



Faculty of Resource Science and Technology

**Direct Chitosan Extraction Using Natural Deep Eutectic Solvent for
Controlled Release Fertilizer Fabrication**

Mohd Hazwan Bin Hamdi

**Master of Science
2025**

Direct Chitosan Extraction Using Natural Deep Eutectic Solvent for Controlled Release Fertilizer Fabrication

Mohd Hazwan Bin Hamdi

A thesis submitted

In fulfillment of the requirements for the degree of Master of Science

(Chemistry)

Faculty of Resource Science and Technology

UNIVERSITI MALAYSIA SARAWAK

2025

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



Signature

Name: Mohd Hazwan Bin Hamdi

Matric No.: 20020415

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

Date : September 11, 2025

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my supervisor, ChM. Dr. Melody Anak Kimi, for her invaluable guidance, support, and encouragement throughout the course of my research. Her expertise and dedication have been instrumental in the successful completion of this thesis.

I am profoundly grateful to my family for their unwavering support, both financially and emotionally. Their belief in my abilities and their constant encouragement provided me with the strength to persevere through the challenges of this journey.

Besides, I would like to appreciate the financial support provided for this research project from the Ministry of Higher Education Malaysia through the Fundamental Research Grant Scheme (FRGS) with grant number of FRGS/1/2020/STG04/UNIMAS/02/1. This research was also funded by Universiti Malaysia Sarawak (UNIMAS).

Last but not least, I also wish to extend my heartfelt thanks to my friends and colleagues from the Faculty of Resource Science and Technology and the Centre of Pre-University Studies of Universiti Malaysia Sarawak (UNIMAS). Their camaraderie, insights, and assistance have enriched my experience and contributed significantly to the completion of this work.

Thank you all for your support and believe in me.

ABSTRACT

Chitosan is a biopolymer commonly found in hard exoskeleton shell animal such as shrimp, snail shell, and crab. Conventional extraction of chitosan biopolymers from natural sources typically involves the use of hazardous chemicals and lengthy processing times. This study presents a sustainable approach for the direct extraction of chitosan from snail shells using a natural deep eutectic solvent (NADES) system. The NADES was formed by combining acetogenin extracted from graviola (hydrogen bond donor) with choline chloride (hydrogen bond acceptor) at various molar ratios and synthesis temperatures. The NADES was characterized for polarity, density, surface tension and DSC, while chitosan extracted was analysed for moisture and ash content, TGA, FTIR-ATR, contact angle, viscosity, XRD, and SEM. The chitosan produced through this method exhibited degree of deacetylation in range of 87.6 - 93.3% and molecular weight in range of 40 - 115 kDa, with a fiber-like morphology. This extracted chitosan is comparable properties to commercial chitosan. The extracted chitosan was further applied as co-polymer for encapsulation of urea with alginate and calcium chloride as crosslinker. Response surface methodology (RSM) was applied to determine the optimal variables (molecular weight of chitosan, amount of alginate, and crosslinker) for achieving optimum encapsulation efficiency (EE%) and sustained release of urea (UR%) fertilizer from chitosan-alginate hydrogel beads. Results shows that the ideal formulation for attaining high EE% and UR% is using low molecular weight chitosan (40 kDa), a moderate quantity of alginate (3.5 wt%), and the maximum level of cross-linker (1.00 M).

Keywords: Alginate, chitosan, NADES, RSM, urea

***Ekstraksi Langsung Kitosan Menggunakan Pelarut Eutekti Semula Jadi untuk
Fabrikasi Baja Lepas Terkawal***

ABSTRAK

Kitosan ialah biopolimer yang lazimnya ditemui dalam kulit eksoskeleton keras haiwan seperti udang, siput, dan ketam. Kaedah pengekstrakan konvensional bagi biopolimer kitosan daripada sumber semula jadi biasanya melibatkan penggunaan bahan kimia berbahaya dan tempoh pemprosesan yang panjang. Kajian ini membentangkan pendekatan mampan untuk pengekstrakan langsung kitosan daripada kulit siput menggunakan sistem pelarut eutektik dalam semula jadi (NADES). NADES ini dibentuk melalui gabungan asetogenin yang diekstrak daripada graviola (penderma ikatan hidrogen) dengan kolina klorida (penerima ikatan hidrogen) pada pelbagai nisbah molar dan suhu sintesis. NADES yang terhasil telah dicirikan dari segi keterkutuban, ketumpatan, tegangan permukaan dan analisis DSC, manakala kitosan yang diekstrak dianalisis dari segi kandungan lembapan dan abu, TGA, FTIR-ATR, sudut sentuhan, kelikatan, XRD, dan SEM. Kitosan yang dihasilkan melalui kaedah ini menunjukkan tahap deasetilasi dalam julat 87.6 - 93.3% dan berat molekul dalam julat 40 - 115 kDa, dengan morfologi berbentuk gentian. Ciri-ciri kitosan yang diekstrak ini adalah setanding dengan kitosan komersial. Kitosan yang diekstrak kemudiannya digunakan sebagai kopolimer untuk pengekapsulan urea dengan alginat dan kalsium klorida sebagai agen penghubung silang. Kaedah gerak balas permukaan (RSM) telah digunakan untuk menentukan pemboleh ubah optimum (berat molekul kitosan, jumlah alginat, dan agen penghubung silang) bagi mencapai kecekapan pengekapsulan (EE%) dan pelepasan urea (UR%) baja secara berterusan daripada bebola hidrogel kitosan-alginat. Hasil kajian menunjukkan bahawa formulasi ideal untuk mencapai EE% dan UR% yang tinggi adalah dengan menggunakan kitosan berat molekul rendah (40

kDa), jumlah alginat yang sederhana (3.5 wt%), dan tahap maksimum agen penghubung silang (1.00 M).

Kata kunci: *Alginat, kitosan, NADES, RSM, urea*

TABLE OF CONTENTS

	Page
DECLARATION	i
ACKNOWLEDGEMENT	ii
ABSTRACT	iii
<i>ABSTRAK</i>	iv
TABLE OF CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	xii
LIST OF ABBREVIATIONS	xiii
CHAPTER 1: INTRODUCTION	1
1.1 Background of Study	1
1.2 Problem Statement	4
1.3 Objectives	6
CHAPTER 2: LITERATURE REVIEW	7
2.1 Food Security and Agriculture	7
2.2 Efficiency of Conventional Nitrogen Fertilizer	9
2.3 Coated Fertilizer	11
2.3.1 Chitosan-Coated Fertilizer	13

2.3.2	Sources of Chitosan	14
2.4	Chitosan Extraction Method	16
2.4.1	Conventional Extraction of Chitosan	16
2.4.2	Natural Deep Eutectic Solvent (NADES)	19
2.5	Chitosan-Copolymer Composites	26
2.5.1	Alginate Copolymer	27
2.5.2	Crosslinkers	29
2.6	Response Surface Methodology (RSM)	33
2.7	Factors Influencing Swelling Behaviour, Encapsulation Efficiency, and Urea Release	35
	CHAPTER 3: METHODOLOGY	38
3.1	Synthesis of Natural Deep Eutectic Solvent (NADES)	38
3.2	Chitosan Extraction using NADES	38
3.3	Characterization of NADES	39
3.3.1	Polarity Measurements	39
3.3.2	Density Measurements	39
3.3.3	Surface Tension Measurements	40
3.3.4	Differential Scanning Calorimetry (DSC) Measurements	40
3.4	Characterization of Extracted Chitosan	41
3.4.1	Chemical Composition Analysis of Chitosan	41

3.4.2	Thermal Gravimetric (TGA) Measurements	41
3.4.3	Fourier Transform Infrared-Attenuated Total Reflection (FTIR-ATR)	41
3.4.4	Contact Angle Measurements	42
3.4.5	Viscosity Measurements	42
3.4.6	X-Ray Powder Diffarctometer (XRD)	43
3.4.7	Scanning Electron Microscopy (SEM)	43
3.5	Synthesis of Chitosan-Alginate Hydrogel Beads with Urea Entrapment	44
3.6	Release Behaviour of Urea From Hydrogel Beads in Water	47
3.6.1	Swelling Study	47
3.6.2	Encapsulation Efficiency	47
3.6.3	Urea Release Evaluation	48
3.7	Response Surface Methodology (RSM) Analysis	49
3.8	Flowchart of Methodology	50
CHAPTER 4: RESULTS AND DISCUSSION		51
4.1	Physicochemical Properties of NADES	51
4.2	Physicochemical Properties of Extracted Chitosan by NADES	60
4.3	Physicochemical Properties of Chitosan-Alginate Hydrogel Beads	73
4.3.1	Hydrogel Particle Size Measurement	74
4.3.2	Fourier Transform Infrared-Attenuated Total Reflection (FTIR-ATR)	75
4.3.3	Scanning Electron Microscope (SEM)	77

4.4	Response Surface Methodology (RSM) analysis	79
4.4.1	Swelling Properties of Hydrogel Beads	79
4.4.2	Encapsulation Efficiency of Hydrogel Bead	87
4.4.3	Urea Release Evaluation	97
4.4.4	Summary of Response Surface Methodology	105
	CHAPTER 5: CONCLUSION AND RECOMMENDATIONS	107
5.1	Conclusion	107
5.2	Recommendations	108
	REFERENCES	109
	APPENDICES	132

LIST OF TABLES

	Page
Table 2.1 Comparison of Conventional Methods of Chitosan Extraction	17
Table 2.2 Chitin Extraction by NADES	21
Table 3.1 Experimental design matrix and dependent variables attributed to the factors used for Box-Behnken Design BBD	45
Table 4.1 Physicochemical properties of NADES	57
Table 4.2 Physicochemical properties of extracted chitosan from different NADES ratio	63
Table 4.3 ANOVA results for the quadratic model of response surface for swelling	84
Table 4.4 Experimental design matrix with response observed (Y) and predicted values (Predicted)	89
Table 4.5 ANOVA results for the quadratic model of response surface for encapsulation efficiency	93
Table 4.6 ANOVA results for the quadratic model of response surface for urea release	100

LIST OF FIGURES

	Page
Figure 1.1 (a) Deacetylation of chitin into (b) chitosan	2
Figure 2.1 Weight of nutrient release from two types of fertilizer	13
Figure 2.2 Role of choline chloride (HBA) and acetogenin (HBD) in the extraction of chitin and chitosan	22
Figure 3.1 Flowchart of methodology	50
Figure 4.1 Synthesized NADES	51
Figure 4.2 Differential scanning calorimetry (DSC) thermograms of (a) choline chloride (HBA) (b) acetogenin (HBD) (c) NADES	58
Figure 4.3 FTIR spectrum of (a) NADES (b) choline chloride (HBA) (c) acetogenin (HBD)	59
Figure 4.4 TGA curve of chitosan extracted by 2:1 80 °C NADES	65
Figure 4.5 FTIR spectra of (a) extracted chitosan from 2:1 80 °C NADES, (b) commercial chitosan and (c) snail shells	68
Figure 4.6 XRD pattern of chitosan extracted by 2:1 80 °C NADES	70
Figure 4.7 SEM micrograph of (a) commercial chitosan and (b) extracted chitosan from 2:1 80 °C NADES	71
Figure 4.8 FTIR spectra of (a) extracted chitosan (b) commercial alginate (c) urea and (d) hydrogel bead from Run 7	75
Figure 4.9 Hydrogel beads (a) air dried condition (b) oven dried condition (c) swelling condition and (d) SEM image of dry bead	78
Figure 4.10 Swelling (%) of each run of the hydrogel beads	80
Figure 4.11 Interaction plot between (a) chitosan (X1) and alginate(X2) for swelling response (b) chitosan (X1) and crosslinker(X3) for swelling response	82
Figure 4.12 (a) Surface plot of concurrent effects of chitosan(X1) and alginate(X2) on the swelling response (b) Contour plot of concurrent effects of chitosan(X1) and alginate(X2) on the swelling response	83
Figure 4.13 (a) Surface plot of concurrent effects of chitosan(X1) and crosslinker(X3) on the swelling response (b) Contour plot of	

	concurrent effects of chitosan(X1) and crosslinker(X3) on the swelling response	83
Figure 4.14	(a) Predicted values for swelling vs observed values (b) Residual plot of predicted swelling (c) Normality of residual for swelling experiments	85
Figure 4.15	Encapsulation efficiency (%) of each run of the hydrogel beads	87
Figure 4.16	Interaction plot between (a) chitosan (X1) and alginate(X2) for encapsulation efficiency response and (b) alginate(X2) and crosslinker(X3) for encapsulation efficiency response	91
Figure 4.17	(a) Predicted values for encapsulation efficiency vs observed values (b) Residual plot of predicted encapsulation efficiency (c) Normality of residual for encapsulation efficiency	94
Figure 4.18	(a) Surface plot of concurrent effects of chitosan(X1) and alginate(X2) on the encapsulation efficiency response (b) Contour plot concurrent effects of chitosan(X1) and alginate(X2) on the encapsulation efficiency response	96
Figure 4.19	Urea release (%) of each run of the hydrogel beads	97
Figure 4.20	Interaction plot between (a) chitosan (X1) and alginate(X2) for urea release response (b) chitosan (X1) and crosslinker(X3) for urea release response	98
Figure 4.21	Predicted values for (a) urea release vs observed values (b) Residual plot of predicted urea release (c) Normality of residual for urea release experiment	102
Figure 4.22	(a) Surface plot of concurrent effects of chitosan(X1) and alginate(X2) on the urea release response (b) Contour plot of concurrent effects of chitosan(X1) and alginate(X2) on the urea release response	104

LIST OF ABBREVIATIONS

BBD	Box-Behnken Design
CaCl ₂	Calcium Chloride
DDA	Degree of Deacetylation
DSC	Differential Scanning Calorimetry
FESEM	Field Emission Scanning Electron Microscopy
FTIR-ATR	Fourier Transform Infrared-Attenuated Total Reflectance
HCl	Hydrochloric Acid
Mw	Molecular Weight
NADES	Natural Deep Eutectic Solvent
NaOH	Sodium Hydroxide
RSM	Response Surface Methodology
TGA	Thermogravimetric Analysis
UV-Vis	Ultraviolet-Visible Spectroscopy
XRD	X-Ray Diffraction

CHAPTER 1

INTRODUCTION

1.1 Background of Study

In the agricultural sector, chitosan holds particular significance owing to its active functional groups, namely hydroxyl and amine groups, which facilitate interactions with water-soluble fertilizers, enabling the formation of coatings for controlled nutrient release. Moreover, the chemical and physical properties of chitosan can be easily modified via chemical methods, further enhancing its utility in agriculture (Islam et al., 2017). Notably, chitosan's distinct characteristics, including water insolubility, cationic biopolymer behaviour, positive charge at biological pH, and propensity for gel formation, contribute to its versatility as an industrial material (Santos et al., 2020).

In the context of ensuring food security, enhancing agricultural yields while minimizing nutrient loss from fertilizers is imperative. Conventional methods of direct fertilizer application are susceptible to leaching and volatilization losses, underscoring the need for controlled-release fertilizers (CRF). CRF has garnered considerable attention for its potential to improve nutrient efficiency and mitigate environmental pollution. By precisely regulating nutrient release, CRF offers a strategic approach to maximize crop yields while minimizing environmental impact (Azeem et al., 2014). Given the varying nutrient requirements of different plant species, the application of CRF becomes paramount in optimizing agricultural productivity. Furthermore, mitigating fertilizer losses to the environment is crucial for preventing environmental contamination and economic losses associated with ineffective nutrient utilization (Tang et al., 2018).

Chitosan presents a promising avenue for developing controlled-release fertilizers (CRF) with enhanced sustainability and efficacy. By encapsulating nutrients within chitosan-based coatings, the controlled-release mechanism can be finely tuned to match plant nutrient uptake requirements, thereby minimizing nutrient leaching and enhancing fertilizer efficiency (Akhter et al., 2021). Chitosan, a polysaccharide derived from the deacetylation of chitin primarily sourced from marine shells, has garnered significant interest across various industries due to its unique chemical properties and versatile applications. As illustrated in Figure 1.1, chitosan exhibits increased chemical reactivity compared to chitin owing to the presence of primary and secondary hydroxyl groups as well as an amine group on each deacetylated unit (Islam et al., 2017). This enhanced chemical activity has positioned chitosan as a valuable polymer in industries ranging from agriculture and medicine to pharmaceuticals, cosmetics, food and beverage, and textiles.

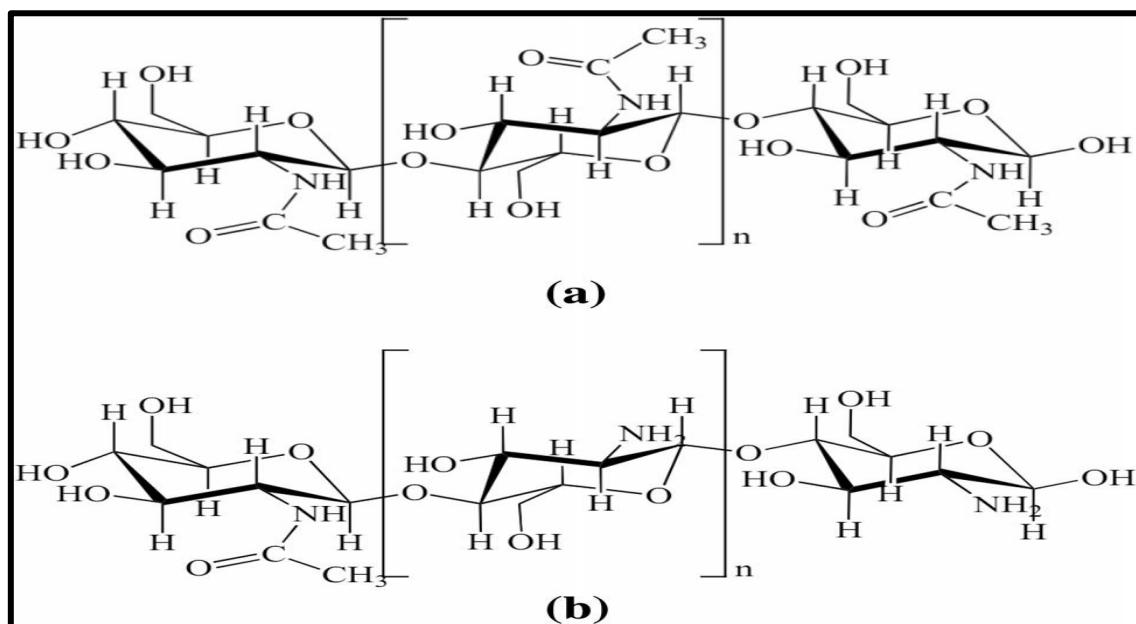


Figure 1.1: (a) Deacetylation of chitin into (b) chitosan (Islam et al., 2017)

Most implemented extraction method of chitin and chitosan are using acid and base involving three main processes which includes demineralization, deproteinization, and deacetylation of chitin to chitosan. Chitin extraction which relied on harsh chemical at elevated temperatures, results in negative environmental impacts and polymer degradation (Yadav et al., 2019). Alternatively, natural deep eutectic solvents (NADES), a subtype of deep eutectic solvents composed of metabolites from primary components, present a promising alternative for chitin extraction. However, chitosan extraction by NADES have not been explored.

NADES exhibit eutectic properties, characterized by lower melting points compared to individual components, making them suitable for demineralizing, deproteinizing, and deacetylating marine shells for chitosan extraction. The extraction mechanism primarily hinges on the strength of hydrogen bond interactions between NADES components and the shell polymer matrix (Zhu et al., 2017). NADES are known for its tunable properties where it can be tailored to improve extraction efficiency, ecofriendly and sustainable, versatile applications in different industry, enhanced extraction efficiency replacing traditional organic solvent, low-volatility and non-flammable. Besides, NADES also comes with its drawbacks in which its high viscosity causes extraction process difficult, challenges in water content and scalability might affect the application efficiency, and variations of components used which varies their performance in different applications.

1.2 Problem Statement

Food waste constitutes a significant portion of daily waste production, necessitating sustainable solutions for its management. Concurrently, shell waste remains largely underutilized, posing environmental challenges due to its disposal in landfills. In light of sustainable production and consumption goals, repurposing food waste compounds as part of composite component materials for controlled release fertilizer application emerges as a promising avenue to mitigate nutrient loss and contribute to agricultural sustainability.

Application of controlled release fertilizer has received great attention from both academia and industry where chitosan is used as a main component in coating or composite materials. Besides, existing controlled release fertilizer (CRF) technology possess limitations in terms of composition materials (Román-Doval et al., 2023), amount of polymer/copolymer, crosslinker availability (Cheng et al., 2017), and molecular weight of polymer, in improving material integrity. Hence, enhancing the mechanical stability and controlled-release properties of chitosan-based fertilizer coatings are crucial for this purpose. The incorporation of crosslinkers and copolymers, such as calcium chloride and alginate, respectively, aims to bolster the chitosan coating's mechanical strength and water retention capabilities (Feyissa et al., 2023).

To proceed with the application process, chitosan must first be extracted. A conventionally used method for extracting chitosan follows three main steps which are deproteinization, demineralization, and deacetylation. Although this method is known effective, the utilization of strong acid and base leads to environmental concern, time-consuming, high-energy input and varied yield and purity (Yi et al., 2024). Hence, this has

prompt researchers to explore and improvise other eco-friendly alternatives including NADES, microbial fermentation, and enzymatic approach (Kozma et al., 2022).

While various extraction methods exist, each presents unique challenges, necessitating careful consideration for optimal chitin extraction. As of now, only NADES system for direct chitin extraction have been developed. NADES system for direct chitosan extraction is yet to be explored. Research by Gopal et al. (2019), has proved that acetogenin from graviola leave extracts are able to direct extract chitosan without the use of harsh chemical. However, this method was time consuming which indirectly increase cost of extraction. Thus, the interest of this study lies in expediting the extraction process by employing NADES system in chitosan extraction. In this study, tailoring NADES system employing acetogenin and choline chloride in varied ratio and synthesis temperature with respect to the characteristics of chitosan extracted.

1.3 Objectives

The objectives of this study were:

- i. To propose a new natural deep eutectic solvent (NADES) consist of choline chloride and acetogenin for direct chitosan extraction from snail shell wastes.
- ii. To determine the relationship between component ratio and synthesis temperature of NADES in affecting the molecular weight, and degree of deacetylation of chitosan extracted.
- iii. To optimize the amount of crosslinker, copolymer and molecular weight of chitosan on the structural behaviour of controlled release fertilizer fabricated.

CHAPTER 2

LITERATURE REVIEW

2.1 Food Security and Agriculture

According to the 2019 Sustainable Development Report, East and South Asia continue to face severe hurdles in achieving the second Sustainable Development Goals (SDG), zero hunger. To guarantee adequate food all year, sustainable agriculture practices that boost production and adaptation in arid locations while gradually improving soil quality must be implemented (United Nation, 2020). Soil fertilization is the most widely used approach for increasing agricultural yield. Urea became one of the most frequently utilized fertilizers due to its high nitrogen content (Iftime et al., 2019). A controlled release mechanism could be used to make urea fertilizer more efficient. Various petroleum-based synthetic polymers, such as polyolefins, polysulfone, polyethylene, and polystyrene, had been widely utilized to extend urea release, but the leftover collected in soil, causing contamination (Liu et al., 2020). As a result, biopolymer-based formulations are being developed as an environmentally beneficial alternative.

The Malaysian Department of Agriculture (DOA) mission are to provide quality and efficient services to agricultural entrepreneurs through the application of advance technologies and agricultural regulatory services to boost production capability and ensure the safety of the country's agriculture sector (DOA, 2021). This shows that the country is committed in elevating the agricultural production to support the local population and exportation demand which indirectly supports the United States Department of Agriculture (USDA) primary objectives is to ensure global food security. Food security is not only crucial for feeding millions of malnourished people around the world, but it is also critical

for the afflicted countries' economic growth (USDA, 2013). To ensure that this goal is met, agriculture and research and development should collaborate to pioneer new technologies for sustainable agriculture practice. In order to secure global food security, agriculture must increase productivity while avoiding the environmental costs associated with artificial nitrogen fertilization (Tang et al., 2022). The main issue in intensive agricultural production systems is to combine intensive production with high nitrogen use efficiency (NUE), because efficient use of nitrogen sources is the foundation for future food security while minimizing negative environmental impact (Rütting et al., 2018). This is difficult, however, because obtaining high productivity and high NUE in agricultural output at the same time necessitates increased food demand, natural resource depletion, and environmental deterioration (Ding et al., 2018).

2.2 Efficiency of Conventional Nitrogen Fertilizer

Nitrogen fertilizer is available in the forms of urea, ammonium, and nitrate (Bucher & Kossmann, 2007). According to the fertilizer business by Rütting et al. (2018), nitrogen is critical for improved crop productivity. Urea fertilizer has been widely employed due of its high nitrogen concentration and cost-effectiveness (Ifime et al., 2019; Tang et al., 2018). While an excess of nitrogen element is environmentally harmful and ineffectual, an undersupply of nitrogen element jeopardizes global food security, creating a main problem in developing an integrated agricultural system (Rütting et al., 2018). As a result of these advantages and disadvantages, the nitrogen supply should be regulated accordingly.

The NUE is a regularly used measure for comparing crop nitrogen uptake to nitrogen provided (Sharma & Bali, 2017). According to Foskolos & Moorby (2018), the NUE can be defined at the animal, farm, and agricultural supply chain levels. Low fertilizer NUE shows the negative impact on environmental, economic, and resource conservation, emphasizing an urgent need to enhance fertilizer NUE (Sharma & Bali, 2017). Improved NUE, on the other hand, can boost crop yields and revenues while having no impact on the environment (Sharma & Bali, 2017). The fundamental issue in efficient agricultural systems, however, is to integrate intense production with an increased NUE, as efficient use of nitrogen sources is intended to promote future food security while limiting negative environmental impact (Rütting et al., 2018).

Given the unevenness in crop yields, nitrogen loss within fields, and fluctuating prices of nitrogen fertilizer and corn, it's essential to develop fertilization methods that optimize the use of nitrogen fertilizer (Sharma & Bali, 2017). Nitrogen utilization efficiency is thought to be fairly low on average in typical agricultural systems around the world, owing

to nitrate leaching or denitrification caused by heavy rainfall (Sharma & Bali, 2017). According to Kusumastuti et al. (2019), only 20–30% of nitrogen-containing fertilizer is absorbed by plants, with the remainder lost through volatilization, nitrification, and leaching. Nitrogen losses owing to volatilization and leaching will result in low efficiency, low crop output, lower economic and agricultural productivity, and environmental contamination.

2.3 Coated Fertilizer

Continuous fertilizer technology progress could alleviate the issue of inadequate NUE by encouraging the usage of coated fertilizer. According to Hiroshi & Yuichi (2019), coated fertilizers are helpful in terms of minimizing fertilizer environmental load, efficient use of fertilizer supplies, and labour savings. To control the release of nutrients, the properties of coated materials are the most significant component in the production of coated fertilizers (Liu et al., 2020). The coating rate of the coating material had a substantial effect on the N release properties of the coated fertilizers, which is consistent with prior research findings. The cumulative N release rate decreased as the weight percent of the covering material increased (Zhang et al., 2017).

Controlled-release fertilizers are classified into three kinds based on the coating materials used: organic compounds, inorganic compounds, and water-soluble fertilizers (Akalin & Pulat, 2020). Sulfur-coated urea (SCU) fertilizer is a form of inorganic and water-soluble fertilizer that has been manufactured for over 50 years (Ibrahim et al., 2014). Gypsum, a sulfur-containing coating substance, can minimize ammonia loss through volatilization (Beig et al., 2020). This is supported by the findings of (Ibrahim et al., 2014), who discovered that increasing the thickness of the coatings improves the strength of gypsum-coated urea fertilizer. This demonstrated that gypsum might be used as a covering material for controlled release fertilizer.

Nonetheless, this alternative has downsides such as high production costs, negative effects on the ecology and soil quality (Fertahi et al., 2021), and non-biodegradability (Beig et al., 2020). This has prompted scientists to develop biopolymer-based coating materials with fertilizer-like properties. Alginate (brown algae), pectin (citrus peel), cellulose (algae),

chitin (insect cuticles), chitosan (crustacean shells), lignin (wood), starch (maize), carrageenan (red algae), guar gum (seeds), and xanthan gum (*Xanthomonas* bacteria) are the most utilized biopolymer coatings (Fertahi et al., 2021). Although these materials are difficult to fully utilize due to the hydrophilicity of most biopolymers, the availability of biomass, and the high cost of production, they must be improved in terms of extraction methods, competition from other fields employing the same biomass, and utilization of waste or by-product biopolymers produced from different biorefineries (Fertahi et al., 2021).

Several factors influence the rate of release, including size, molecular weight, coating film composition and structure, as well as the nature and polymeric solution strength (Beig et al., 2020). The mechanical strength and release behaviour of a coating material are directly proportional to its molecular weight (Liu et al., 2020). This is corroborated by the findings of (Gumelar et al., 2020), who claimed that the mechanical strength of the coating material may influence the small amount of NPK nutrients released by chitosan-coated fertilizer. High molecular weight polymer produced a denser coat with a smooth surface when used as a coating material in the gradual release of urea.

Furthermore, the application of a high concentration of polymer has been shown to increase the thickness of the coating layer, promoting gradual nutrient release (Fertahi et al., 2021). The presence of a crack reduces the urea holding capacity as a result of the polymer's decreased molecular weight due to its short chain structure, allowing water to seep through the membrane (Liu et al., 2020). In contrast, a polymer with a high molecular weight reduces film porosity and produces a smoother surface, which reduces fracture formation (Liu et al., 2020).

2.3.1 Chitosan-Coated Fertilizer

Coating materials based on natural polysaccharides have received a lot of attention recently because of their biodegradability, recyclability, cheap cost, and renewability (Lü et al., 2016), which includes chitosan, cellulose, and starch as natural polymeric coating materials (Akalin & Pulat, 2020). Chitosan is a highly deacetylated derivative of chitin that is one of nature's most abundant and biodegradable polymers. Chitosan has been utilized in the coating of coated fertilizer. Previous research discovered that chitosan and salicylaldehyde combine to generate a hydrogel for controlled release fertilizer with strong water retention capabilities (Ifime et al., 2019). This is supported by Wu & Liu (2008) results that chitosan coated NPK fertilizers have a high-water retention capacity. The use of chitosan particles in NPK fertilizer increases fertilizer efficiency, as indicated by a slower rate of N, P, and K nutrient release (Gumelar et al., 2020). Figure 2.1 shows the lower weight of nutrient release from chitosan coated granules (slow-release fertilizer, SRF) compared to uncoated granules (NPK fertilizer).

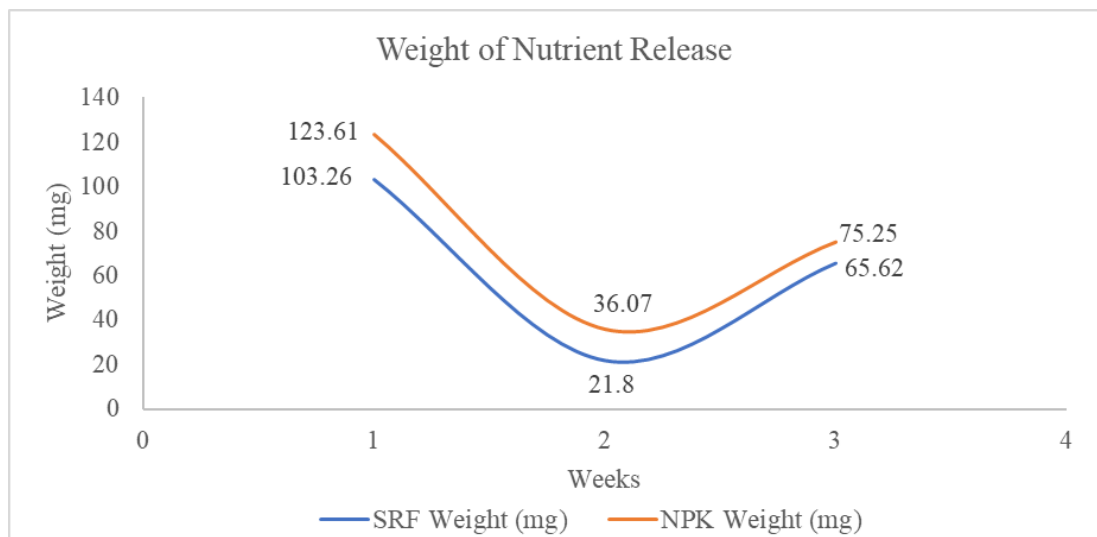


Figure 2.1: Weight of nutrient released from two types of fertilizer (Gumelar et al., 2020)

2.3.2 Sources of Chitosan

Given its nature as a deacetylated derivative of chitin, chitosan can be found in practically all chitin sources. Chitin is the primary substance found in crab exoskeletons (D'Angelo et al., 2018). As a result, chitosan might be made from crustacean trash, and the possible source would be industrial waste, lowering costs (Mesias et al., 2019). Crab and shrimp shell wastes are currently used as the primary industrial source of biomass for large-scale chitin and chitosan synthesis (Islam et al., 2017).

Cerithidea obtuse is a common snail found in muddy coastal areas in Malaysia. They are known locally as 'siput sedut' and are used as a food source, with the shells later disposed. The protein content of soft muscular tissues was found to be 11.2 g, total fat 1.8 g, carbohydrate 1.5 g, and energy 287 kJ or 67 kcal per 100 g serving (Onn et al., 2017). Chitin made up 21.65 % of the dry weight base of *Conus inscriptus* sea snail shell (Mohan et al., 2019), which corresponds to other chitin marine sources (Hong et al., 2018). Aside from having a high chitin content, the use of sea snail shells allows for the utilization of a major source of trash in the seafood sector.

Chitin is extracted from crustacean shells using acid demineralization followed by alkali deproteinization in industrial processing. Acid-base wastewater generated during this technique poses major environmental issues (Zhou et al., 2019). Chitosan's high susceptibility to processing conditions such as heating might produce stress on its structure and polymer breakdown (Szymańska & Winnicka, 2015). Besides, chitosan's molecular weight changes according to the difference in DDA and the source of chitosan (Varma & Vasudevan, 2020). This is consistent with the assertions made by (Szymańska & Winnicka, 2015), who say that the properties of chitosan material from various sources vary

substantially in terms of degree of deacetylation, molecular weight, purity level, and molecular weight distribution. Knowledge of the source of chitin/chitosan, its nature, water content, and impurity level are also required for accurate calculation of the degree of deacetylation of chitosan values (Kumirska et al., 2010), which is one of the important parameters to target the desired properties of chitosan material.

Most marine shells are composed of numerous components, the most common of which are calcium carbonate, chitin, and protein. Crustacean shells, for example, contain 15% to 20% chitin, 25% to 40% protein, and 40% to 55 % calcium carbonate, the latter of which is important for crustacean stiffness (Riaz Rajoka et al., 2019). This is consistent with the findings of (Santos et al., 2020), who discovered that crustacean shells are composed of proteins (30–40%), calcium carbonate (30–50%), and chitin (20–30%). As a result, it is critical to extract and segregate the chitin from other components before deacetylating it into chitosan in a step-by-step process.

2.4 Chitosan Extraction Method

2.4.1 Conventional Extraction of Chitosan

This time-consuming and environmentally unfriendly traditional method encouraged scientists to look for a more direct and environmentally favourable way. This involves using biological techniques such as microbial fermentation and enzymatic reactions (Hong et al., 2018; Zhu et al., 2017). For example, microbial fermentation using lactic acid-producing bacteria outperformed the chemical technique in eliminating minerals from shells and extracting and recovering chitin and its derivatives, chitosan. Chitin deacetylase (CDA), the first identified enzyme that catalyses the conversion of chitin to chitosan (Kaur et al., 2012), chitinases, and chitosanases are extensively utilized in enzymatic reactions (Kaczmarek et al., 2019). This enzymatic reaction offers various advantages over typical chemical procedures, the most significant of which is the generation of chitosan with a higher molecular weight and the required degree of deacetylation (Kaczmarek et al., 2019). However, the inadequate removal of minerals and proteins limits the implementation of enzymatic processes and microbial fermentation methods, even though both approaches have less negative environmental consequences (Zhao et al., 2019).

Other chemical methods of interest include ionic liquids (ILs), deep eutectic solvents (DES), and natural deep eutectic solvents (NADES). Ionic liquids were utilized to extract chitin directly from raw materials (Zhu et al., 2017). However, synthesis of ionic liquids is often challenging because it requires a large number of organic solvents, is difficult to purify, and is difficult to remove the leftover ions and water (Chen et al., 2018). DESs are a new generation of green-designed solvents with reduced cost and environmentally friendly specialty as compared to traditional ILs (Hong et al., 2018). The comparison of traditional chitosan extraction are portrayed in Table 2.1.

Table 2.1: Comparison of Conventional Methods of Chitosan Extraction

No.	Extraction Method	Advantages	Disadvantages	Extraction Performance	Molecular Weight (kDa)	DDA (%)	Citation
1	Chemical Extraction	Cost-effective; High purity and efficiency	Environmental hazards; Harsh chemicals can degrade chitin	High-purity chitin; may degrade polymer chains	300–360	~80% (microwave-assisted)	Younes & Rinaudo (2015)
2	Biological Extraction (Microbial Fermentation)	Eco-friendly; Preserves high molecular weight	Costly; Incomplete demineralization or deproteinization	High molecular weight chitin but slower and incomplete in some cases	Higher than chemical (strain-dependent)	Higher than chemical (strain-dependent)	Kozma et al. (2021)
3	Enzymatic Extraction	Mild and selective; High polymer preservation	Expensive enzymes; Not easily scalable	Preserved molecular integrity, but slower	Typically higher than chemical	Higher DDA; enzyme-dependent	Kozma et al. (2021)
4	Microwave-Assisted Extraction	Fast and efficient; Reduces solvent use	High equipment cost; Difficult to scale	Improved yield and speed	300–360	>80% in 10 mins	Younes & Rinaudo (2015)
5	Ultrasound-Assisted Extraction	Efficient energy usage; Enhances solvent access	Equipment requirements; Scale-up issues	Efficient and fast; needs optimization	Typically higher than conventional	Higher DDA than conventional	Younes & Rinaudo (2015)

Table 2.1: continued

6	Natural Deep Eutectic Solvent (NADES) Extraction	Biodegradable; Mild conditions preserve structure	High viscosity; Limited industrial-scale data	High-quality chitin; green method	Preserves native Mw (e.g., ~392)	High DDA possible; varies by NADES type	Zhang et al. (2022); Morgan et al. (2021)
7	Fungal Fermentation	Direct chitosan from fungi; Sustainable	Lower purity; Variable yield	Sustainable source, but purity is species-dependent	Rhizopus oryzae: ~100–400 (varies)	~60–85%, species and growth dependent	Goy et al. (2009); Synowiecki & Al-Khateeb (2003)

The discovery of DES sparked a lot of research interest in the chemistry community, which has been bolstered by recent findings of its analogous solvent, NADES, in which many bio-based substances were found to change their state from solid to liquid at certain temperatures when mixed in the right ratio (Gómez et al., 2019). As a result, NADES is a more biocompatible solvent than DES. In comparison to organic solvents and ionic liquids, NADES biodegradability aids in the avoidance of potential environmental dangers (Liu et al., 2018) due to its ability to be easily biosynthesized by most organisms. As a result of NADES' promising features, this work is focusing on using NADES in the direct and immediate extraction of chitosan.

2.4.2 Natural Deep Eutectic Solvents (NADES)

Natural deep eutectic solvents (NADES) are a type of deep eutectic solvent (DES) solvent consists of two or more natural components, namely chlorine derivatives, organic acids or bases, amino acids, and sugars (Santana et al., 2019). This NADES interact via strong hydrogen bonds and have a lower melting temperature than its individual components (Smirnov et al., 2020). NADES is a natural deep eutectic solvent formed by hydrogen bonding between natural metabolites have the ability of serving as hydrogen bond acceptor (HBA) or hydrogen bond donor (HBD) (Zhou et al., 2019). Several parameters have a substantial influence on the creation and stability of NADES, including the number of hydrogen-bond donors (HBD) or hydrogen-bond acceptors (HBA), the position of the bonds that emerge, and the spatial arrangement of those groups (Savi et al., 2019).

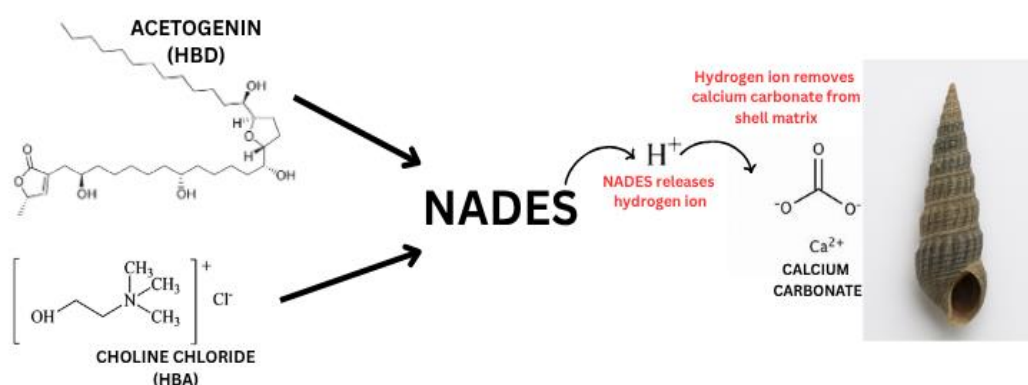
NADES has been effectively used in the extraction of chitin from marine sources (Hong et al., 2018). Huang et al. (2018) found that the chitin extracted using NADES is proportional to the conventional approach in terms of shape, crystallinity, and thermal stability. Findings from previous researchers on chitin extraction by NADES is shown in Table 2.2. The NADES system used in this work consists of choline chloride (ChCl), a biodegradable, inexpensive, non-toxic quaternary ammonium chloride salt derived from biomass as the HBA (Jablonský et al., 2019). Choline chloride has long been employed as a component of the NADES system. This is because choline chloride is a common ammonium salt due to its low cost, biodegradability, and low toxicity (González-Rivera et al., 2020). Acetogenin, a secondary metabolite identified in graviola leaves extract, is the HBD employed in this NADES system as it shows that graviola leaves extract yielded 72-87% of DDA from sources such crab, shrimp shells, and squid pens (Gopal et al., 2019). The role of choline chloride (HBA) and acetogenin (HBD) in the extraction of chitin and chitosan is portrayed in Figure 2.2.

Table 2.2: Chitin Extraction by NADES

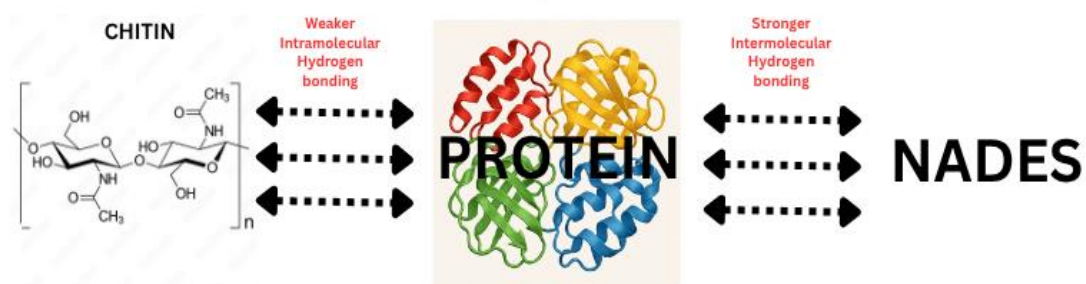
No	NADES Composition	Source Material (Shell)	Molecular Weight (kDa)	Degree of Deacetylation (DDA%)	Reference
1	Choline chloride–oxalic acid	Crab	392	N.S	Huang et al. (2022)
2	Choline chloride–glucose–formic acid	Crab	392	N.S	Sun et al. (2022)
3	Choline chloride–oxalic acid	Shrimp	N.S	Minimal deacetylation observed	Lei et al. (2024)
4	Choline chloride with various organic acids	Lobster	91–312	N.S	Hong et al. (2018)
5	Choline chloride–oxalic acid	Crustacean	N.S	N.S	Huang et al. (2018)

*N.S = Not specified

DEMINERALIZATION



DEPROTEINIZATION



DEACETYLATION

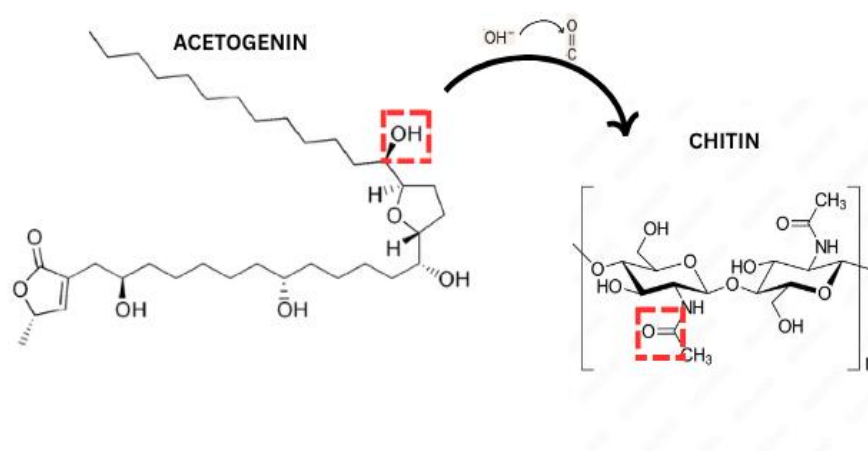


Figure 2.2: Role of choline chloride (HBA) and acetogenin (HBD) in the extraction of chitin and chitosan

The minerals in chitin, such as calcium carbonate, are eliminated by the hydrogen ions generated by NADES. Mineral removal reduces the connections between protein and chitin. This is obvious when more than 99 % of the calcium carbonate was removed from the shrimp shells by the treatment of NADES after 9 minutes of microwave irradiation (Huang et al., 2018). Proteins in chitin are eliminated by NADES-induced intermolecular hydrogen bonds, which disrupt the intramolecular hydrogen bond produced in protein-chitin fibres (Zhou et al., 2019). As a result, chitin is dissolving in NADES and being separated from the proteins (Huang et al., 2018). This is because the deproteinization process includes the breakdown of chemical interactions between proteins and chitin, necessitating the use of chemicals to depolymerize the biopolymer, and the removal of related proteins is an important step in the polysaccharide purification process (Santos et al., 2020). Protein hydroxyl and carboxyl groups competed for chloride anion electrostatic interactions by generating hydrogen ions. As a result, the hydrogen bonds between NADES are disrupted, and new links between NADES and protein are established (Huang et al., 2018).

While NADES can extract chitin, its effectiveness in deacetylating chitin during the extraction process to produce chitosan in a single step has not been documented. Chitosan is preferred over chitin for material fabrication because it has both a hydroxyl and an amino group. This is owing to chitosan's hygroscopic characteristics, which allow it to create more hydrogen bonds with water (formed with both hydroxyl and amino groups) than chitin (Szymańska & Winnicka, 2015). Unlike chitin, chitosan contains a high fraction of a highly protonated free amino group, which attracts ionic substances (Varma & Vasudevan, 2020). The protonated amino group (Liu et al., 2020) contains cationic charge that can be functionalized (Mokhtar et al., 2020).

A recent study discovered that the nucleophilic attack of hydroxyl groups from acetogenin on the carbonyl group of chitins facilitates the deacetylation process of chitin to chitosan (Gopal et al., 2019). However, utilizing acetogenin as the only extraction solvent was time demanding. The notion of using acetogenin as HBD in direct extracting solvent by generating NADES with HBA to minimize the time for direct extraction of chitosan was discovered as a result of these exploratory experiments.

The interactions of hydrogen bonds and solubility between NADES and the biopolymer matrix in the shells have a significant impact on the extraction efficiency of chitosan. Polarity, density, surface tension, melting temperature, bonding interactions, and viscosity all exhibit interactions. The interactions are likely to differ depending on the molar ratios of choline chloride and acetogenin, as well as the synthesis temperature (Jablonský et al., 2019). According to Huang et al. (2018), increasing the shells-to-NADES ratio from 1:5 to 1:20 makes NADES more efficient for deproteinization.

Furthermore, the high temperature utilized in chitin extraction resulted in chitin with a low molecular weight (Hong et al., 2018). To obtain higher molecular weight chitosan, low temperature should be employed in NADES extraction to achieve a low rate of breakdown. This is especially true because the molecular weight of chitosan is impacted not only by high temperature, but also by reaction duration, base concentration, previous treatment of chitin, chitin concentration, particle size, shear stress, and dissolved oxygen content (Varma & Vasudevan, 2020).

The physicochemical properties and structure of chitosan resulting from NADES treatment will be described in this work using significant metrics to measure its features and functionality, such as purity, molecular weight, and degree of deacetylation of extracted

chitosan. The reusability of NADES is assessed, improving the possibility of chitosan extraction at a larger scale. This is achievable since, according to Zhou et al. (2019), the reusability of NADES was easily obtained by the addition of some ethanol.

2.5 Chitosan-Copolymer Composites

Chitosan by itself may not be ideal as standalone beads due to its inherent characteristics, which can influence its mechanical stability and nutrient release performance. Chitosan beads might not have sufficient mechanical strength to endure handling and environmental conditions, potentially leading to fragmentation or breakdown. Jantrawut et al. (2015) found that chitosan beads exhibited inadequate mechanical properties, including low compressive strength and high friability, which can limit their effectiveness as standalone carriers. Chitosan tends to swell and degrade in aqueous environments, compromising its structural integrity over time. Research by Rabea et al. (2003) and Kumar et al. (2014) indicated that chitosan beads are prone to significant swelling and rapid degradation, particularly in acidic conditions, which affects their ability to maintain controlled release of encapsulated nutrients or agrochemicals.

Chitosan alone may not provide the most effective controlled release properties for encapsulated substances. Although chitosan has natural mucoadhesive qualities and can support controlled release to some extent, it may not achieve the desired release kinetics or duration without further modification or the addition of other materials. Agnihotri et al. (2004) showed that chitosan-based formulations often exhibit burst release kinetics, which limits their efficacy for sustained nutrient delivery. The degradation products of chitosan may impact soil and water quality, raising environmental concerns. Bakshi et al. (2020) and Prajapati et al. (2022) emphasized the biodegradability of chitosan and its potential to release nitrogen and other byproducts, which could alter soil properties and nutrient dynamics.

2.5.1 Alginate Copolymer

While chitosan offers numerous benefits as a biopolymer for encapsulation and controlled release purposes, its limitations in mechanical stability, swelling behavior, release kinetics, and environmental impact may require combining it with other materials or using it in composite formulations to address these challenges. To function effectively as a composite, chitosan needs specific molecular modifications with the help of copolymers and crosslinkers. Multifunctional formulations that can deliver nutrients and moisture to the soil in a controlled manner are desirable for improving agricultural practices. Alginate, a natural polysaccharide derived from brown algae, is an example of a copolymer that pairs well with chitosan.

Alginate possesses several properties that make it an ideal copolymer for use with chitosan in various applications, such as controlled release systems and biomedical scaffolds. In the presence of divalent cations like calcium ions, alginate undergoes gelation, forming stable hydrogels under mild conditions. Research by Lee & Mooney (2012) and Zhang et al. (2019) has clarified the gelation process of alginate and its potential in controlled release applications. The ability to form hydrogels enables the encapsulation of bioactive agents within the chitosan-alginate matrix, supporting sustained release and protection of the encapsulated materials.

In addition, alginate undergoes ionic crosslinking with divalent cations, resulting in the formation of physically crosslinked networks. Studies by Choi et al. (2022) and Pawar & Edgar (2012) have emphasized the role of calcium ions in facilitating crosslinking between alginate chains, leading to the development of stable hydrogel structures. This ionic

crosslinking mechanism complements the polycationic nature of chitosan, aiding in the formation of chitosan-alginate complexes with customizable properties.

Moreover, alginate exhibits excellent water retention and swelling behavior, which can improve the mechanical properties and stability of chitosan-based formulations. These properties are particularly beneficial for controlled-release fertilizers. Research by Zhang et al. (2019) and ALSamman & Sánchez (2022) has demonstrated alginate's ability to absorb and retain water, resulting in increased hydration and swelling of chitosan-alginate hydrogels. This feature is advantageous for applications requiring moisture retention, such as wound dressings and tissue engineering scaffolds.

Additionally, the degradation rate of alginate can be adjusted by modifying its composition and crosslinking density, providing control over the release profile of encapsulated agents. Studies by Lee & Mooney (2012) and Pawar & Edgar (2012) have investigated methods to regulate the degradation rate of alginate-based hydrogels for sustained release purposes. This adjustability allows for the creation of chitosan-alginate copolymers with tailored release kinetics to meet specific therapeutic needs. These distinct features of alginate such as biocompatibility, gelation properties, ionic crosslinking, water retention, and tunable degradation rates, making it an excellent copolymer for chitosan in controlled release applications.

2.5.2 Crosslinkers

Aside from copolymer, chitosan rigidity depends on the presence of crosslinker. This is supported by claims of (Negm et al., 2020) where crosslinked chitosan biopolymer membranes showed stronger hydrophobic features than uncrosslinked chitosan biopolymer membranes. Crosslinkers have a role in chitosan strength by inhibiting breakdown, allowing nutrients to be released. Alternative green and natural crosslinkers are becoming more popular as a way to lessen the detrimental impact of chemical crosslinkers. Genipin (Mi et al., 2000), protein, starch, and plant extracts (polyphenols and aldehyde compounds) are examples of naturally occurring crosslinkers (Tomadoni et al., 2019).

Vanillin, a natural crosslinker derived from aldehyde compounds (Tomadoni et al., 2019), is often employed in the formulation of chitosan films. The Schiff reaction between the aldehyde group of vanillin and the amino group of chitosan is used in the creation of chitosan films (Wang et al., 2011). Vanillin's aldehyde group ($-CHO$) reacts with chitosan's amino group ($-NH_2$) to form the imino group ($N=C$), and its hydroxyl ($-OH$) reacts with chitosan's hydroxymethyl ($-CH_2OH$) to form hydrogen bonds (Zhang et al., 2015).

However, Hunger & Domalik-pyzik (2020) stated that while vanillin has generally minimal cytotoxicity effect, the crosslinker residues may be harmful. According to Zhang et al. (2015), vanillin crosslinker concentrations of up to 50 % in chitosan film seemed irregularly structured with some granules and folds on the surface of chitosan when examined under scanning electron microscope (SEM). This could be due to the excess vanillin did not react with the chitosan and hence remained on the surface during the drying process (Zhang et al., 2015).

Prior to the emergence of in-demand natural crosslinkers, chemical crosslinkers were predominantly used. Dialdehydes (such as glutaraldehyde or glyoxal) and genipin are the commonly used chemical crosslinkers for chitosan (Szymańska & Winnicka, 2015). Garnica & Sánchez (2016) studied the relationship between the structural and mechanical properties of chitosan/poly(vinyl alcohol), CS/PVA, crosslinked with two distinct crosslinkers, genipin and glutaraldehyde. Although both crosslinkers show a decrease in swelling capacities from the initial swelling capacity of the CS/PVA blend, the genipin-crosslinked CS/PVA (270 %) is proven to have slightly higher swelling capacities, at 270 %, than glutaraldehyde-crosslinked CS/PVA, which is at 190 %, according to this study. This demonstrates that genipin is a good alternative crosslinker for increasing the mechanical properties of the CS/PVA blend, and it has the potential to be employed in agriculture (e.g., water-controlled release systems), medicine, food, and the environment (Garnica & Sánchez, 2016). Unfortunately, these crosslinkers have certain undesired properties, including the fact that dialdehydes and genipin are poisonous in nature and cause colour shifts from translucent to blue (Szymańska & Winnicka, 2015).

Citric acid is a frequently studied crosslinker for chitosan. Mesias et al. (2019) showed significant improvements in fertilizer encapsulation using citric acid-crosslinked chitosan composites. Nevertheless, using citric acid as a crosslinker in chitosan composites comes with several drawbacks. The crosslinking process typically necessitates an acidic environment, which can restrict its use in applications that involve sensitive biomolecules or biological systems. Goy & Assis (2014) emphasized the importance of maintaining controlled pH levels during citric acid crosslinking to avoid adverse effects on chitosan's properties. Additionally, chitosan composites crosslinked with citric acid may exhibit weaker mechanical properties compared to those crosslinked with other agents. Arca et al.

(2018) and Sharmin et al. (2022) reported that citric acid-crosslinked chitosan films tend to have reduced tensile strength and elasticity, due to less robust intermolecular interactions and incomplete crosslinking.

Moreover, the effectiveness of citric acid crosslinking can fluctuate depending on factors such as pH, temperature, and concentration, leading to inconsistent crosslinking and varying material properties. Arca et al. (2018) and Yang et al. (2020) pointed out the need to optimize reaction conditions to achieve the desired crosslinking density and mechanical performance. Although citric acid is Generally Recognized As Safe (GRAS) by regulatory bodies, there are still concerns about its biocompatibility, particularly in biomedical and pharmaceutical contexts, due to the possibility of adverse reactions or cytotoxic effects from residual citric acid and its degradation products. Song et al. (2021) underscored the importance of thorough cytotoxicity evaluations of citric acid-crosslinked chitosan materials before they are used in clinical settings. In addition, citric acid, like other organic acids, can present environmental challenges during production, disposal, and degradation. The large-scale synthesis of citric acid may involve energy-intensive processes and generate waste byproducts, contributing to environmental pollution. The need for more sustainable and eco-friendly crosslinking methods to minimize environmental impact should be highlighted.

When creating alginate hydrogels, divalent ions are necessary for crosslinking, and calcium chloride is frequently used in this process. Calcium chloride is highly water-soluble and dissociates into calcium (Ca^{2+}) and chloride (Cl^-) ions. The high ionic strength of CaCl_2 solutions enhances interactions with alginate molecules, facilitating rapid gelation. Choi et al. (2022) and Lee & Mooney (2012) demonstrated that CaCl_2 provides superior crosslinking efficiency compared to other divalent cations, making it a preferred choice for alginate

gelation. Calcium ions interact with the guluronic acid (G) blocks in alginate chains, forming egg-box structures and crosslinked networks. This rapid crosslinking mechanism enables the immediate formation of alginate hydrogels when they come into contact with CaCl_2 solutions. Pawar & Edgar (2012) and Zhang et al. (2019) have clarified the kinetics of calcium-alginate gelation, which depends on factors such as alginate concentration and calcium ion concentration.

Additionally, the mechanical properties and swelling behavior of alginate hydrogels can be modified by adjusting the concentration of calcium chloride. Higher concentrations of CaCl_2 result in denser crosslinking networks and stiffer hydrogels, whereas lower concentrations produce softer and more pliable gels. This adjustability allows for the customization of hydrogel properties to suit specific application needs. Lee & Mooney (2012) and Zhang et al. (2019) examined how varying calcium ion concentrations influence the rheological and mechanical characteristics of alginate hydrogels.

2.6 Response Surface Methodology (RSM)

Response surface methodology (RSM) has been described as an analysis tool for determining the best reaction conditions between variables and responses (Tang et al., 2018). According to Tomadoni et al. (2019), the RSM approach using Box-Behnken design and the desirability function was discovered to be an effective tool for investigating changes in film properties and searching for the optimal active chitosan film formulation with a natural antioxidant cross-linking agent, such as vanillin. According to Beig et al. (2020), the following factors influence the release rate and process: (i) solid dispersion/inflammation; (ii) urea coating deterioration; and (iii) coating rupture, water penetration, and finally disintegration. As a result, variables could be used to determine the release of nutrients from fertilizers. In this study, a series of systematically designed experiments will be performed to determine the effects of (i) crosslinker loading (wt%), (ii) copolymer loading (wt%), and (iii) percentage of molecular weight of chitosan (%) as independent variables on the release behaviour of coated fertilizers as dependent variables. To ensure that adequate nutrients are given, the release behaviour of fertilizer and water from the coatings must be examined. The impact of the crosslinker and copolymer on the release performance of these qualities should be quantified.

Aside from nutrients, the primary necessity for plant growth is water retention in soil, which is especially important in arid locations. As a multifunctional coating material, the capacity to retain water is particularly significant. As a result, the water retention and absorbance properties of this coating material should be evaluated. Molecular weight (Mw) and degree of deacetylation (DDA) of chitosan both shows significant effects towards its controlled release properties. This is supported by (Jabrail, 2018) findings in which the chitosan microspheres with high Mw (2227 kg/mol) and high DDA (75 wt%) encapsulating

the isoniazid compounds released more isoniazid in a controlled manner. Thus, the mode of action in customizing the purity, molecular weight, and degree of deacetylation of chitosan by tweaking the solvent characteristics is the subject of this study. The component ratio and synthesis temperature regulate the solvent characteristics, which are mostly determined by hydrogen bond interactions. There is still a dearth of research on the release behaviour of modified chitosan copolymerized with alginate\ for fertilizer. The outcome of this investigation will be compared to the projected outcomes based on RSM. The association between variables influencing release behaviour could be scaled up for controlled release fertilizer and soil water retention in sustainable agriculture methods.

2.7 Factors Influencing Swelling Behaviour, Encapsulation Efficiency, and Urea Release

Hydrogel-based fertilizer delivery systems, particularly those incorporating biopolymers such as chitosan and alginate, have garnered significant attention due to their environmental compatibility and ability to modulate nutrient release in agricultural applications. Their performance is typically evaluated based on three interdependent parameters: swelling percentage, encapsulation efficiency (EE%), and urea release rate (UR%). These parameters are influenced by the physicochemical characteristics of the formulation components as well as external environmental conditions.

Swelling behavior plays a vital role in determining the nutrient absorption and diffusion characteristics of hydrogels. The degree of swelling is closely linked to the type, molecular weight, and concentration of the biopolymers used. Alginate, a hydrophilic, anionic polysaccharide, typically enhances water uptake due to its affinity for polar solvents (Rudzinski et al., 2002). However, excessive alginate content can reduce the porosity of the matrix, leading to a decline in swelling capacity (Zohuriaan-Mehr & Kabiri, 2008). Chitosan, on the other hand, exhibits pH-sensitive swelling due to the protonation of its amino groups under acidic conditions, and its swelling ability is modulated by its molecular weight and degree of deacetylation (DDA) (Kumar et al., 2004). Low molecular weight chitosan tends to form more flexible matrices with higher swelling potential but may compromise mechanical strength. Additionally, the use of crosslinking agents such as calcium chloride is essential for gel formation. High crosslinker concentrations create a more rigid and tightly bound network, reducing swelling by restricting polymer chain movement, while insufficient crosslinking can result in unstable gels (Murata et al., 1993). Environmental factors, such as temperature and pH, further influence swelling behavior; higher temperatures may enhance

polymer chain mobility and increase swelling, but excessive thermal exposure can lead to hydrogel degradation (Li & Mooney, 2016).

Encapsulation efficiency (EE%) reflects the effectiveness of the hydrogel matrix in retaining urea during the bead formation process. EE% is greatly influenced by polymer concentration and the relative ratio between chitosan and alginate. An optimal ratio facilitates the formation of an interpenetrating polymer network that entraps the active compound efficiently. High alginate content may induce premature urea leaching during crosslinking in calcium solutions (Giri et al., 2012), whereas chitosan provides additional electrostatic interactions that improve encapsulation stability. The molecular weight of chitosan also plays a role in which low molecular weight chitosan can better penetrate the alginate matrix, improving encapsulation, but if too low, may reduce viscosity and gel strength (Goy et al., 2009). Crosslinking time and concentration are additional critical factors; extended or intense crosslinking can produce dense outer surfaces that trap air bubbles or prevent full diffusion of urea into the core (Patel et al., 2016). Furthermore, higher initial urea concentrations may saturate the matrix, reducing the relative encapsulation due to diffusion losses during gelation (Li et al., 2008).

The release profile of urea from hydrogel systems is a function of swelling, polymer degradation, and environmental conditions. Generally, increased swelling promotes rapid nutrient diffusion, while denser or more crosslinked matrices retard release (Wu et al., 2012). The structure and degradation behavior of the polymer network are crucial where alginate matrices degrade via ion exchange, particularly in the presence of monovalent cations like Na^+ , leading to the release of encapsulated compounds (Draget et al., 2006). Chitosan, due to its pH-responsive behavior, shows limited solubility in neutral and alkaline soils, which

can slow nutrient release and enhance longevity (Elsabee & Abdou, 2013). The presence of stronger crosslinking agents or multilayer coatings can prolong release duration by creating diffusion barriers. However, excessive crosslinking may induce brittleness, resulting in mechanical failure under soil conditions (Liu et al., 2007). Additionally, environmental factors such as soil pH, moisture content, and microbial activity accelerate polymer degradation and influence urea leaching patterns. In coated fertilizer systems, coating thickness plays a critical role in modulating release. Thicker coatings provide extended release durations but may delay nutrient availability, which may not align with crop demand cycles (Trenkel, 2010).

In conclusion, the formulation of hydrogel-based fertilizers requires careful optimization of multiple parameters to achieve desired performance in terms of swelling, encapsulation efficiency, and nutrient release. Parameters such as polymer concentration, molecular weight, crosslinker type and dosage, and environmental pH must be synergistically tailored. Response Surface Methodology (RSM) has proven to be an effective tool in modeling and optimizing these variables simultaneously to develop more efficient and sustainable fertilizer delivery systems (Montgomery, 2017). Understanding the interplay among these factors is essential for advancing the development of controlled-release fertilizers that can reduce nutrient losses, minimize environmental impact, and improve crop productivity.

CHAPTER 3

METHODOLOGY

3.1 Synthesis of Natural Deep Eutectic Solvent (NADES)

NADES was prepared using the method previously described (Shafie et al., 2019). Choline chloride, ChCl, the hydrogen bond acceptor, and acetogenin, Aceto, the hydrogen bond donor, were mixed in a mole ratio of 1:1 respectively and heated in a round bottom flask at 80 °C in an oil bath with magnetic stirring for 1 to 2 hours until a homogenous solution was formed. After that, the solution was cooled to ambient temperature and placed in a desiccator to decrease moisture absorption. The processes were repeated with HBA to HBD mole ratios of 1:2 and 2:1, respectively. The temperature was also varied between 40 °C and 60 °C.

3.2 Chitosan Extraction using NADES

The sea snail (*Cerithidea obtuse*) waste shells came from Kuching, Sarawak, Malaysia. The boiled shell waste were rinsed with tap water and dried in the oven at 100 °C (Hong et al., 2018). Dried shells were ground to a fine powder with a mixing mixer. Chitosan was extracted using NADES in the same way that chitin was extracted, with some adjustments (Hong et al., 2018). A 1:10 (w/v) ratio of sea snail shell powder and NADES solvent was combined in an oil bath with magnetic stirring at room temperature until supernatant was detected. After centrifuged, supernatant's precipitate was recovered and rinsed with MiliQ water to neutral pH. The precipitate was oven-dried at 40 °C until it reached a consistent weight.

3.3 Characterization of NADES

3.3.1 Polarity Measurements

NADES polarity was determined using the solvatochromic dye Nile red as a probe, as previously described (Gaspar et al., 2020). In brief, a 1 mg/mL stock solution of Nile red was produced in methanol. A 5 μ L Nile red stock solution was added to a quartz cuvette with 1 mL of NADES. The spectra were collected using a Shimadzu UV-2600i spectrophotometer. Equation 3.1 uses Planck's constant ($6.626 \times 10^{-34} \text{ m}^2\text{kgs}^{-1}$), the speed of light in a vacuum ($3.0 \times 10^8 \text{ ms}^{-1}$), and Avogadro's constant ($6.022 \times 10^{23} \text{ particles mol}^{-1}$) to calculate the molar transition energies (ENR). Polarity was calculated by comparing the ENR to a reference solution.

$$\text{ENR (kcal/mol)} = 28591 / \lambda_{\text{max, NR}} \quad \text{Equation 3.1}$$

3.3.2 Density Measurements

The density was calculated simply by pouring the NADES into a 10 mL volumetric flask until it reaches the calibrated mark (Shafie et al., 2019). The weight of NADES was measured in a volumetric flask at 25 °C. The density was computed using Equation 3.2, where m is the weight (g) of NADES in the volumetric flask and V is the volume of NADES (mL).

$$\text{Density, } \rho = m / V \quad \text{Equation 3.2}$$

3.3.3 Surface Tension Measurements

The surface tension measurements of intermolecular forces intensity in NADES were carried out using a force tensiometer (Kruss K20). The surface tensions of the NADES were measured at 25 °C and compared with distilled water.

3.3.4 Differential Scanning Calorimetry (DSC) Measurements

Differential scanning calorimetry (DSC) curve was recorded using NETZSCH DSC 214 Polyma with heating of approximately 8 mg sample in an aluminium holder under nitrogen atmosphere at a temperature ramping of 10 °C min⁻¹ for the heating and cooling processes from -40 °C to 200 °C.

3.4 Characterization of Extracted Chitosan

3.4.1 Chemical Composition Analysis of Chitosan

The ash and moisture were determined as previously reported (Hong et al., 2018). The ash content was determined gravimetrically by incinerating the sample (1–2 g) until a constant weight in a muffle furnace at 600 °C. The moisture content was determined by drying the sample in oven at 105 °C until the weight is constant.

3.4.2 Thermal Gravimetric (TGA) Measurements

Thermal analysis was performed on a Perkin Elmer Thermal Analysis (Pyris Diamond TG/ DTA). The samples were heated from 40 °C to 800 °C with a constant heating rate of 10 °C min⁻¹ under nitrogen atmosphere.

3.4.3 Fourier Transform Infrared-Attenuated Total Reflection (FTIR-ATR)

FTIR-ATR is used to determine molecular interactions, such as hydrogen bond formation between the various NADES and chitosan components. A background spectrum was captured before acquisition and served as a reference. The samples' spectra were recorded at room temperature, between 4000 and 500 cm⁻¹, with a resolution of 4 cm⁻¹. Chitosan's degree of deacetylation (%) was evaluated using the ATR-FTIR based on Equation 3.3 (Gopal et al., 2019), with 1655 cm⁻¹ representing the amide bond and 3450 cm⁻¹ for the hydroxyl bond.

$$\text{Degree of deacetylation (\%)} = A_{1655} / A_{3450} \times 115 \quad \text{Equation 3.3}$$

3.4.4 Contact Angle Measurements

Water contact angle measurements were performed on NADES (in contact with snail shells) and chitosan (in contact with distilled water) using a static contact angle goniometer (Dataphysic (Germany) Dcat 21). A 5 μL drop of sample was applied on the surface for characterization.

3.4.5 Viscosity Measurements

The viscosity of NADES can help us understand the nature of their intermolecular interactions. The viscosity of NADES was determined at temperatures ranging from 25 to 60 $^{\circ}\text{C}$. From these viscosity values, the intrinsic viscosity $[\eta]$ was determined by extrapolating the specific viscosity-concentration curve to zero concentration using standard procedures. The molecular weight (MW) of chitosan was estimated using a viscometric method involving a digital rotational viscometer. The molecular weight (Mw) of the extracted chitosan was estimated using the Mark–Houwink equation based on intrinsic viscosity $[\eta]$ measurements performed in 0.30 M acetic acid/0.20 M sodium acetate solution at 25 $^{\circ}\text{C}$. Equation 3.4 shows how obtained viscosity was applied to the Mark-Houwink equation (Costa et al., 2015). The chitosan solution was produced in 0.30 M CH_3COOH /0.20 M CH_3CHOONa with the values $\alpha = 0.76$ and $K = 0.93$.

$$[\eta] = KM_w^{\alpha} \quad \text{Equation 3.4}$$

3.4.6 X-Ray Powder Diffractometer (XRD)

The purity of extracted chitosan was verified through XRD results. X-ray diffraction data 2θ angle were scanned between 5° and 30° . The crystalline index (%) was calculated according to Equation 3.5 where I_{110} is the maximum intensity at $2\theta = 20^\circ$ and I_{am} is the intensity of amorphous diffraction at $2\theta = 16^\circ$ (Hong et al., 2018).

$$\text{Crystalline index (\%)} = [(I_{110} - I_{am}) / I_{110}] \times 100 \quad \text{Equation 3.5}$$

3.4.7 Scanning Electron Microscopy (SEM)

The surface morphology of extracted chitosan was observed by scanning electron microscope using JSM-6390 (JEOL, USA). Before testing, the samples were given a thin gold coating using a sputter coater in a vacuum environment to improve their electrical conductivity.

3.5 Synthesis of Chitosan-Alginate Hydrogel Beads with Urea Entrapment

The hydrogel beads were synthesized following the method outlined by Ablouh et al. (2019), with some modifications. The 17 formulations prepared, based on chitosan, alginate, and a crosslinker, are detailed in Table 3.1. The chitosan–alginate beads were created using the ionotropic gelation technique, with calcium ions serving as the crosslinking agent. Specifically, for Run 1, a crosslinking solution was prepared at room temperature, consisting of 0.55 M CaCl_2 , 5% (w/v) chitosan, and 1% (v/v) acetic acid, which was stirred continuously for 30 minutes to achieve a uniform suspension. Separately, an emulsion (10 mL) containing 2.5 wt% alginate and 1 wt% urea was prepared by dissolving sodium alginate and urea powder in deionized water.

This alginate-urea solution was then administered dropwise into 10 mL of the chitosan-crosslinker solution using a syringe with an 18 G gauge blunt needle, while being stirred mechanically at 500 rpm. The resulting spherical beads were stirred continuously for 2 hours, after which they were collected by centrifugation, washed with deionized water and ethanol, and air-dried overnight. These dried beads were subsequently used in the urea release experiment.

For each formulation, beads were randomly selected, and their diameters were measured using a digital vernier calliper (± 0.01 mm precision) under uniform lighting and magnification conditions to ensure consistency. The average diameter was calculated and reported in micrometres (μm). This method was used consistently across all 17 formulations, and the influence of the syringe gauge and formulation composition on particle size was assessed.

Table 3.1: Experimental design matrix and dependent variables attributed to the factors used for Box-Behnken Design (BBD)

Run	Chitosan molecular weight (kDa)		Alginate (wt%)		Crosslinker (M)	
	X ₁		X ₂		X ₃	
	Coded	Actual	Coded	Actual	Coded	Actual
1	-1	Lowest	-1	2.5	0	0.55
2	1	Highest	-1	2.5	0	0.55
3	-1	Lowest	1	4.5	0	0.55
4	1	Highest	1	4.5	0	0.55
5	-1	Lowest	0	3.5	-1	0.10
6	1	Highest	0	3.5	-1	0.10
7	-1	Lowest	0	3.5	1	1.00
8	1	Highest	0	3.5	1	1.00
9	0	Medium	-1	2.5	-1	0.10
10	0	Medium	1	4.5	-1	0.10
11	0	Medium	-1	2.5	1	1.00
12	0	Medium	1	4.5	1	1.00
13	0	Medium	0	3.5	0	0.55
14	0	Medium	0	3.5	0	0.55
15	0	Medium	0	3.5	0	0.55
16	0	Medium	0	3.5	0	0.55
17	0	Medium	0	3.5	0	0.55

The selection of sodium alginate concentration (2.5–4.5 wt%) and CaCl_2 crosslinker concentration (0.1–1.0 M) was based on preliminary trial experiments and literature guidelines that focus on hydrogel bead formation, structural integrity, and encapsulation performance. Sodium alginate concentrations below 2.5 wt% produced soft, unstable beads with poor mechanical strength and shape retention. In contrast, concentrations above 4.5 wt% led to extremely viscous solutions that were difficult to extrude and tended to clog the syringe nozzle during bead formation. This range (2.5–4.5 wt%) ensures adequate gel strength and bead uniformity while maintaining practical ease of processing. Similar alginate concentrations have been used in fertilizer encapsulation and drug delivery studies to ensure optimal gel formation and swelling behavior (Li et al., 2016; Murata et al., 2020).

The selected CaCl_2 concentration range of 0.10 to 1.00 M ensures sufficient ionic crosslinking of alginate chains via Ca^{2+} –G (guluronic acid) interactions. Concentrations below 0.1 M typically led to incomplete crosslinking and weak bead structure, while concentrations above 1.0 M can result in excessive shrinkage and overly dense beads due to rapid external gelation. The selected range allows tuning of the crosslinking density, porosity, and mechanical rigidity, which are critical for regulating swelling behavior and urea release. This range is also supported by literature, where CaCl_2 concentrations between 0.05 and 1.0 M are commonly used for alginate-based hydrogel synthesis (George & Abraham, 2006; Chan et al., 2011). These specific ranges were also selected to suit the Box-Behnken Design (BBD) framework used for optimization, allowing for reliable modelling of interaction effects across practical low-to-high thresholds for both alginate and crosslinker concentrations.

3.6 Release Behaviour of Urea from Hydrogel Beads in Water

3.6.1 Swelling Study

Swelling studies were conducted at room temperature by immersing 1.0 g of dried beads in 5 mL of distilled water for 2 hours. Afterward, the beads were retrieved, gently blotted with a paper towel, and reweighed. The degree of swelling (S_w) was determined by calculating the dynamic change in the weight of the beads over time, using Equation 3.6.

$$S_w (\%) = (W_t - W_0)/W_0 \times 100 \quad \text{Equation 3.6}$$

where W_t is the weight of the beads in the swollen state at time t and W_0 is the initial weight of the dried beads (Saber et al., 2015).

3.6.2 Encapsulation Efficiency

The encapsulation efficiency of urea in the chitosan–alginate hydrogel beads was calculated by the formula shown in Equation 3.7.

$$EE (\%) = (W_u/W_t) \times 100 \quad \text{Equation 3.7}$$

where W_u is the actual loading of urea and W_t is the theoretical loading of urea in the beads (Takka & Gürel, 2010).

3.6.3 Urea Release Evaluation

After 2 hours of swelling, the solution was filtered to remove any polymeric particles. A 0.5 mL sample of the clear filtrate from the swelling test was analyzed using a UV-visible spectrophotometer to determine the urea content, following the method described by Liu et al. (2020). The urea content was measured at a wavelength of 416 nm, using 4-dimethylaminobenzaldehyde as the coloring agent, based on a standard calibration curve. Each experiment was performed in triplicate.

3.7 Response Surface Methodology (RSM) Analysis

The interactions among variables and optimization of synthesis conditions for chitosan-alginate urea-loaded hydrogel were investigated using Response Surface Methodology (RSM) with a Box-Behnken design (BBD) in R Studio software., as described by Yousefi et al. (2019). The response variables evaluated were encapsulation efficiency and urea release, both measured using a UV-visible spectrophotometer. Three independent variables were selected for the synthesis: (X1) molecular weight of chitosan (mPa.s), (X2) alginate loading (wt%), and (X3) crosslinker concentration (M).

The independent variables were evaluated at three levels: -1 , 0 , and $+1$, with the respective ranges established based on cost factors and initial experiments that set the minimum and maximum values for the process variables. The specific process parameters and their coded levels are outlined in Table 3.1. Chitosan samples with molecular weights of 40 kDa, 74 kDa, and 115 kDa were used, and the amount of alginate added was 2.5%, 3.5%, and 4.5% by weight. Crosslinker concentration was investigated at 0.10, 0.55, and 1.00 M.

Seventeen experiments were conducted in total, which included three replicates at five centre points for the RSM analysis. This approach ensured that the predictions were comparable to the experimental results, facilitating the evaluation of the selected quadratic model. Statistical analysis was performed using analysis of variance (ANOVA) to estimate the interactions between the process variables and the response variables.

3.8 Flowchart of Methodology



Figure 3.1: Flowchart of Methodology

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Physicochemical Properties of NADES

The nine synthesized NADES were evaluated physicochemically. Different composition and synthesis temperature only slightly affected the physical characteristics. The results are presented in Table 4.1.

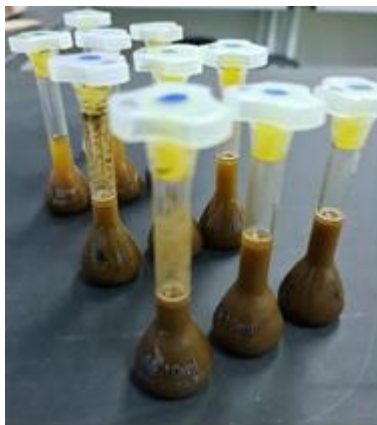


Figure 4.1: Synthesized NADES

The synthesized NADES exhibited a slightly viscous nature at ambient temperature, as shown in Figure 4.1. This higher viscosity compared to water is primarily attributed to extensive hydrogen bonding between the hydrogen bond acceptor (HBA), choline chloride, and the hydrogen bond donor (HBD), acetogenin. These interactions form highly ordered liquid structures with strong directional bonding, resulting in internal cavities and increased resistance to flow (Dai et al., 2013a; Santana et al., 2019; Craveiro et al., 2021). Consequently, the viscosity and density of NADES are higher than those of conventional solvents like water. The results also indicate that synthesis temperature plays a key role in modulating viscosity. As the temperature increases, the viscosity of NADES decreases due

to the disruption of hydrogen bonding networks and reduced intermolecular resistance, allowing the liquid to flow more freely. This inverse relationship between temperature and viscosity is consistent with observations by Mbous et al. (2017), who reported that elevated temperatures reduce structural rigidity in NADES, enhancing their fluidity.

The densities of the nine synthesized NADES were found to be slightly higher than that of water, with values exceeding 1.00 g cm^{-3} . These findings are consistent with previous research by Che Zain et al. (2021) and Hammond et al. (2016), which also observed higher density values for NADES compared to water. The increased density is attributed to the strong intermolecular hydrogen bonding and higher number of -OH functional groups in the hydrogen bond donor (HBD), reducing the free volume. However, the narrow temperature range used in the synthesis of NADES did not significantly affect their density. While synthesis temperature had only a mild effect on density, changes in HBD:HBA ratio had a more noticeable impact.

The polarity of NADES is closely related to their solubilizing ability, which is influenced by the hydrogen bonds between the hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA). The polarity of NADES, indicated by the ENR parameter, showed that all nine NADES had similar polarities, close to that of methanol at $51.98 \text{ kcal mol}^{-1}$. The ENR polarity values of the NADES, ranging close to methanol, indicate moderately high polarity, which is crucial for efficient solvation and interaction with biopolymer substrates like chitin (Abbott et al., 2011). The polarity of NADES is lower than that of water ($48.21 \text{ kcal mol}^{-1}$) (Dai et al., 2013b) due to the presence of organic components in NADES. The polarity of natural deep eutectic solvents (NADES) plays a vital role in the efficiency and selectivity of chitosan extraction from natural sources such as snail shells. The polarity's

role in disrupting hydrogen bonds within chitin matrices, aiding demineralization and deproteinization, has also been emphasized by Zdanowicz et al. (2018) and Qiu et al. (2020).

Chitin, the precursor to chitosan, is a highly crystalline and tightly hydrogen-bonded biopolymer, rendering it insoluble in most conventional solvents. To effectively extract chitosan, the NADES system must possess a polarity high enough to disrupt the intermolecular hydrogen bonds in chitin and break down the surrounding matrix of proteins and minerals such as calcium carbonate (Dai et al., 2013; Zdanowicz et al., 2018). NADES typically consist of a hydrogen bond acceptor (HBA), such as choline chloride, and a hydrogen bond donor (HBD), like organic acids or polyols. The overall polarity of the solvent system determines its ability to interact with polar functional groups ($-OH$ and $-NH_2$) in the chitin structure through hydrogen bonding and dipole–dipole interactions (Abbott et al., 2011). Moreover, a polar NADES system enhances deproteinization and demineralization processes by facilitating the dissolution of proteins and calcium salts from the shell matrix, often eliminating the need for harsh acids like HCl.

However, the polarity must be balanced solvents that are too polar may risk partial degradation of the chitosan polymer, while those with insufficient polarity may fail to disrupt the biomolecular matrix effectively. Therefore, NADES with moderate to high polarity, such as choline chloride–lactic acid or choline chloride–oxalic acid, have demonstrated promising results in extracting chitosan while preserving favorable properties such as high degree of deacetylation and adequate molecular weight (Qiu et al., 2020). However, excessively polar solvents may degrade the polymer; thus, the use of a moderately polar NADES system like 2:1 at 80 °C represents a balanced solution. In summary, optimizing the polarity of NADES is essential not only for enhancing extraction efficiency but also for maintaining the

structural integrity and functional quality of the extracted chitosan in a sustainable and environmentally friendly manner.

Surface tension is a measure of the attractive forces between molecules at the liquid's surface. The surface tension of NADES is lower than that of water, which has a surface tension of 71.7 mN m^{-1} , indicating a reduction in cohesive forces. The lower surface tension observed in NADES compared to water aligns with trends reported by Smith et al. (2014). Three representative NADES samples with a 1:2 ratio, prepared at 40°C , 60°C , and 80°C , exhibited contact angles of 74.7° , 60.3° , and 57.0° , respectively, when placed on snail shells. The contact angles being less than 90° suggest a strong affinity between NADES and the snail shells. This result confirms that NADES have stronger adhesive forces with snail shells but reduced cohesive forces between their molecules.

The observed decrease in contact angle with increasing temperature indicates enhanced wettability and stronger adhesive interaction between the NADES and the snail shell surface. This trend is attributed to the reduction in solvent surface tension and viscosity at elevated temperatures, which improves the spreading behavior of the liquid and facilitates better surface contact (Yuan & Lee, 2013; Drelich et al., 2019). Enhanced wetting behavior can promote more effective penetration of NADES into the shell matrix, thereby improving the potential for chitosan extraction (Smith et al., 2014).

Initially, the 1:2 choline chloride-acetogenin NADES was selected as a representative formulation for contact angle analysis due to its balanced polarity and moderate viscosity, which are ideal for assessing surface interaction with the snail shell substrate. Contact angle measurements are typically used to evaluate the wettability and surface affinity of solvents toward solid materials. The 1:2 ratio was observed to maintain

consistent polarity values and lower surface tension compared to other tested ratios, enabling clearer interpretation of adhesive behavior (Smith et al., 2014; Craveiro et al., 2021).

Additionally, this NADES ratio produced visually stable and homogeneous liquids at all synthesis temperatures tested, which is important for ensuring consistent droplet formation during contact angle measurements (Dai et al., 2013). The temperatures of 40 °C, 60 °C, and 80 °C were chosen to examine thermal effects on wettability without degrading the solvent or altering the substrate surface. These temperature points allowed observation of trends in interfacial behavior, where increasing temperature led to lower contact angles, indicating stronger surface interaction and improved spreadability over the snail shell surface (Hammond et al., 2016). Thus, the 1:2 NADES was selected not for extraction efficiency, but as a stable, representative system to evaluate the surface wetting behavior of NADES on the raw shell matrix.

The thermal properties of NADES were also examined. A representative DSC curve for 2:1 NADES prepared at 80 °C is shown in Figure 4.2. The DSC curves were analyzed to determine the glass transition temperatures (T_g) of NADES at different HBD to HBA ratios and synthesis temperatures. The evaluation revealed that the T_g of NADES ranged from 11.7 °C to 15.3 °C. The results indicated that NADES do not exhibit any of the characteristic peaks of their individual components. DSC analysis confirmed the formation of new eutectic structures through shifts in thermal transition peaks, supporting findings by Francisco et al. (2012), who reported that eutectic systems show thermal properties distinct from their individual components. The DSC thermograms of choline chloride and acetogenin showed endothermic peaks (T_c) at 73.6 °C and 98.9 °C, respectively, which correspond to a phase transition involving crystallographic rearrangement.

Additionally, the endothermic transition of NADES occurs at a lower temperature than that of their components. A shoulder is observed on the high-temperature side of this peak, indicating the evaporation of crystallization water. It has been reported that after the release of crystallization water, NADES convert to their anhydrous form, which melts at 150 °C (Craveiro et al., 2021). Therefore, the thermal stability of NADES suggests they are suitable solvents for extracting chitosan under mild temperature conditions below 100 °C. The increased thermal stability of NADES compared to their individual components, such as choline chloride and acetogenin, is primarily due to the enhanced intramolecular interactions, especially hydrogen bonding, which require more energy to break.

Table 4.1: Physicochemical properties of NADES

NADES (ChCl:Aceto X °C)	Viscosity (mPa.s)	Density (g cm⁻³)	Polarity (kcal mol⁻¹)	Surface tension (mN m⁻¹)	T_g (°C)
1:1 40 °C	12.8	1.0068	51.79	38.7	12.3
1:1 60 °C	39.9	1.0035	51.79	39.2	13.7
1:1 80 °C	18.1	1.0011	51.79	39.7	13.6
1:2 40 °C	12.1	1.0173	51.70	36.0	12.0
1:2 60 °C	44.0	1.0275	51.70	39.3	12.9
1:2 80 °C	23.6	1.0353	51.70	39.3	11.7
2:1 40 °C	16.5	1.0163	51.89	33.4	15.3
2:1 60 °C	25.4	1.0114	51.89	36.5	13.5
2:1 80 °C	55.5	1.0585	51.89	38.5	13.3

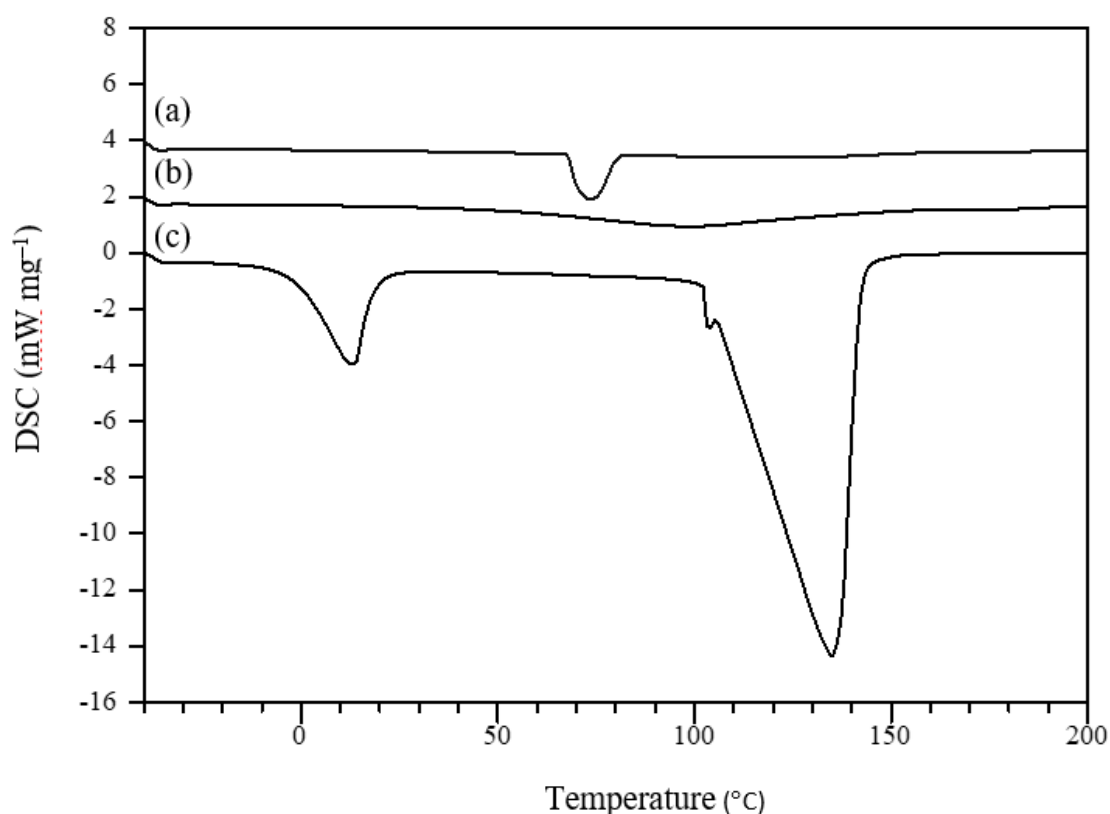


Figure 4.2: Differential scanning calorimetry (DSC) thermograms of (a) choline chloride (HBA) (b) acetogenin (HBD) (c) NADES 2:1 80 °C

Two distinct changes in the FTIR spectrum of the representative NADES, shown in Figure 4.3, compared to the pure components, indicate the formation of a hydrogen bond network. The pure choline chloride exhibits OH bands at 3214 cm^{-1} , while pure acetogenin shows a peak at 3274 cm^{-1} . In the 2:1 NADES prepared at $80\text{ }^{\circ}\text{C}$, these peaks are replaced by a broad band at 3322 cm^{-1} , attributed to a more extensive hydrogen bonding network (Nguyen et al., 2020). Additionally, the overlapping bands from 1500 cm^{-1} to 900 cm^{-1} , present in the pure components, disappear in the NADES mixture.

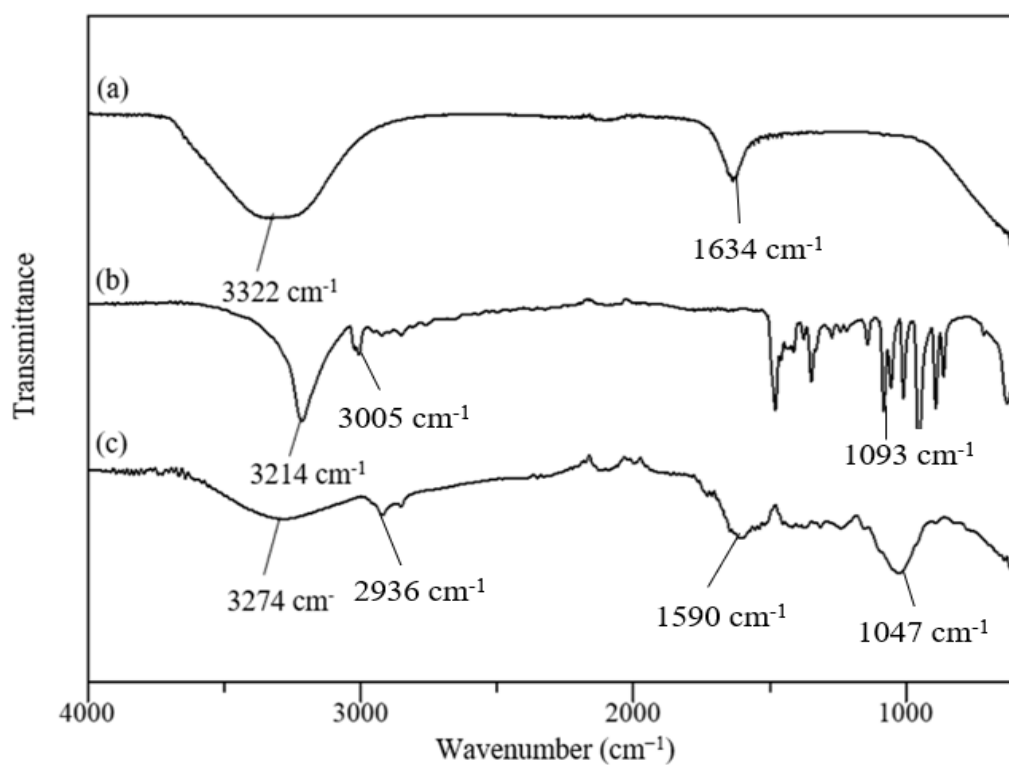


Figure 4.3: FTIR spectrum of (a) NADES 2:1 80 °C (b) choline chloride (HBA) (c) acetogenin (HBD)

4.2 Physicochemical Properties of Extracted Chitosan by NADES

The shells of *Cerithidea obtusa*, a common mud-creeper sea snail found in the mangrove ecosystems of Kuching, Sarawak, are predominantly composed of calcium carbonate (CaCO_3), typically accounting for 85–95% of the total shell weight. The calcium carbonate exists mainly in the form of aragonite or calcite crystals (Zailan et al., 2022). In addition to CaCO_3 , the shells contain 1–5% organic matter, which includes proteins and polysaccharides that contribute to the structural integrity of the shell matrix (Raut & Suryawanshi, 2020). Minor constituents such as magnesium (0.3–1%), phosphorus (0.1–0.5%), and trace elements like sodium, potassium, and strontium (<1%) are also present, influenced by environmental conditions such as salinity, pollution, and the snail's diet (Jusoh et al., 2021). This composition makes *Cerithidea obtusa* shells a viable source for bio-based applications, including the extraction of chitin and chitosan.

The ash and moisture contents of the chitosan samples are presented in Table 4.2. Ash content serves as an essential parameter for assessing the purity of chitosan and the effectiveness of the demineralization process. The ash content of chitosan extracted using NADES ranges from 26.5% to 58.2%. This content largely depends on the source material and its composition (El-araby et al., 2022). The relatively high ash content may be attributed to the use of snail shells, as well as the possibility that the mild conditions of NADES may not completely eliminated minerals from the shells.

The moisture content of the extracted chitosan ranged between 0.4% and 2.9%, significantly lower than the moisture content of shrimp-derived chitosan, which was 9.34% (Puvvada et al., 2012). This difference may be due to variations in the extraction method, which could lower the moisture content in the extracted chitosan. Szymańska and Winnicka

(2015) also recommend that chitosan's moisture content remain low, ideally between 6-10%, to improve its hydrogen-bonding ability. Additionally, a higher water content in the chitosan structure accelerates polymer degradation through hydrolysis reactions (El-araby et al., 2022).

Chitosan is a naturally hydrophilic biopolymer due to the presence of abundant hydroxyl ($-OH$) and amino ($-NH_2$) functional groups along its molecular backbone. This characteristic facilitates excellent water absorption and swelling behavior, which is advantageous in applications such as drug delivery, hydrogels, and wound healing. However, in applications requiring moisture resistance or structural stability, such as controlled release fertilizers, biodegradable films, or tissue scaffolds, this high hydrophilicity becomes a limitation. Excessive water uptake can lead to undesirable effects such as rapid dissolution, loss of mechanical integrity, or premature release of encapsulated compounds (Fan et al., 2021; Zhou et al., 2020).

To overcome this limitation, various surface modification strategies have been employed to tailor the hydrophilicity of chitosan. One common approach is hydrophobic modification, where chitosan is chemically grafted with long-chain fatty acids (e.g., stearic acid) or alkyl groups to reduce its affinity toward water. These modifications decrease the number of free polar groups, thereby lowering its water solubility and swelling capacity (Fan et al., 2021). Another widely used method is crosslinking, in which crosslinking agents such as glutaraldehyde, genipin, or sodium tripolyphosphate (TPP) form intermolecular bridges between chitosan chains. This results in a denser network structure that limits water diffusion and enhances stability in aqueous environments (Zhou et al., 2020; Hafemann et al., 2020).

In addition, blending chitosan with other biopolymers or synthetic polymers such as alginate, polyvinyl alcohol (PVA), or gelatin, has proven effective in modulating its water interaction properties while maintaining biocompatibility (Mujtaba et al., 2019). Another emerging technique involves layer-by-layer deposition or coating of chitosan with hydrophobic polymers or nanoparticles to improve its surface barrier properties without altering its core structure (Fan et al., 2021). Overall, although chitosan's hydrophilicity is intrinsic to its chemical structure, these modification strategies are essential to broaden its application potential in environments where controlled water interaction is critical.

Table 4.2: Physicochemical properties of extracted chitosan from different NADES

ratio

Chitosan from different NADES ratio (ChCl:Aceto X °C)	Ash content (%)	Moisture content (%)	Viscosity	Molecular weight (kDa)	Crystallinity index (%)	Degree of Deacetylation (%)
1:1 40 °C	53.7	2.9	3101	43	25.0	91.9
1:1 60 °C	58.2	1.4	5477	91	22.6	93.3
1:1 80 °C	46.3	0.6	3703	55	27.5	91.8
1:2 40 °C	37.4	1.6	2934	40	30.2	87.6
1:2 60 °C	36.6	1.2	5667	96	18.8	87.9
1:2 80 °C	44.9	0.9	4668	74	18.5	88.1
2:1 40 °C	26.5	0.6	3442	50	36.9	92.9
2:1 60 °C	57.9	0.6	5280	87	26.2	90.4
2:1 80 °C	39.0	0.4	6553	115	22.2	91.0

Chitosan samples were dissolved in an aqueous solution of 0.30 M acetic acid and 0.20 M sodium acetate buffer to determine their intrinsic viscosity. The viscosity and corresponding molecular weight data are summarized in Table 4.2. The calculated molecular weight was found to be approximately 40–115 kDa, which is within the medium molecular weight range and is suitable for hydrogel formation and coating applications. This molecular weight range is consistent with previously reported values for chitosan derived from mollusk or crustacean shells under mild deacetylation conditions (Roberts, 1992). The NADES extraction method successfully produced chitosan with low to medium molecular weight. The results indicate that the degree of deacetylation does not influence the molecular weight of the extracted chitosan.

Based on the FTIR spectrum, the degree of deacetylation, which reflects the percentage of acetyl groups removed during chitosan preparation and the substitution by reactive amino groups, was identified. The extracted chitosan showed a high degree of deacetylation, ranging from 87.6% to 93.3%, indicating that NADES was effective in deacetylating chitin. Previous studies have shown that variations in the degree of deacetylation (DD) of chitosan are influenced by the extraction method and the starting material used (El-araby et al., 2022).

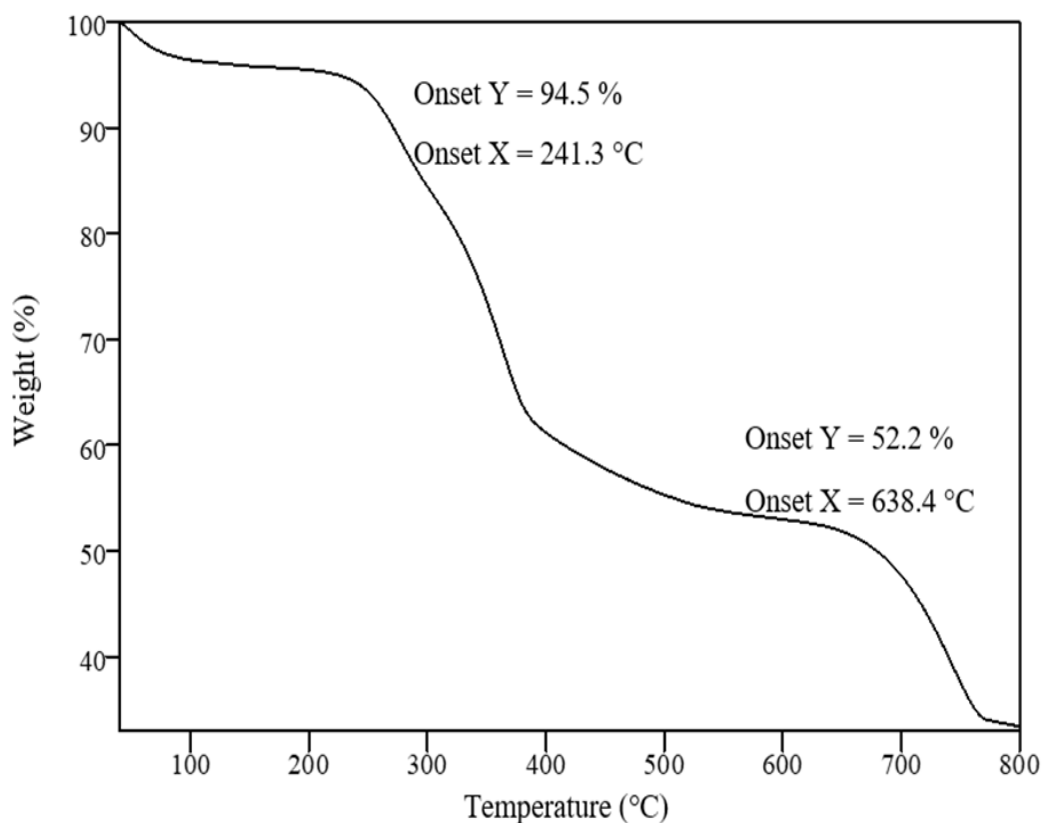


Figure 4.4: TGA curve of chitosan extracted by 2:1 80 °C NADES

The TGA curve in Figure 4.4 for the representative chitosan sample from 2:1 80 °C NADES reveals two degradation stages. The first occurs at 241 °C, with a 5.5% weight loss, corresponding to the loss of water. The weight loss observed between 300 °C and 500 °C is associated with the degradation and deacetylation of chitosan. A third weight loss occurs above 639 °C, with a 47.8% loss, attributed to the oxidative degradation of carbonaceous residues. These findings are consistent with those reported in previous studies (Mohktar et al., 2020).

Three chitosan samples prepared using NADES in a 1:2 ratio at 40 °C, 60 °C, and 80 °C exhibited contact angles of 80.8°, 74.6°, and 75.9°, respectively, when water was applied

to their surface. Since all contact angles are below 90° , it indicates the hydrophilic nature of the extracted chitosan surface.

The characteristic bands in the FTIR spectrum of the extracted chitosan (Figure 4.5(a)) closely match the pattern of commercial chitosan (Figure 4.5(b)). The FTIR results of the extracted chitosan align with those from earlier studies (Ali et al., 2018). The spectrum of extracted chitosan shows a broad band with multiple peaks between $3400\text{--}3000\text{ cm}^{-1}$, representing the stretching of --OH overlapping with --NH_2 groups, as well as intra- and intermolecular hydrogen bonds. A similar broad band is also seen in commercial chitosan.

The peaks between $3000\text{--}2800\text{ cm}^{-1}$ indicate alkyl C-H stretching from --CH_2 groups, as shown by the peaks at 2918 cm^{-1} and 2851 cm^{-1} . Peaks at 1651 cm^{-1} and 1615 cm^{-1} correspond to the carbonyl (C=O) stretching of secondary amide from the residual N-acetyl group and N-H bending of the primary amide (--NH_2). The absorption band at 1149 cm^{-1} represents asymmetric stretching of the C-O-C bridge, while the stretching band of C-O appears at 1030 cm^{-1} in the chitosan spectrum. The peak at 857 cm^{-1} indicates the presence of carbonate groups, although with lower intensity compared to the snail shells. In Figure 4.5(c), the main absorption bands at 1455 cm^{-1} and 857 cm^{-1} in the snail shells are associated with C-O vibrations of the carbonate group (Osseni et al., 2018).

The degree of deacetylation (DDA%) of chitosan was determined using Fourier Transform Infrared (FTIR) spectroscopy by analyzing the relative absorbance intensities of specific functional groups. Conventionally, the amide I band associated with the C=O stretching of acetyl groups appears near 1655 cm^{-1} , while the broad O-H stretching vibration is observed around 3450 cm^{-1} and serves as an internal reference. In this study, however, the FTIR spectrum of the extracted chitosan showed a slight shift in these characteristic peaks,

with the amide I band appearing at 1651 cm⁻¹ and the O–H stretching band at 3326 cm⁻¹. Such shifts are commonly attributed to changes in the hydrogen bonding environment and structural conformation of the chitosan chains following extraction, particularly when using green solvents like NADES. The reduction of acetyl groups during deacetylation affects the electron distribution around the C=O bonds, while residual solvent interactions and film preparation conditions can also influence vibrational frequencies, leading to bathochromic shifts. Despite these shifts, the peaks at 1651 cm⁻¹ and 3326 cm⁻¹ were still suitable for DDA calculation using the Equation 4.1 proposed by Brugnerotto et al. (2001), where 115 is a calibration constant derived from standard chitin-chitosan spectra, as below:

$$DDA(\%) = 100 - \left[\left(\frac{A_{1651}}{A_{3326}} \right) \times 115 \right] \quad \text{Equation 4.1}$$

This method provided a reliable estimation of DDA and confirmed the effectiveness of the NADES-based extraction process. Overall, FTIR analysis supports the feasibility of using NADES for chitosan extraction, promoting the idea of employing NADES as an environmentally friendly alternative to mineral acids in chitosan extraction.

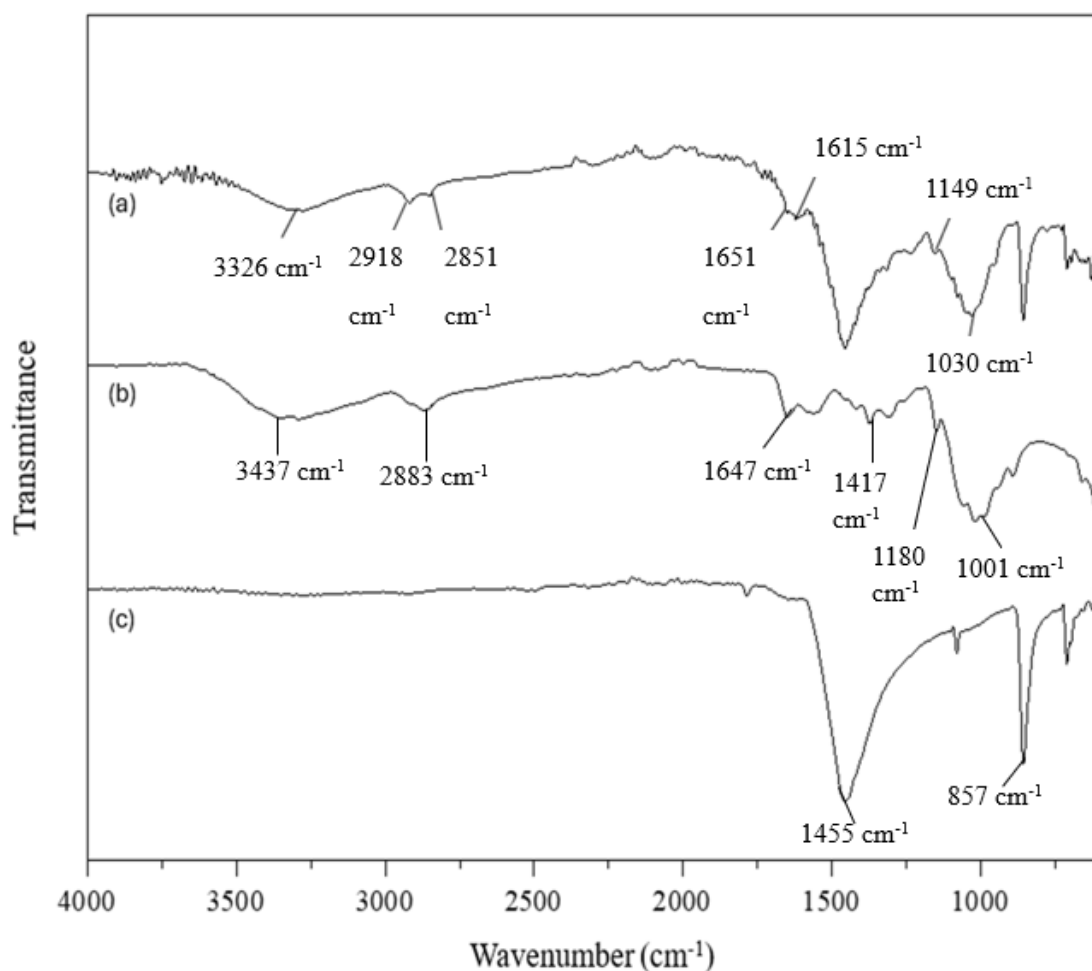


Figure 4.5: FTIR spectra of (a) extracted chitosan from 2:1 80 °C NADES, (b) commercial chitosan and (c) snail shells

The crystalline structures of the extracted chitosan were examined using X-ray diffraction (XRD) within the 2θ range of 5–25°, as shown in Figure 4.6. All samples displayed characteristic broad diffraction peaks at 2θ values around 10.0° and 21.5°, corresponding to the crystalline reflections of (020) and (110), respectively (Hao et al., 2021). These peaks are characteristic of semi-crystalline chitosan, and their intensity decreases as the degree of deacetylation increases. This observation aligns with the α -crystalline structure of chitosan derived from swimming crab (Hao et al., 2021).

XRD analysis was used to assess the crystallinity of the extracted chitosan, with the Crystallinity Index calculated from the intensity of both crystalline and amorphous regions. The extracted chitosan showed low crystallinity, ranging from 18.5% to 36.9%. This reduced crystallinity is attributed to the high degree of deacetylation (DDA) in the chitosan, where the removal of acetyl groups and incorporation of amino groups disrupt the orderly chitin structure, resulting in a more amorphous chitosan structure. Lower crystallinity contributes to several beneficial properties, such as improved solubility in acidic solutions, enhanced biocompatibility, and increased flexibility (Yadav et al., 2023).

The effect of different NADES ratios on the crystallinity profile of the extracted chitosan was examined using X-ray Diffraction (XRD) analysis. Chitosan typically exhibits characteristic peaks around $2\theta \approx 10^\circ$ and 20° , which correspond to its semi-crystalline nature arising from residual ordered regions and amorphous phases (Younes & Rinaudo, 2015). In this study, chitosan extracted using NADES at 1:1 and 2:1 molar ratios (HBA:HBD) exhibited variations in peak intensity and sharpness, which indicate differences in crystallinity.

Specifically, chitosan extracted with the 2:1 ratio at 80°C showed weaker and broader peaks, particularly at 20° , suggesting a greater disruption of the crystalline domains and higher amorphous content compared to the 1:1 sample. This reduction in crystallinity can be attributed to the more efficient hydrogen bonding interactions and improved solvent penetration achieved at the 2:1 ratio, which facilitated enhanced deacetylation and loosening of the chitin structure (Dai et al., 2013). The more amorphous structure obtained is beneficial for subsequent applications such as hydrogel formation, as it correlates with improved solubility, swelling, and interaction with crosslinking agents (Mourya et al., 2010). These

findings support that NADES composition plays a critical role in modulating the structural properties of extracted chitosan, with the 2:1 ratio showing optimal performance in reducing crystallinity.

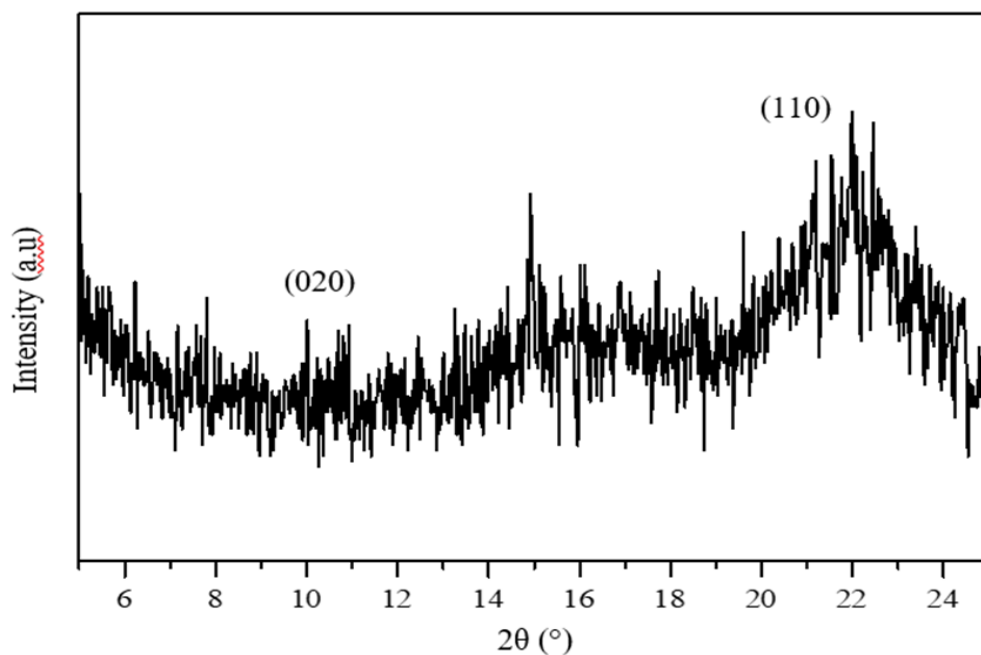


Figure 4.6: XRD pattern of chitosan extracted by 2:1 80 °C NADES

The SEM micrographs displayed in Figure 4.7 illustrate the morphological features of both extracted and commercial chitosan. Both samples revealed similar surface morphologies, exhibiting non-homogeneous, non-porous structures with randomly oriented grains or fiber-like shapes, similar to chitosan derived from horn snail (Rajathy et al., 2021). However, the commercial chitosan had a higher bulk density compared to the extracted chitosan, which may be due to the difference in the source material used for the commercial sample.

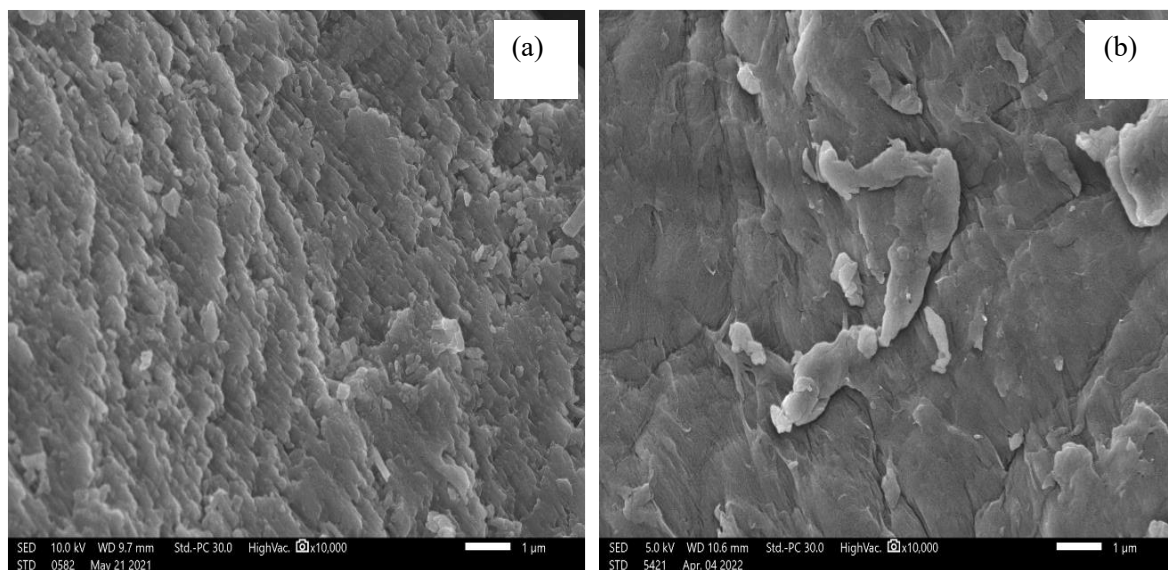


Figure 4.7: SEM micrograph of (a) commercial chitosan and (b) extracted chitosan from 2:1 80 °C NADES

A comparative analysis was conducted to evaluate the properties of chitosan extracted using different choline chloride:acetogenin-based NADES systems, synthesized at varied molar ratios (1:1, 1:2, and 2:1) and temperatures (40 °C, 60 °C, and 80 °C). Each formulation influenced the extraction outcome in terms of yield, molecular weight (MW), and degree of deacetylation (DDA). For instance, chitosan extracted using the 2:1 NADES at 80 °C yielded the high DDA (91%) and molecular weight (115 kDa), indicating efficient deacetylation and minimal polymer degradation. In contrast, lower-performing formulations like 1:2 at 40 °C produced chitosan with noticeably lower DDA and MW, reflecting incomplete matrix disruption and weaker demineralization.

This variation can be attributed to differences in viscosity, polarity, and hydrogen bonding strength across NADES types. The 2:1 NADES provided an optimal hydrogen bond network between choline chloride and acetogenin, contributing to better solubilization and structural penetration during extraction (Dai et al., 2013; Qiu et al., 2020). Structural

characterization supported this trend such as FTIR spectra of chitosan from the 2:1 system showed sharper amide and hydroxyl bands, XRD analysis revealed reduced crystallinity, and SEM images displayed smoother, more porous morphology compared to chitosan from other NADES systems. These findings align with literature where higher HBA content and elevated temperatures have been shown to enhance extraction efficiency by facilitating the breakdown of the chitinous shell matrix (Zdanowicz et al., 2018; Craveiro et al., 2021). Thus, while 2:1 at 80 °C was selected for further analysis due to its superior performance, the comparative results across all formulations provided insight into how NADES composition and synthesis conditions influence the physicochemical quality of extracted chitosan.

4.3 Physicochemical Properties of Chitosan-Alginate Hydrogel Beads

Although a total of 17 hydrogel bead formulations were synthesized and analyzed, only a subset was selected for in-depth discussion based on their relevance to the study's objectives and statistical significance. This selective focus is methodologically justified, particularly in studies involving Response Surface Methodology (RSM), where the goal is to identify and analyze optimal factor combinations rather than evaluate each formulation individually. Including detailed discussions of all 17 formulations would risk overwhelming the core findings with redundant or statistically non-significant data, potentially diluting the clarity and interpretability of the results (Bezerra et al., 2008).

Scientifically, not all formulations exhibited meaningful differences in terms of performance metrics such as particle size, encapsulation efficiency (EE), urea release (UR), or swelling behavior. Many displayed only minor variations within experimental error, making extended discussion unnecessary from a comparative standpoint. Moreover, formulations at the lower or upper extremes of the factor ranges often served only to define the design space boundaries and were not intended to yield optimal results. Hence, the discussion was concentrated on formulations that demonstrated significant improvements and that informed the selection of optimal conditions. This approach aligns with best practices in formulation optimization studies, where detailed interpretation is typically reserved for statistically relevant or practically superior formulations (Montgomery, 2017; Liu et al., 2020).

Lastly, the practical implications of the research such as developing an efficient and sustainable slow-release fertilizer system, necessitated a focus on hydrogel formulations with superior performance. Highlighting only the most effective combinations ensures that

the findings are directly applicable and relevant to potential agricultural applications, rather than burdening the discussion with suboptimal or inconsistent data sets.

4.3.1 Hydrogel Particle Size Measurement

The average particle size of the 17 hydrogel bead formulations varied between 1909 to 6121 μm , with noticeable but moderate differences across the formulations. One of the primary contributing factors to this variation was the use of an 18-gauge syringe during bead formation, which produced larger droplets that gelled into beads exceeding 1.00 mm in diameter. As highlighted by Saber-Samandari and Khezri (2015), bead size is highly dependent on the droplet formation method, where larger syringe diameters or pipettes typically yield larger particles. This method-dependent influence is crucial in ionotropic gelation, where the droplet size entering the crosslinking solution directly dictates the final bead dimensions.

Beyond the syringe diameter, other formulation parameters also played significant roles in particle size variation. For instance, higher sodium alginate concentrations led to increased solution viscosity, resulting in slower droplet detachment and larger bead formation. Meanwhile, varying concentrations of calcium chloride altered the gelation rate and bead rigidity, higher concentrations promoted stronger crosslinking, potentially enlarging the beads through structural expansion. These combined factors, including droplet size, alginate viscosity, and crosslinking intensity, contributed synergistically to the observed size differences across formulations (Natarajan et al., 2018; Hu et al., 2021). Therefore, while the syringe size had a major influence, the interplay of all these parameters was critical in shaping the final hydrogel particle sizes.

4.3.2 Fourier Transform Infrared-Attenuated Total Reflection (FTIR-ATR)

FTIR spectroscopy was conducted to analyze the characteristics of each polymer used in the hydrogel formulation, as well as potential intermolecular interactions between chitosan and alginate that could influence the physical properties of the hydrogel. The FTIR spectra of the extracted chitosan, commercial alginate, urea, and the hydrogel bead are presented in Figure 4.8.

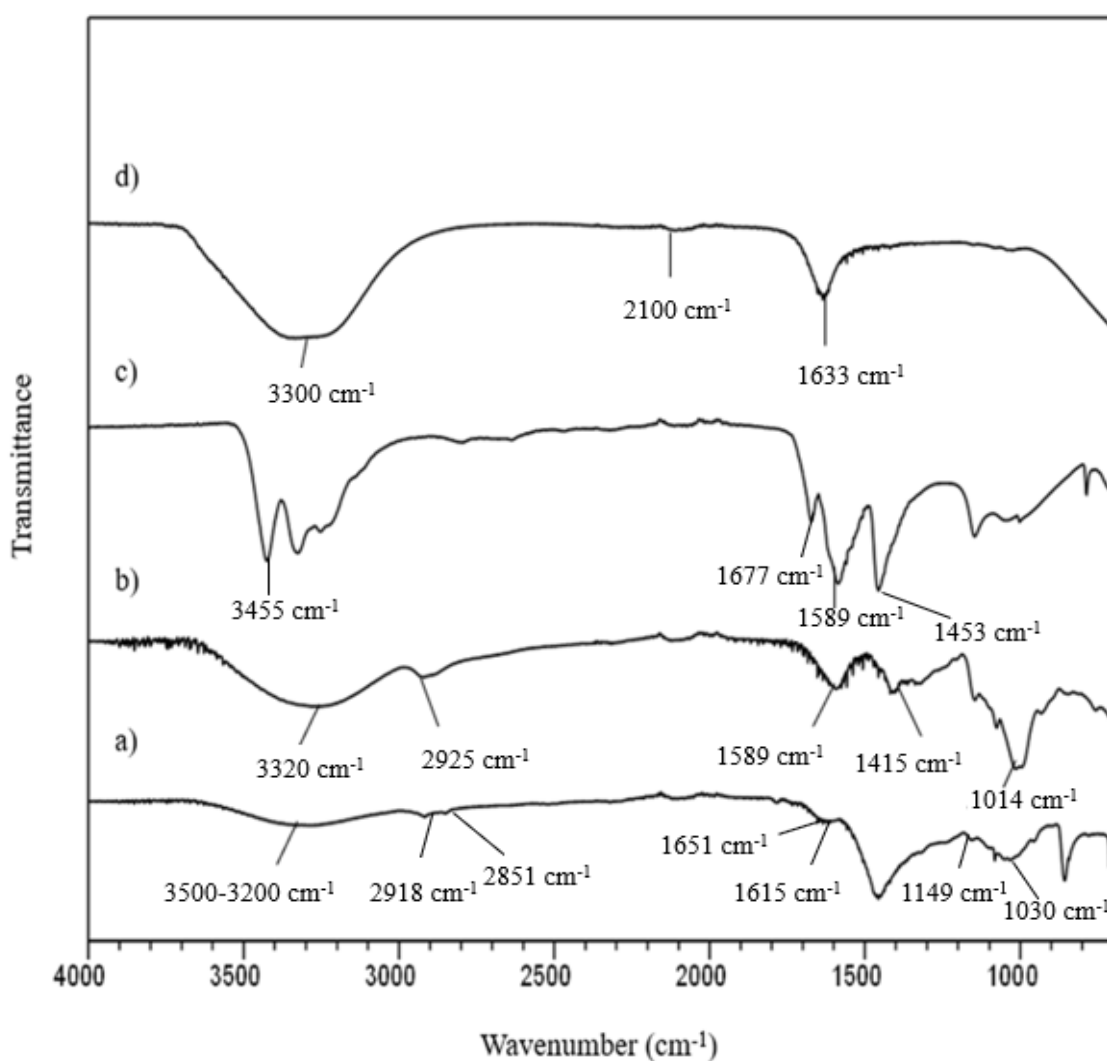


Figure 4.8: FTIR spectra of (a) extracted chitosan (b) commercial alginate (c) urea and (d) hydrogel bead from Run 7

The FTIR spectrum of the chitosan with the lowest molecular weight (Figure 4.8(a)) displayed a broad band between 3500-3200 cm^{-1} , indicating the stretching of $-\text{OH}$ groups overlapping with $-\text{NH}_2$ groups, as well as intermolecular and intramolecular hydrogen bonds. The chitosan characteristic peaks were observed at 2924 cm^{-1} (C-H stretching), 1646 cm^{-1} (C=O stretching of amide I), 1459 cm^{-1} ($-\text{OH}$ bending), and 1100 - 990 cm^{-1} (C-O stretching) (Franco et al., 2020). Figure 4.8(b) shows the characteristic peaks for sodium alginate, where the $-\text{OH}$ group displays a broadened band at 3320 cm^{-1} and 1415 cm^{-1} due to hydrogen bonding. The C-H stretching of alkyl vibrations is noted at 2925 cm^{-1} , with $-\text{COO}$ asymmetric stretching at 1589 cm^{-1} indicating carbonyl groups, and 1014 cm^{-1} for carboxyl and ether vibrations. These absorption features were also reported in alginate-cellulose-kaolin composites (Sunardi et al., 2021).

All hydrogel bead formulations exhibited similar FTIR patterns. For instance, Run 7, as shown in Figure 4.8(d), demonstrates two prominent peaks indicative of alginate and urea, each with varying transmittance intensities. The FTIR spectrum displays a strong, broad absorption band around 3300 cm^{-1} , corresponding to $-\text{OH}$ group stretching vibrations akin to those in alginate, and suggests a high-water content associated with these groups (Zhuo et al., 2017). The peak at 2100 cm^{-1} , characteristic of urea (Figure 4.8(c)), was also present in the hydrogel spectrum, indicating urea's incorporation into the hydrogel. Crosslinking between chitosan and alginate occurred through CaCl_2 , with the asymmetric band of carboxylate anions at 1589 cm^{-1} shifting to 1633 cm^{-1} in the alginate-chitosan hydrogels. This shift indicates electrostatic interactions between the negatively charged carboxyl groups in alginate and the positively charged amino groups in chitosan.

4.3.3 Scanning Electron Microscope (SEM)

Figure 4.9 shows digital and scanning electron microscope (SEM) images of chitosan-alginate beads. Immediately after preparation, the Run 7 beads (Figure 4.9(a)) displayed a regular shape with homogeneous dimensions immediately following preparation. They exhibit transparency and a consistently smooth surface. Hydrogel beads instantly swell and maintain their form when water is added. This can be explained by the interactions between the chitosan and alginate chains that form a crosslinked structure, which provides mechanical strength, restricts the absorption of fluid, and prevents the structure from breaking down. The beads retain a consistent weight throughout the duration of the test. Drying did not alter the bead shape but resulted in reduced dimensions and introduces changes to the surface properties, making it uneven and wrinkled, as shown in the digital image (Figure 4.9(b)) and SEM images (Figure 4.9(d)). This size reduction is due to water loss, which decreases the distance between polymer chains previously occupied by water (Digatis et al., 2022). As a result, the drying process changes the spherical shape of the beads, making them smaller and rougher which probably because of the sticky nature of chitosan (Mati-Baouche et al., 2014).

The fully swollen beads from Run 7 are shown to have a regular shape and a smooth surface in Figure 4.9(c). When the chitosan-alginate hydrogel systems are dry but come into contact with water, they are capable of rehydrating and swelling. This swelling is primarily due to the hydration of the hydrophilic groups in the polymers. Water penetrates the polymer chains, causing the beads to expand without breaking apart (Lukitowati & Indrani, 2018). The significant degree of crosslinking within the hydrogel provides effective resistance against the leakage of urea molecules through the hydrogel membrane (Lum et al., 2016).

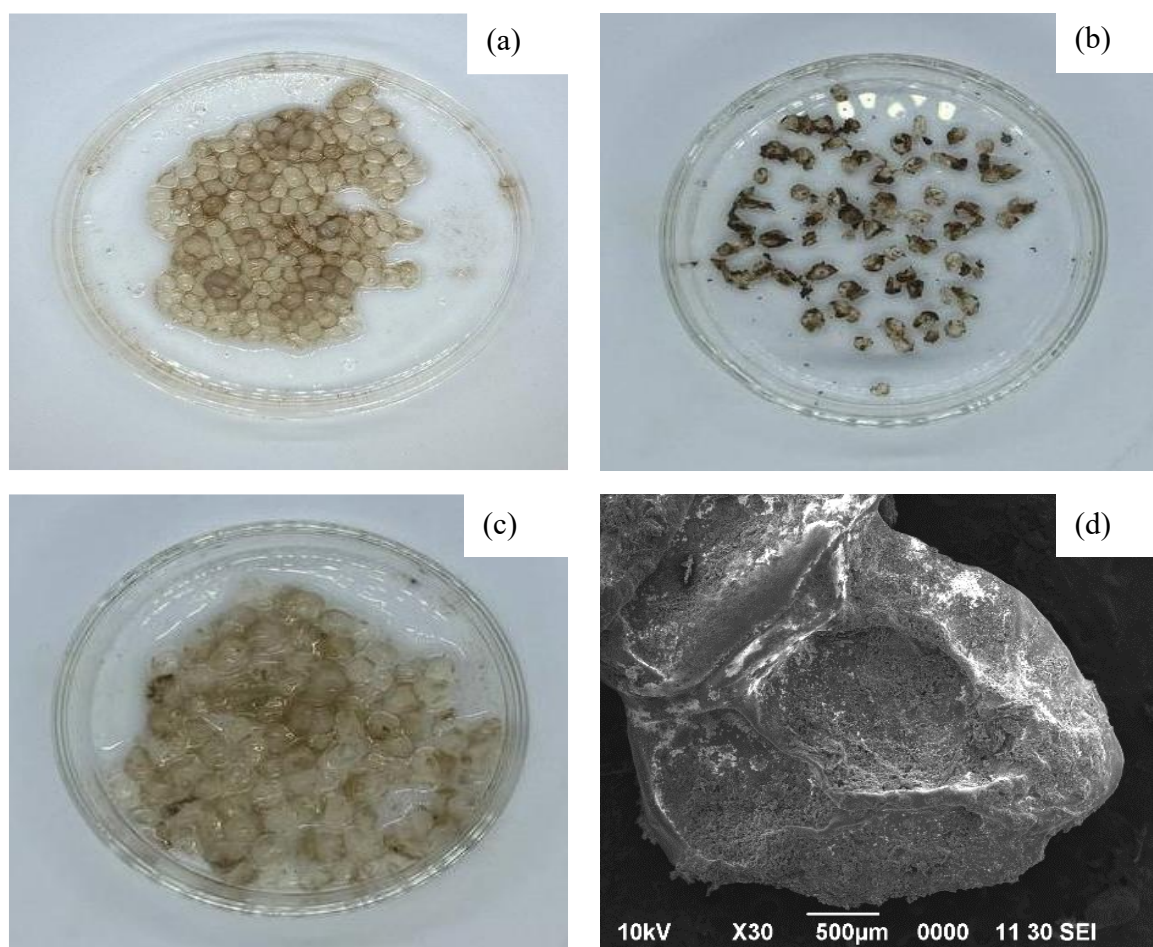


Figure 4.9: Hydrogel beads (a) air dried condition (b) oven dried condition (c) swelling condition and (d) SEM image of dry bead

4.4 Response Surface Methodology (RSM) Analysis

The hydrogel beads from all 17 runs are evaluated in terms of their swelling ability, encapsulation efficiency, and urea release activity.

4.4.1 Swelling Properties of Hydrogel Beads

The swelling behavior varies among different formulations, with Run 11 achieving the highest swelling degree of 77% after 2 hours, as shown in Figure 4.10. Limited swelling in some cases can be attributed to the formation of hydrogen bonds, which enhance structural stability and hinder the penetration of additional fluid into the deeper layers of the particles, thereby restricting their expansion. Upon contact with water, the hydrogel beads swell immediately while retaining their structure. This is due to the interactions between chitosan and alginate crosslinking chains, which contribute to mechanical resistance, limit fluid absorption, and prevent structural disintegration. The beads maintain a constant weight throughout the test period. The hydrogels show significant variation in water absorption capacity, ranging from 11% (Run 10) to 77% (Run 11). Similar variations have been observed in other studies, potentially linked to the degree of deacetylation of the chitosan used (Remedio et al., 2019). Due to differences in chitosan molecular weight, alginate amount, and crosslinker concentration in the hydrogels, isolating the effect of each variable on swelling capacity proves challenging.

The swelling capacity differs for each formulation, with Run 11 reaching the highest swelling degree of 77% after 2 hours, as illustrated in Figure 4.10. The hydrogels demonstrate significant differences in their water absorption capacity, with values ranging from 11% (Run 10) to 77% (Run 11). The limited swelling behavior of these hydrogels may be due to the formation of hydrogen bonds, which enhance structural stability and hinder the

infiltration of additional fluid into the deeper layers of the particles, thus restricting their swelling. As Run 7 has the highest encapsulation efficiency and lowest urea release, it can be deduced that the reduced diffusivity of urea correlates with the low water content in Run 7 hydrogel. It was previously observed that the release of plasmid from hydrogels was slower when the hydrogels were more resistant to deformation and had a lower water content (Comez and Ozbas, 2023).

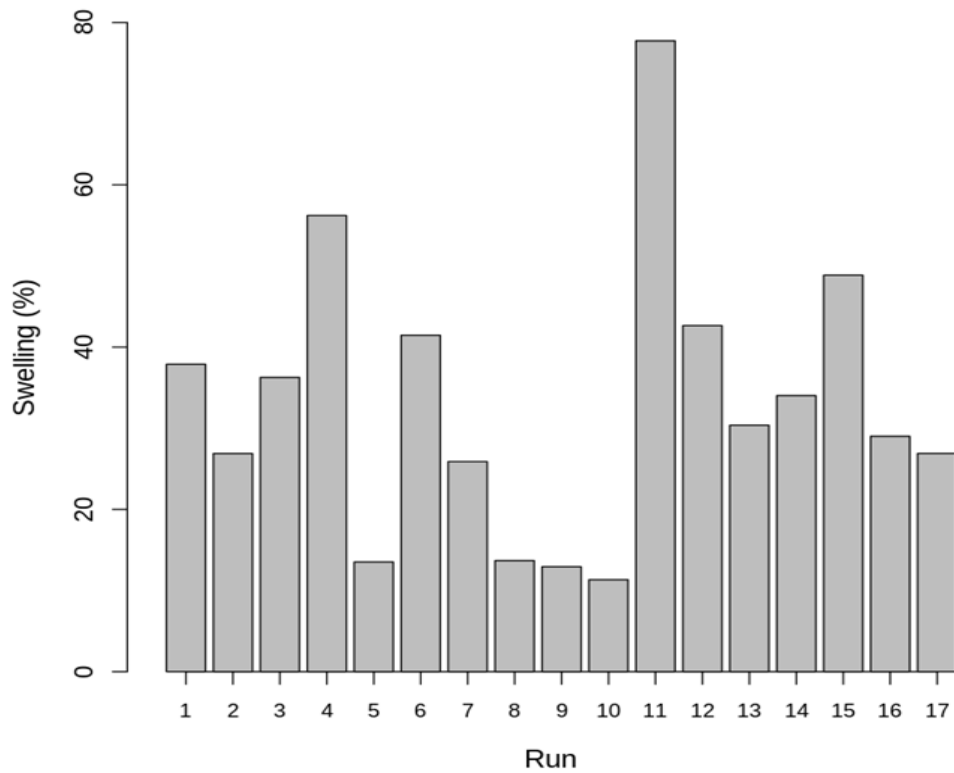


Figure 4.10: Swelling (%) of each run of the hydrogel beads

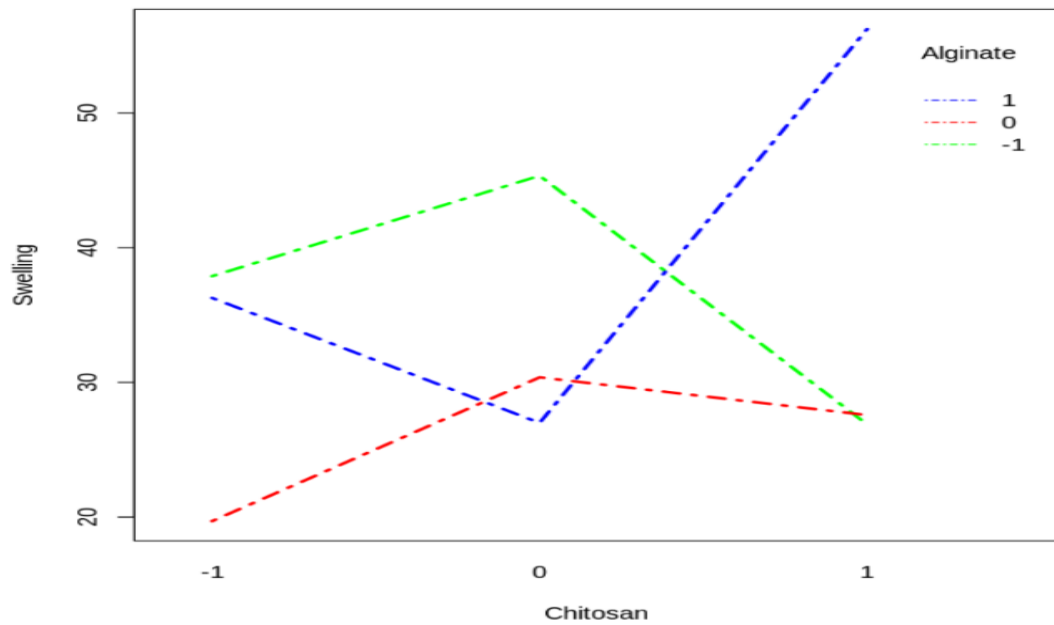
Based on the interaction plot in Figure 4.11, the effect of each variable on swelling changes its trend when highest amount is used in experimental run. In Figure 4.11(a), adding amount of chitosan from lowest to medium initially improves the swelling ability when alginate of 2.5 wt(%) (lowest) and 3.5 wt(%) (medium) were used but degrades to about 25% when alginate of 4.5 wt% (highest) was used. In addition, the swelling response

suddenly spikes to highest value when highest level of chitosan molecular weight was used together with 4.5 wt% of alginate. Moving to crosslinker, the swelling ability magnifies drastically from 10% to 30% when the crosslinker increases from 0.10 M to 0.55 M. By adding chitosan molecular weight, the swelling capacity were not improved when these amount of crosslinker were used. The effect of chitosan towards swelling only surfaced when the crosslinker of 1.00 M was used. The acute drop of swelling when crosslinker of 1.0 M was used indicates that the medium level of chitosan molecular weight is sufficient to maintain swelling ability of urea. Figure 4.12 and Figure 4.13 show these interactions in the form of concurrent effects.

Given the uncertain trend in swelling responses produced by different combination of chitosan molecular weight, amount of alginate, and amount of crosslinker in the prepared hydrogels, isolating the impact of each variable on the swelling capacity is difficult. Results in Table 4.3 further supports the findings with statistically insignificance of model with p-value at 0.6481 and a statistical significance in the lack of fit ($p = 0.03 < 0.05$) with best of fit Equation 4.2 given as:

$$Y_{SW} = 33.8357 + 3.0860X_1 - 1.1225X_2 + 10.0922X_3 + 7.7385X_1X_2 - 10.0358X_1X_3 - 8.3733X_2X_3 - 3.5241X_1^2 + 9.0031X_2^2 - 6.6725X_3^2 \quad \text{Equation 4.2}$$

(a)



(b)

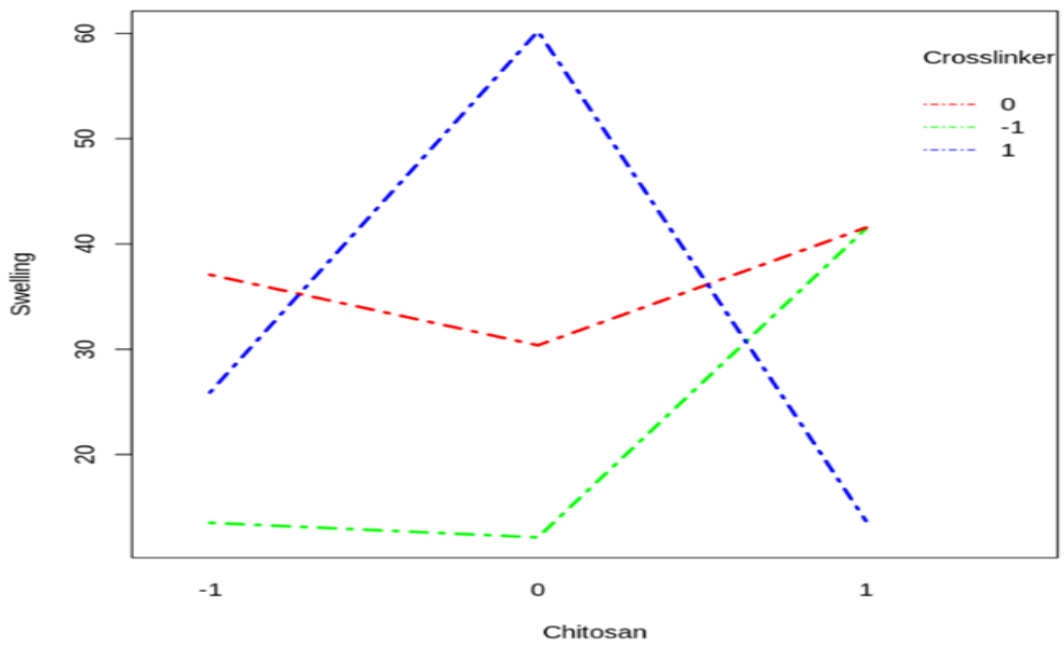


Figure 4.11: Interaction plot between (a) chitosan (X1) and alginate(X2) for swelling response (b) chitosan (X1) and crosslinker(X3) for swelling response

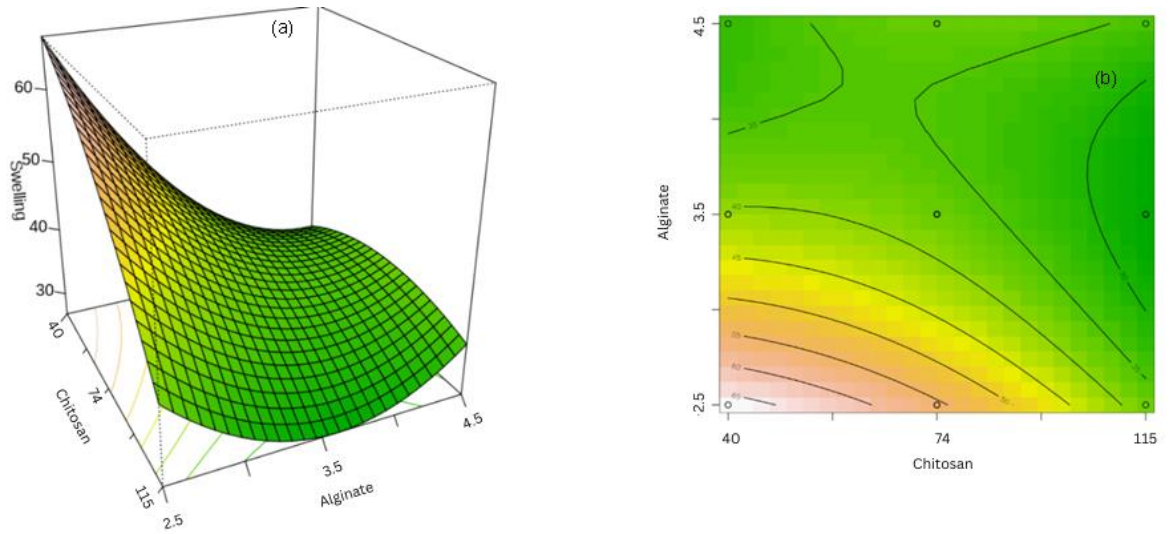


Figure 4.12: (a) Surface plot of concurrent effects of chitosan(X1) and alginate(X2) on the swelling response (b) Contour plot of concurrent effects of chitosan(X1) and alginate(X2) on the swelling response

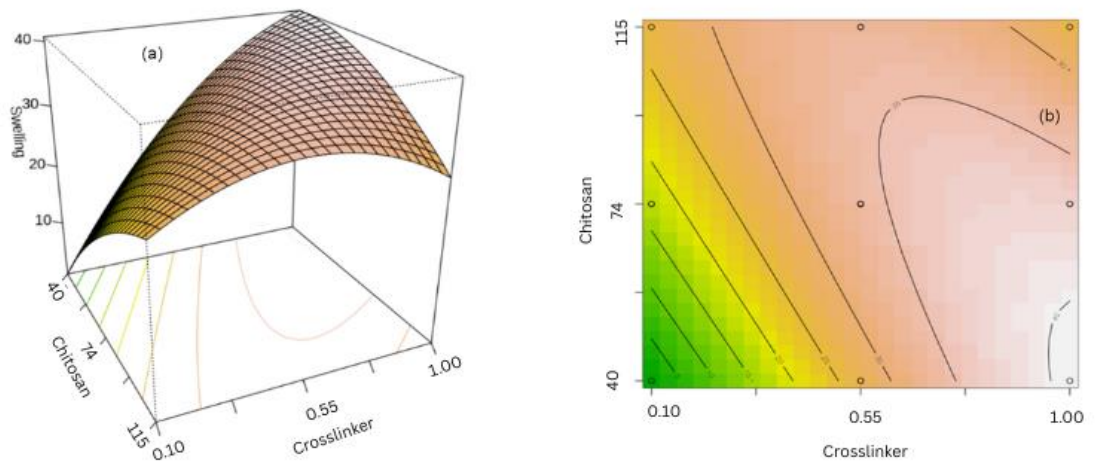


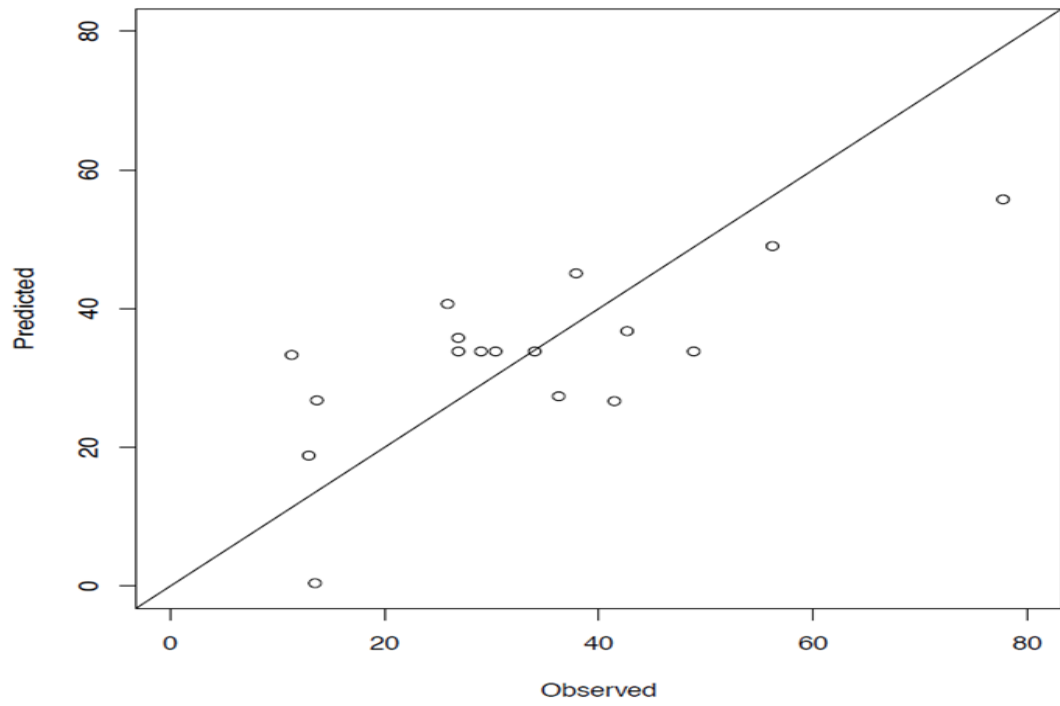
Figure 4.13: (a) Surface plot of concurrent effects of chitosan(X1) and crosslinker(X3) on the swelling response (b) Contour plot of concurrent effects of chitosan(X1) and crosslinker(X3) on the swelling response

Table 4.3: ANOVA results for the quadratic model of response surface for swelling

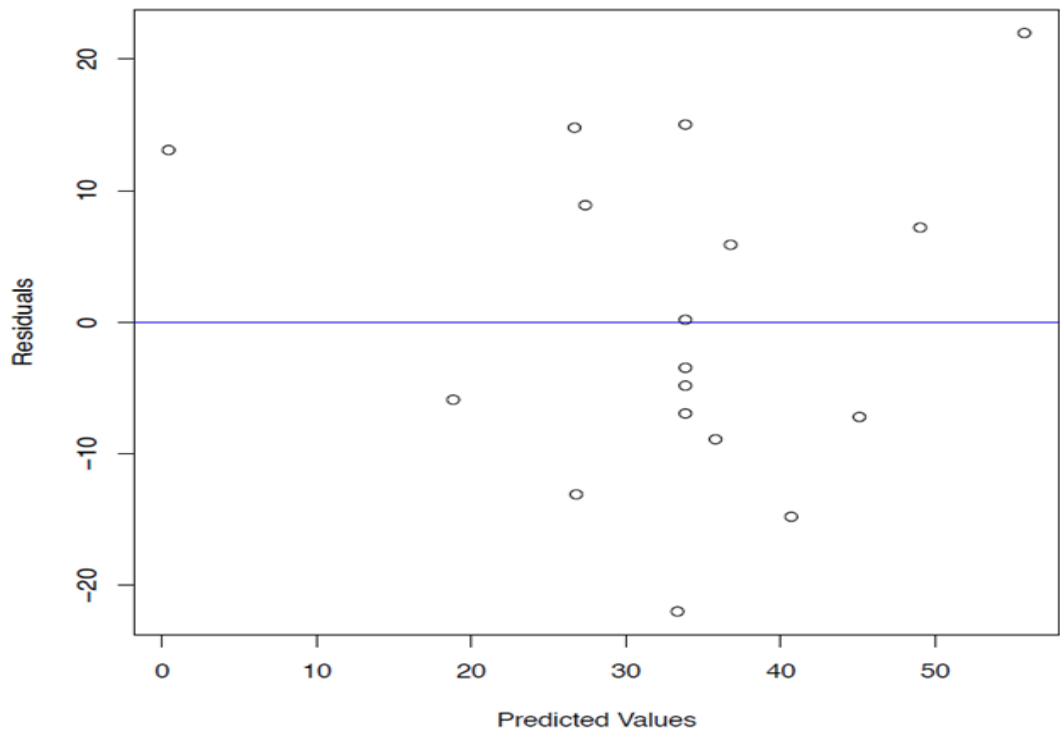
Source	Sum of Square	Degree of Freedom	Mean Square	F Value	p-value
Swelling Model		7		0.7738	0.6481
X_1, X_2, X_3	901.08	3	425.42	0.8803	0.4959
$X_1 X_2$	922.85	3	217.71	0.9016	0.4868
X_1^2, X_2^2	552.33	3	865.36	0.5396	0.6702
Residuals	2388.34	7	107.35		
Lack of Fit	2079.02	3	144.49	8.9618	0.03006
Pure Error	309.32	4	51.63		

Since the value of adjusted R^2 for the swelling model is low, the swelling response is not statistically dependent to the three variables studied. The discrepancy between observed data and predicted data together with residual studies are shown in Figure 4.14.

(a)



(b)



(c)

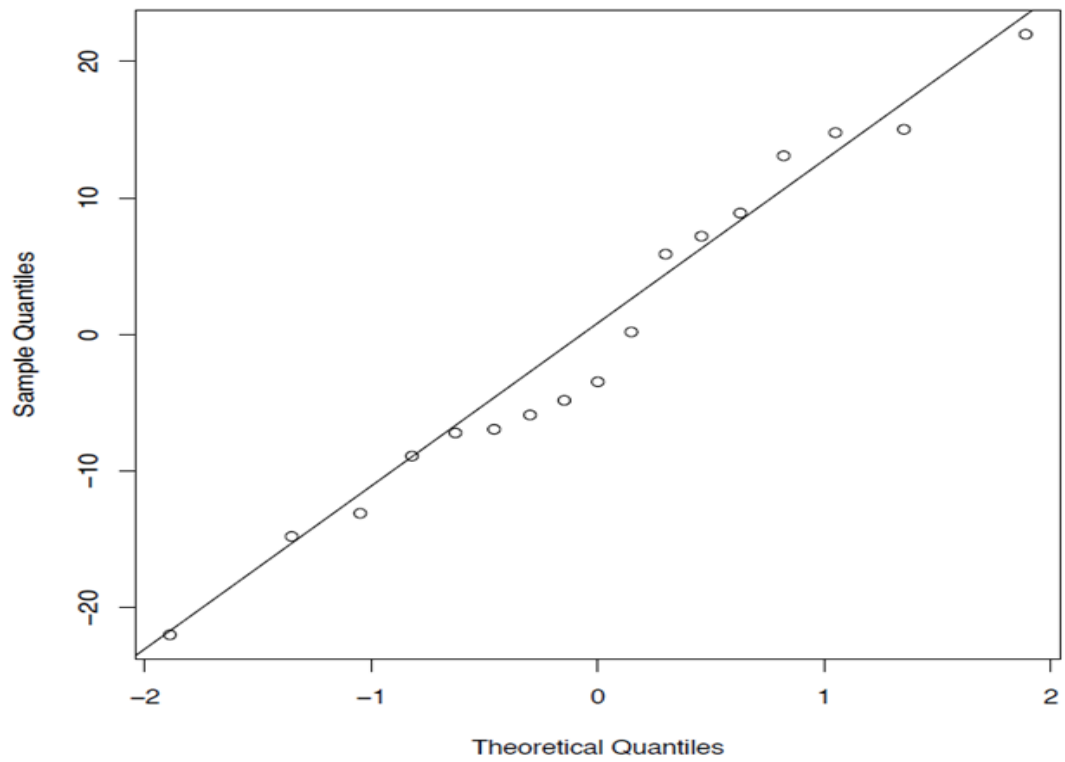


Figure 4.14: (a) Predicted values for swelling vs observed values (b) Residual plot of predicted swelling (c) Normality of residual for swelling experiments

4.4.2 Encapsulation Efficiency of Hydrogel Beads

All formulations demonstrate a high level of urea encapsulation, as shown in Figure 4.15. Generally, the urea is evenly distributed throughout the excipient matrix. However, two formulations (Run 3 and Run 10) show lower encapsulation efficiency. Both runs involve the use of 4.5 wt% of alginate. An increase of alginate from 2.5 wt% to 4.5 wt% magnifies the effect of molecular weight of chitosan and crosslinker on encapsulation efficiency, increasing it from 55% to 85%, as shown in Figure 4.15. Alginate concentrations higher than 2.5 wt% tend to maximize the encapsulation efficiency.

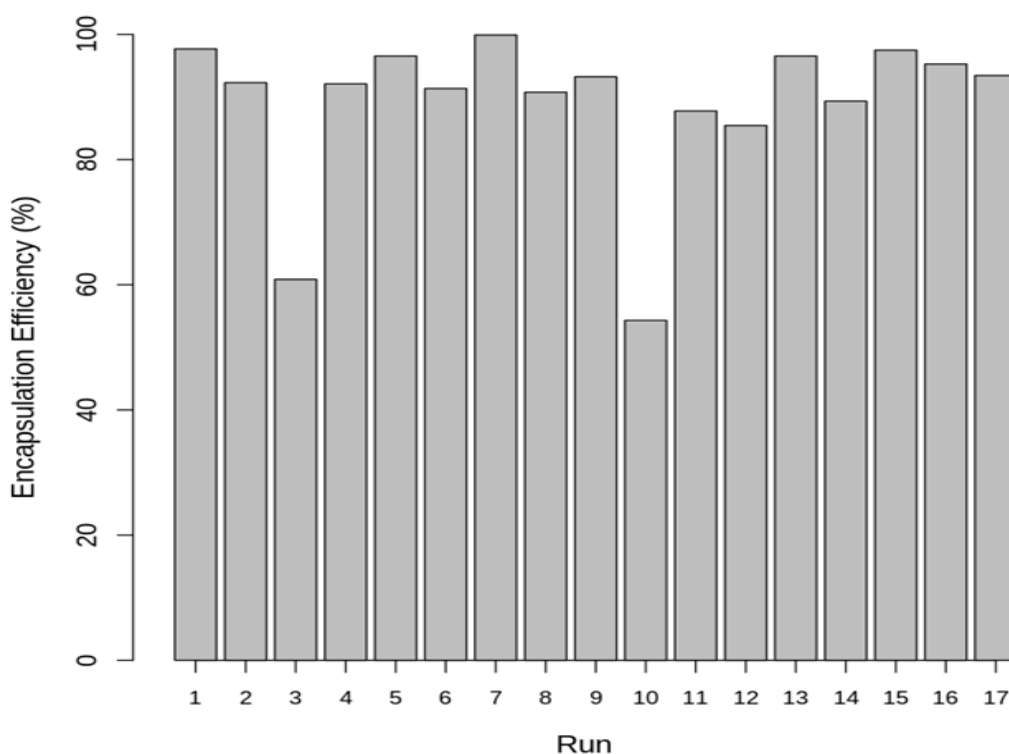


Figure 4.15: Encapsulation efficiency (%) of each run of the hydrogel beads

Table 4.4 presents the complete matrix of the Box-Behnken experimental design for the three variables tested, along with the observed responses for encapsulation efficiency. To analyze the interactions between the independent variables and the responses of interest, a quadratic polynomial model was employed. By integrating response surface methodology with the Box-Behnken design and performing numerical optimization of the overall desirability function, the optimal conditions for maximizing encapsulation efficiency were determined.

For the encapsulation efficiency response, the Equation 4.3 with the best fit is expressed in terms of the following variables: (X1) molecular weight of chitosan (mPa.s), (X2) alginate loading (wt%), and (X3) crosslinker loading (M).

$$Y_{EE} = 94.51950 - 9.78213X_2 + 9.15446X_1X_2 + 9.15180X_2X_3 - 11.55596X_2^2 \quad \text{Equation 4.3}$$

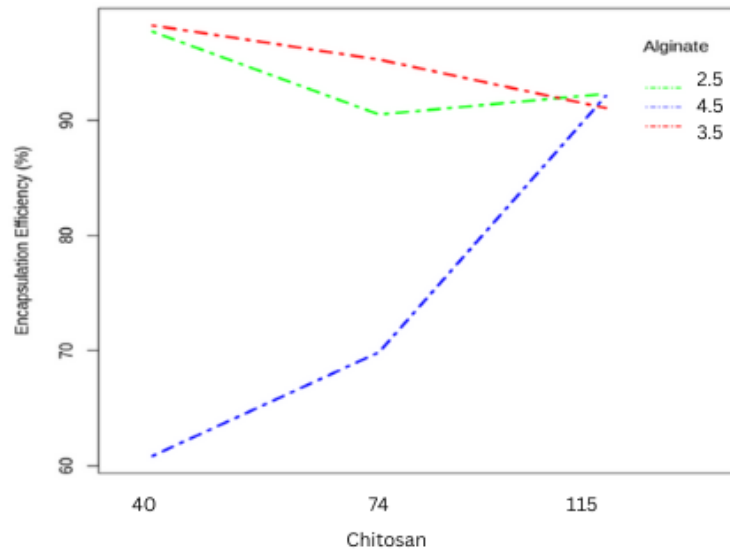
Table 4.4: Experimental design matrix with response observed (Y) and predicted values (Predicted)

Run	X ₁ (kDa)	X ₂ (wt%)	X ₃ (M)	Y _{EE} (%)	Predicted Y _{EE} (%)	Y _{UR} (%)	Predicted Y _{UR} (%)	Y _{SW} (%)	Predicted Y _{SW} (%)
1	40	2.5	0.55	97.6855	101.9001	16.8233	10.5005	37.8875	45.0897
2	115	2.5	0.55	92.2966	83.5912	4.2982	13.0861	26.8856	35.7848
3	40	4.5	0.55	60.8710	64.0269	53.1831	41.8317	36.2669	27.3678
4	115	4.5	0.55	92.0992	82.3358	11.1479	14.9073	56.2189	49.0168
5	40	3.5	0.10	96.5504	89.9781	3.0646	18.7885	13.5174	0.42512
6	115	3.5	0.10	91.3589	91.9521	6.8646	6.6191	41.4580	26.6688
7	40	3.5	1.00	99.9062	99.0608	2.1664	4.1168	25.8918	40.6811
8	115	3.5	1.00	90.7667	97.0868	4.2491	-8.0526	13.6892	26.7815
9	74	2.5	0.10	93.2342	98.3431	41.2017	33.8326	12.9332	18.8233
10	74	4.5	0.10	54.3076	60.4752	53.3910	50.4088	11.3336	33.3250
11	74	2.5	1.00	87.7663	87.1482	14.2568	19.1609	77.7456	55.7542
12	74	4.5	1.00	85.4469	85.8875	25.1628	35.7371	42.6528	36.7627
13	74	3.5	0.55	96.5504	94.5195	21.5113	20.0714	30.3744	33.8357
14	74	3.5	0.55	89.3455	94.5195	10.6544	20.0714	34.0251	33.8357
15	74	3.5	0.55	97.4881	94.5195	20.7215	20.0714	48.8665	33.8357
16	74	3.5	0.55	95.2674	94.5195	21.6592	20.0714	29.0147	33.8357
17	74	3.5	0.55	93.4415	94.5195	30.9371	20.0714	26.8980	33.8357

*EE: encapsulation efficiency; UR: urea release; SW: swelling study

For the encapsulation efficiency response, the linear term of alginate (X2) was identified as the most significant factor ($p = 0.001264$), followed by its quadratic term ($p = 0.003417$). While the molecular weight of chitosan and the amount of crosslinker did not directly impact the response, they influenced how the encapsulation efficiency varied with changes in alginate concentration, as demonstrated in Figure 4.16(a) and (b). Interaction terms also had notable effects, with the interaction between chitosan and alginate being particularly significant ($p = 0.014918$), closely followed by the interaction between alginate and crosslinker ($p = 0.014939$). Notably, a higher encapsulation efficiency was noted as the amount of alginate increased while keeping the crosslinker at its maximum level. Additionally, when the alginate concentration was fixed at 3.5 wt%, increasing the chitosan molecular weight resulted in more consistent encapsulation efficiency, indicating a potential optimal point.

(a)



(b)

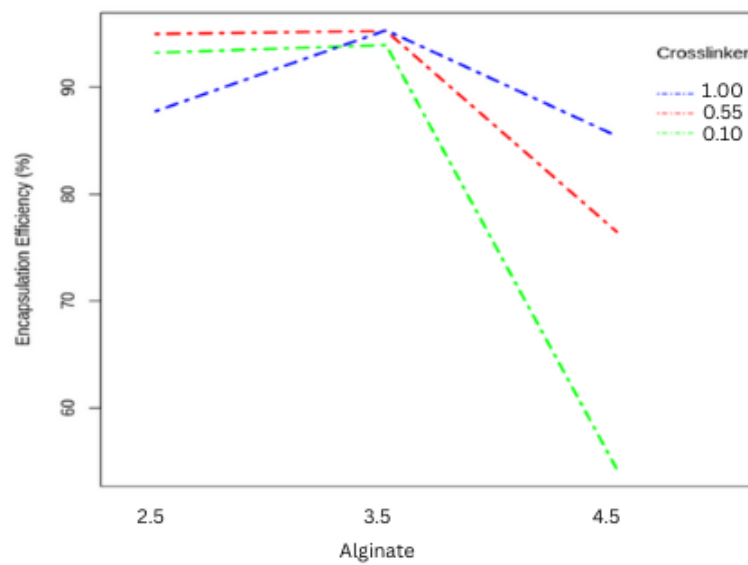


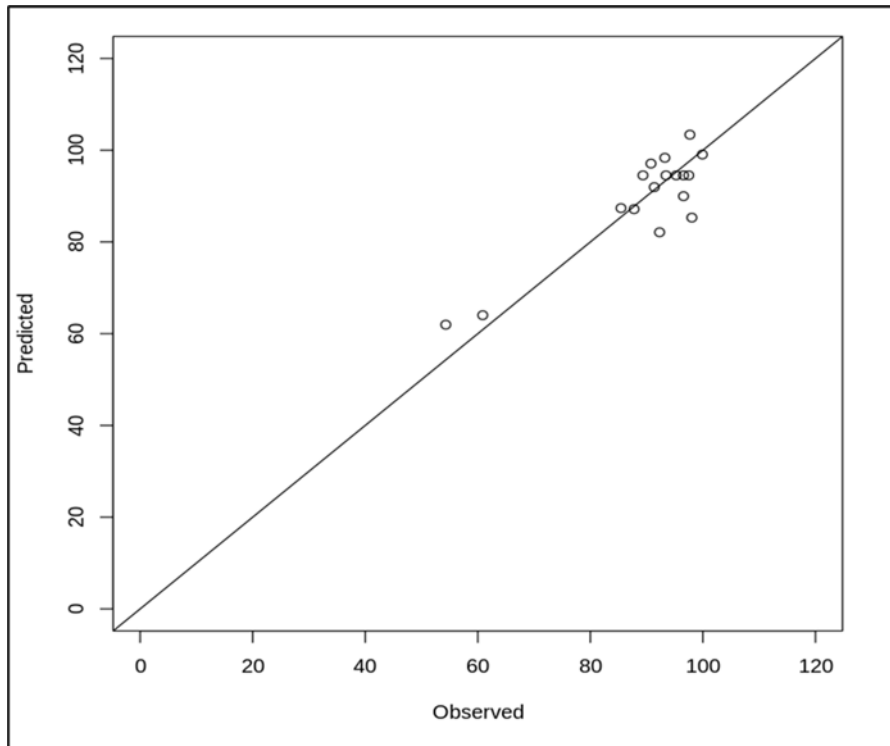
Figure 4.16: Interaction plot between (a) chitosan (X1) and alginate(X2) for encapsulation efficiency response and (b) alginate(X2) and crosslinker(X3) for encapsulation efficiency response

The determination coefficients for the encapsulation efficiency model, specifically the multiple R^2 and adjusted R^2 , were found to be 0.844 and 0.7505, respectively. The difference between these values was less than 0.2, and the model's p-value was 0.001468 ($p < 0.01$), with a high p-value for lack of fit ($p = 0.593438$). These statistical values suggest that the model accurately describes the behavior of encapsulation efficiency with high statistical significance, as summarized in Table 4.5. The random distribution of residuals, shown in Figure 4.17(a)-(c) for the encapsulation efficiency experimental results, supports this assessment. The combined effects of chitosan and alginate on the response, while keeping the crosslinker at its maximum level, are illustrated in the 3D surface plots in Figure 4.18(a) and (b).

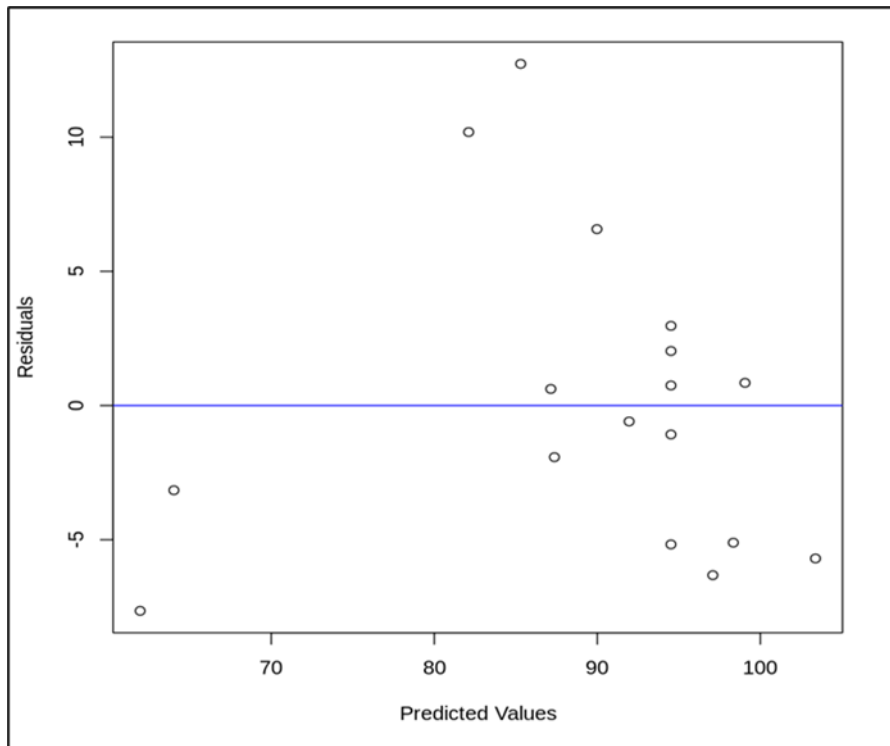
Table 4.5: ANOVA results for the quadratic model of response surface for encapsulation efficiency

Source	Sum of Square	Degree of Freedom	Mean Square	F Value	p-value
Encapsulation efficiency model		10			0.001468
X_2, X_3	866.59	2	433.29	11.1331	0.002859
X_1X_2, X_1X_3, X_2X_3	674.13	3	224.71	5.7737	0.014815
X_2^2	565.58	1	565.58	14.5321	0.003417
Residuals	389.20	10	38.92		
Lack of Fit	129.55	4	32.39	0.7484	0.593438
Pure Error	259.65	6	43.27		

(a)



(b)



(c)

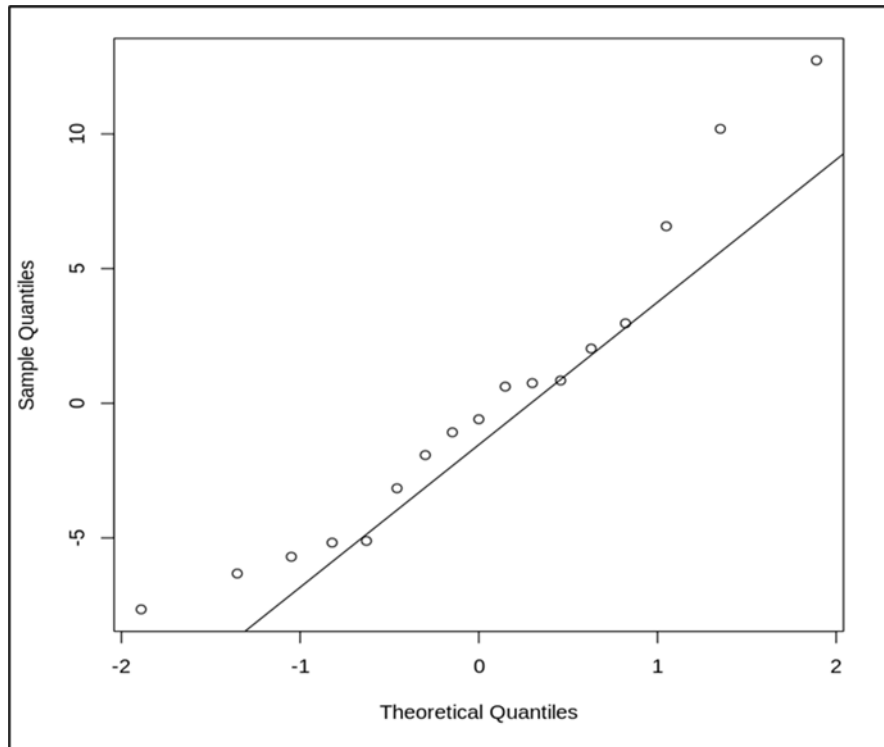
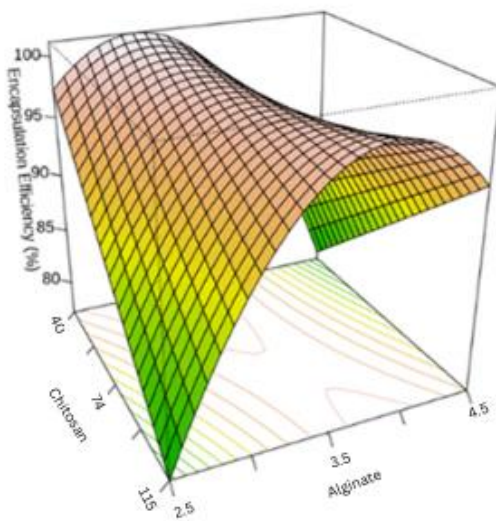


Figure 4.17: (a) Predicted values for encapsulation efficiency vs observed values
(b) Residual plot of predicted encapsulation efficiency (c) Normality of residual for encapsulation efficiency

(a)



(b)

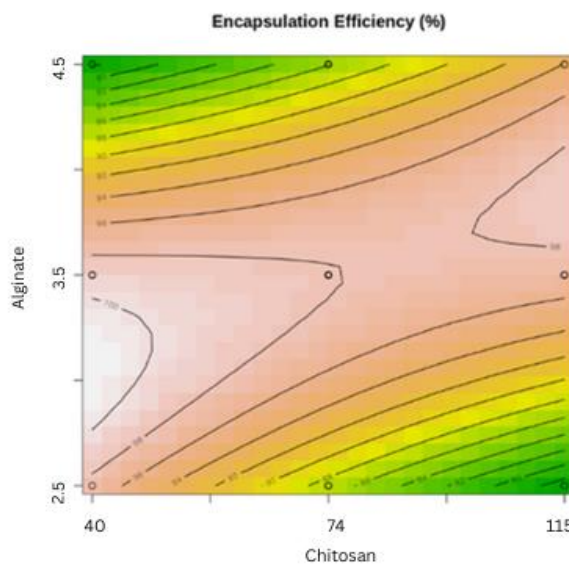


Figure 4.18: (a) Surface plot of concurrent effects of chitosan(X1) and alginate(X2) on the encapsulation efficiency response (b) Contour plot concurrent effects of chitosan(X1) and alginate(X2) on the encapsulation efficiency response

4.4.3 Urea Release Evaluation

Urea release was assessed for the 17 different formulations over a period of 2 hours to evaluate the hydrogel's ability to act as a barrier and control the immediate release of urea, as depicted in Figure 4.19. Run 5 and run 7 produce the lowest urea release response. Both run commonly have lowest level of amount for chitosan molecular weight and alginate. Further increase of chitosan with alginate held constant at that level would further reduce the urea release as shown in Figure 4.20(a). This drop is more obvious in Figure 4.20(b) when crosslinker is 0.55 M (medium level).

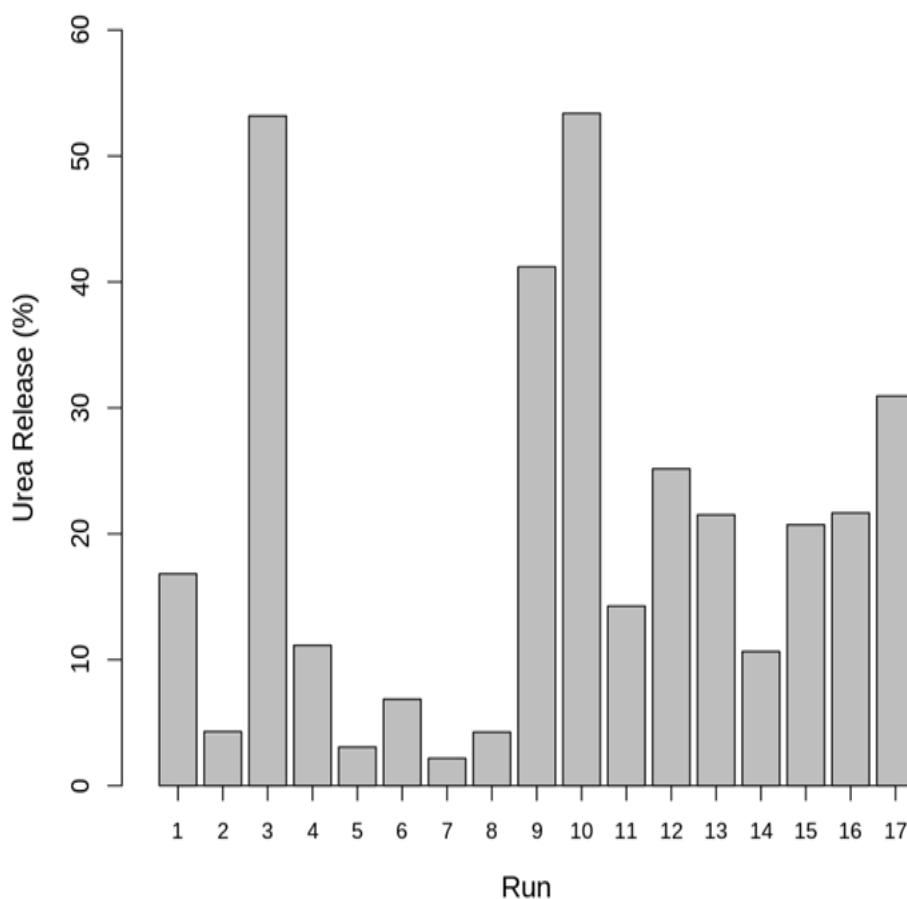
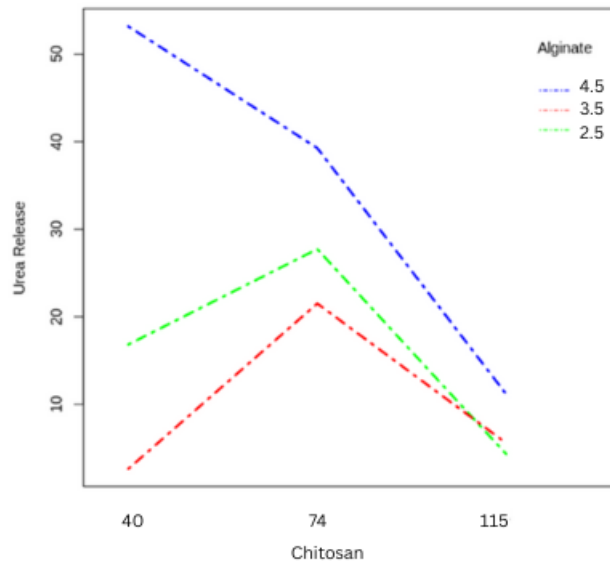


Figure 4.19: Urea release (%) of each run of the hydrogel beads

(a)



(b)

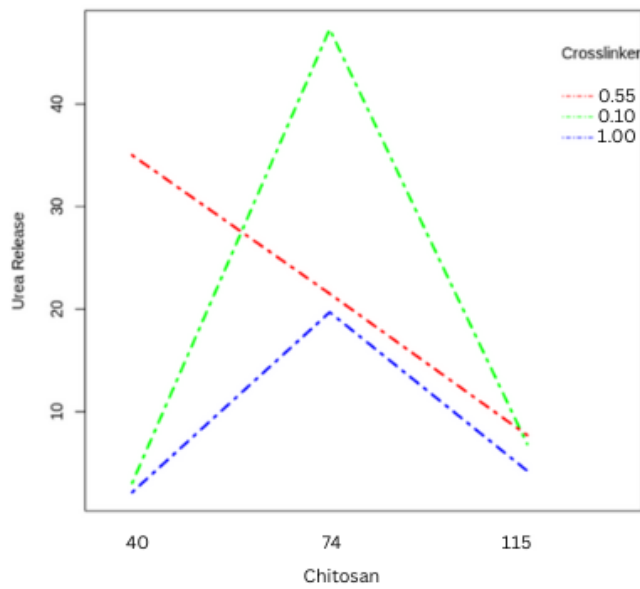


Figure 4.20: (a) Interaction plot between chitosan (X1) and alginate(X2) for urea release response (b) chitosan (X1) and crosslinker(X3) for urea release response

The Equation 4.4 with best fit for urea release is expressed as below:

$$Y_{UR} = 20.0714 + 8.2881X_2 - 7.3359X_3 - 14.7034X_1^2 + 14.7135X_2^2 \quad \text{Equation 4.4}$$

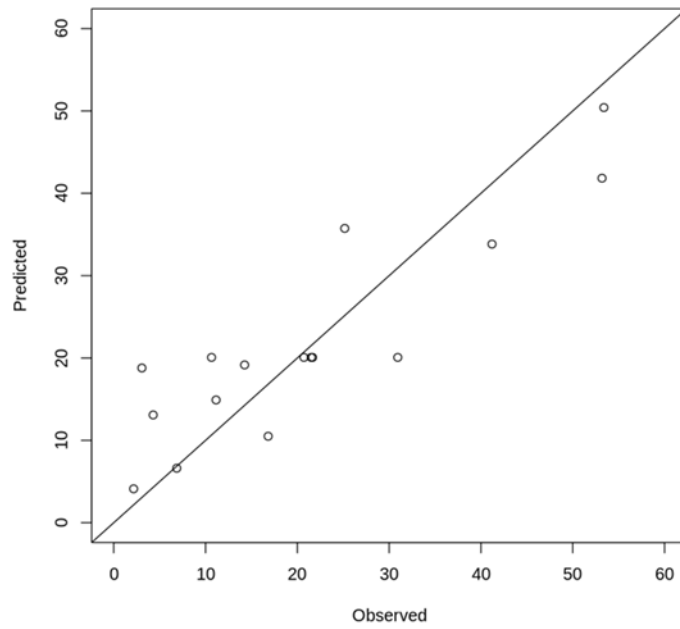
The results in Table 4.6 indicate that the quadratic term of alginate (X2) had the most significant impact on urea release ($p = 0.01535$), followed closely by the quadratic term for the molecular weight of chitosan (X1) ($p = 0.01540$). The linear term of alginate also significantly affected urea release ($p = 0.04234$), whereas the effect of the crosslinker was less pronounced ($p = 0.07306$). Notably, the interactions between these variables were not significant ($p = 0.18486$).

Table 4.6: ANOVA results for the quadratic model of response surface for urea release

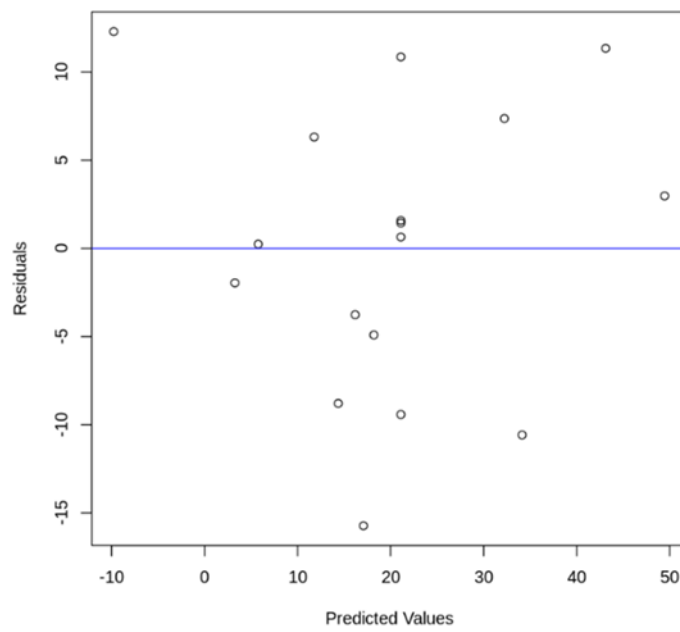
Source	Sum of Square	Degree of Freedom	Mean Square	F Value	p-value
Urea Release Model		10		5.007	0.01282
X_1, X_2, X_3	1276.25	3	425.42	3.9631	0.04234
$X_1 X_2$	217.71	1	217.71	2.0281	0.18486
X_1^2, X_2^2	1730.71	2	865.36	8.0614	0.00822
Residuals	1073.45	10	107.35		
Lack of Fit	866.95	6	144.49	2.7988	0.16920
Pure Error	206.50	4	51.63		

Despite the relatively low determination coefficients for the urea release model, namely the multiple R^2 and adjusted R^2 being 0.7503 and 0.6004, respectively, the difference between these two values is less than 0.2 (Yuan et al. 2018). The model's p-value is 0.01282 ($p < 0.05$), and the p-value for lack of fit is high ($p = 0.16920$), indicating that the model effectively describes the urea release behavior with a high degree of statistical significance. The random distribution of residuals, shown in Figure 4.21(a)-(c) for the urea release experimental results, supports this conclusion. The 3D surface plot and contour plot are shown in Figure 4.22(a) and (b) also confirm minimal interaction between these variables as there is a saddle point in the plot. The variations in the variables do not lead to a simple increase or decrease in urea release, resulting in a statistically insignificant interaction within the regression model.

(a)



(b)



(c)

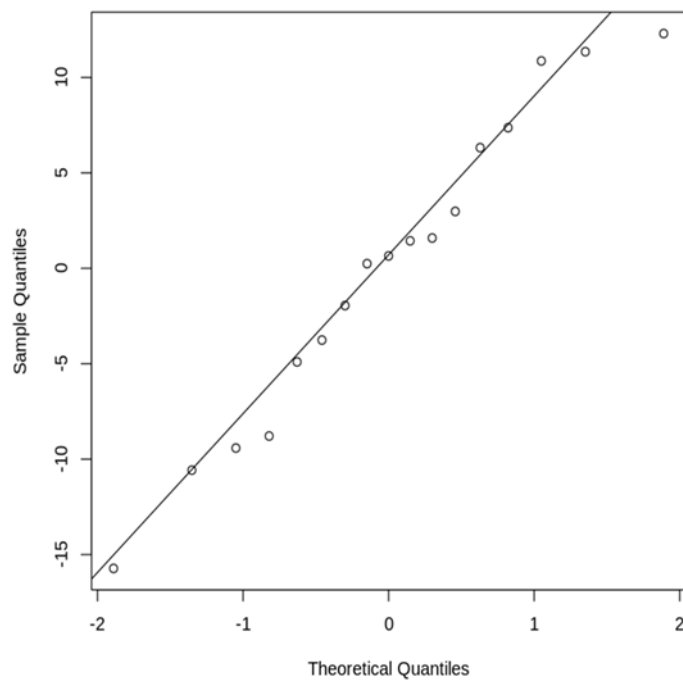
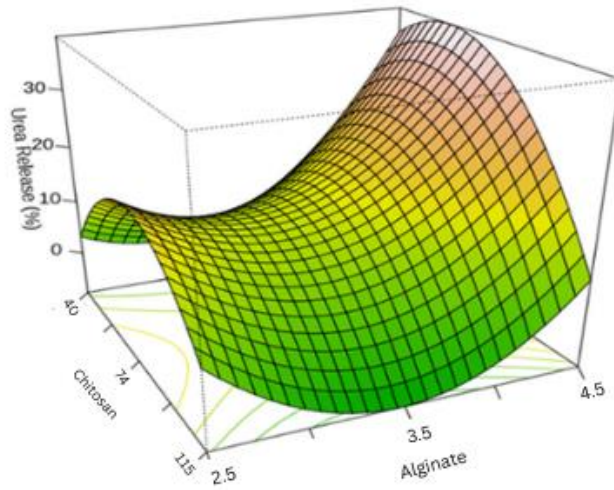


Figure 4.21: Predicted values for (a) urea release vs observed values (b) Residual plot of predicted urea release (c) Normality of residual for urea release experiments

(a)



(b)

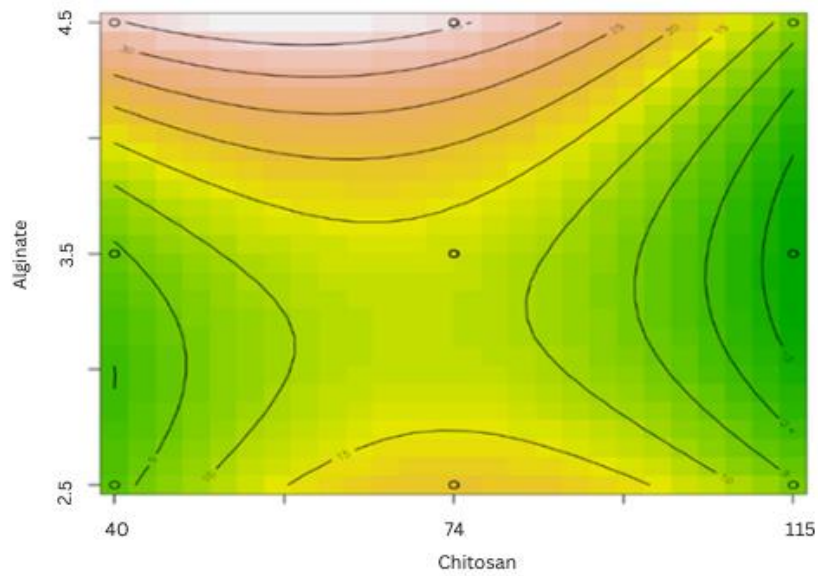


Figure 4.22: (a) Surface plot of concurrent effects of chitosan(X1) and alginate(X2) on the urea release response (b) Contour plot of concurrent effects of chitosan(X1) and alginate(X2) on the urea release response

4.4.4 Summary of Response Surface Methodology

Swelling behavior (SW) reflects the hydrogel's ability to absorb water and is closely related to the structural stability and porosity of the bead. Excessive swelling can lead to uncontrolled nutrient diffusion, while minimal swelling may restrict urea release. In this study, the average swelling percentage was $33.27\% \pm 17.26\%$, indicating a moderately crosslinked structure with adequate water uptake. This was achieved through the combination of 3.5 wt% sodium alginate and 1.00 M CaCl_2 , which created a well-balanced hydrogel matrix. The low molecular weight chitosan also contributed to reduced water penetration by forming a more compact bead structure. These findings align with prior research emphasizing the importance of gel density and polymer interactions in moderating hydrogel swelling for controlled-release applications (Halimi et al., 2020).

Encapsulation efficiency (EE) is a key parameter in evaluating the effectiveness of hydrogel beads as a controlled-release fertilizer system. In this study, EE was strongly influenced by the molecular weight of chitosan, alginate concentration, and the calcium chloride crosslinker. The use of low molecular weight chitosan (40 kDa) promoted a denser polymer matrix due to better solubility and higher mobility of polymer chains, allowing for more uniform interaction and crosslinking with alginate. A tighter matrix structure enhances the entrapment of urea molecules within the bead, reducing diffusion loss during synthesis and leading to a high average EE of $89.43\% \pm 12.66\%$ (Liu et al., 2020). Additionally, a higher CaCl_2 concentration of 1.00 M facilitated stronger ionic crosslinking with alginate, reinforcing bead integrity and further improving encapsulation.

Urea release (UR) from hydrogel beads is a critical factor for assessing their efficiency as a slow- or controlled-release fertilizer. The optimized formulation significantly

minimized urea leaching, with an average urea release percentage of $20.08\% \pm 16.39\%$. This low UR value can be attributed to the synergistic effect of the selected parameters, particularly the strong ionic gel network formed between 3.5 wt% alginate and 1.00 M CaCl_2 , as well as the denser matrix achieved with 40 kDa chitosan. Such tightly packed hydrogel structures limit pore size and nutrient diffusion pathways, allowing for prolonged nutrient availability in the soil (Saber-Samandari et al., 2015).

Among all tested combinations, the optimal formulation was identified as 40 kDa chitosan, 3.5 wt% sodium alginate, and 1.00 M calcium chloride. This composition delivered the most favorable response values across all three-performance metrics: high encapsulation efficiency (89.43%), moderate swelling (33.27%), and low urea release (20.08%). These characteristics meet the requirements for an effective controlled-release fertilizer system, offering sustained nutrient delivery, minimal environmental leaching, and structural durability during soil exposure.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study successfully synthesized NADES using choline chloride and acetogenin, with varying molar ratios and extraction temperatures replacing traditional acid and base method. The chitosan extracted through this process exhibits physicochemical properties comparable to commercial chitosan. Among the tested conditions, the 2:1 molar ratio at 80 °C was identified as the most effective formulation for chitosan extraction. Under this condition, the extracted chitosan achieved a molecular weight of approximately 115 kDa, indicating minimal degradation and effective preservation of polymer integrity. Additionally, this condition yielded high DDA%, reduced crystallinity, and favourable surface morphology, as confirmed through FTIR, XRD, and SEM analyses. The improved performance is attributed to the optimal polarity and hydrogen bonding capacity of the 2:1 NADES at elevated temperature, which enhanced the solubilization and deacetylation of chitin.

Additionally, the study underscores the effectiveness of a three-factor three-level Box-Behnken design in characterizing and optimizing chitosan-alginate hydrogel beads. The optimum formulation of chitosan–alginate hydrogel beads was achieved using chitosan with a low molecular weight of 40 kDa, moderate quantity of sodium alginate at 3.5 wt%, and maximum level of CaCl₂ at 1.00 M, corresponding to (-1, 0, 1) in the experimental design. This combination resulted in an average swelling percentage of $33.274 \pm 17.257\%$, encapsulation efficiency of $89.430 \pm 12.660\%$, and urea release of $20.076 \pm 16.390\%$. These results fulfil the objective of developing an effective hydrogel formulation with balanced

swelling, nutrient entrapment, and release properties suitable for controlled-release fertilizer applications. Additionally, it was found that the amount of alginate is a critical factor, significantly influencing both encapsulation efficiency ($p = 0.001264$) and urea release ($p = 0.04716$). The cross-linker plays a key role in reinforcing the chitosan-alginate beads, thereby enhancing sustained urea release.

5.2 Recommendations

For future work, it is recommended to explore the scale-up potential of the NADES-based extraction method for larger batch processing. Further mechanical testing of the hydrogel beads, such as strength and durability, should also be considered to assess their performance in real agricultural settings. Long-term urea release studies under soil conditions would provide better insights into their practical effectiveness. Besides, other NADES formulations using different natural components could be explored to further improve chitosan extraction efficiency and sustainability. Environmental impact assessments and biodegradability studies are also suggested to support broader applications in green agriculture and controlled-release systems.

REFERENCES

- Abbott, A. P., Capper, G., Davies, D. L., Rasheed, R. K., & Tambyrajah, V. (2003). Novel solvent properties of choline chloride/urea mixtures. *Chemical Communications*, (1), 70–71. <https://doi.org/10.1039/B210714G>
- Ablouh, E.H., Hanani, Z., Eladlani, N., Rhazi, M., and Taourirte, M. (2019). Chitosan microspheres/sodium alginate hybrid beads: an efficient green adsorbent for heavy metals removal from aqueous solutions. *Sustainable Environment Research*, 29, 5. <https://doi.org/10.1186/s42834-019-0004-9>
- Agnihotri, S. A., Mallikarjuna, N. N., & Aminabhavi, T. M. (2004). Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *Journal of Controlled Release*, 100(1), 5-28.
- Akalin, G. O., & Pulat, M. (2020). Controlled release behavior of zinc-loaded carboxymethyl cellulose and carrageenan hydrogels and their effects on wheatgrass growth. *Journal of Polymer Research*, 27(1). <https://doi.org/10.1007/s10965-019-1950-y>
- Akhter, M., Shah, G. A., Niazi, M. B. K., Mir, S., Jahan, Z., & Rashid, M. I. (2021). Novel water-soluble polymer coatings control NPK release rate, improve soil quality and maize productivity. *Journal of Applied Polymer Science*, 138(42), 1–17. <https://doi.org/10.1002/app.51239>
- Ali M. E. A., Aboelfadi M. M .S., Selim A. M., Khalil H. F., and Elkady, G. M. (2018). Chitosan nanoparticles extracted from shrimp shells, application for removal of Fe(II) and Mn(II) from aqueous phases. *Separation Science and Technology*, 53 (18), 2870-2881. <https://doi.org/10.1080/01496395.2018.1489845>

- ALSamman, M. T., & Sánchez, J. (2022). Chitosan- and alginate-based hydrogels for the adsorption of anionic and cationic dyes from water. *Polymers*, *14*(8), 1498. <https://doi.org/10.3390/polym14081498>
- Arca, H. C., Mosquera-Giraldo, L. I., Bi, V., Xu, D., Taylor, L. S., & Edgar, K. J. (2018). Pharmaceutical applications of cellulose ethers and cellulose ether esters. *Biomacromolecules*, *19*, 2351-2376.
- Azeem, B., Kushaari, K., Man, Z. B., Basit, A., & Thanh, T. H. (2014). Review on materials & methods to produce controlled release coated urea fertilizer. *Journal of Controlled Release*, *181*(1), 11–21. <https://doi.org/10.1016/j.jconrel.2014.02.020>
- Bakshi, P. S., Selvakumar, D., Kadirvelu, K., Kumar, N. S. (2020). Chitosan as an environment friendly biomaterial – A review on recent modifications and applications. *International Journal of Biological Macromolecules*, *150*, 1072-1083.
- Beig, B., Niazi, M. B. K., Jahan, Z., Hussain, A., Zia, M. H., & Mehran, M. T. (2020). Coating materials for slow release of nitrogen from urea fertilizer: A review. *Journal of Plant Nutrition*, *43*(10), 1510–1533. <https://doi.org/10.1080/01904167.2020.1744647>
- Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, *76*(5), 965–977. <https://doi.org/10.1016/j.talanta.2008.05.019>
- Brugnerotto, J., Lizardi, J., Goycoolea, F. M., Argüelles-Monal, W., Desbrières, J., & Rinaudo, M. (2001). An infrared investigation in relation with chitin and chitosan characterization. *Polymer*, *42*(8), 3569–3580. [https://doi.org/10.1016/S0032-3861\(00\)00713-8](https://doi.org/10.1016/S0032-3861(00)00713-8)
- Bucher, M., & Kossmann, J. (2007). Molecular physiology of the mineral nutrition of the

- potato. *Potato Biology and Biotechnology: Advances and Perspectives*, 311–329.
<https://doi.org/10.1016/B978-044451018-1/50057-9>
- Chan, L. W., Lee, H. Y., & Heng, P. W. S. (2011). Mechanisms of external and internal gelation and their impact on the functions of alginate as a coat and delivery system. *Carbohydrate Polymers*, 83(3), 1265–1273.
- Cheng, B., Pei, B., Wang, Z., & Hu, Q. (2017). Advances in chitosan-based superabsorbent hydrogels. *Royal Society of Chemistry*, 7, 42036-42046.
- Chen, W., Xue, Z., Wang, J., Jiang, J., Zhao, X., & Mu, T. (2018). Investigation on the thermal stability of deep eutectic solvents. *Wuli Huaxue Xuebao/ Acta Physico - Chimica Sinica*, 34(8), 904–911. <https://doi.org/10.3866/PKU.WHXB201712281>
- Che Zain, M. S., Yeoh, J. X., Lee, S. Y., Lee, S. Y., & Shaari, K. (2021). Physicochemical properties of choline chloride-based natural deep eutectic solvents (NADES) and their applicability for extracting oil palm flavonoids. *Sustainability*, 13(23), 12981. <https://doi.org/10.3390/su132312981>
- Choi, I., Lee, Y., Lyu, J. S., Lee, J. S., & Han, J. (2022). Characterization of ionically crosslinked alginate films: Effect of different anion-based metal cations on the improvement of water-resistant properties. *Food Hydrocolloids*, 131, 107785. <https://doi.org/10.1016/j.foodhyd.2022.107785>
- Comez, B., & Ozbas, S. (2023). Alginate-chitosan hydrogels containing shRNA plasmid for inhibition of CTNNB1 expression in breast cancer cell. *Gels*, 9(7), 541. <https://doi.org/10.3390/gels9070541>
- Costa, C. N., Teixeira, V. G., Delpech, M. C., Souza, J. V. S. & Costa, M. A. S. (2015). Viscometric study of chitosan solutions in acetic acid/sodium acetate and acetic

- acid/sodium chloride. *Carbohydrate Polymer*, 133, 245-250.
<https://doi.org/10.1016/j.carbpol.2015.06.094>
- Craveiro, R., Castro, V. I. B., Viciosa, M. T., Dionisio, M., Reis, R. L., Duarte, A. R. C., and Paiva, A. (2021). Influence of natural deep eutectic systems in water thermal behavior and their applications in cryopreservation. *Journal of Molecular Liquids*, 329, 115533. <https://doi.org/10.1016/j.molliq.2021.115533>
- Dai, Y., Spronsen, J. V., Witkamp, G-J., Verpoorte, R., & Choi, Y. H. (2013a). Natural deep eutectic solvents as new potential media for green technology. *Analytica Chimica Acta*, 766, 61-68. <https://doi.org/10.1016/j.aca.2012.12.019>
- Dai, Y., Spronsen, J. V., Witkamp, G. J., Verpoorte, R., & Choi, Y. H. (2013b). Ionic liquids and deep eutectic solvents in natural products research: mixtures of solids as extraction solvents. *Journal of Natural Products*, 76, 2162–2173.
<https://pubs.acs.org/doi/10.1021/np400051w>
- D'Angelo, G., Elhussieny, A., Faisal, M., Fahim, I. S., & Everitt, N. M. (2018). Mechanical behavior optimization of chitosan extracted from shrimp shells as a sustainable material for shopping bags. *Journal of Functional Biomaterials*, 9(2), 1–10.
<https://doi.org/10.3390/jfb9020037>
- Department of Agriculture. (2021). *Vision and Mision*. [online] Available at: <http://www.doa.gov.my/index.php/pages/view/495?mid=27> [Assessed on 21 August 2021].
- Digatis, R., Falkman, P., Oltner, V., Briggner, L-E., & Kocherbitov, V. (2022). Hydration and dehydration induced changes in porosity of starch microspheres. *Carbohydrate Polymer*, 291, 119542. <https://doi.org/10.1016/j.carbpol.2022.119542>
- Ding, W., Xu, X., He, P., Ullah, S., Zhang, J., Cui, Z., & Zhou, W. (2018). Improving yield

- and nitrogen use efficiency through alternative fertilization options for rice in China: A meta-analysis. *Field Crops Research*, 227(1), 11–18.
<https://doi.org/10.1016/j.fcr.2018.08.001>
- Draget, K. I., Smidsrød, O., & Skjåk-Bræk, G. (2006). Alginates from algae: Chemistry, structure, and applications. *Biomaterials Science*.
- Drelich, J., Marmur, A., & Miller, J. D. (2019). Contact angles: History of over 200 years of open questions. *Surface Innovations*, 7(1), 3–20.
- El-araby, A., Ghadraoui, L. E., and Errachidi, F. (2022). Physicochemical properties and functional characteristics of ecologically extracted shrimp chitosan with different organic acids during demineralization step. *Molecules*, 22(23), 8285.
<https://doi.org/10.3390/molecules27238285>
- Elsabee, M. Z., & Abdou, E. S. (2013). Chitosan based edible films and coatings: A review. *Materials Science and Engineering*, 33(4), 1819–1841.
- Fertahi, S., Ilsouk, M., Zeroual, Y., Oukarroum, A., & Barakat, A. (2021). Recent trends in organic coating based on biopolymers and biomass for controlled and slow release fertilizers. *Journal of Controlled Release*, 330(12), 341–361.
<https://doi.org/10.1016/j.jconrel.2020.12.026>
- Feyissa Z, Edossa GD, Gupta NK, Negera D. (2023). Development of double crosslinked sodium alginate/chitosan based hydrogels for controlled release of metronidazole and its antibacterial activity. *Heliyon*, 9(9), 20144. doi: 10.1016/j.heliyon.2023.e20144.
- Focher, B., Beltrame, P. L., Naggi, A., & Torri, G. (1990). Alkaline N-deacetylation of chitin enhanced by flash heating. *Carbohydrate Polymers*, 12(4), 405–418.
[https://doi.org/10.1016/0144-8617\(90\)90058-X](https://doi.org/10.1016/0144-8617(90)90058-X)
- Foskolos, A., & Moorby, J. M. (2018). Evaluating lifetime nitrogen use efficiency of dairy

- cattle: A modelling approach. *PLOS One*, 13(8), 1–22.
<https://doi.org/10.1371/journal.pone.0201638>
- Francisco, M., van den Bruinhorst, A., & Kroon, M. C. (2012). Low-transition-temperature mixtures (LTTMs): A new class of designer solvents. *Angewandte Chemie International Edition*, 52(11), 3074–3085.
- Franco, T. S., Amezcua, R. M. J., Rodríguez, A. V., Enriquez, S. G., Urquiza, M. R., Mijares, E. M., and de Muniz, G. B. (2020). Carboxymethyl and nanofibrillated cellulose as additives on the preparation of chitosan biocomposites: their influence over films characteristics. *Journal of Polymers and the Environment*, 28, 676–688.
<https://doi.org/10.1007/s10924-019-01639-0>
- Gaspar, B., Rodrigues, L. A., Pereira, C. V., Silva, J. M., Reis, R. L., Duarte, A. R. C., Paiva, A., & Matias, A. A. (2020). Terpene-based natural deep eutectic systems as efficient solvents to recover astaxanthin from brown crab shell residues. *ACS Sustainable Chemistry & Engineering Journal*, 8(5), 2246–2259.
- George, M., & Abraham, T. E. (2006). Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan: A review. *Journal of Controlled Release*, 114(1), 1–14.
- Giri, T. K., Thakur, D., Alexander, A., Ajazuddin, & Badwaik, H. (2012). Alginate based hydrogel as a potential biopolymeric carrier for drug delivery and cell delivery systems: Present status and applications. *Current Drug Delivery*, 9(6), 539–555.
<https://doi.org/10.1021/acssuschemeng.9b06283>
- Gómez, A. V., Biswas, A., Tadini, C. C., Furtado, R. F., Alves, C. R., & Cheng, H. N. (2019). Use of natural deep eutectic solvents for polymerization and polymer reactions.

Journal of the Brazilian Chemical Society, 30(4), 717–726.

<https://doi.org/10.21577/0103-5053.20190001>

González-Rivera, J., Husanu, E., Mero, A., Ferrari, C., Duce, C., Tinè, M. R., D'Andrea, F., Pomelli, C. S., & Guazzelli, L. (2020). Insights into microwave heating response and thermal decomposition behavior of deep eutectic solvents. *Journal of Molecular Liquids*, 300. <https://doi.org/10.1016/j.molliq.2019.112357>

Gopal, J., Muthu, M., Dhakshanamurthy, T., Kim, K. J., Hasan, N., Kwon, S. J., & Chun, S. (2019). Sustainable ecofriendly phytoextract mediated one pot green recovery of chitosan. *Scientific Reports*, 9(1), 1–12. <https://doi.org/10.1038/s41598-019-50133-z>

Goy, R. C., & Assis, O. B. G. (2014). Antimicrobial analysis of films processed from chitosan and N,N,N-trimethylchitosan. *Brazilian Journal of Chemical Engineering*, 31(3), 643- Goy, R. C., de Britto, D., & Assis, O. B. G. (2009). A review of the antimicrobial activity of chitosan. *Polímeros*, 19(3), 241–247.648.

Gumelar, M. D., Hamzah, M., Hidayat, A. S., Saputra, D. A., & Idvan. (2020). Utilization of chitosan as coating material in making NPK slow release fertilizer. *Macromolecular Symposia*, 391(1), 1–4. <https://doi.org/10.1002/masy.201900188>

Hafemann, B., Souza, G. P., & Petzhold, C. L. (2020). Crosslinking of chitosan: Current status and perspectives. *Polímeros*, 30(3), e2020019. <https://doi.org/10.1590/0104-1428.04020>

Halimi, M. S., Zain, N. M., & Samsudin, M. W. (2020). Controlled-release urea fertilizer using biodegradable polymers: A review. *Journal of Environmental Chemical Engineering*, 8(5), 104174. <https://doi.org/10.1016/j.jece.2020.104174>

- Hammond, O. S., Bowron, D. T., & Edler, K. J. (2016). Liquid structure of the choline chloride–urea deep eutectic solvent. *Angewandte Chemie International Edition*, 55(1), 491–495.
- Hammond, O. S., Bowron, D. T., & Edler, K. J. (2016). The effect of hydrogen bonding on NADES structure. *Green Chemistry*, 18(10), 2736–2744.
- Hao, G., Hu, Y., Shi, L., Chen, J., Cui, A., Weng, W., and Osako, K. (2021). Physicochemical characteristics of chitosan from swimming crab (*Portunus trituberculatus*) shells prepared by subcritical water pretreatment. *Scientific Reports*, 11 (1646), 1-9. <https://doi.org/10.1038/s41598-021-81318-0>
- Hiroshi, A., & Yuichi, S. (2019). Development and application of coated fertilizer in Japan. *Journal of Food Science and Engineering*, 9(4), 142–152. <https://doi.org/10.17265/2159-5828/2019.04.004>
- Hong, S., Yuan, Y., Yang, Q., Zhu, P., & Lian, H. (2018). Versatile acid base sustainable solvent for fast extraction of various molecular weight chitin from lobster shell. *Carbohydrate Polymers*, 201, 211–217. <https://doi.org/10.1016/j.carbpol.2018.08.059>
- Huang, W. C., Zhao, D., Guo, N., Xue, C., & Mao, X. (2018). Green and facile production of chitin from crustacean shells using a natural deep eutectic solvent. *Journal of Agricultural and Food Chemistry*, 66(45), 11897–11901. <https://doi.org/10.1021/acs.jafc.8b03847>
- Huang, C., Ma, J., Liu, W., Liu, Y., Zhang, H., & Deng, Y. (2022). An efficient method for chitin production from crab shells by a natural deep eutectic solvent. *Carbohydrate Polymers*, 291, 119553. <https://doi.org/10.1016/j.carbpol.2022.119553>
- Hunger, M., & Domalik-pyzik, P. (2020). Double crosslinking of chitosan/vanillin hydrogels

- as a basis for mechanically strong gradient scaffolds. *Engineering of Biomaterials*, 155, 2-11. <https://doi.org/10.34821/eng.biomat.155.2020.2-11>
- Ibrahim, K. R. M., Babadi, F. E., & Yunus, R. (2014). Comparative performance of different urea coating materials for slow release. *Particuology*, 17, 165–172. <https://doi.org/10.1016/j.partic.2014.03.009>
- Iftime, M. M., Ailiesei, G. L., Ungureanu, E., & Marin, L. (2019). Designing chitosan based eco-friendly multifunctional soil conditioner systems with urea controlled release and water retention. *Carbohydrate Polymers*, 223(July), 115040. <https://doi.org/10.1016/j.carbpol.2019.115040>
- Islam, S., Bhuiyan, M. A. R., & Islam, M. N. (2017). Chitin and chitosan: Structure, properties and applications in biomedical engineering. *Journal of Polymers and the Environment*, 25(3), 854–866. <https://doi.org/10.1007/s10924-016-0865-5>
- Jablonský, M., Škulcová, A., & Šima, J. (2019). Use of deep eutectic solvents in polymer chemistry – A review. *Molecules*, 24(21), 1–33. <https://doi.org/10.3390/molecules24213978>
- Jabrail, F. H., & Gupta, K. C. (2008). Effect of molecular weight and degree of deacetylation on controlled release of isoniazid from chitosan microspheres. *Polymers for Advanced Technologies*, 19(5), 432-441. <https://doi.org/10.1002/pat.1035>
- Jantrawut, P., Chabin, O., & Ruksiriwanich, W. (2015). Scavenging activity of rutin encapsulated in low methoxyl pectin beads. *Cellulose Chemistry and Technology*, 49(1), 51-54.
- Jusoh, S., Yahya, A., Mohd, N., & Ibrahim, N. (2021). Characterization of seashell waste for potential use in biocomposite materials. *Malaysian Journal of Analytical Sciences*, 25(4), 643–651. <https://doi.org/10.17576/mjas-2021-2504-08>

- Kaczmarek, M. B., Struszczyk-swita, K., Li, X., Szczesna-antczak, M., & Daroch, M. (2019). Enzymatic modifications of chitin, chitosan, and chitooligosaccharides. *Frontiers in Bioengineering and Biotechnology*, 7(243). <https://doi.org/10.3389/fbioe.2019.00243>
- Kasaai, M. R. (2007). A review of several reported procedures to determine the degree of N-acetylation for chitin and chitosan using IR spectroscopy. *Carbohydrate Polymers*, 68(4), 477–488. <https://doi.org/10.1016/j.carbpol.2006.11.022>
- Kaur, K., Dattajirao, V., Shrivastava, V., & Bhardwaj, U. (2012). Isolation and characterization of chitosan-producing bacteria from beaches of Chennai, India. *Enzyme Research*, 2012. <https://doi.org/10.1155/2012/421683>
- Kozma, M., Acharya, B., & Bissessur, R. (2022). Chitin, chitosan, and nanochitin: Extraction, synthesis, and applications. *Polymers*, 14(19), 3989. <https://doi.org/10.3390/polym14193989>
- Kumar, M. N. V. R. (2004). A review of chitin and chitosan applications. *Reactive and Functional Polymers*, 46(1), 1–27.
- Kumar, S., Kumari, M., Dutta, P. K., & Koh, J. (2014). Chitosan biopolymer based Schiff base: Preparation, characterization, optical & antibacterial activity. *International Journal of Polymeric Materials and Polymeric Biomaterials*, 63(4), 173-177.
- Kumirska, J., Czerwicka, M., Kaczyński, Z., Bychowska, A., Brzozowski, K., Thöming, J., & Stepnowski, P. (2010). Application of spectroscopic methods for structural analysis of chitin and chitosan. *Marine Drugs*, 8(5), 1567–1636. <https://doi.org/10.3390/md8051567>
- Kusumastuti, Y., Istiani, A., Rochmadi, & Purnomo, C. W. (2019). Chitosan-based polyion

- multilayer coating on NPK fertilizer as controlled released fertilizer. *Advances in Materials Science and Engineering*, 2019. <https://doi.org/10.1155/2019/2958021>
- Lee, K. Y., & Mooney, D. J. (2012). Alginate: Properties and biomedical applications. *Progress in Polymer Science*, 37(1), 106-126. <https://doi.org/10.1016/j.progpolymsci.2011.06.003>
- Lei, Y., Peng, H., Chen, J., Li, Y., & Liu, X. (2024). Pretreatment of shrimp shells with an acidic deep eutectic solvent system for chitin extraction and its enhanced performance as a carrier for immobilized lipase. *International Journal of Biological Macromolecules*, 262, 129245. <https://doi.org/10.1016/j.ijbiomac.2024.129245>
- Li, J., & Mooney, D. J. (2016). Designing hydrogels for controlled drug delivery. *Nature Reviews Materials*, 1, 16071.
- Li, X., Zhang, Y., Wang, X., & Li, Y. (2008). Preparation and release characteristics of slow-release fertilizer encapsulated by alginate and bentonite. *Journal of Rare Earths*, 26(3), 389–392.
- Li, Y., Liu, Y., & Chu, L. (2016). Design and performance evaluation of controlled-release fertilizer using calcium alginate hydrogel beads. *Journal of Applied Polymer Science*, 133(12), 43120.
- Liu, L., Ni, Y., Zhi, Y., Zhao, W., Pudukudy, M., Jia, Q., Shan, S., Zhang, K., & Li, X. (2020). Sustainable and biodegradable copolymers from SO₂ and renewable eugenol: A novel urea fertilizer coating material with superior slow release performance. *Macromolecules*, 53(3), 936–945. <https://doi.org/10.1021/acs.macromol.9b02202>
- Liu, M., Liang, R., Zhan, F., Liu, Z., & Niu, A. (2020). Synthesis and evaluation of chitosan–alginate hydrogel beads for controlled release of urea. *Carbohydrate Polymers*, 249, 116867. <https://doi.org/10.1016/j.carbpol.2020.116867>

- Liu, Y., Friesen, J. B., McAlpine, J. B., Lankin, D. C., Chen, S. N., & Pauli, G. F. (2018). Natural deep eutectic solvents: Properties, applications, and perspectives. *Journal of Natural Products*, 81(3), 679–690. <https://doi.org/10.1021/acs.jnatprod.7b00945>
- Lü, S., Feng, C., Gao, C., Wang, X., Xu, X., Bai, X., Gao, N., & Liu, M. (2016). Multifunctional environmental smart fertilizer based on l-aspartic acid for sustained nutrient release. *Journal of Agricultural and Food Chemistry*, 64(24), 4965–4974. <https://doi.org/10.1021/acs.jafc.6b01133>
- Lukitowati, F., and Indrani, D. J. (2018). Water absorption of chitosan, collagen, and chitosan/collagen blend membranes exposed to gamma-ray irradiation. *Iranian Journal of Pharmaceutical Sciences*, 14, 57–66.
- Lum, Y-H., Shaaban, A., Mohamad, N., Dimin, F., and Yatim, N. M. (2016). Boric acid modified starch polyvinyl alcohol matrix for slow release fertilizer. *e-Polymers*. 16. 151–158. <https://doi.org/10.1515/epoly-2015-0259>
- Mati-Baouche, N., Elchinger, P-H., de Baynast, H., Pierre, G., Delattre, C., and Michaud, P. (2014). Chitosan as an adhesive. *European Polymer Journal*, 60, 198-212. <https://doi.org/10.1016/j.eurpolymj.2014.09.008>.
- Mbous, Y. P., Hayyan, M., Hayyan, A., & Wong, W. F. (2017). Applications of deep eutectic solvents in biotechnology. *Critical Reviews in Biotechnology*, 37(7), 912–929.
- Mesias, V. S. D., Agu, A. B. S., Benablo, P. J. L., Chen, C. H., & Penaloza, D. P. (2019). Coated npk fertilizer based on citric acid-crosslinked chitosan/alginate encapsulant. *Journal of Ecological Engineering*, 20(11), 1–12. <https://doi.org/10.12911/22998993/113418>
- Mohan, K., Ravichandran, S., Muralisankar, T., Uthayakumar, V., Chandirasekar, R., Rajeevgandhi, C., Karthick Rajan, D., & Seedeve, P. (2019). Extraction and

- characterization of chitin from sea snail *Conus inscriptus* (Reeve, 1843). *International Journal of Biological Macromolecules*, 126, 555–560.
<https://doi.org/10.1016/j.ijbiomac.2018.12.241>
- Mokhtar, A., Abdelkrim, S., Djelad, A., Sardi, A., Boukoussa, B., Sassi, M., & Bengueddach, A. (2020). Adsorption behavior of cationic and anionic dyes on magadiite-chitosan composite beads. *Carbohydrate Polymers*, 229, 115399.
<https://doi.org/10.1016/j.carbpol.2019.115399>
- Montgomery, D. C. (2017). *Design and Analysis of Experiments (9th ed.)*. Wiley.
- Morgan, K., Conway, C., Faherty, S., & Quigley, C. (2021). A comparative analysis of conventional and deep eutectic solvent (DES): Mediated strategies for the extraction of chitin from marine crustacean shells. *Molecules*, 26(24), 7603.
<https://doi.org/10.3390/molecules26247603>
- Mourya, V. K., Inamdar, N. N., & Tiwari, A. (2010). Carboxymethyl chitosan and its applications. *Advanced Materials Letters*, 1(1), 11–33.
<https://doi.org/10.5185/amlett.2010.3101>
- Mujtaba, M., Majeed, A., Arshad, M., & Khalid, N. (2019). Functionalization of chitosan and its blends for controlled release fertilizer formulations: A review. *International Journal of Biological Macromolecules*, 133, 122–136.
<https://doi.org/10.1016/j.ijbiomac.2019.04.071>
- Murata, Y., Hirai, S., & Kondo, T. (2020). Alginate–calcium hydrogel beads as a platform for fertilizer delivery: Effect of crosslinking on release properties. *Materials Today Communications*, 24, 101161.
- Murata, Y., Jinno, D., Liu, D., Isobe, T., & Nakai, Y. (1993). Preparation of chitosan-reinforced alginate gel beads and control of release rates of indomethacin. *Journal of*

Controlled Release, 23(1), 29–35.

- Natarajan, D., Krithica, N., & Rajendran, N. (2018). Evaluation of physical parameters affecting size and shape of calcium alginate beads prepared using Box-Behnken design. *International Journal of Biological Macromolecules*, 108, 989-995. <https://doi.org/10.1016/j.ijbiomac.2017.11.055>
- Negm, N. A., Hefni, H. H. H., Abd-Elaal, A. A. A., Badr, E. A., & Abou Kana, M. T. H. (2020). Advancement on modification of chitosan biopolymer and its potential applications. *International Journal of Biological Macromolecules*, 152, 681–702. <https://doi.org/10.1016/j.ijbiomac.2020.02.196>
- Nguyen, H. V. D., De Vries, R. and Stoyanov, S.D. (2020). Natural deep eutectics as a “green” cellulose cosolvent. *ACS Sustainable Chemistry & Engineering Journal*, 8 (37), 14166–14178. <https://doi.org/10.1021/acssuschemeng.0c04982>
- Onn, M., Zuraidah, R., Mohd, R., Zainuddin, A., Sidik, N., & Tukiman, N. (2017). Concentration of heavy metal and nutrients fact of Pontian Johor *Cerithidea obtus*. *Journal of Applied Environmental and Biological Sciences*, 7(12), 49–54.
- Osseni, S., Bonou, S. A. S., Sagbo, E. V., Ahouansou, R., Agbahoungbata, M. Y., Neumeyer, D., Verelst, M., and Mauricot, R. (2018). Synthesis of calcium phosphate bioceramics based on snail shells: Towards a valorization of snail shells from Republic of Benin. *American Journal of Chemistry*. 8 (4), 90-95. <https://doi.org/10.5923/j.chemistry.20180804.02>
- Patel, V. F., Hardin, J. N., Mastro, J. M., & Law, J. R. (2016). Designing polymeric drug delivery systems. *Polymers*, 8(3), 71.
- Pellis A, Guebitz GM, Nyanhongo GS. (2022). Chitosan: Sources, processing and modification techniques. *Gels*. 8(7), 393. doi: 10.3390/gels8070393.

- Pawar, S. N., & Edgar, K. J. (2012). Alginate derivatization: A review of chemistry, properties and applications. *Biomaterials*, 33(11), 3279-3305.
<https://doi.org/10.1016/j.biomaterials.2012.01.007>
- Prajapati, D., Pal, A., Dimkpa, C., Harish, Singh, U., Devi, K. A., Jagdish, L. C., & Saharan, V. (2022). Chitosan nanomaterials: A prelim of next-generation fertilizers; existing and future prospects. *Carbohydrate Polymers*, 288, 119356.
<https://doi.org/10.1016/j.carbpol.2022.119356>
- Puvvada, Y. S., Vankayalapati, S., and Sukhavasi, S. (2012). Extraction of chitin from chitosan exoskeleton of shrimp for application in the pharmaceutical industry. *International Journal of Current Pharmaceutical Research*, 1 (9), 258-263.
<https://doi.org/10.3329/icpj.v1i9.11616>
- Qiu, H., Li, W., Gong, Y., Zhang, M., & Song, L. (2020). Extraction and characterization of chitosan from snail shells using deep eutectic solvents. *International Journal of Biological Macromolecules*, 152, 244–251.
<https://doi.org/10.1016/j.ijbiomac.2020.02.285>
- Rabea, E. I., Badawy, M. E-T., Stevens, C. V., Smagghe, G., & Steurbaut, W. (2003). Chitosan as antimicrobial agent: Applications and mode of action. *Biomacromolecules*, 4(6), 1457-1465.
- Rajathy, T. J., Srinivasan, M., and Mohanraj, T. (2021). Physicochemical and functional characterization of chitosan from horn snail gastropod *Telescopium telescopium*. *Journal of Applied Pharmaceutical Science*, 11(2), 52-58.
<https://doi.org/10.7324/JAPS.2021.110207>

- Raut, N., & Suryawanshi, V. (2020). Characterization of marine snail shell as a calcium-rich biowaste for industrial applications. *International Journal of Engineering Research & Technology*, 9(6), 1081–1086.
- Remedio, L. N., Silva dos Santos, J. W., Vieira Maciel, V. B., Yoshida, C. M. P., and Aparecida de Carvalho, R. (2019). Characterization of active chitosan films as a vehicle of potassium sorbate or nisin antimicrobial agents. *Food Hydrocolloids.*, 87, 830–838. <https://doi.org/10.1016/j.foodhyd.2018.09.012>
- Riaz Rajoka, M. S., Zhao, L., Mehwish, H. M., Wu, Y., & Mahmood, S. (2019). Chitosan and its derivatives: Synthesis, biotechnological applications, and future challenges. *Applied Microbiology and Biotechnology*, 103(4), 1557–1571. <https://doi.org/10.1007/s00253-018-9550-z>
- Roberts, G. A. F. (1992). *Chitin Chemistry*. Macmillan Press.
- Román-Doval, R., Torres-Arellanes, S. P., Tenorio-Barajas, A. Y., Gómez-Sánchez, A., & Valencia-Lazcano, A. A. (2023). Chitosan: Properties and its application in agriculture in context of molecular weight. *Polymers*, 15(13), 2867. <https://doi.org/10.3390/polym15132867>
- Rudzinski, W. E., Aminabhavi, T. M. (2002). Chitosan as a carrier for targeted delivery of bioactive agents. *Journal of Controlled Release*, 79(1-3), 1–16.
- Rütting, T., Aronsson, H., & Delin, S. (2018). Efficient use of nitrogen in agriculture. *Nutrient Cycling in Agroecosystems*, 110(1), 1–5. <https://doi.org/10.1007/s10705-017-9900-8>
- Saber-Samandari, S., Yekta, H., and Saber-Samandari, S. (2015). Effect of iron substitution in hydroxyapatite matrix on swelling properties of composite bead. *Journal of Mineral Metal and Material Engineering*, 1. 19-25.

- Santana, A. P. R., Andrade, D. F., Mora-Vargas, J. A., Amaral, C. D. B., Oliveira, A., & Gonzalez, M. H. (2019). Natural deep eutectic solvents for sample preparation prior to elemental analysis by plasma-based techniques. *Talanta*, 199(January), 361–369. <https://doi.org/10.1016/j.talanta.2019.02.083>
- Santos, V. P., Marques, N. S. S., Maia, P. C. S. V., de Lima, M. A. B., Franco, L. de O., & de Campos-Takaki, G. M. (2020). Seafood waste as attractive source of chitin and chitosan production and their applications. *International Journal of Molecular Sciences*, 21(12), 1–17. <https://doi.org/10.3390/ijms21124290>
- Savi, L. K., Carpiné, D., Waszczynskyj, N., Ribani, R. H., & Haminiuk, C. W. I. (2019). Influence of temperature, water content and type of organic acid on the formation, stability and properties of functional natural deep eutectic solvents. *Fluid Phase Equilibria*, 488, 40–47. <https://doi.org/10.1016/j.fluid.2019.01.025>
- Shafie, M. H., Yusof, R., & Gan, C. Y. (2019). Synthesis of citric acid monohydrate-choline chloride based deep eutectic solvents (DES) and characterization of their physicochemical properties. *Journal of Molecular Liquids*, 288. <https://doi.org/10.1016/j.molliq.2019.111081>
- Sharma, L. K., & Bali, S. K. (2017). A review of methods to improve nitrogen use efficiency in agriculture. *Sustainability (Switzerland)*, 10(1), 1–23. <https://doi.org/10.3390/su10010051>
- Sharmin, N., Rosnes, J. T., Prabhu, L., Böcker, U., & Sivertsvik, M. (2022). Effect of citric acid cross linking on the mechanical, rheological and barrier properties of chitosan. *Molecules*, 27 (16), 5118. <https://doi.org/10.3390/molecules27165118>
- Smirnov, M. A., Nikolaeva, A. L., Vorobiov, V. K., Bobrova, N. V., Abalov, I. V., Smirnov, A. V., & Sokolova, M. P. (2020). Ionic conductivity and structure of chitosan films

- modified with lactic acid-choline chloride NADES. *Polymers*, 12(2).
<https://doi.org/10.3390/polym12020350>
- Smith, E. L., Abbott, A. P., & Ryder, K. S. (2014). Deep eutectic solvents (DESs) and their applications. *Chemical Reviews*, 114(21), 11060–11082.
- Song, Z., Ma, T., Zhi, X., & Du, B. (2021). Cellulosic films reinforced by chitosan-citric complex for meat preservation: Influence of nonenzymatic browning. *Carbohydrate Polymers*, 272, 118476. <https://doi.org/10.1016/j.carbpol.2021.118476>
- Sunardi, Faramitha, G. N., and Santoso, U. T. (2021). Characterization of alginate-cellulose-kaolin composites for slow-release urea fertilizer. *Indonesian Journal of Chemical Research*, 8(3):219-227. <https://doi.org/10.30598/ijcr.2021.8-sun>
- Sun, L., Zhao, Y., Wu, D., & Xu, Y. (2022). In-depth study on the extraction and mechanism of high-purity chitin based on NADESs method. *Sustainable Chemistry and Pharmacy*, 22, 100500. <https://doi.org/10.1016/j.scp.2021.100500>
- Synowiecki, J., & Al-Khateeb, N. A. A. Q. (2003). Production, properties, and some new applications of chitin and its derivatives. *Enzyme and Microbial Technology*, 35(1), 93–102. [https://doi.org/10.1016/S0141-0229\(03\)00141-5](https://doi.org/10.1016/S0141-0229(03)00141-5)
- Szymańska, E., & Winnicka, K. (2015). Stability of chitosan - A challenge for pharmaceutical and biomedical applications. *Marine Drugs*, 13(4), 1819–1846.
<https://doi.org/10.3390/md13041819>
- Takka, S., and Gürel, A. (2010). Evaluation of chitosan/alginate beads using experimental design: Formulation and *in vitro* characterization. *AAPS PharmSciTech*, 11(1), 460-466. <https://doi.org/10.1208/s12249-010-9406-z>.
- Tang, J., Hong, J., Liu, Y., Wang, B., Hua, Q., Liu, L., & Ying, D. (2018). Urea controlled-release fertilizer based on gelatin microspheres. *Journal of Polymers and the*

- Environment*, 26(5), 1930–1939. <https://doi.org/10.1007/s10924-017-1074-6>
- Tang, Q., Cotton, A., Wei, Z., Xia, Y., Daniell, T., & Yan, X. (2022). How does partial substitution of chemical fertiliser with organic forms increase sustainability of agricultural production?. *Science of the Total Environment*, 803, 149933. <https://doi.org/10.1016/j.scitotenv.2021.149933>
- Tomadoni, B., Ponce, A., Pereda, M., & Ansorena, M. R. (2019). Vanillin as a natural cross-linking agent in chitosan-based films: Optimizing formulation by response surface methodology. *Polymer Testing*, 105935. <https://doi.org/10.1016/j.polymertesting.2019.105935>
- Trenkel, M. E. (2010). *Slow- and Controlled-Release and Stabilized Fertilizers: An Option for Enhancing Nutrient Use Efficiency in Agriculture*. International Fertilizer Industry Association.
- USDA. (2013). *U.S. Department of Agriculture*. [online] Available at <https://www.usda.gov/topics/food-and-nutrition/food-security> [Assessed on 21 August 2021]
- Varma, R., & Vasudevan, S. (2020). Extraction, characterization, and antimicrobial activity of chitosan from horse mussel modiolus modiolus. *ACS Omega*, 5(32), 20224–20230. <https://doi.org/10.1021/acsomega.0c01903>
- Wang, G., Li, P., Peng, Z., & Huang, M. (2011). Formulation of vanillin cross-linked chitosan nanoparticles and its formulation of vanillin cross-linked chitosan nanoparticles and its characterization. *Advanced Materials Research*, 335-336, 474-477. <https://doi.org/10.4028/www.scientific.net/AMR.335-336.474>
- Wu, L., & Liu, M. (2008). Preparation and properties of chitosan-coated NPK compound fertilizer with controlled-release and water-retention. *Carbohydrate Polymers*, 72(2),

240–247. <https://doi.org/10.1016/j.carbpol.2007.08.020>

- Wu, Y., Wang, L., & Xu, H. (2012). Controlled-release urea encapsulated by starch-g-polyvinyl alcohol and polyacrylic acid. *Carbohydrate Polymers*, 89(2), 479–484.
- Yadav, M., Kaushik, B., Rao, G. K., Srrivastava, C. M., and Vaya, D. (2023). Advances and challenges in the use of chitosan and its derivatives in biomedical fields: A review. *Carbohydrate Polymer Technologies and Application*, 5, 100323. <https://doi.org/10.1016/j.carpta.2023.100323>
- Yadav, M., Goswami, P., Paritosh, K., Kumar, M., Pareek, N., & Vivekanand, V. (2019). Seafood waste: a source for preparation of commercially employable chitin/chitosan materials. *Bioresources and Bioprocessing*, 6(1). <https://doi.org/10.1186/s40643-019-0243-y>
- Yang, X. Li, Z., Jiang, Z., Wang, S., Liu, H., Xu, X., Wang, D., Miao, Y., Shang, S., & Song, Z. (2020). Mechanical reinforcement of room-temperature-vulcanized silicone rubber using modified cellulose nanocrystals as cross-linker and nanofiller. *Carbohydrate Polymers*, 229, 115509. <https://doi.org/10.1016/j.carbpol.2019.115509>
- Yi, K., Miao, S., Yang, B., Li, S., & Lu, Y. (2024). Harnessing the potential of chitosan and its derivatives for enhanced functionalities in food applications. *Foods*, 13(3), 439. <https://doi.org/10.3390/foods13030439>
- Younes, I., & Rinaudo, M. (2015). Chitin and chitosan preparation from marine sources. *Marine Drugs*, 13(3), 1133–1174. <https://doi.org/10.3390/md13031133>
- Yousefi, M., Khorshidian, N., Mortazavian, A. M., & Khosravi-Darani, K. (2019). Preparation optimization and characterization of chitosan-tripolyphosphate microcapsules for the encapsulation of herbal galactagogue extract. *International*

Journal of Biological Macromolecules, 140, 920–928.

<https://doi.org/10.1016/j.ijbiomac.2019.08.122>

Yuan, Y., & Lee, T. R. (2013). *Contact Angle and Wetting Properties*. In: Bracco, G., Holst, B. (eds) *Surface Science Techniques*. Springer.

Yuan, Z., Xu, Z., Zhang, D., Chen, W., Zhang, T., Huang, Y., Gu, L., Deng, H., & Tian, D. (2018). Box-Behnken design approach towards optimization of activated carbon synthesized by co-pyrolysis of waste polyester textiles and MgCl₂. *Applied Surface Science*, 427, 340–348. <https://doi.org/10.1016/j.apsusc.2017.08.241>

Zailan, M. R., Nayan, N., & Sapuan, S. M. (2022). Chemical composition and potential utilization of gastropod shells from Sarawak mangrove ecosystems. *Borneo Journal of Marine Science*, 6(2), 87–93.

Zdanowicz, M., Spychaj, T., & Wilpiszewska, K. (2018). A review of polysaccharide-based materials modified with poly(ethyleneglycol)s. *Carbohydrate Polymers*, 200, 361–380. <https://doi.org/10.1016/j.carbpol.2018.07.087>

Zdanowicz, M., Wilpiszewska, K., & Spychaj, T. (2018). Deep eutectic solvents for polysaccharides processing. *Carbohydrate Polymers*, 200, 361–380. <https://doi.org/10.1016/j.carbpol.2018.07.086>

Zhang, B., Hu, B., Nakauma, M., Funami, T., Nishinari, K., Draget, K. I., Phillips, G. O., & Fang, Y. (2019). Modulation of calcium-induced gelation of pectin by oligoguluronate as compared to alginate. *Food Research International*, 116, 232–240. <https://doi.org/10.1016/j.foodres.2018.08.020>

Zhang, J., Wang, M., & Wang, X. (2022). Extraction of chitin using NADES: An efficient and green approach. *Journal of Cleaner Production*, 365, 132847.

<https://pubmed.ncbi.nlm.nih.gov/35337528/>

- Zhang, S., Yang, Y., Gao, B., Li, Y. C., & Liu, Z. (2017). Superhydrophobic controlled-release fertilizers coated with bio-based polymers with organosilicon and nano-silica modifications. *Journal of Materials Chemistry A*, 5(37), 19943–19953. <https://doi.org/10.1039/c7ta06014a>
- Zhang, Z., Han, Z., Zeng, X., Xiong, X., & Liu, Y. (2015). Enhancing mechanical properties of chitosan films via modification with vanillin. *International Journal of Biological Macromolecules*, 81, 638-643. <https://doi.org/10.1016/j.ijbiomac.2015.08.042>
- Zhao, D., Huang, W. C., Guo, N., Zhang, S., Xue, C., & Mao, X. (2019). Two-step separation of chitin from shrimp shells using citric acid and deep eutectic solvents with the assistance of microwave. *Polymers*, 11(3). <https://doi.org/10.3390/polym11030409>
- Zhou, P., Li, J., Yan, T., Wang, X., Huang, J., Kuang, Z., Ye, M., & Pan, M. (2019). Selectivity of deproteinization and demineralization using natural deep eutectic solvents for production of insect chitin (*Hermetia illucens*). *Carbohydrate Polymers*, 225(February), 115255. <https://doi.org/10.1016/j.carbpol.2019.115255>
- Zhou, Y., Yang, D., He, Y., Liu, Z., & Xu, Q. (2020). Modification of chitosan for biomedical applications: A review. *Materials Science and Engineering*, 113, 110985. <https://doi.org/10.1016/j.msec.2020.110985>
- Zhu, P., Gu, Z., Hong, S., & Lian, H. (2017). One-pot production of chitin with high purity from lobster shells using choline chloride–malonic acid deep eutectic solvent. *Carbohydrate Polymers*, 177(6), 217–223. <https://doi.org/10.1016/j.carbpol.2017.09.001>

- Zhuo, X., Liu, C., Pan, R., Dong, X., and Li, Y. (2017). Nanocellulose mechanically isolated from *Amorpha fruticosa* Linn. *ACS Sustainable Chemistry & Engineering Journal*, 5, 4414–4420. <https://doi.org/10.1021/acssuschemeng.7b00478>
- Zohuriaan-Mehr, M. J., & Kabiri, K. (2008). Superabsorbent polymer materials: A review. *Iranian Polymer Journal*, 17(6), 451–477.

APPENDICES

Appendix 1: FTIR Method for Degree of Deacetylation (DDA) Calculation

1. Chitin is a natural polysaccharide consisting of N-acetyl-D-glucosamine units, while chitosan is the deacetylated form containing D-glucosamine units. The degree of deacetylation (DDA) is the percentage of deacetylated units ($-NH_2$) in the polymer chain and is a crucial parameter affecting chitosan's solubility, charge density, biological activity, and film-forming ability. Fourier-transform infrared spectroscopy (FTIR) is a non-destructive and rapid technique for estimating DDA. It works by analyzing the relative intensity of characteristic absorption peaks related to specific functional groups:

- 1655 cm^{-1} (Amide I band): Corresponds to $C=O$ stretching from the acetyl group of chitin/chitosan.
- 3450 cm^{-1} (O–H stretch): Broad band corresponding to hydroxyl groups in the polysaccharide backbone.

As deacetylation proceeds, the amide band at 1655 cm^{-1} decreases, while the O–H band remains relatively constant. This makes the A_{1655}/A_{3450} ratio a reliable indicator of acetyl content.

2. DDA Calculation Formula:

$$DDA(\%) = 100 - \left[\left(\frac{A_{1655}}{A_{3450}} \right) \times K \right]$$

where:

A_{1655} : Absorbance at 1655 cm^{-1} (Amide I band)

A_{3450} : Absorbance at 3450 cm^{-1} (O–H stretching band)

K : Empirical constant, commonly taken as 115 (Focher et al., 1990).

3. Reason why the 3450 cm⁻¹ band was used:

The 3450 cm⁻¹ O–H stretch is present in both chitin and chitosan and is not significantly affected by the deacetylation process. Therefore, it is used as an internal reference to normalize changes in peak intensities caused by differences in sample preparation or instrument sensitivity.

4. Calculation Example based on Chitosan extracted from NADES 2:1 80 °C from Figure 4.5.

Suppose absorbance at 1651 cm⁻¹ and 3326 cm⁻¹ are 0.0666 and 0.8500 respectively, the calculation would be as below:

$$DDA(\%) = 100 - \left[\left(\frac{0.0666}{0.8500} \right) \times 115 \right]$$

$$DDA(\%) = 100 - 9.0106$$

$$DDA(\%) = 91\%$$

This indicates approximately 91% of acetyl groups were removed, classifying the sample as moderately deacetylated.

Appendix 2: Journal Publication

1. Kimi, M. & **Hamdi, M. H.** (2023). Direct extraction of chitosan from snail shells by natural deep eutectic solvent. *Current Chemistry Letters*, 12(2), 275-280.