use of any mineral acids, organic acids, mineral bases, high boiling solvents and metal salts. The reaction conditions are also benign, green and only water is used as solvent.
- N-containing chemicals were synthesized from the chitosan using solid acid catalysts.

Future perspective:

Stable and recyclable solid acid catalysed systems for chitosan and chitin conversion and $GlcNH_2$ dehydration are reported for the first time in this work. However, there are lots of scopes to work in these field and develop chitin chemistry in future.

- More development of catalysts and processes are needed to enhance the yield of 5-HMF from chitosan and $GlcNH_2$. The designing of the catalysts should contemplate the structural rigidity, stability under reaction condition, recyclability and product specific.
- More extensive research is needed on identification of other unknown products forming in the both reactions (chitosan conversion and $GlcNH_2$ dehydration) because the unidentified products may have many applications.
- Detailed analysis of reaction mixtures using proper analytical tools and deep investigation of reaction pathways can be a very interesting and important topic to work in future.

- The future research directions should be aimed at synthesizing nitrogen containing chemicals from both chitin and chitosan as these bio-polymers only have the special structural feature of having nitrogen in their structure.
- Moreover, detailed investigation and research is required to make this valorisation process more viable on large scale and economic in future.

Overall, a detailed study on chitin chemistry was performed using solid acid catalysts in the present work which includes

- ***Development of HPLC method for simultaneous separation and quantification of chitin derived compounds***
- ***Catalysis of dehydration of chito-monomers ($GlcNH_2$ and GlcNAc) using solid acid catalysts***
- ***Understanding the properties of chitin and chitosan using physico-chemical techniques***
- ***Extraction of pure chitin from shell waste followed by its complete characterization***
- ***Catalysis of conversion of different chitosan and chitin substrates using heterogeneous acid catalysts and water as solvent***

Abstract

Name of the Student : Neha Ghosh **Registration No. :**10CC16J26009
Faculty of Study : Chemical Science **Year of Submission:** 2022
AcSIR academic centre/CSIR Lab: CSIR-National Chemical Laboratory
Name of Supervisor : Dr. Paresh L. Dhepe
Title of the thesis : Understanding N-containing saccharides, their valorisation by solid acids and HPLC method development for separation and identification of products

Chitin is abundant and potential feedstock for value added chemicals, especially nitrogen containing chemicals due to its special structural feature of having biologically fixed nitrogen in its structure which is different from other biomass sources. Detailed literature study showed that valorisation of chitin/chitosan/monomers was done in last decade but the reports are associated with several drawbacks like use of homogeneous acid catalysts, organic acids, metal salts, use of high boiling solvents, use of acids and bases as solvent, use of mechanical energy or microwave heating. Moreover, proper analytical method is not reported to simultaneously separate or quantify reaction products. In this context, I have developed and discussed a simple, rapid and precise HPLC method was developed for simultaneous separation and quantification of chitin derived compounds in the first working chapter (Chapter 2). After setting the analytical method, synthesis of 5-HMF from chito-monomers ($GlcNH_2$ and GlcNAc) using solid acid catalysts is represented in 3rd Chapter. A stable and recyclable solid acid catalysed system for dehydration of $GlcNH_2$ to 5-HMF was developed in presence of water as reaction medium for the first time where no mineral acids and organic acids were used as solvent. After that, detailed physico-chemical characterizations were carried out to understand the structural and physical properties of commercially procured chitosan and chitin samples (Chapter 4). Eight different samples of chitosan and two samples of chitin were studied in detail to know the similarities and differences in their structural and physical properties which is very necessary before starting catalytic conversion experiments. The different shell wastes were collected from different coastal state of India and pure chitin was extracted from them. The comparative study on physico-chemical properties was performed between the extracted and commercial chitin samples using various analytical techniques. Moreover, the effect of different species, habitat and geographical location on the properties of chitin was studied (5th Chapter). For the first time, stable and recyclable solid acid catalytic system is developed for the conversion of different types of chitosan and chitin without the use of any mineral acids, organic acids, mineral bases, high boiling solvents and metal salts (Chapter 6). The reaction conditions are also ambient and only water is used as solvent. N-containing chemicals were synthesized from the chitosan using solid acid catalysts and also discussed in 6th chapter.

List of publication(s) in SCI Journal(s) (published and accepted) emanating from the thesis work with bibliographic details

- HPLC method development for separating the chitin & chitosan chemistry, **Neha Ghosh**, Paresh L. Dhepe*, *Carbohydrate Polymer Technologies and Applications*, Volume 2, **2021**, 100139, DOI: https://doi.org/10.1016/j.carpta.2021.100139

Other publications and patent (emanating from thesis work)

- Employment of catalysts with acidic functionality in the valorization of animal derived biomass, Patent, Application no.-202011031311, 2020
- Synthesis of 5-HMF from chito-monomers using solid acid catalysts, Neha Ghosh, Paresh L. Dhepe*, manuscript submitted
- Shell waste valorization to chemicals: Methods and Progress, Lavanya K, Neha Ghosh, Paresh L. Dhepe*- manuscript prepared
- Valorisation of chitosan and chitin using recyclable solid acid catalysts and ambient reaction conditions, Neha Ghosh, Paresh L. Dhepe*- manuscript preparation phase

List of papers with abstract presented (oral/poster) at national/international conferences/seminars with complete details

1. Poster presentation at **"57th Annual convention of Chemists, 2020"** and **"International conference on recent trends in Chemical Sciences (RTCS-2020)"** organized by Indian Chemical Society, Kolkata (Virtual mode: online presentation) (26th-29th December, 2020).
   Title: "Understanding the properties of different types of chitosan and their value addition"
2. Poster presented at **"National Science Day"** at CSIR-NCL, Pune (26th-28th February, 2020).
   Title: "Understanding the properties of different types of chitosan and their value addition"
3. Poster presented at Symposium on **"Catalysis & Multiphase Reactor Engineering (CATMUR)"** at CSIR-NCL, Pune (23rd December, 2019).
   Title: "Understanding the properties of different types of chitosan and their value addition"
4. Poster Presentation at **"The 8th Asia Pacific Congress on Catalysis (APCAT-8)"** held at Bangkok, Thailand (4th-7th August, 2019)
   Title: "Understanding the properties of different types of chitosan and their value addition"
5. Poster presented at **"DST annual review meeting"** at Ahmedabad (25th July, 2019).
   Title: "Understanding the properties of different types of chitosan and their value addition"
6. Poster presented at **"National Science Day"** at CSIR-NCL, Pune (26th-28th February, 2019).
   Title: "Understanding the properties of different types of chitosan and their value addition"

List of Conferences attended with details

1. **"The 7th Asia Pacific Congress on Catalysis (APCAT-7)"** held at Mumbai, India (17th-21st January, 2017)
2. **"23rd National Symposium on Catalysis (CATSYMP-23)"** held at Bangaluru (17th-19th January, 2018)

Award Received

Received "**Indian Chemical Society Research Excellence Award**" in RTCS-2020 and 57th Annual Convention of Chemists organized by Indian Chemical Society during 26th-29th December, 2020 for poster presentation.

Title: "Understanding the properties of different types of chitosan and their value addition"

Carbohydrate Polymer Technologies and Applications 2 (2021) 100139

Contents lists available at ScienceDirect

Carbohydrate Polymer Technologies and Applications

journal homepage: www.sciencedirect.com/journal/carbohydrate-polymer-technologies-and-applications

HPLC method development for chitin and chitosan valorisation chemistry

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ARTICLE INFO

Keywords:
High Performance Liquid Chromatography
Method development
Chitin and Chitosan chemistry
Separation and quantification

ABSTRACT

Recently, chitin/chitosan chemistry is gaining huge attention to yield chemicals like oligomers, monomers, N-containing chemicals and furan derivatives. However, due to lack of proper analytical technique to separate them due to their almost similar size and polarity hamper the further progress in this domain. In this work, a high performance liquid chromatography method with a variable wavelength and refractive index detector is optimised for simultaneous separation and quantification of amino sugars (D (+)- glucosamine, N-acetyl-D-glucosamine, Glucosamine hydrochloride dimer), other sugars (D (+)-glucose, D-(+)-fructose, 2-Deoxy-D-glucose), N-containing chemicals (D-glucosaminic acid, 2-Aminoethanol, N-acetylethanolamine, Acetamide) and furan derivative (5-Hydroxymethylfurfural). While non-polar C18 column with isocratic elution [acetonitrile/water (1/9v/v)] could show separation of few compounds, but best results were achieved with RPM-Monosaccharide Pb2+ column with water as a mobile phase. The regression equation revealed good linear relationship (R2=0.9904-0.9974) within test ranges. Repeatability of the method and column stability was found to be within acceptable limits.

Introduction

Marine biomass/oceanic biomass is the waste generated from the crustacean species, i.e., shrimps, lobsters, prawns, squids, etc., which contains calcium carbonate (20-50%), protein (20-40%), and chitin (15-40%) (Yan & Chen, 2015). Valorisation of marine biomass can bring both economic and environmental benefits to industries, including the fishery industry (Chen, Yang, & Yan, 2016). Chitin, one of the important constituents of crustacean waste, is a polysaccharide made up of several N-Acetyl-D-Glucosamine (GlcNAc) units liked via β-1,4 linkages. From an abundance point of view, it is the second most plentiful bio-polymer available on earth after cellulose, with 10^{11} tonnes of annual production (Jardine & Sayed, 2016). Native chitin up on alkali catalysed deacetylation yields chitosan, a polymer with amino sugars as backbone. To be precise, chitosan is a polymer consisting of D-(+) Glucosamine ($GlcNH_2$) and N-Acetyl-D-Glucosamine (GlcNAc) units and has potential applications in various industries, i.e., agriculture, water purification, biomedical materials, and catalysis (El Kadib, 2015; Elwakeel, 2010; Katiyar, Hemantaranjan, & Singh, 2015; Nivethaa, Martin, Frank-Kamenetskaya, & Kalkura, 2020). Unlike other plant biomass feedstocks, the importance of chitin and chitosan is that it contains 7% biologically fixed nitrogen in its structure, which can be harnessed to make nitrogen containing chemicals, those are otherwise very tedious to prepare (Chen, Chew, Kerton, & Yan, 2014; Kim, 2010). A simplistic illustration of acid catalyzed depolymerisation of chitin/chitosan to monomers and their further dehydration to furan derivative(s) is presented in Figure 1. Nevertheless, it is possible to obtain various other products either from chitin/chitosan or monomers or furan derivative(s) in presence of acid and base or even up on thermal treatment.

While, researchers are engaged in the development of various catalytic systems to yield monomers, N-containing chemicals and furan derivatives from chitin and chitosan, either via biochemical or catalytic routes, it is often observed that the analysis of the reaction mixture is restricted to either oligomers formation or monomer formation or furan formation. This is mainly due to lack of knowledge in the domain of chitin/chitosan chemistry regarding thorough investigations on the analytical method developments for the separation and quantification of potential products formed in these reactions. Since $GlcNH_2$, GlcNAc and other N-containing chemicals are sugar derivatives with very high and close polarity, those are completely soluble in water and thus, it is absolutely necessary to develop and use proper analytic method(s) to separate and quantify these compounds to take work in this area to the next level. Moreover, besides these reported products, it is often seen that catalytic or thermal reactions may yield few other products which

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https://doi.org/10.1016/j.carpta.2021.100139
Received 23 June 2021; Received in revised form 13 August 2021; Accepted 16 August 2021
Available online 18 August 2021

are until now gone undetected or simply not separated or identified due to lack of optimum analytical conditions.

Literature reports, several analytical methods such as chromatographic (gas chromatography (GC) (Matsagar, Munshi, Kelkar, & Dhepe, 2015; Tzeng, Bhaumik, & Chung, 2019; Zhang & Yan, 2017), column chromatography, high performance liquid chromatography (HPLC) (Pandit, Deshpande, Patil, Jain, & Dandekar, 2020; Tzeng et al., 2019; Yu et al., 2016), chemical method (Fehling's test, gravimetric analysis) (Erb & Zerban, 1938), and colorimetric method (DNS method) (Garriga, Almaraz, & Marchiaro, 2017; Li et al., 2015; Pandit et al., 2020; Yu et al., 2016) for the separation and quantification of chitin/chitosan derived chemicals.

Gas chromatography (GC), as is known, requires compounds to be volatile or having boiling points less than their degradation temperature (s) for their successful analysis. This method is suitable to be used for the detection and quantification of organic compounds having above properties and it is seen that it can be very well used to detect and quantify 5-HMF and other volatile compounds formed in chitin/chitosan chemistry (Tzeng et al., 2019; Zhang & Yan, 2017). Nonetheless, amino sugars such as $GlcNH_2$ and GlcNAc do not have very clear boiling points and are also non-volatile and thus cannot be detected by the GC method unless those are derivatized. Moreover, these amino sugars also undergo degradation at higher temperatures employed during GC analysis and thus make GC method unsuitable for their separation and quantification. Additionally, chitin/chitosan hydrolysis reactions are carried out in water media and water based samples are not recommended to be injected in the GC system since water can extinguish the flame (in flame ionization detector) and water at higher temperature may dissolve silica in capillary column. Therefore, simultaneous analysis and separation of $GlcNH_2$, GlcNAc, N-containing chemicals, furan derivatives and 5-HMF is not probable using GC technique.

Column chromatography is another good analytical technique which is used for the analysis of products in most of organic reactions. Amino sugars, N-containing chemicals and furan derivatives are considered to be having very comparable polarity due to their structural and functional groups similarity. Thus, this can lead to difficulty in the separation of these compounds. Additionally, this technique requires tedious process to follow and hence running a column for every reaction/sample is also time-consuming process and is not recommended for fast and quantitative analysis. In consideration of this, column chromatography may not be a good technique for separating these compounds.

Another method by which reducing sugars can be quantified is a gravimetric analysis method by measuring the weight of CuO_2 precipitate formed in the reaction of reducing sugar with copper(II) sulfate in an alkaline medium (Erb & Zerban, 1938). However, the drawback of this method is that only reducing sugars can be quantified and thus, organic compounds such as furan derivatives and any other N-containing chemicals cannot be detected. Moreover, as the main drawback of this method, the total amount of reducing sugars can be calculated, but the separation of two or more different reducing sugars and their separate quantification is not possible. Also, gravimetric analysis is very much sensitive of reaction time, temperature, pH and concentration of both sugars and copper(II) sulfate.

Reducing sugars can also be quantified by the colorimetric technique that consists of a redox reaction between 3,5-dinitrosalicylic acid and the reducing sugars present in the sample, then quantified by UV-Visible

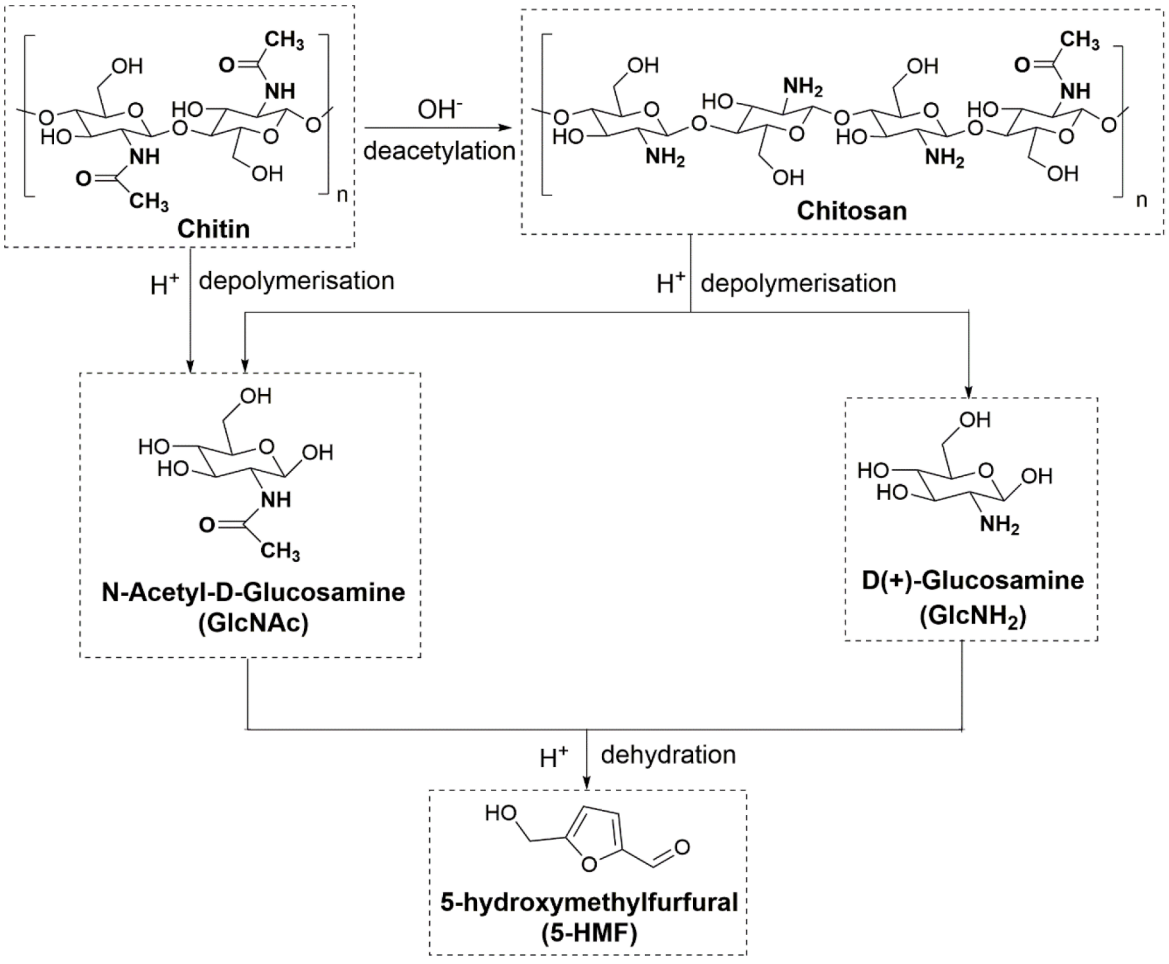

Fig. 1. Depolymerisation of chitin/chitosan into $GlcNH_2$ and GlcNAc and further dehydration to 5-HMF.

spectroscopy at 540 nm. The intensity of the colour is proportional to the concentration of sugars and thus the total concentration of sugars can be easily quantified. Nevertheless, this method too has the same drawbacks as like previously discussed chemical method. Colorimetric method is also used by researchers in chitin/chitosan field for quantifying reducing sugars (Kalane, Krishnan, Yadav, Jain, & Dandekar, 2019; Li et al., 2015; Yu et al., 2016). Further, brief on the drawbacks are summarized in Table S1 (ESI).

HPLC, for a very long time, is a well-known technique used for the separation and quantification of sugar compounds. HPLC is also an easy, rapid, sensitive analytical technique that can separate water soluble sugar compounds as well as organic compounds.

While researchers are working on the chitin/chitosan chemistry, they have extensively used HPLC as a prime methodology to detect the products formed in their reactions, nevertheless with few restrictions. A quick observation made from Table S2 (ESI) suggests that, in most of the works, HPLC system is used along with RID/VWD detector and variety of columns are used. However, from the sugar chemistry originated from plant biomass, it is known that non-polar columns cannot separate sugar compounds very well as the non-polar stationary phase cannot have strong interaction with polar sugar compounds. It is acknowledged from the sugar chemistry originated from plant biomass that the best HPLC column for the separation and quantification of glucose, fructose, xylose, arabinose etc., along with furfural, 5-hydroxymethylfurfural (5-HMF) etc. are ion-exchanged columns such as Pb^{2+}, H^{+}, Ca^{2+} etc. These columns are preferred due to their tolerance towards a wide range of pH (1-8) and solvents (<10% organic solvent with water as base eluent). However, it is seen from Table S2 (ESI) that in chitin/chitosan chemistry, rarely ion-exchange columns are used for their efficiencies to detect and quantify amino sugars along with N-containing chemicals and furan derivatives. Additionally, in most of the works (Table S2, ESI), simultaneous analysis of all the chemicals is not reported due to the lack of efforts put in the direction for the separation of these compounds by altering analytical parameters (Ma et al., 2020; Pierson, Chen, Bobbink, Zhang, & Yan, 2014; Tzeng et al., 2019).

Besides researchers working in this area, HPLC column manufacturing companies also report the use of several methods for the analysis of amino sugars and 5-HMF. The details on those are given in Table S3 (ESI).

As observed from Table S3 (ESI), even the HPLC column manufacturers have not put much effort to separate chemicals that can be obtained in chitin/chitosan chemistry. Most of the manufacturers have typically used amino or C18 columns except by Agilent and Hamilton; those have shown the possibility for the use of ion-exchange columns. Additionally, in sugar chemistry originated from plant biomass, few of the researchers have used C18, amino (-NH_2) and cyano (CN) columns for the separation and quantification of products, but the main drawback of these columns is overlapping of peaks for few of sugar compounds. Thus those may possibly give wrong results. From the above, it is very clear that a detailed study on the separation of various chemicals which can form in chitin/chitosan chemistry is not done.

In view of the constraints observed to detect, separate and quantify products formed in the recently developing chitin/chitosan chemistry, this work aimed to investigate and optimize various HPLC parameters, which must be suitable for simultaneous separation and quantification of sugars, N-containing chemicals and furan derivatives. In this work, columns with varying properties such as polarity, length, stationary phase, along with the mobile phase of different polarity and detectors, were evaluated to achieve better separation of these compounds. A simple, rapid and reproducible method is developed, which can give simultaneous separation and quantification of the compounds which can be formed from chitin and chitosan.

Experimental

Materials and reagents

D-(+)-glucosamine ($GlcNH_2$•HCl, 99%) and dimer of D-(+)-glucosamine (99%) were purchased as pure hydrochloride salt from Sigma-Aldrich, USA. N-Acetyl-D-glucosamine (GlcNAc, 99%) 5-hydroxymethylfurfural (5-HMF, 99%), Levulinic acid (98%), 2-Aminoethanol (>98%), N-Acetylethaolamine (technical grade), 2-Deoxy-D-glucose (99%) Acetamide (99%) were also purchased from Sigma-Aldrich, USA. D-Glucosaminic acid (98%) is procured from TCI chemicals. D-(+)-glucose (99%), D-(+)-fructose (99%), Formic acid (98%) and Glacial acetic acid were purchased from Loba Chemie. HPLC grade acetonitrile, methanol and Millipore Milli-Q water (Millipore, Milford, USA) were used for the separation and sample preparations. HPLC grade acetonitrile and methanol were purchased from Merck. Methanol (AR grade), isopropyl alcohol (AR grade), ethyl acetate (AR grade), dichloromethane (AR grade), methyl-iso-butyl ketone (AR grade), acetone (AR grade), hexane (AR grade) are procured from Loba Chemie and used for solubility study. Ethanol (99.9% AR) is procured from Analytical CSS reagent and used for solubility study.

Preparation of standards

Preparation of $GlcNH_2$.HCl, GlcNAc, 5-HMF

Standard solutions of all the chemicals used in this study were prepared in Milli-Q water. Standards were prepared by dissolving a known quantity of compounds in water. The details of prepared samples are summarized in Table S4a-S4c (ESI). These solutions were used immediately after their preparations or otherwise stored in the refrigerator at 4°C to avoid any degradation. For their further use, those were allowed to warm up to room temperature (32±3 °C). All the samples were injected three times to check the accuracy and precision of the results.

Preparation of standard mixture ($GlcNH_2$.HCl + GlcNAc + 5-HMF)

Standard mixture solution of $GlcNH_2$.HCl, GlcNAc and 5-HMF were prepared by dissolving 13.78 mg $GlcNH_2$.HCL, 10.86 mg GlcNAc and 8.76 mg 5-HMF in Milli-Q water (30.02). Standard was used immediately after its preparations or otherwise stored at 4°C in the refrigerator to avoid degradation. For their further use, those were allowed to warm up to room temperature (32±3 °C).

Preparation of other standards for checking effectiveness of the developed method

Standard solutions of D-glucosaminic acid, Glucosamine Hydrochloride dimer, Acetic acid, Levulinic acid, Formic acid, 2-Aminoethanol, N-Acetylethanolamine, 2-Deoxy-D-glucose, D-(+)-glucose, D-(+)-fructose and Acetamide were prepared by dissolving 5.00±0.05 mg compound in 10.00±0.2g water. These solutions were used immediately after their preparations or otherwise stored at 4°C in the refrigerator or to avoid degradation. For their further use, those were allowed to warm up to room temperature (32±3 °C).

Solubility study

In a bid to develop optimum analytical conditions, solubility of the standard compounds was checked in different solvents, e.g., water, methanol, ethanol, isopropyl alcohol, ethyl acetate, dichloromethane, methyl-iso-butyl ketone, acetone, hexane. In a typical solubility experiment, 50±0.05 mg ('X') compound was weighed, and to it, 30±0.02 g solvent was added at room temperature (32±3 °C). The resultant mixture was stirred for 4 h at 200 rpm to make sure enough time is given for the solubility of compounds in the solvents. Subsequently, the solution was filtered through Whatman filter paper (grade no. 41). The filtrate was taken in a round bottom flask, and the solvent was evaporated using rotary-evaporator. Further, to remove the solvent

completely, RB was evacuated at 50±3 °C under vacuum for 2 h. The solubility of compounds was calculated based on the weight of the compound observed in the RB ('Y'). Following formulae were used for the calculation.

$$Solubility\ of\ compound\ (\%) = \frac{Initial\ weight\ of\ compound\ taken\ (X) - Weight\ of\ compound\ observed\ in\ RB\ (Y)}{\text{Intial weight of compound taken (X)}} x100$$

$$Weight\ of\ compound\ observed\ in\ RB(Y) = \text{Weight of RB after removal of solvent} - \text{Weight of blank RB}$$

Thin layer chromatography (TLC)

TLC was carried out to understand the polarity trend of the compounds. Methanol/Dichloromethane (DCM) system was used as mobile phase in different *v/v* ratios to achieve satisfactory separation of compounds. Following formula was used for calculation retention factor of the compounds.

$$Retention\ factor = \frac{\text{Distance travelled by the compound from origin (mm)}}{\text{Distance travelled by the solvent from origin (mm)}}$$

HPLC equipment

For the work, two separate HPLC systems were used viz. Shimadzu LC-9A HPLC system equipped with variable wavelength detector (SPD-6A) and Agilent 1260 Infinity HPLC equipped with G1310 quaternary pump, G1367 sample autosampler, G1314 variable wavelength detector, G1362 refractive index detector and OpenLab software. For sample injection in Shimadzu make HPLC, Rheodyne manual injector 7725i equipped with 20 uL loop was used. For the sample injection in Agilent HPLC, autosampler G1367 with 10 uL sample injection volume was chosen.

Chromatographic conditions

For chromatographic separation, three different types of columns were used based on their packing and polarity. The details of different columns are summarized in Table 1.

As summarized in the Table 1, columns used for optimization were normal phase polar column, i.e, amino (250 mm x 4.6 mm i.d., 5.0 um, Nucleosil, Macherey-Nagel), mid-polar ion exchange column, i.e., RPM-Monosaccharide Pb^{2+} (300 mm x 7.8 mm i.d., 8.0 um, 8 % cross-linking, Rezex™ Phenomenex), and non-polar reversed phase column C18 (250 mm x 4.6 mm i.d., 5.0 um, Agilent Zorbax). The separation of investigated compounds was tested by changing the ratio of acetonitrile and methanol with water. Mobile phase was prepared by mixing acetonitrile/methanol and water with varying *v/v* ratios, followed by sonication to remove air bubbles and proper mixing of two solvents. Column temperature was also an important factor to achieve better separation and minimize the viscosity of mobile phase and column pressure; column temperature was set to 80°C while using Pb^{2+} column and 25°C while using C18 column. The flow rate of the pump was varied from 0.4-0.7 mL/min. Injection volume was kept at 10 uL for Agilent HPLC autosampler G1367 and 20 uL sample was manually injected using Rheodyne manual injector 7725i equipped with 20 uL loop in case of Shimadzu LC-9A HPLC system. Wavelength of the variable wavelength detector (VWD) was selected after doing UV-Vis spectrophotometric experiment. Temperature of the refractive index detector was kept at 40°C.

Calibration curves

The standard solutions tabulated in Table S4a-S4c (ESI) were prepared and injected for the construction of calibration curves. This process was repeated thrice to get reproducible results and then standard deviation was calculated. The calibration curves were plotted by the peak area versus concentration of each compound.

Repeatability and precision

Repeatability of the optimized method was evaluated by injecting same sample ($GlcNH_2$: 1192 mg/L, GlcNAc: 926 mg/L and 5-HMF: 733 mg/L) three times. Then percentage of relative standard deviation (% RSD) was calculated. The precision of injection was demonstrated by three replicative injections of the standard solutions. Measurements of retention time and peak areas were used to check the repeatability and reproducibility of the developed method.

Results and discussion

Understanding structural and chemical properties of the investigating compounds

Before initializing the analytical method development studies for these compounds, understanding of their properties is very important (Table 2 and Table S5, ESI). Kinetic dimensions of these compounds were calculated using Chem3D software after energy minimization, and the results are presented in Table 2. Kinetic diameters of these compounds were also derived using theoretical formula using molecular weight (Eq. 1) (Wang & Frenklach, 1994).

Table 2
Properties of the compounds.

Properties	$GlcNH_2$	GlcNAc	5-HMF
Width (Å) (from Chem 3D)	5.039	5.341	3.179
Length (Å) (from Chem 3D)	7.139	8.388	7.321
Kinetic diameter (Å) (from M. W)	6.954	7.460	6.186
M.W (g/mol)	179	221	126
Melting Point (°C)	150	211	34
Boiling point (°C)	-	-	115
Degradation temperature (°C)	190	230	-
Solubility (%)	Water (100) Methanol (35)	Water (100) Methanol (44)	Water (100) Methanol (95)

Table 1
Details on the columns used in this study.

Column	Dimensions (l x d in mm), packing, particle size (um), pH range	Polarity and phase	Analysis conditions	Detector used
Agilent Zorbax C18	250 mm x 4.6 mm I.D, ODS, 5, 2-9	Non-polar and reverse	Column temp: 25 °C, Acetonitrile: Water (1:9 *v/v*), Flow rate: 0.5 mL/min	VWD
Agilent C18	100 mm x 4.6 mm I.D, ODS, 5, 2-9	Non-polar and reverse	Column temp: 25 °C, Acetonitrile: Water (1:9 *v/v*), Flow rate: 0.5 mL/min	VWD
Rezex Phenomenex Pb^{2+}	300 mm x 7.8 mm I.D, Sulfonated Styrene Divinyl Benzene, 8, Neutral	Mid-polar and reverse	Column temp: 80 °C, Water, Flow rate: 0.4-0.7 mL/min	VWD, RID
Machery-Nagel Amino	250 mm x 4.6 mm I.D, Silica, 5, 2-8	Polar and normal	Column temp: 30 °C, Acetonitrile: Water (8:2 *v/v*), Flow rate: 0.5-1.0 mL/min	VWD, RID

$$Kinetic\ diameter(\sigma) = 1.234x(Molecular\ weight)^{1/3} \quad (1)$$

Data on the size of these compounds clearly shows that there is a minimal difference in the size of these molecules, and this suggests that size based separation (size exclusion or typical HPLC column) of these compounds is not possible.

Solubility data of these compounds also show that those are completely soluble in water and are partially soluble in methanol. This indicates that all the compounds are polar in nature and thus only based on polarity those may not be able to separate from each other.

Since both, GlcNAc and $GlcNH_2$ do not have clear boiling points and degrade very close to their melting points; those will be impossible to analyse using GC as in this technique, typically higher temperatures (injector, oven and detector) are used to vaporize the samples. Moreover, their restricted solubility in organic solvents restricts their injection in GC as with water as a solvent (in which 100% solubility is observed), FID cannot be used for their detection. In view of these restrictions, it would be essential to find out a technique, which gives fast results and thus, HPLC seems to be the most suitable technique.

Properties of other N-containing chemicals and furan derivatives in chitin/chitosan chemistry were also tabulated in Table S5 (ESI). Sugar derivatives like D-(+)-Glucose, D-(+)-Fructose, 2-deoxy-D-glucose, Glucosamine hydrochloride dimer do not have boiling points; thus, they will degrade and are impossible to analyse using GC technique. D-Glucosaminic acid has very high boiling point and hence GC will not be a good method to analyse all these compounds which are derived from chitin and chitosan.

Polarity trend of main three compounds, $GlcNH_2$, GlcNAc and 5-HMF is understood from TLC experiment (Figure S1, ESI). As amino sugars are only soluble in methanol solvent (at least partially), methanol/DCM solvent system was used as a running solvent.

TLC plate of pure methanol shows that compounds were not moving from the origin. For changing the polarity of methanol, DCM was added. The ratio of methanol/DCM was varied from 1/9 *v/v* to 1/1 *v/v* to check under which solvent system best separation of these compounds is possible. 5-HMF is found to be least polar as the spot of 5-HMF runs along with the solvent system. As the polarity of solvent system was increased by the addition of methanol, the spot of GlcNAc got separated from $GlcNH_2$. From the TLC plate, it is understood that $GlcNH_2$ is most polar, GlcNAc is mid-polar and 5-HMF is least polar. Retention factors of each compound in different solvent systems were tabulated in Table S6, (ESI).

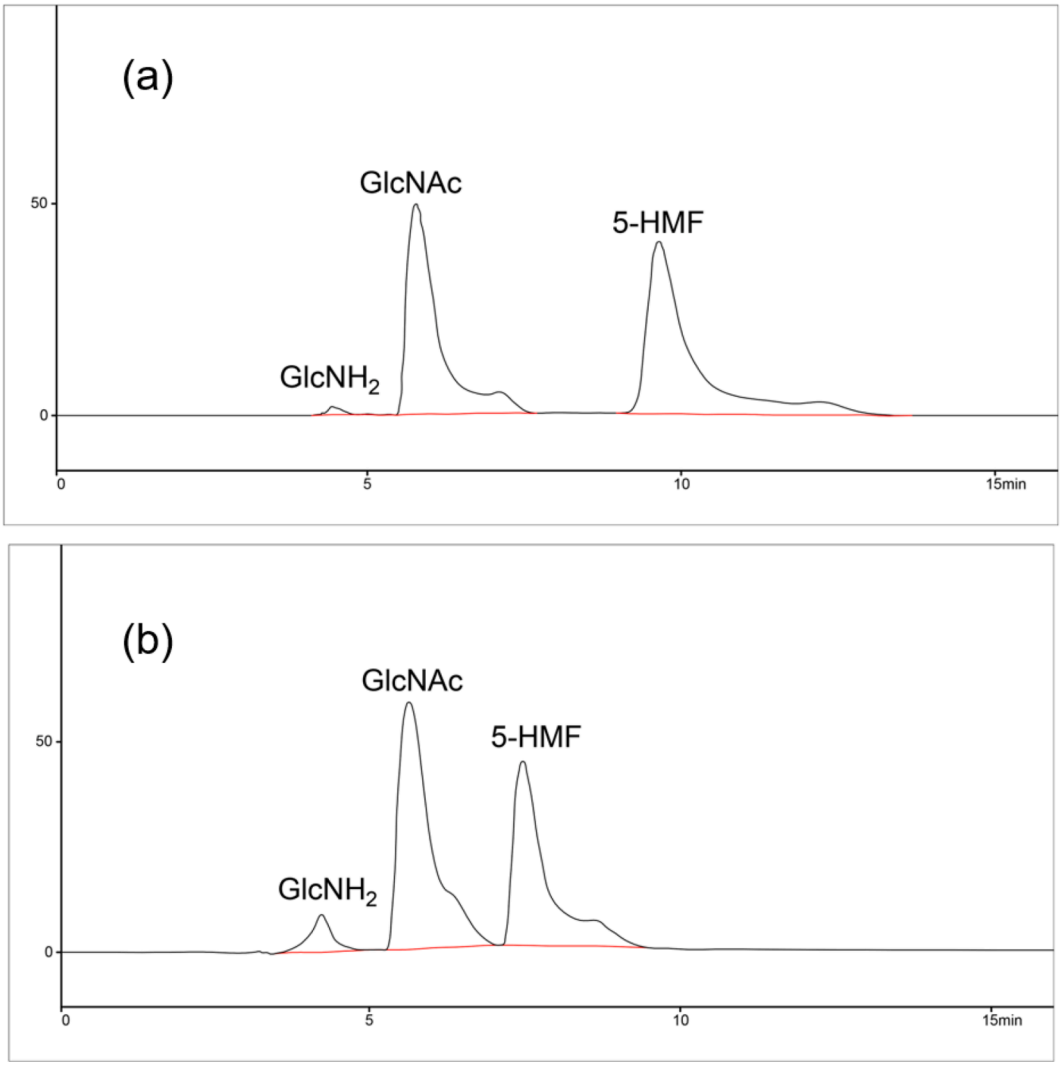

Fig. 2. Chromatogram for Mixture ($GlcNH_2$+ GlcNAc+5-HMF) (a) Analysis conditions: Column: C18 (250 mm x 4.6 mm i.d., 5.0 um, Agilent Zorbax), Mobile phase: Methanol/water: 2.3/7.7 *v/v*, column temperature: 25°C, Flow rate: 0.5 mL/min, VWD wavelength: 195 nm (b) Analysis conditions: Column: C18 (250 mm x 4.6 mm i.d., 5.0 um, Agilent Zorbax), Mobile phase: acetonitrile/water: 2.3/7.7 *v/v*, column temperature: 25°C, Flow rate: 0.5 mL/min, VWD wavelength: 195 nm.

HPLC method development

Wavelength selection for VWD and validation for separating compounds

The mixture of $GlcNH_2$, GlcNAc and 5-HMF (sample details given in section 2.2.2) was first injected in HPLC using previously reported method for analysing 5-HMF [C18 column (250 mm x 4.6 mm, 5.0 um, Agilent Zorbax) and methanol/water: 2.3/7.7 *v/v* ratio, VWD wavelength was kept at 284 nm] (Yu et al., 2016). The chromatogram for the analysis is shown in Figure S2, (ESI). As seen, only one peak for 5-HMF, confirmed by injecting standard for 5-HMF was observed in the chromatogram. The absence of peaks for $GlcNH_2$ and GlcNAc showed that this method may not be suitable for the analysis of all the compounds.

To understand the reason behind absence of peaks for $GlcNH_2$ and

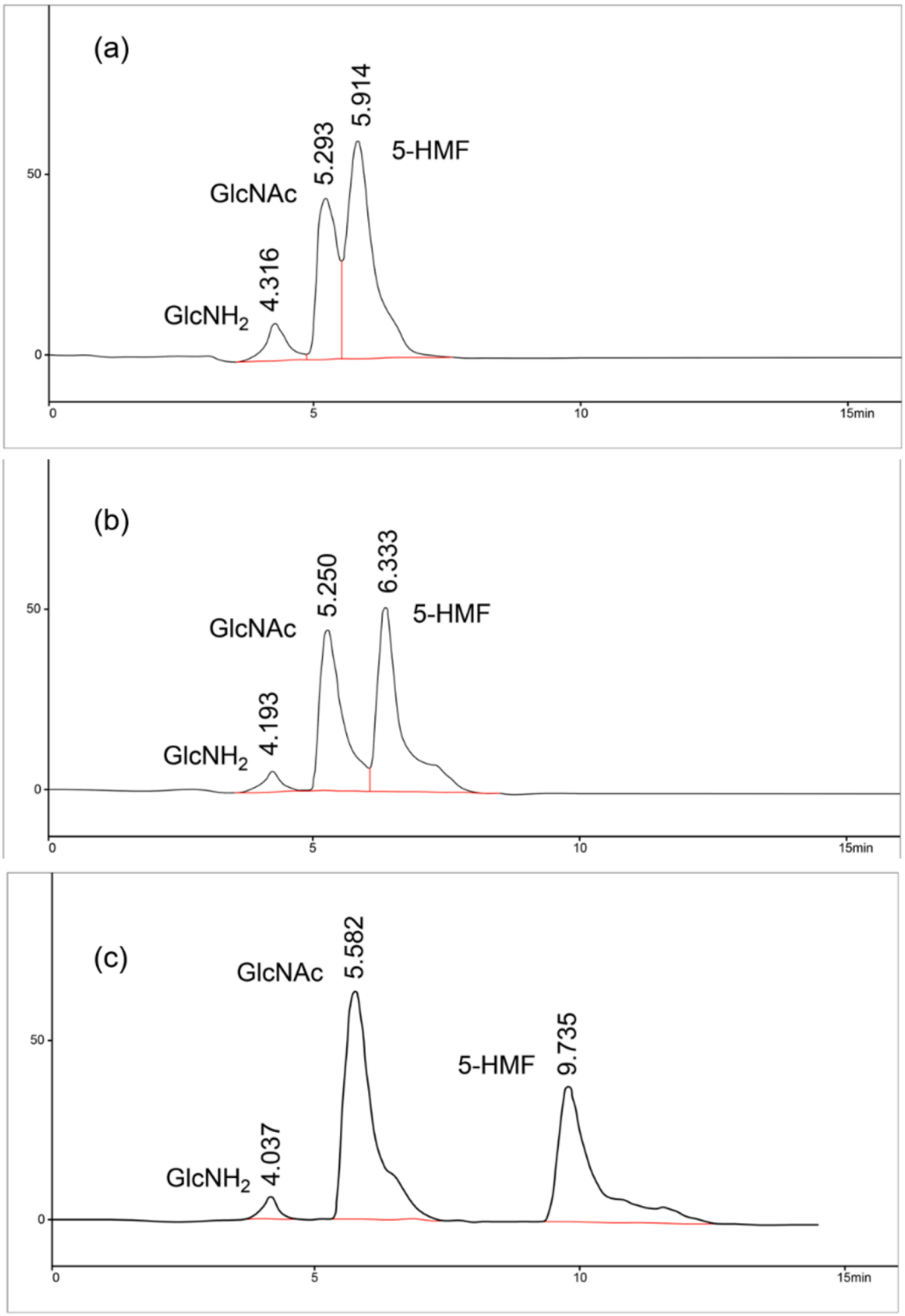

Fig. 3. Chromatogram for Mixture ($GlcNH_2$+ GlcNAc+5-HMF) in acetonitrile/water.

GlcNAc, UV-Visible spectra of these three compounds were recorded to find out the λ_{max} for all the compounds (Figure S3, ESI).

UV-Visible spectra of these compounds clearly show that $GlcNH_2$ and GlcNAc have λ_{max} at 195 nm and 5-HMF has λ_{max} at 284 nm. As lower wavelength corresponds to higher energy, more energy is required to excite the electrons in the case of amino sugars, so they are not detected at higher wavelength, i.e., 284 nm. Then, the detector wavelength was set at 195 nm keeping other conditions same as previous and three peaks were found in the chromatogram (shown in Figure 2a). Peaks were identified by injecting separate standard samples. Energy associated with lower wavelength at 195 nm is able to excite electrons for all the compounds. In an another report, formation of 5-HMF from chitosan was analyzed using VWD as a detector with fixed wavelength at 283 nm and hence compounds which are not UV active at this wavelength can be missed (Kalane et al., 2019). Recently, formation of $GlcNH_2$ (calorimetric) and 5-HMF (HPLC) from chitosan using two separate methods is reported, which eventually increases the time required for the analysis (Pandit et al., 2020). In the work, VWD wavelength was fixed at 283 nm, which would hamper detection of $GlcNH_2$, if formed in the reaction. The colorimetric method is also a specific method to quantify total reducing sugars, which cannot quantify 5-HMF. Thus, it is essential to use the correct wavelength to detect all the compounds in the sample.

Separation in non-polar C18 column

Selection of mobile phase. Previously researchers have used methanol/water systems for the analysis of reaction mixtures and hence they required to keep the VWD above 205 nm wavelength since methanol has a UV cut-off at 205 nm (Li et al., 2015; Pandit et al., 2020; Yu et al., 2016). From the literature, it was found that every solvent has their specific UV cut-off. UV cut-off value of methanol is 205 nm (Corradini, 2016). If measurement wavelength is less than this cut-off value, then mobile phase also has some contribution in absorbance (Corradini, 2016). As detector wavelength must be kept at 195 nm to detect all the compounds, methanol has to be replaced by another solvent which has less UV cut off than 195 nm. Acetonitrile was found to have less UV cut off, i.e., <190 nm. Then acetonitrile/water-2.3/7.7 *v/v* was used as mobile phase keeping column and VWD wavelength same as section 3.2.1. The chromatogram (Figure 2b) shows clear separation for all the compounds in acetonitrile/water- 2.3/7.7 *v/v*.

Effect of acetonitrile and water ratio. Acetonitrile/water (2.3/7.7 *v/v*) mobile phase system shows less difference in retention time of GlcNAc and 5-HMF. Polarity of the mobile phase system was then altered by addition of more polar solvents, i.e., water. The ratio of acetonitrile/water was varied from 1/1 *v/v* to 1/9 *v/v* and the separation profiles are shown in Figures 3a-3c. It was observed that as the polarity of the mobile phase increased by the addition of water, separation of the compounds is better. As 5-HMF is least polar in nature, 5-HMF showed strong interaction with non-polar stationary phase. In the case of least polar solvent system, i.e., acetonitrile/water (1/1 *v/v*), 5-HMF eluted fast. Because of this, peaks of GlcNAc and 5-HMF were observed to be merging with each other. Acetonitrile/water (1/9 *v/v*) mobile phase system shows best separation of three compounds.

Analysis conditions: Column: C18 (250 mm x 4.6 mm i.d., 5.0 um, Agilent Zorbax), column temperature: 25°C, Flow rate: 0.5 mL/min, VWD wavelength: 195 nm, Mobile phase: (a) acetonitrile/water: 1/1 *v/v*, (b) acetonitrile/water: 3/7 *v/v*, (c) acetonitrile/water: 1/9 *v/v*

Effect of column length

To reduce the analysis time, separation of the compounds was checked in the shorter column. C18 column of 100 mm with 4.6 mm i.d, 5 um particle size was used for the purpose. But with the decrease of column length, compounds had shorter time to interact with stationary phase and that resulted in the merging of peaks of $GlcNH_2$ and GlcNAc (Figure S4, ESI). These results imply that for a better separation, longer columns are better.

Separation of other sugars, N-containing chemicals, furan derivatives in C18 column

Detection and separation of glucose and fructose were also checked using optimized HPLC condition, i.e., C18 column (250 mm x 4.6 mm i.d., 5.0 μm, Agilent Zorbax), Mobile phase: acetonitrile/water-1/9 *v/v*, column temperature: 25°C, Flow rate: 0.5 mL/min, VWD wavelength: 195 nm. There is very small difference in the retention times of glucose, fructose, GlcNAc and acetic acid (Figure S5, ESI). Peaks of glucosaminic acid, $GlcNH_2$ and glucosamine hydrochloride dimer are also merging with each other under this analytical condition. The difference in retention time of N-acetylethanolamine and 2-deoxy-D-glucose is also very less (Figure S5, ESI). So, it is very obvious that this method will be unable to separate these compounds.

Separation in polar amino column

It was thought that polar stationary phase can give better separation as polar compounds will have strong interactions with polar stationary phase. The Amino column was also used in previous reports to analyse amino sugar, $GlcNH_2$ and N-containing acid (Dai, Gözaydın, Hu, & Yan, 2019). In view of this, polar amino column was used for potential separation of the investigating compounds. The chromatogram (Figure 4a) shows that polar compounds have very strong interaction with stationary phase of the column, which leads to peak tailing. Nevertheless, these compounds retained on the stationary phase strongly that peak tailing was observed even when the flow rate was increased from 0.5 mL/min to 1.0 mL/min (Figure 4b) with an increment of 0.1 mL/min. Further increase in flow rate would merge the peaks with each other as with 1.0 mL/min flow too peaks were not resolved completely (as observed from the baseline) and hence, amino column is not a good choice for separating amino sugars.

Analysis conditions: Column: Amino (250 mm x 4.6 mm i.d, 5.0 um, Nucleosil, Macherey-Nagel), Mobile phase: acetonitrile/water: 8/2 *v/v*, column temperature: 25°C, VWD wavelength: 195 nm, Flow rate: 0.5 mL/min (a), Flow rate: 1.0 mL/min (b)

Separation of other sugars and chitin/chitosan derived various products were checked, which is shown in Figure S6 (ESI). It was found that peak of Glucosaminic acid, Acetamide and 5-HMF is merging with each other under employed conditions. Peak of fructose and GlcNAc were also merged with each other.

In view of peak merging and peak tailing in amino column, it is considered that this column is not suitable for the separation of these compounds.

Separation in mid-polar ion-exchange column

Ion-exchange column, monosaccharide Pb^{2+} column has mid-polarity as the matrix of the column is a sulfonated styrene divinyl benzene polymer. It is a cation exchange column with Pb^{2+} ions. Typically for separation of compounds on this column, water is used as mobile phase and in our study, 0.5 mL/min flow was observed as best flow to achieve better separation. In this work, column temperature was kept at 80°C (which is also recommended by manufacturers as optimum for separation of various sugar compounds) to minimize the viscosity of the mobile phase and column pressure. Mixture of $GlcNH_2$, GlcNAc and 5-HMF was injected and good separation was observed, as seen in Figure 5.

Analysis condition: Column: RPM-Monosaccharide Pb^{2+} (300 mm x 7.8 mm i.d, 8.0 um, 8 % cross linking, Rezex™ Phenomenex), Mobile phase: water, column temperature: 80°C, Flow rate: 0.5 mL/min, VWD wavelength: 195 nm

With the use of this column, better separation of $GlcNH_2$, GlcNAC and 5-HMF was observed in comparison to other columns used. Also,

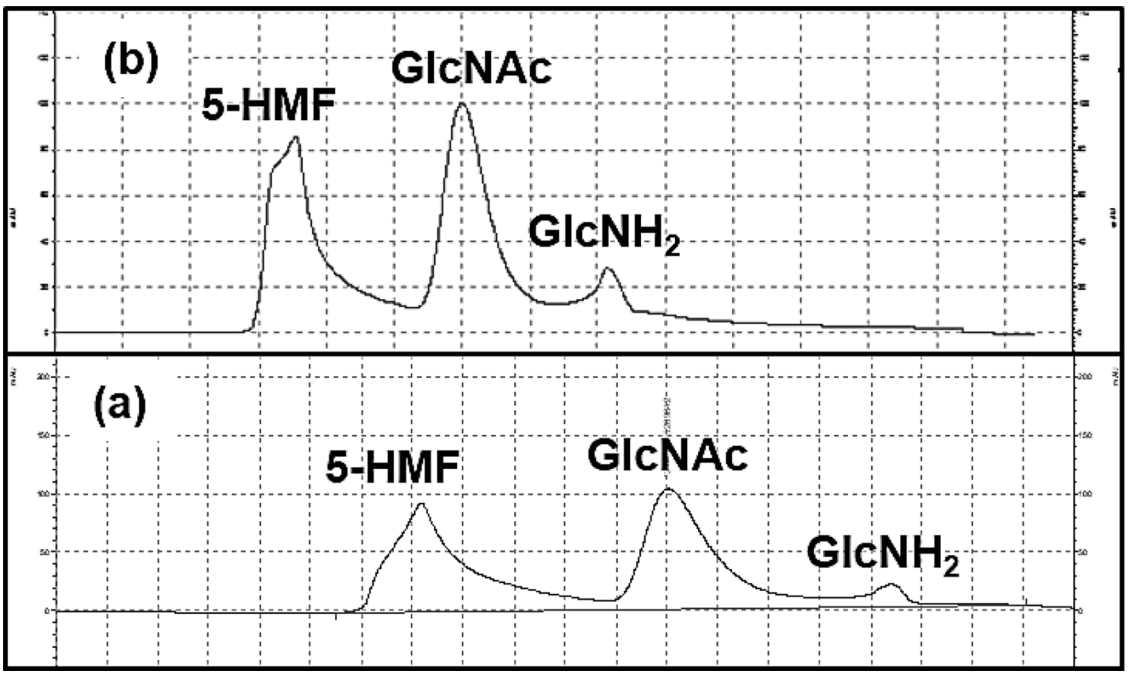

Fig. 4. Chromatogram for mixture ($GlcNH_2$+ GlcNAc+5-HMF) in amino column.

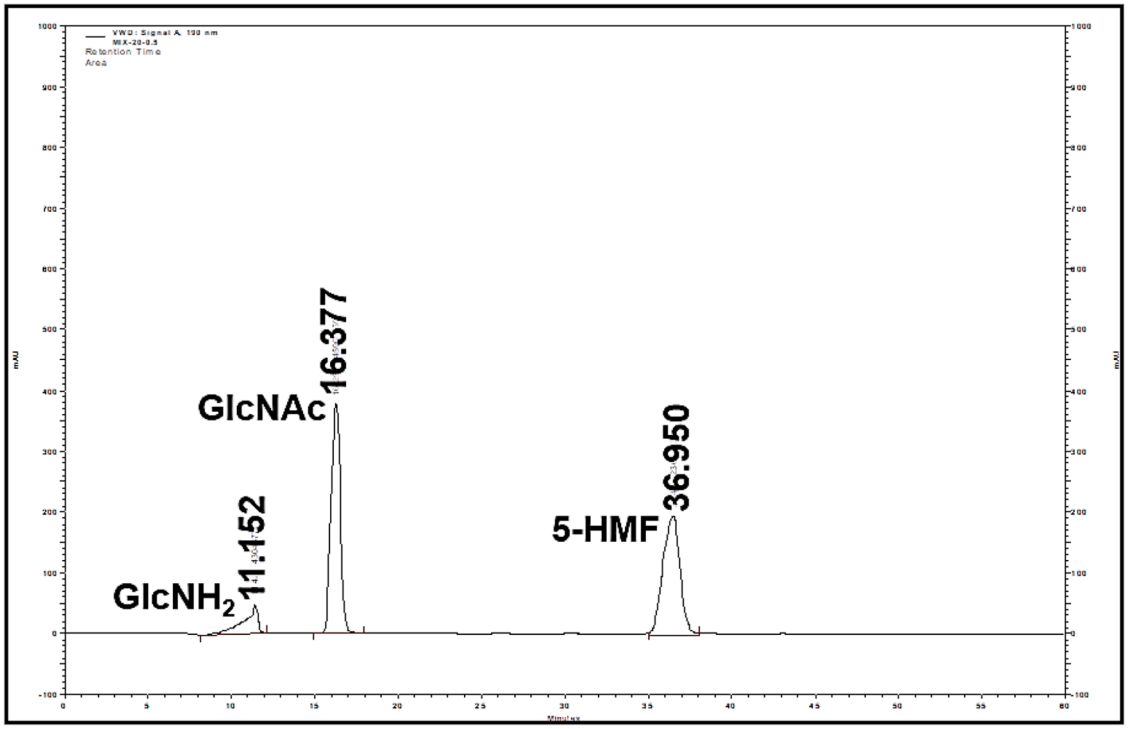

Fig. 5. Chromatogram for mixture ($GlcNH_2$+ GlcNAc+5-HMF) in Pb^{2+} column.

except for $GlcNH_2$, sharp peaks were observed for 5-HMF and GlcNAc. Subsequent to this observation, separation of other sugars, N-containing chemicals and furan derivatives were checked and clear separation of all the compounds was seen with the analytical conditions employed (Figure 6). Since the packing material in this column has mid-polarity, it has played a key role in optimized interaction of different compounds with the phase, which in turn has yielded clear separation of the chemicals.

Analysis condition: Column: RPM-Monosaccharide Pb^{2+} (300 mm x 7.8 mm i.d, 8.0 um, 8 % cross linking, Rezex^{TM} Phenomenex), mobile phase: water, column temperature: 80°C, flow rate: 0.5 mL/min, VWD wavelength: 195 nm

As seen from Figure 6, within 39 min run time, separation of all compounds can be completed and quantification can be done by using calibration curve. Nevertheless, it is seen that few of the peaks can overlap in actual analysis, but this is the best possible separation achievable using this column and also best with any other system. In order to reduce the time of analysis, flow rate of mobile phase was

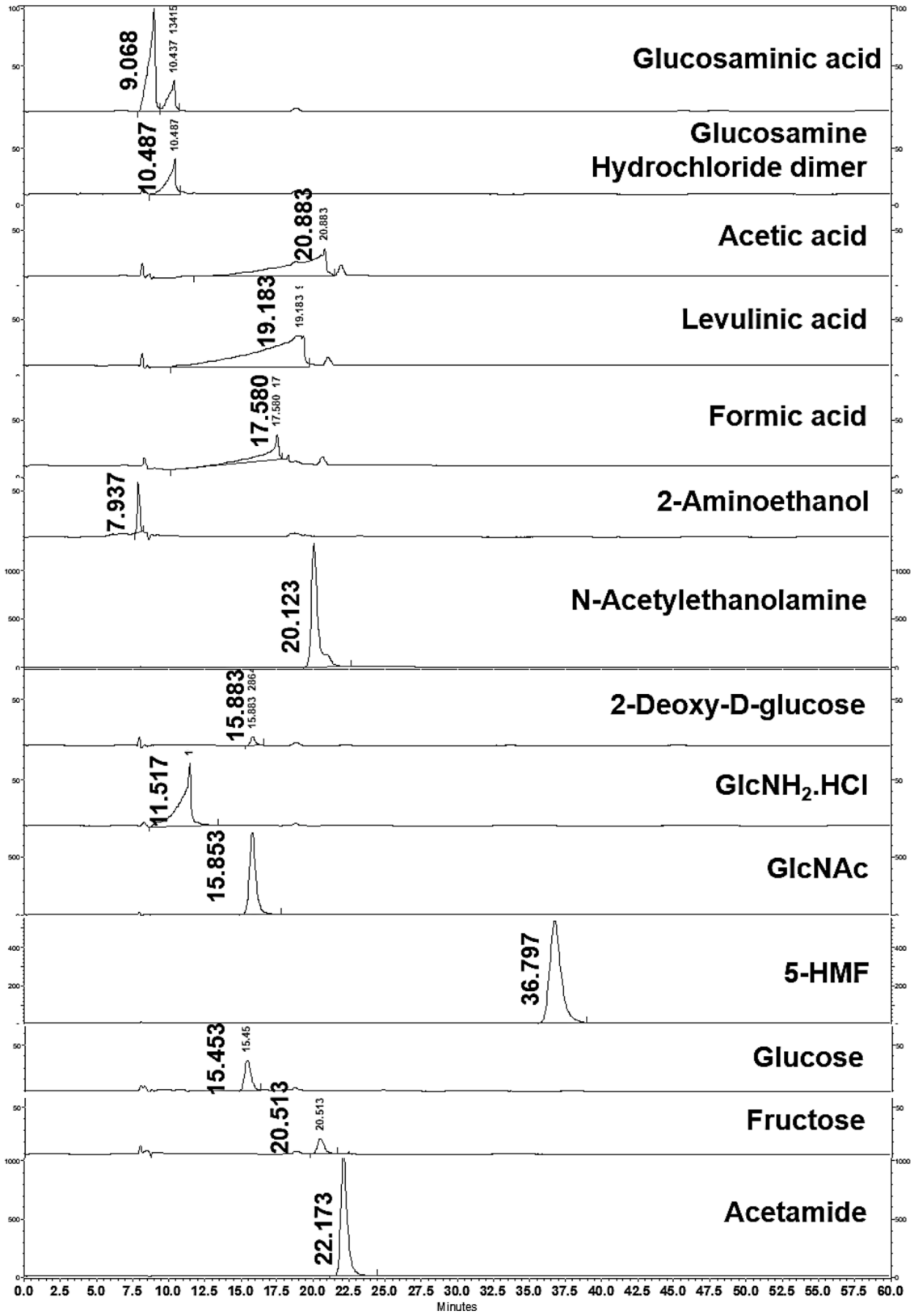

Fig. 6. Chromatograms for Amino sugars, other sugars, N-containing chemicals and furan derivatives in Pb^{2+} column.

increased from 0.5 mL/min to 0.7 mL/min. The analysis could be completed within 30 min with sharp peaks for the compounds if the flow rate is kept at 0.7 mL/min (Figure S7, ESI). Although this result is good but with increase in flow, separation of other compounds can hamper as some peaks may overlap. Higher flow rate also creates high column pressure, which is detrimental for this column as maximum pressure for this column is 1000 psi.

Method validation

The reliability of the method in terms of precision was evaluated by injecting four standards of each compound. All calibration curves showed good linear regression. R^2 values and regression equation is tabulated in Table S7 (ESI). R^2 values are in the range of 0.9904-0.9982 for both the detectors. This observation proves that the method is precise and reliable.

Day to day variations of retention times were determined on three days using same standard sample mixture and it was observed that results are satisfactory (Table S8, ESI). Therefore, the developed HPLC method is precise, accurate and enough sensitive for simultaneous separation and quantification of $GlcNH_2$, GlcNAc, 5-HMF and other compounds.

The repeatability of the optimized method is evaluated by analysing same sample ($GlcNH_2$: 1190 mg/L, GlcNAc: 920 mg/L and 5-HMF: 700 mg/L) for four times. Areas under the peak in the four repeated injections fall in a same range which proves that the results are reproducible. %RSD is also calculated and tabulated in Table S9 (ESI). Low value of %RSD also proves that the method is able to quantify precisely.

Data for another detector, i.e., RID, was also checked and same trend was observed like with VWD (Tables S7-S9, ESI).

Lead leaching

According to organometallic chemistry, lead has a tendency to form chelate complexes with nitrogen containing ligands and thus, it becomes essential to check whether it is possible to use Pb^{2+} column for this analysis. To check the leaching of lead, Mixture of $GlcNH_2$ and GlcNAc was injected 72 times on the column. Elution of these two compounds was completed within 20 min. At the end of the analysis, HPLC waste was collected, and ICP-OES was done to check the presence of lead. This experiment is done for two mixture samples.

- Dilute sample (0.031 *wt*% of amino sugars)
- Concentrated sample (0.30 *wt*% of amino sugars)

For dilute sample amount of lead was not detected, but the concentrated sample showed 0.1152 ppm leaching of lead at the end of 72 samples. This indicates that although amino groups can bind with Pb^{2+}, no much change of Pb^{2+} concentration was seen on the column and that the reproducibility of the results was achieved.

Conclusion

To assist researchers working in the area of chitin and chitosan chemistry, it was necessary to develop a HPLC methodology for the separation of compounds. From the study, it was observed that Non-polar C18 and mid-polar cation exchange Pb^{2+} columns are best suitable for separation of $GlcNH_2$, GlcNAc, other N-containing chemicals and furan derivatives. Non-polar C18 column, although it showed better separation than NH_2 column but was unable to separate other sugars like glucose, fructose etc., from each other and other chemicals injected. On the other hand, Pb^{2+} column could separate most of the compounds from each other because it possesses optimum polarity to allow rapid adsorption-desorption of the compounds. This developed HPLC method was demonstrated to be suitable to separate and quantify $GlcNH_2$, GlcNAc and 5-HMF simultaneously within 39 min. The regression equation showed very good linear relationship with $R^2 = 0.9904\text{-}0.9974$. Therefore, the developed HPLC method is simple, rapid, accurate, precise, and specific and has the ability to separate and quantify amino sugars ($GlcNH_2$, GlcNAc) and 5-HMF simultaneously.

Acknowledgement

Authors thank CSIR-NCL for research facilities and NG thank DST-Inspire for research fellowship.

Funding

Funding support was provided by Council of Scientific and Industrial Research (CSIR) and doctoral fellowship of Neha Ghosh was given by Innovation of Science Pursuit for Inspire Research (INSPIRE) by Department of Science and Technology (DST), Govt. of India.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.carpta.2021.100139.

References

Chen, X., Chew, S. L., Kerton, F. M., & Yan, N. (2014). Direct conversion of chitin into a N-containing furan derivative. *Green Chemistry, 16*(4), 2204–2212.
Chen, X., Yang, H., & Yan, N. (2016). Shell biorefinery: dream or reality? *Chemistry–A European Journal, 22*(38), 13402–13421.
Corradini, D. (2016). *Handbook of HPLC*. CRC Press.
Dai, J., Gözaydın, G.k., Hu, C., & Yan, N. (2019). Catalytic conversion of chitosan to glucosaminic acid by tandem hydrolysis and oxidation. *ACS Sustainable Chemistry & Engineering, 7*(14), 12399–12407.
El Kadib, A. (2015). Chitosan as a sustainable organocatalyst: a concise overview. *ChemSusChem, 8*(2), 217–244.
Elwakeel, K. Z. (2010). Environmental application of chitosan resins for the treatment of water and wastewater: a review. *Journal of dispersion science and technology, 31*(3), 273–288.
Erb, C., & Zerban, F. (1938). Determination of total reducing sugars and of dextrose and levulose in cane molasses. *Industrial & Engineering Chemistry Analytical Edition, 10*(5), 246–250.
Garriga, M., Almaraz, M., & Marchiaro, A. (2017). Actas de Ingeniería. *Actas de ingeniería, 3*, 173–179.
Jardine, A., & Sayed, S. (2016). Challenges in the valorisation of chitinous biomass within the biorefinery concept. *Current Opinion in Green and Sustainable Chemistry, 2*, 34–39.
Kalane, N. D., Krishnan, R. A., Yadav, V. D., Jain, R., & Dandekar, P. (2019). Synergistic effect of hetero-and homo-catalysts on the 'green'synthesis of 5-hydroxymethylfurfural from chitosan biomass. *Cellulose, 26*(4), 2805–2819.
Katiyar, D., Hemantaranjan, A., & Singh, B. (2015). Chitosan as a promising natural compound to enhance potential physiological responses in plant: a review. *Indian Journal of Plant Physiology, 20*(1), 1–9.
Kim, S.-K. (2010). *Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications*. CRC Press.
Li, M., Zang, H., Feng, J., Yan, Q., Yu, N., Shi, X., & Cheng, B. (2015). Efficient conversion of chitosan into 5-hydroxymethylfurfural via hydrothermal synthesis in ionic liquids aqueous solution. *Polymer Degradation and Stability, 121*, 331–339.
Ma, X., Gözaydın, G., Yang, H., Ning, W., Han, X., Poon, N. Y., & Zhou, K. (2020). Upcycling chitin-containing waste into organonitrogen chemicals via an integrated process. *Proceedings of the National Academy of Sciences, 117*(14), 7719–7728.
Matsagar, B., Munshi, M., Kelkar, A., & Dhepe, P. (2015). Conversion of concentrated sugar solutions into 5-hydroxymethyl furfural and furfural using Brönsted acidic ionic liquids. *Catalysis Science & Technology, 5*(12), 5086–5090.
Nivethaa, E., Martin, C. A., Frank-Kamenetskaya, O. V., & Kalkura, S. N. (2020). Chitosan and chitosan based nanocomposites for applications as a drug delivery carrier: A review. *Processes and Phenomena on the Boundary Between Biogenic and Abiogenic Nature* (pp. 23–37).
Pandit, A., Deshpande, C., Patil, S., Jain, R., & Dandekar, P. (2020). *Mechanistic insights into controlled depolymerization of Chitosan using H-Mordenite. Carbohydrate polymers, 230*, Article 115600.
Pierson, Y., Chen, X., Bobbink, F. D., Zhang, J., & Yan, N. (2014). Acid-catalyzed chitin liquefaction in ethylene glycol. *ACS Sustainable Chemistry & Engineering, 2*(8), 2081–2089.
Tzeng, T.-W., Bhaumik, P., & Chung, P.-W. (2019). Understanding the production of 5-hydroxymethylfurfural (HMF) from chitosan using solid acids. *Molecular Catalysis, 479*, Article 110627.
Wang, H., & Frenklach, M. (1994). Transport properties of polycyclic aromatic hydrocarbons for flame modeling. *Combustion and flame, 96*(1-2), 163–170.
Yan, N., & Chen, X. (2015). Sustainability: Don't waste seafood waste. *Nature News, 524*(7564), 155.
Yu, S., Zang, H., Chen, S., Jiang, Y., Yan, B., & Cheng, B. (2016). Efficient conversion of chitin biomass into 5-hydroxymethylfurfural over metal salts catalysts in dimethyl sulfoxide-water mixture under hydrothermal conditions. *Polymer Degradation and Stability, 134*, 105–114.
Zhang, J., & Yan, N. (2017). Production of Glucosamine from Chitin by Co-solvent Promoted Hydrolysis and Deacetylation. *ChemCatChem, 9*(14), 2790–2796.