

## ABSTRACT

LONDONO ZULUAGA, CAROLINA. Fundamental and Applied Aspects of the Valorization of Seafood Waste: Water Remediation and Nanofiber Generation. (Under the direction of Dr. Lucian Lucia, Dr. Hasan Jameel and Dr. Ronalds Gonzalez).

Crustacean shells have long been known for being an important source of chitin, a fibrous polysaccharide of N-acetylglucosamine chains that next to cellulose is the most abundant polysaccharide on earth. Chitin has been used as an enforcement material due to its mechanical strength, low thermal expansion coefficient, and high elasticity. In addition, chitin is the precursor for chitosan production via deacetylation. Despite the outstanding properties of these natural polymers, industrial scale production is expensive. For chitin, the price range is between 3-6 USD/kg while for chitosan it is between 15-20 USD/kg depending on the grade of purity and applications. Part of those high prices is the low yield in the production process due to minerals and protein being present in considerable quantities within marine exoskeletons.

In this work, crustacean shell have been characterized and used for two purposes: Heavy metal ion removal from water and nanofiber production. Among the methods for metal ion removal, adsorption is one of the most reliable, robust, and hence prevalent. The technical feasibility and cost effectiveness of this process is highly related to the materials used. Crustacean shells, a waste from the seafood industry, have been identified as a viable material for the biosorption of lead, cadmium, chromium and zinc with removal of heavy metals up to 99%. This biomaterial is a low-cost alternative for water remediation.

Alternatively, a mechanical treatment for chitin/chitosan production is proposed in order to totally or partially avoid the chemical treatment in the production of these biopolymers at the nano scale. In this study, production of nano-chitin, non-protein crustacean shells and non-mineral

crustaceans shells was evaluated. Results showed the production of crustacean shell-based nanofibers and potential applications in blood clotting and co-grinding with cellulose.

Further studies need to be completed in rheology of the co grinded materials as well as the formation of foam-like materials need to be characterized in terms of mechanical and thermal properties.

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Fundamental and Applied Aspects of the Valorization of Seafood Waste: Water Remediation and Nanofiber Generation

by  
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## **DEDICATION**

*“To my mom, my friend, my angel,*

*A mother’s love is a fuel that enables a normal*

*human being to do the impossible”*

Marion C Garretty

## **BIOGRAPHY**

Carolina was born and raised in the city of eternal Spring: Medellin, Colombia. After finishing school at Liceo Francisco Restrepo Molina, she went to college where she started pursuing her Chemical Engineering degree at Universidad Pontificia Bolivariana in Medellin. Before graduation, she worked as innovation intern at Euroceramica group in the production of new formulations for ceramic enamels, along with this, she worked in her undergraduate project at Akzonobel Colombia. After graduation, in 2015, she worked in the same university that she graduated from as a Research Assistant in adding value to waste residues from the silk industry. In 2016, she decided to go to NC State University, Department of Forest Biomaterials to work as a visitor scholar. Being a visitor scholar, she was invited to stay as a PhD student under the supervision of Dr. Lucian Lucia and Dr. Ronalds Gonzalez. Since then she has been working in different projects with a focus on the use of waste materials such as lignin and crab shell to produce materials such as foams, bioadsorbents and nanofibers.

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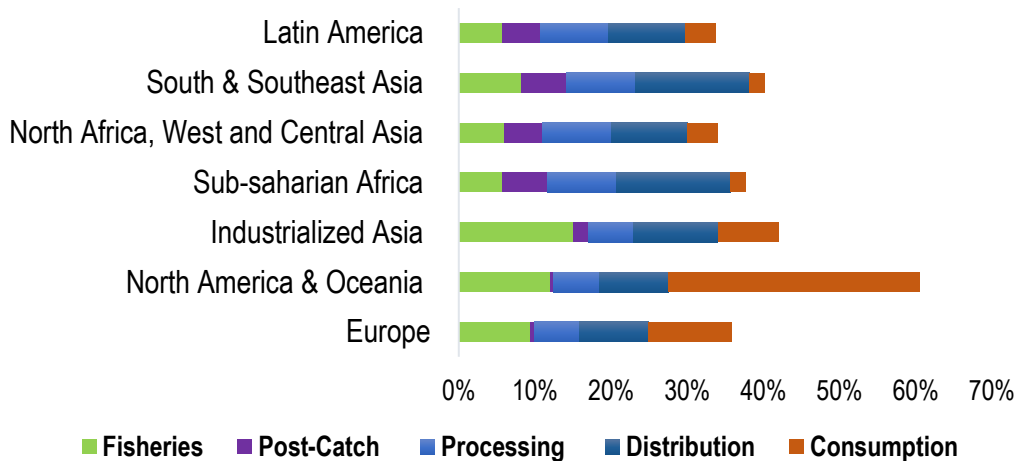
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# 1. INTRODUCTION

## 1.1. Seafood waste generation

Every year an average of 47 million tons of seafood waste are disposed globally, costing the global economy 50 billion dollars [1]. This cost is due to a poor management of our aquaculture environment, non-sustainable fishing, and a huge quantity of residues that are commonly disposed in landfill, discarded back to the ocean; whereas, only a miniscule amount is used for value added products [2].

Along with a poor management and sustainability practices, losses along the supply chain characterize the seafood industry as a large waste generator. As seen in **Figure 1.1**, the percentage of losses also change depending on the location. Most of this is due to cultural habits in seafood consumption, fishing practices, and the perishability of fish, and its derivatives [3].

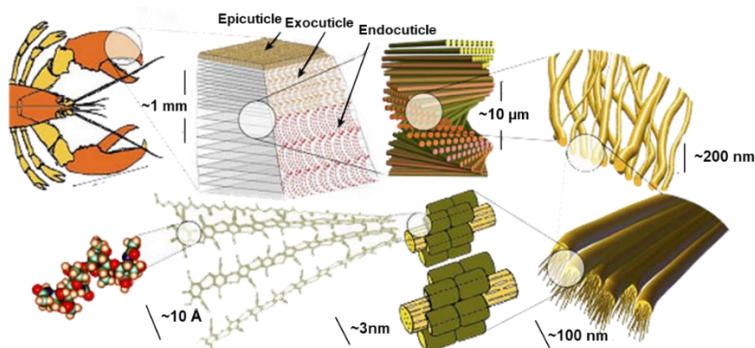


**Figure 1.1.** Seafood waste generation along supply chain. Adapted from: [3].

## 1.2. Crustaceans shells

Out of the 47 million tons of seafood waste, 6 to 8 million correspond to crab, shrimp and lobster shells [4], which are commonly discarded in landfill and a very small amount goes to

production of valuable products as chitin. Yet, shells have an enormous potential as a source of different chemical for multiple applications [4].



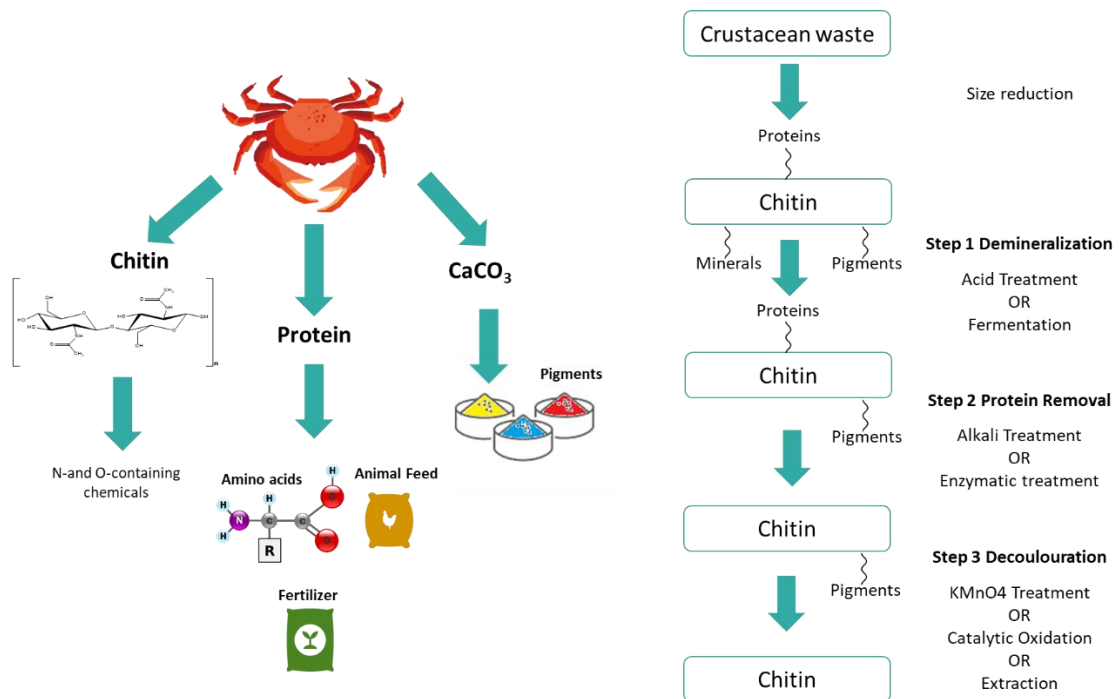
**Figure 1.2.** Crustacean shells structure. Adapted from [5].

Crustacean shells have three main components: chitin (5-50%), protein (6-48%) and minerals (4-90%) for which the variability for components depends on the species of crustaceans; for example, squid pen contains a high level of chitin relative to the other components, while cuttlefish pen have a higher level of minerals such as calcium carbonate [6]. At the molecular level, chitin is characterized by polymeric chains of N-acetyl glucosamine aligned in an antiparallel manner to give rise to the  $\alpha$ -chitin crystals, which comprise nanofibers having diameters of ~ 2-5 nm. Then, the fibers are wrapped in protein layers that help to form the chitin- protein bundles of 50-300 nm; finally, the planar woven and branched network is embedded in a variety of proteins and minerals of which calcium carbonate is the most common mineral [7]. (See **Figure 1.2**)

Out of the three main components, chitin is the most valuable because it is considered as the second most abundant natural polysaccharide [8][9] being only surpassed by cellulose [9]. It occurs in nature as a complexed substance with proteins and other polysaccharides while remaining a valuable component for crustacean exoskeleton because it helps to provide a harder shell partially due to the calcium carbonate [10].

### 1.3. Shell biorefinery concept: Crustacean shell components extraction

As mentioned above, many products can be obtained out of shell biomass, which have opened up a new concept for the way crustacean shells are treated. Different authors have followed up on the idea of a shell biorefinery [11]–[13] (Figure 1.3). This concept relies on the manufacturing of multiple products via fractionation of the crustacean shell in the same way woody biomass biorefinery does, leading to a greener and sustainable production of fuel, chemicals, and materials [13].



**Figure 1.3.** Shell biorefinery concept. Adapted from [11] and [13]

In addition to chitin, calcium carbonate is commonly used as a filler in the paper industry, while also being used in construction, pharmaceutical, pigments, plastics, etc. Finally, proteins are always a good source of amino acids and show good function as fertilizers and animal feeds [4].



### 1.3.1. Chitin

So far, most research studies have been oriented to demonstrate that chitin is the most valuable ingredient, whereas the other components are discarded. A typical extraction process for chitin includes a chemical treatment that does not allow a co-production isolation; in fact, minerals and proteins create byproducts that are waste [13]. The most common method includes particle size reduction followed by a demineralization with hydrochloric acid in which carbon dioxide is produced due to decomposition of calcium carbonate. Afterwards, protein removal with sodium hydroxide takes place in which proteins need to be denatured with an alkali treatment and temperature to be removed; in a final stage, carotenoids and color associated compounds are removed by a mixture of organic solvents [14].

A greener method for chitin extraction has been proposed: deproteinization is performed by placing the raw material with deionized water in an autoclave. The demineralization is carried out in autoclave at room temperature with deionized water and carbon dioxide after which carbon dioxide is released and the calcium is re-precipitated [15]. Other processing options include biological fractionation in which different microorganisms are used for obtaining a liquid fraction rich in proteins, minerals, astaxanthin, a solid fraction of chitin [16], while solvent extraction with ionic liquids is used to remove different components [17].

Once chitin is removed from shells, it can be transformed into multiple chemicals including 5-chloromethylfurfural, (5-CMF)[18], [19], 5-(formyloxymethyl) ,N-acetylglucosamine (GlcNAc), acetic acid, pyrrole[20], furfural (FMF) and chitosan among others [12]. Out of these chemicals, chitin is typically converted into chitosan via N-deacetylation with an alkali treatment under controlled conditions of time and temperature [21]. Besides chemicals production, chitin possess multiple characteristics including non-toxicity, biodegradability, biological activity and

film and fiber forming properties [22]. These properties allow chitin to have applications in cosmetics, pharmaceuticals, textiles, water treatment as metal ion chelates (See Table 2.2) [4] and nanofiber production [23] among others.

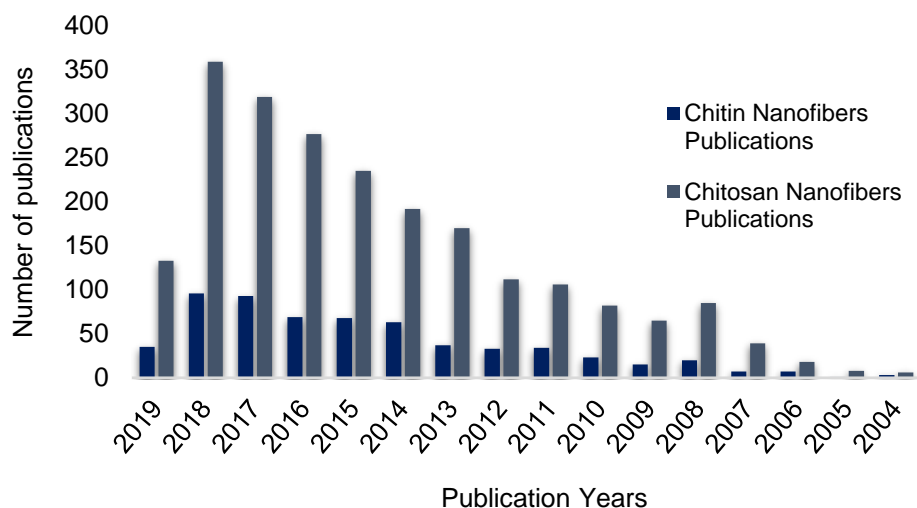
However, despite all its properties and potential applications, the arrangement of chitin within the chitin-protein bundles makes it an expensive product [24] due to its low yields and extensive chemical treatment needed [24]. Besides low yields, both chitin polymorphs ( $\alpha$  and  $\beta$  chitin) are insoluble in all usual solvents, which makes it a hard to process to be applied [25] [26]. For this reason, modified chitin (reduced degree of acetylation) or chitosan are the preferred option for industrial applications. As mentioned before, chitosan is the product of chitin deacetylation. When chitin losses about 50% of the acetyl groups, it becomes soluble in aqueous acidic median and is referred to as chitosan [26].

#### **1.3.1.1.Nanofibers**

In general, nanofiber production has been a trend since its first production back in 1930's [27]; indeed, interest in manmade fibers has grown exponentially specially within the last three decades [28]. Nanofibers have shown to be great materials in the development of sophisticated applications due to its high surface area, small pore size, and the possibility of producing three-dimensional structures [27]. Different materials have been used in its production including synthetic and natural polymers.

Among natural polymers, the most used is cellulose; however, recent research has also been focusing on chitin and chitosan nanofiber production due to their unique properties although chitosan is the most explored (See

Figure 1.4). This is due to the differences in solubility, especially since electrospinning is the most common technique used in the production of nanofibers and requires solubility in volatile solvents [29].



**Figure 1.4.** Chitin and Chitosan nanofibers publications. Source: Web of Science

Chitin nanofiber production follows two approaches: top down and bottom up [23]. In the top down approach, chitin is isolated from crustacean shells and later broken down into small pieces via mechanical treatment or acid hydrolysis. Common equipment used for breakdown include Masuko grinding [30], high water pressure jetting [31], [32] and dynamic high pressure homogenization [33]. Before mechanical processing, the protonation of primary amines is important to preserve the stability of the colloidal suspension with small fibres and facilitate dispersion and nanofiber formation upon grinding [23]. For this reason the slurry is adjusted at pH 3 with acetic acid [33]–[35]; also, once the chitin is extracted, it is kept under wet conditions in order to avoid strong hydrogen bonding formation upon drying [31].

The bottom up approach on the other hand considers the individual molecules that are assembled in a product [23]. This approach uses electrospinning as the main technique for chitin nanofiber production. As discussed previously, chitin solubility is very important in this process;

therefore, chitin is soluble in specific solvents. As detailed in Table 1.1, mixtures of N, N-dimethylacetamide (DMAC) with Lithium Chloride (LiCl), 1,1,1,3,3,3-hexa- fluoro-2-propanol (HFIP), mixtures of sodium hydroxide with urea and ionic liquids have been prepared.

**Table 1.1.** Summary of the most common techniques for chitin nanofibers production.

Raw Material	Fibrillation			Reference
	Solvent	Method	Parameters	
<b>Black Tiger Pawn Shells</b>	Water Slurry 1wt% pH 3, Pretreatment using Ultraturrax (10,000 rpm) and ultrasonic treatment for 5 min	Grinder	Masuko 1500 rpm one pass	[30]
<b>Crab Shells, mushrooms</b>		Grinder /HPWJ*	Masuko 1500 rpm gauge 0.15 mm	[31], [32]
<b>Lobster</b>		Grinder		[34]
<b>Shrimp Shell</b>		Grinder /HPWJ		[35]
<b>Lobster</b>		Dynamic high pressure homogenizing	1000 bar	[33]
<b>Crab Shells, shrimp shells and squid pen</b>	3N HCL boiling for 90 min.	Acid Hydrolysis/ Dialysis	Dialysis until pH=4/	[36],[37][38]
<b>Squid Pen</b>	TEMPO**			[39]
<b>Pure chitin</b>	HFIP***	Electrospinning	>0.002 wt% HFIP	[40], [41]
<b>Squid Pen</b>	LiCl/DMAC****		>0.02 wt%	[40]
<b>Pure chitin</b>	Ionic Liquids			[17] [42][43]
<b>Pure chitin</b>	NaOH-Urea			[44]

\*High Pressure Water Jet System

TEMPO: 2,2,6,6-tetramethylpiperidine 1-oxyl radical, \*\*\*\*DMAC: N, N-dimethylacetamide, HFIP:1,1,1,3,3,3-hexa- fluoro-2-propanol

In the same way as the production of chitin, both approaches for chitin nanofiber production are costly. In this case, in addition to chitin isolation, there are cost associated with either energy consumption (grinder) or solvent use. Perspectives and new research in the optimization of isolation and nanofiber solution are needed.

### **1.3.1.2. Crustacean shells as a whole**

As mentioned before, common crustacean shell applications include products of shell fractionation resulting in individual components; however, the properties and applications of crustacean shells as a whole (no chemical fractionation) remain somehow unknown. Few efforts have evaluated mechanical and physical properties [7], [45], [46], while fewer works have focused on the use of crustacean shells in different applications including pollutants removal from water (see Chapter 1), animal feed [47], fertilizer [48], while limited work has been done for crustacean shell-based nanofiber production [49].

Based on the properties and uses described before, we have decided to focus and explore potential applications of the crustacean shells as a whole. This document is divided in five sections. Section 2 corresponds to a literature review on the applications of crab shell as a heavy metal ion removal. In this section, cost effectiveness and challenges of the crustacean shells as biosorbents are considered. In Section 3, crab shell as a biosorbent is evaluated for lead removal and optimization of the adsorption parameters including adsorbent amount, pH, temperature and residence time was performed. In Section 4, a design of experiments was executed to evaluate the performance of crab shell in the removal of zinc, cadmium, and chromium. Finally, with the objective of evaluating other potential applications, Section 5 is dedicated to the production, characterization, and applications of crustacean shell-based nanofibers. Section 6 is related to waste from other sources (lignin) from the pulp & paper industry and a literature review on the application of this material was performed.

## 2. CRUSTACEAN SHELL-BASED BIOSORPTION WATER REMEDIATION PLATFORMS: STATUS AND PERSPECTIVES<sup>1</sup>

### 1. Abstract

The importance of water pollutants on human health has been the subject of intense study and constitutes perhaps the most significant grand challenge for the future of human society. Water remediation faces many challenges in effectively combating pollution, especially for low income populations where poor water sanitation and little to no access to technically competent and cost effective remediation are nearly insurmountable issues.

In an effort to provide low-cost adsorbents, research over the last few years has focused on biological residual materials from plants and animal biomass to not only to add value, but to remediate water at a lower cost with the same or improved efficiency as commercially available option. Crustacean shells are among a class of biological residues that are commonly treated as a waste product of the seafood industry. Potential valorization by remediation of heavy metal ions, organic matter, and anionic species is a topic of high interest in the current eco-friendly environment. The aim of this review is to provide insight on the state of the art of crustacean shells for addressing water remediation and to offer some perspective regarding challenges and the future of this type of biomass.

**Keywords:** Biosorption, Crustaceans shells, Shrimp shells, Crab shells, Lobster shell, Heavy metal

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## 2.1. Introduction

Water sanitation and hygiene are much more significant and intractable problems than water scarcity on a global scale. For example, nearly 2.4 billion people do not currently have access to adequate water cleaning facilities [50]. As a consequence, 1,800 children under five years of age die each day [51]. This situation is a much larger problem in developing countries where clean water is unaffordable because few can afford the cost of efficient cleaning methods or people need to walk long distances in order to get clean drinking water [51].

Heavy metals are ubiquitous in environmental water systems and account for a large fraction of water pollution [52]. They enter water from anthropogenic point sources that include industrial and agricultural activities and fossil-fuels combustion as the main culprits amongst others and minimally as a consequence of natural point sources such as volcanic activity and rock erosion. In general, heavy metals are defined as either metal or metalloids with a “relatively high density” (3.5-7 g/cm<sup>3</sup>) that can be toxic at low concentrations [53].

Despite several heavy metals being essential in human, animal, and plants nutrition, only small amounts are needed [54] and exceeding these amounts can be toxic. Common heavy metals include Ag (silver), As (Arsenic), Be (Beryl), Cd (Cadmium), Cr (Chromium), Cu (Copper), Hg (Mercury), Ni (Nickel), Pb (lead), Sb (Antimony), Se (Selenium), Ti (Titanium), and Zn (Zinc) [55]. These metals have a tendency to accumulate in the environment and as a result are hard to remove from the food chain with the consequence being that they can exert a heavy toll on human health [53].

Examples of heavy metal impacts on human health are brain and kidneys damage, liver, teeth and bone deterioration from lead [56]. Mercury can damage an in utero child

along by compromising developing skin, kidneys, eyes, lungs and digestive, and the immune system [57]. Arsenic can cause cancer while also damaging cardiovascular, nervous, renal, respiratory and endocrine systems [58].

Thus, international organizations, environmental agencies, and governments have encouraged and supported programs for development of new technologies for water remediation, especially for heavy metal removal. Common methods used include precipitation and coagulation, ion exchange, membrane filtration, bioremediation, photocatalysis and adsorption [53] [59].

Among the methods for metal ion removal, adsorption is the most common. The process is based on mass transfer: the metal ions in the liquid phase are transferred to the solid phase (sorbent) whose interactions are modulated by physical and chemical forces [60]. The technical feasibility and cost effectiveness is highly related to the type of adsorbent used. Some include chelating resins, activated carbon, activated alumina, kaolinite, and iron-oxide coated sand [61]. Other low-cost adsorbents from natural sources have been developed. These include zeolites, clay, fly ash, natural oxide, agricultural wastes, and crustacean shells and its derivatives [62].

Crustacean shells and associated chitin/chitosan products are known to show heavy metal removal properties that are made more attractive by biodegradability, bioactivity, biocompatibility, low cost, and nontoxicity [63]. Their performance is to a large extent dependent on physical treatments such as boiling, drying, grinding, or acid and alkali processes [64]. Also, the nature of the material will affect the adsorption depending on its chemical composition, the number of binding sites, particle size, and surface area [65].



Besides the type of material and its quality and properties, physicochemical conditions have an important impact in the performance and effectiveness of adsorption of heavy metal ions [64]. Parameters affecting biosorption include pH, contact time, adsorbent dosage, temperature, binding competition and initial pollutant concentration [64], [65].

The most relevant parameter is pH. Because pH affects the interaction chemistry of pollutants, the speciation and mobility of each metal are likely different as a function of pH. As a result, each metal can have a different oxidation state depending on the redox conditions and pH [66] which are critical to their solubility and concentration on the surface of the biosorbent. [67].

Contact time and dosage of the adsorbent are a function of sequestration/capture efficiency and materials cost. For crustacean shells, contact time necessary to achieve acceptable heavy metal sequestration may vary from 1 min [68] to 6 h [69]. The difference is due to the kinetics of the reaction between the adsorbent and the specific heavy metals. Typically, the kinetics of the reaction is fitted to a pseudo first or pseudo second order depending on the metal and the types of interaction with the material [70].

Temperature also plays a key role in the thermodynamics of the adsorption mechanism. The effect exerted by the temperature depends on the type of metal and it is also related to pH. The process might be endothermic or exothermic; for example, for an endothermic process, an increase in temperature will increase the metal adsorption [71], while for an exothermic process, an inverse relation might be observed, i.e., increased temperature will decrease the adsorption. [72].

## 2.2. Crustacean shells and components for biosorption

Crustaceans shells are biomineralized structures composed of chitin, protein and inorganic minerals as calcium carbonate [46] in the form of calcite [73]. Their high content of calcium carbonate gives crustacean shells a high degree of rigidity that is enhanced by the hierarchical structure formed by the chitin fibrils that are enmeshed with proteins and assembled into fibers [73].

Crustaceans are a major food source for humans and animals. Common crustaceans include shrimp, lobster and crab with an estimated annual production of 8 million tons [4]. The most valuable ingredient is the meat despite the possible value of the exoskeleton which the seafood industry commonly landfills during processing although a small fraction is used to extract chitin [4].

Crustacean shells by virtue of their components have different potential applications in industry. As a whole, crustacean shells, specifically crab shells, have been used as animal feed [47], fertilizer [74] [48], and for removal of organic compounds in water [75], anionic metal species [76], [77], [78] and heavy metal ions [79]–[88]. With respect to shrimp and lobster shells, there is little data on their applications (see **Table 2.1**)

**Table 2.1.** Summary of literature search for crustacean shells as biosorbents

Biomass	Pollutant	Comments	Reference
Crab Shell	Lead		[79],[80],[88], [89], [90],[82],[91], [92],
	Zinc		[88], [89],[82],
	Cadmium		[81],[92]
	Copper	acid washed Crab Shell	[81],[86]
	Chromium (III)		[81], [82],
	Chromium (VI)	Acid washed crab shell	[93]
	Nickel	Possibly chitosan	[94]
	Arsenic (V)	Acid washed crab shell	[84]
	Mercury		[85]
	Silver		[95]
Shrimp Shell	Toluene		[75]
	Selenate, chromate and vanadate	Acid washed crab shell	[76], [78]
	Dye 25		[96], [97]
	Fluoride		[98]
	Nickel		[99]
Crawfish	Arsenic (III)		[100]
	Chromium (III)		[101]
Crawfish	Chromium, Lead, Selenium		[102]

Lead [79], [80],[103], zinc [81],[88], Cadmium [81], Copper (II) [103] [81], Chromium [81], cobalt (II) [86], arsenic (V) [104] [84] and silver ions have been successfully removed from water using pure crab shell.

Crab shell particles have been used to remove lead from aqueous solutions. The results showed an adsorption capacity of 1300 mg/g at an optimum pH between 5.5-11 [79]. Under similar conditions, ion-strength effect, contact time, and crab shell dose was studied. The results showed that after 2 hours of contact time, ~ 99% of the lead was removed from an lead aqueous solution[80].

In another study, crab shell showed a higher absorption capacity for lead, cadmium, copper, and chromium from water compared to a cation exchange resin, zeolite, and activated carbon [81]. The same crab shell species was used for heavy metal removal of mixed heavy metal ions in aqueous solutions. An equimolar solution of all heavy metal ions was tested for single (one heavy metal), binary (two heavy metal) and ternary (three heavy metal) systems from single systems, chromium and lead were adsorbed largely than cadmium. For binary systems, lead removal was not affected by the presence of other heavy metal ions, while chromium showed severe inhibition by the presence of lead as well as cadmium [82].

Each component in a crab shell may display a role in the process of heavy metal ion sequestration; thus, removal of each of the components may affect final adsorbent performance. Because lead is easiest to sequester compared to copper, zinc and cadmium [81] [69], studies with this heavy metal are more common. When minerals were removed from crab shell by acid, lead removal decreased, while alkali treatment did not considerably change any results [83]. However, if calcium carbonate is removed, the adsorption capacity for the metal ions significantly dropped [86]. In a similar manner, calcium carbonate free crab shells were used to remove arsenic (V). Unlike other studies, arsenic mainly interacts with the amine groups present in the chitin structure, while for other metals, calcium carbonate has a far bigger effect on water remediation. It is important to highlight that for the crab shell species used in this study, the chitin content was ~ 53%, while for other species it was ~ 15-20% [84]. Similar studies were reported for lead and zinc removal [89].

Pure shrimp shell as a heavy metal ion removal has been poorly studied. Since shrimp shell has a higher chitin content (30-40%)[22] compared to other crustacean species such as crab shell (15-30%) [22], it is more common to find studies using the chitin or chitosan extracted from shrimp

[105],[100]. Shrimp shell has been used for biosorption of dyes [97],[96], heavy metals [101],[100], and fluoride [98]; shrimp head (no modification) has been used for nickel removal [99]. However, shrimp shell use has been directed towards optimization and extraction of chitin. For this reason, it was common to find that for shrimp shell and crab shell, the claimed use of the biomass as a whole, but after more intense discovery, the adsorbent was either chitin or a less pure form of it [94],[105]. As shown previously, even when crustacean shells applications on water treatment have been studied, few efforts have been made to improve or explain the mechanisms behind adsorption of heavy metal ions.

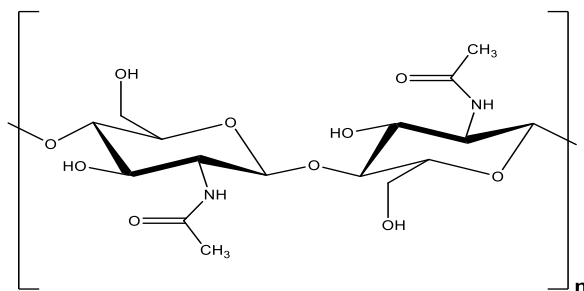
In study performed by Fabbicino, shrimp shell was used to remove chromium (III) from tannery waste water. A 99% removal of chromium by non-modified or non-pre-treated shrimp shell was found. With respect to the mechanistic interpretation of the chromium removal, no studies were available. Yet, from isotherm and kinetic analyses, it was concluded that both weak and strong adsorption sites play a role in the removal although a residence time of 2 h was determined as optimum[101]

### **2.3.Chitin biosorbency**

Chitin is a natural polymer with a high chemical similitude to cellulose. Its structure is based on 2-acetamido-2-deoxy- $\beta$ -D-glucose residues covalently bonded through linear  $\beta$  (1 $\rightarrow$ 4) linkages. (see

Figure 2.1) [25]. This natural polymer is highly abundant in the shells of crustaceans such as crab, lobster, and shrimp [106]. It is therefore by virtue of its ubiquitous nature, inexpensiveness, non-toxic material which endows this natural polysaccharide with a high potential for applications for heavy metal elimination from water [107]. Within the realm of water treatment,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ , and  $\text{As}^{3+}$  have been successfully removed through application of either chitin or

its derivatives. A summary of metal ion chelation/sequestration through the use of chitin and modified chitin has already been reported [67]–[72], [108]–[117].



**Figure 2.1.** A chemical representation of the major repeat unit in a chitin polymer.

Unmodified chitin has been widely used for metal ions chelation [68], [69], [72], [108], [109], [112]–[116]. However, to achieve a higher adsorption capacity, chitin modifications must be pursued. Chitin chemical modifications include the use of L-cysteine [114], humic acid [117], bentonite [67], polypyrrole [71] [70], acrylonitrile [110], and thiol [111].

L-cysteine modification has been studied for Zn<sup>2+</sup>, Cd<sup>2+</sup>, and Pb<sup>2+</sup> removal from water and shows higher adsorption capacity against unmodified chitin [114]. Humic acid modification has been used for Cr<sup>3+</sup> adsorption [117]. Compared to unmodified chitin, Cr<sup>3+</sup> adsorption capacity increased with humic acid modification from 7.74 mg/g [113] to 35.57 mg/g [117]. Chitin modification with bentonite has been used for chromium (III) showing improved adsorption capacity relative to unmodified chitin [72] and polypyrrole-chitin modification [67].

Polypyrrole modification has been used for adsorption of Cr<sup>6+</sup> [71], Pb<sup>2+</sup>, and Cd<sup>2+</sup> [70]. Compared to other methods this modification does not show improved adsorption capacity for heavy metals, but instead a moderate adsorption by the modified product [71] [70]. Grafting of acrylonitrile on chitin surface has also been used for arsenic (III) removal showing promising results by completely removing the arsenic from water. It is important to highlight that the adsorption was done using a packed bed of the material, which increases the contact area and as a

result improved adsorption is obtained [110]. Chitin/thiol nanofibers have also been used for arsenic removal in which thiol modification shows improved adsorption capacity of arsenic compared to commercially available adsorbents as activated carbon [111].

Other composites have used chitin/cellulose fibers for metal ions absorption. The composite was chemically modified using ethylenediaminetetraacetic acid (EDTA) to capture  $\text{Hg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Ni}^{1+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Ca}^{2+}$  [118]. Table 2.2 shows a summary of chitin a modified chitin studies for heavy metal ion removal

**Table 2.2.** Chitin and modified chitin as heavy metal ion removal

Material	Metal Ion	Metal concentration	Solution Volume	Adsorbent amount	Contact time	Adsorption capacity (mg/g)	pH Conditions	Reference
Chitin L-Cystin	Cu <sup>2+</sup> , Cd <sup>2+</sup> , Pb <sup>2+</sup> , Zn <sup>2+</sup> , Ni <sup>2+</sup>	25 mg/mL	10 mL	0.1 g		86.10, 351.50, 214.60, 107.00, 11.20 respectively	5-6.5	[114]
Chitin	Cu <sup>2+</sup> , Cd <sup>2+</sup> , Pb <sup>2+</sup> , Zn <sup>2+</sup> , Ni <sup>2+</sup>	25 mg/mL	10 mL	0.1 g		57.90, 108.00, 132.40, 46.90, 21.30 respectively	5-6.5	
Chitin	Fe <sup>3+</sup>	14 mg/L	50 mL	10 mg	8 min	32.35	3	[115]
Chitin	Zn <sup>2+</sup>	300 mg/L	100 mL	0.1 g	24 h	8.21	4.5	[116]
Chitin-humic acid	Cr <sup>3+</sup>	8mg/L	10 mL	10 mg	3h	10.20	3-4.5	[117]
Chitin	Cd <sup>2+</sup>	0.1 g/L	30 mL	40-85 mg	N/A	93.90	5.41	[108]
Calcareous chitin	Cd <sup>2+</sup>	474 mg/L	15 mL	50 mg	15-90 min	71.40	4-7	[68]
Chitin	Cd <sup>2+</sup>	475 mg/L	16 mL	51 mg	15-90 min	32.40	4-7	[68]
Chitin	Zn <sup>2+</sup>	50-500 mg/L	250 mL	0.5-10 g	180 min	270.27	7	[109]
Chitin/Bentonite	Cr <sup>6+</sup>	50-1000 mg/L	100 mL	1 g	45 min	394.00	4	[67]
Chitin-polypyrrole	Cr <sup>6+</sup>	50 mg/L	50 mL	0.1 g	60 min	28.92-35.22	2-5	[71]
Chitin from Bargi fish	Cr <sup>6+</sup>	50 mg/L	50 mL	0.1 g	60 min	37.04	6-8	[72]
Chitin-acrylonitrile	As <sup>3+</sup>	65-650 mg/L	200 mL	3g	24 h	19.72	N/A	[110]
Chitin-polypyrrole	Pb <sup>2+</sup> , Cd <sup>2+</sup>	10 mg/L	50 mL	0.1 g	60 min	13.45 and 8.11 respectively	6	[70]
Chitin	Cu <sup>2+</sup> , Cd <sup>2+</sup> , Zn <sup>2+</sup>	100 mg/L	300 mL	0.6 g	6h	20.50, 13.27 and 5.35 respectively	7	[69]
Chitin-Thiol	As <sup>3+</sup>	100 ppm (Na <sub>3</sub> AsO <sub>3</sub> )	N/A	0.5 wt%	?	149.00	7	[111]
Chitin	Cu <sup>2+</sup>	150-300 mg/L	100 mL	0.2 g	1-90 min	58.67	7	[112]
Chitin	Cr <sup>3+</sup>	50 mg/L	50 mL	100 mg	4 h	7.74	pH 3-5	[113]



## **2.4. Calcium carbonate biosorbency**

Calcium carbonate ( $\text{CaCO}_3$ ) is a widespread mineral commonly found within the crust and marine sediments [119]. It is also known for being part of bones, teeth, and shells [73]. In nature, it can be found in six different structures having the same composition: calcite, aragonite, vaterite, calcium carbonate monohydrate, calcium carbonate hexahydrate, and amorphous calcium carbonate [120]. Among those structures, calcite, aragonite and amorphous calcium carbonate are most common in crustacean shells providing high mechanical strength [73]. Calcite and aragonite are two of the most common minerals in seawater reactions. They exhibit polymorphism that allows them to chelate divalent cations other than  $\text{Ca}^{2+}$ ; which explains its trend to exchange cations with heavy metal ions [119].

With respect to heavy metal ion removal, calcium carbonate has been virtually ignored despite its significance during the adsorption process of heavy metal ions by crustacean shells. Calcium carbonate have been used for the elimination of cadmium in wastewaters using its aragonite form [121]. As part of this study, it was concluded that the metal uptake was caused by precipitation of cadmium-carbonate compounds on the surface as a type of calcite-type structure [121]. In another study, calcium carbonate was used for groundwater remediation of arsenic, zinc, and nickel. Results regarding the calcium carbonate showed high efficiency for the removal of nickel and zinc (~95%), but poor with respect to arsenic (~50%) [122].

## **2.5. Cost effectiveness**

In general, biosorption is a practical economic method for selectively removing heavy metal ions. When assessing the implementation of a new biosorbent, costs associated to operation and processing of the biomass need to be addressed [123]. Regarding operational cost, adsorption is known for having a low energetic requirement and facile operation, which makes the process

per se a low-cost procedure [124]. However, one adsorption process deficiency is a secondary stage as filtration or solid-liquid separation to obtain clean water [124].

On the other hand, biomass cost will be related to availability and is treated either as a byproduct or a waste; also, for biosorption, the costs might vary depending on the processing and physico-chemical treatment needed to activate binding sites, increase surface area, or desorption/reuse steps [125]. Specifically, for crustacean shells, biomass availability is estimated to be 8 million tons, most of which goes to landfill and just a small part goes for added value products [4]; landfilling cost can be removed from seafood processing companies, crustacean shell applications can not only increase profitability, but reduce environmental impact and operational cost of this type of organizations.

Crab shell processing for biosorption is straightforward and does not require pretreatment or modification. From the work cited in this paper it can be concluded that for low-cost efficient material, only drying and sieving are necessary to obtain good overall performance of the material. Additional chemical treatments might improve heavy metal ion removal, but this may not always be globally applicable.

## **2.6.Challenges of crustacean shells as biosorbents**

Biosorption is versatile, flexible, cost effective process [125]. However, multiple challenges need to be overcome to achieve commercialization [64], [65], [123], [125]. While a large market is available for water remediation materials, it is mainly dominated by a well established industry of ion exchange resins and traditional methods that have well developed technology and standardized processes [123],[126]. Permeability into this market and lack of solid capitalization of new technologies have not vigorously allowed biosorbents to be widely studied nor commercialized. On the other hand, to make competitive biosorbents, technical feasibility

issues need to be addressed. Those issues include improved adsorption capacity and selectivity towards specific or multiple pollutants, adaptability to existing facilities and effluent handling, which include proper and cheap disposal of the sludge produced after water remediation [125]

### 3. DYNAMICS AND KINETICS STUDIES FOR THE BIOSORPTION OF Pb (II) ONTO CRAB SHELLS<sup>2</sup>

#### Abstract

Crustacean shells, a waste from the seafood industry, have been identified as a viable biomaterial for the adsorption of lead. The dynamics and kinetics of its performance has been evaluated in batch experiments for heavy metals under pH, temperature, initial concentration, and time. A key finding was that among the native components of crab shell matrix, i.e., chitin, protein, and calcium carbonate; calcium carbonate was instrumental in sequestration of smaller heavy metals. The role of protein was minimal whereas the efficiency of chitin in the complexation of heavy metal ions was linked to the radius and the size of the contaminant. Bigger contaminants such as lead, for example, were more likely to interact. The optimal set of conditions for lead removal were found to be 1-hour residence time, pH 4, and temperature of 30 °C, and 200 mg of crab shell.

**Keywords:** *Dynamics, kinetics, biosorption, lead, Pb(II)*

#### 3.1. Introduction

Increasing human activity and industrialization are currently the main point sources of pollutants that are found in the environment. Large volumes of effluents that contain heavy metals, radioactive waste, and organic pollutants are discharged into natural watersheds that eventually contaminate soils, wildlife, fish, and humans [65]. Heavy metals are among the most persistent and intractable contaminants compared to organic compounds and petroleum derivatives [127]

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<sup>2</sup>The material in this chapter has been submitted as:

Carolina Londono-Zuluaga, Hasan Jameel, Ronalds W. Gonzalez, Lucian Lucia. Dynamics and kinetics of marine exoskeleton-based lead bio sorption. *Journal of Environmental Chemical Engineering*

with a tendency to bio-accumulate, thus facilitating transit through the food chain [53] eventually resulting in heavy metal poisoning in humans.

In humans, heavy metal poisoning is consequence of exposure to contaminated water, food, or air [127]. Although several heavy metals are needed for human life (e.g., Se), higher levels can have severe adverse effects. Today, thirty-five metals have been classified as a threat for human health of which twenty-three are heavy metals [128] whose intake through drinking water can lead to cancer, heart diseases, anemia, kidney and liver damage, among others [58] [57].

Even though the consequences of heavy metals consumption by human are known, removing them from water is a challenge. Common water remediation technologies include chemical precipitation and electrochemical treatment; however, these methods are only amenable to high heavy metal concentration. Alternative technologies include membrane filtration and activated carbons, zeolites or synthetic polymers; yet, these are expensive because they are highly dependent on availability of raw material [129]. Adsorption with activated carbon is commonly used because it provides a wide pore size distribution and large surface area; nonetheless, large energy consumption and low yields conspire to deliver an expensive product for treating large amounts of water [129]. Consequently, activated carbon, even though effective, is unfeasible for large scale applications nor is it affordable for low-to-medium income communities [130].

New materials for heavy metals removal have been studied to assess technical feasibility and economics. Biosorption, understood as the use biological material for adsorption purposes [125], has been an intense object of recent study for a number of reasons [131]. Biomaterials have been studied as potential low cost adsorbents because they potentially have high metal binding capacities and are ubiquitous [60]. Examples of such biomaterials include algae [130],

microorganisms [131], bacteria [125] and biomass from fruit peel, bagasse, black liquor, and crustacean shells [129].

Crustaceans shells have been used as heavy metal sequesters because they are a low-cost option and are available in large quantities as a by-product of the sea food industry[132]. It is estimated that 6 to 8 million tons of crab, shrimp, and lobster shell are produced every year. [4] Typically, crustacean shells are disposed in landfills because they are not exploited for valorization; however, landfilling costs in several countries are high [4]. Alternative uses have been considered that include chemical treatment for the production of fertilizers [49],[48], animal feed [47] and heavy metal ion sequesters [79]. The current work has focused on the removal of lead through crushed crab shells. Their performance was evaluated at high and low concentrations with concomitant material characterization.

## **3.2. Materials and Methods**

### **3.2.1. Materials**

Crab shells were purchased from a seafood processing company. Sodium hydroxide, hydrochloric acid, and lead nitrate were used for pH adjustment, protein and mineral removal, and heavy metal stock solution preparation, respectively.

### **3.2.2. Methods**

**Shell cleaning and particle size reduction.** Crab shells were washed and cleaned using deionized water and oven dried at 60 °C for 24 h. Particle size reduction was performed using a Wiley mill until a 40 mesh particle size was achieved.

**Composition Analysis.** Raw material (crab shell) characterization was performed to determine chemical composition (protein, calcium carbonate, and chitin content). Mass

balance was performed by removing each component. Protein was removed using sodium hydroxide 1 M at 65 °C for 2 hours. Subsequently, calcium carbonate and other minerals were removed by an acid treatment with hydrochloric acid 1.5 M at room temperature for 5 hours. Elemental analysis was used to measure the amount of Nitrogen and Calcium present. The results can be translated into protein content using the following equation:

$$\%P = (\%N - 6.9) \times 6.25 \quad (1)$$

Where % P is the fraction of protein and % N is the fraction of nitrogen reported by elemental analysis. 6.9 corresponds to the theoretical percentage of nitrogen on fully acetylated chitin and 6.5 corresponds to the theoretical percentage of nitrogen in proteins [133].

**Fourier transform infrared spectroscopy (FTIR).** The analysis of functional groups present in the composite material was accomplished using UATR in a PerkinElmer Frontier IR single range spectrometer. The range used for the analysis is 4000-650  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$  and 32 scans.

**X-Ray Diffraction (XRD).** Rigaku Smart Lab X-Ray diffractometer was used for XRD measurements of powdered samples. The experiments are carried out at  $2\theta=5-40^\circ$ . [14]. According to the intensity of the peaks, it is expected to calculate the crystallinity index (CI) after mathematical manipulation of the data. The CI is calculated as follows.

$$CI = \frac{I_{110} - I_{am}}{I_{110}} \quad (2)$$

Where  $I_{110}$  corresponds to the intensity peak at 110 lattices and  $I_{am}$  corresponds to the intensity peaks of the amorphous part. Chitin is expected to obtain peaks  $\sim 20^\circ$  and  $16^\circ$ .

**Scanning electron microscopy (SEM).** Morphological analyses of the samples was done using SEM with a Field Emission Scanning Electron Microscope – FEI Verios 460L. For sample microphotographs, a voltage of 1.00 kV and 13 pA current was used. For higher magnification samples, a 500 V bias was used to reduce sample charging.

**Energy-dispersive X-ray spectroscopy (EDS)** Elemental Analysis was performed using an Oxford energy dispersive X-ray within a Variable pressure scanning electron microscope (VP-SEM) Hitachi S3200N.

**Brunauer–Emmett–Teller (BET)** The specific surface area (BET) for the samples was measured with a Micromeritics Gemini VII 2390p, Norcross, USA instrument, using the adsorption of N<sub>2</sub> at at 77 K.

**Batch Experiments.** Once the raw material was characterized, the crab shell was grounded and used as an adsorbent for the removal of lead. We determined removal efficiency for initial concentrations of 100 mg/L and 10 mg/L whose results are shown in the following sections.

Metal uptake (mg/g) was used to assess performance of the material. In order to know the parameters that affect product performance, the effect of pH, adsorbent dose, contact time, and temperature were evaluated. Crab shell was used for the removal of lead (Pb<sup>2+</sup>). Metal ion concentration was measured using an ICP-MS (Inductively coupled plasma mass spectrometry) Perkin Elmer Elan DRCII.

$$q_e = \frac{V \times (C_0 - C_e)}{W} \quad (3)$$

$$\%Removal = \frac{C_0 - C_e}{C_0} \times 100\% \quad (4)$$



Where  $q_e$  is the metal uptake (mg/g),  $V$  is the volume of the solution (mL),  $W$  is the weight of the adsorbent (g),  $C_0$  is the concentration of metal ions before adsorption (mg/mL), and  $C_e$  is the concentration of metal ions after adsorption (mg/mL).

**Effect of pH.** The metal uptake was determined at different pH of the aqueous solution. pH was controlled using diluted solutions of hydrochloric acid (HCl) and sodium hydroxide (NaOH).

**Effect of adsorbent dose.** Amounts of adsorbent (0.1-1.0 g) were placed in contact with the metal ion solution during 1 h and metal uptake was determined.

**Effect of contact time.** Standard amounts of adsorbent were put in contact with the heavy metal solution between 10-240 min.

**Effect of temperature.** In order to analyze the thermodynamic behavior of the adsorption process, the effect of the temperature in the adsorption process was measured from 20-40 °C.

**Adsorption Isotherms.** The adsorption process was evaluated using a variable concentration of heavy metal from 10 mg/L to 1000 mg/L, and the data obtained was fitted to Langmuir and Freundlich isotherm models. The adsorption of a monomolecular adsorption of a single metal from a liquid phase to a solid was modeled using Langmuir and Freundlich adsorption isotherm models detailed below [134]:

$$q_e = \frac{q_{max}bC_e}{1 + bC_e} \quad (5)$$

Reorganizing equation (5), we have a linear relationship as follows:

$$\frac{1}{q_e} = \frac{1}{bq_{max}C_e} + \frac{1}{q_{max}} \quad (5)$$

Where  $q_{\max}$  is the maximum metal uptake (mg/g),  $b$  is the Langmuir equilibrium constant (L/mg), and  $C_e$  is the final concentration of the metal in the solution.

$$q_e = K_f C_e^{1/n} \quad (6)$$

In order to obtain the parameters  $K_f$  and  $1/n$ , the equation was modified to give a linear relation:

$$\text{Log}(q_e) = \text{Log}(K_f) + \frac{1}{n} \text{Log}(C_e) \quad (7)$$

Where  $q$  is the metal uptake (mg/g),  $K_f$  is the adsorption capacity at unit concentration (L/mg),  $1/n$  is the strength of adsorption, and  $C_e$  is the final concentration of the metal in the solution.

### 3.3. Results and discussion

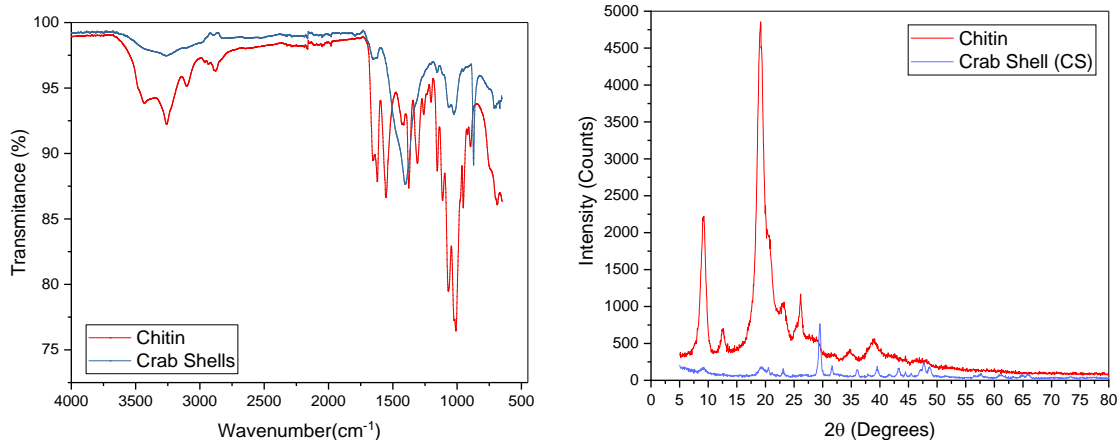
**Characterization.** Part of the characterization studies was to recognize the main constituents of the raw material. Acid and alkali treatments were used for calcium carbonate and protein removal from the crab shell, respectively. In theory, crab shell and crustacean shells are composed of mainly calcium carbonate, protein, and chitin [6]. The amount of each changes depending of the species. After acid extraction with hydrochloric acid (1.5 M), the calcium carbonate was determined to be 68% of total mass. The protein content was determined using bicinchoninic acid assay (BCA) protein analysis and elemental analysis after alkali treatment (1 M). As a result, 8.42% of protein was determined. Chitin content was determined as 15.10% and water content as 6.45%. All the values are reported on a dry basis. The values for the crab shell composition are comparable to what was previously reported [135].

**Fourier transform infrared spectroscopy (FTIR).** FTIR spectra (**Error! Reference source not found.**a) were used to identify key functional groups for chitin and crab shell. For chitin,

distinct peaks at 3443, 3259, 1656, 1625 and 1553  $\text{cm}^{-1}$  were observed. These peaks are attributed to the stretching of OH groups, NH groups and amide I and amide II, respectively [71][136]. In crab shell, peaks at 3262, 1635, 1420, 1052 and 872 are observed. The peaks are attributed to the NH stretching, -N-H-CO stretching, NO<sub>2</sub> groups in protein, and calcium carbonate [95]. [137].

**X-Ray Diffraction (XRD).** Among crab shell components, calcium carbonate and chitin crystal structures are capable to diffract X-Rays. The peaks with higher intensity are related to one of these latter structures. In , X-Ray diffraction patterns of crab shell and chitin can be observed. In the samples of pure crab shell the peak at  $2\theta \sim 30^\circ$  is related to the presence of calcium carbonate in its polymorphic structures; calcite [138], which at room temperature, is the most thermodynamically stable form of  $\text{CaCO}_3$  [139].

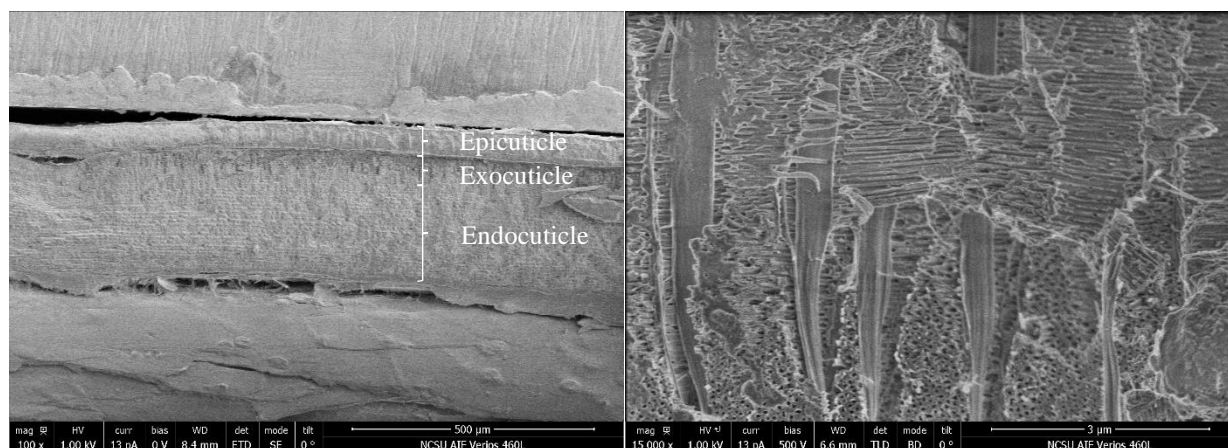
On the other hand, for pure chitin samples, the main peaks are found at  $2\theta \sim 9.15$ - $9.25^\circ$  and  $19.05$ - $19.15^\circ$  that are related to the amorphous and crystalline diffraction of chitin, respectively. These peaks serve to determine the crystallinity index of the chitin structure based on eq (2), which for chitin was found to be 0.54. Together with the results from FTIR and what has been reported, it is conjectured that the structure present in the crab shell corresponds to the  $\alpha$ -chitin polymorph [140].



**Figure 3.1.** a) FTIR spectra of chitin and crab shells. b) X-Ray diffraction for pure crab shell and pure chitin.

### Scanning electron microscopy (SEM)

Scanning electron microscopy was used to determine the material morphology. As seen in Figure 3.2 a, crab shell is composed of three external layers. The first one is the epicuticle that is mostly a waterproof barrier followed by the exocuticle that helps support mechanical loads, and finally the endocuticle that comprises ~ 90% of the shell [7]. In a more detailed image, Figure 3.2 b, the chitin fibers can be observed with orientation. In addition, a porous structure, responsible for nutrient transport within the structure can also be seen.

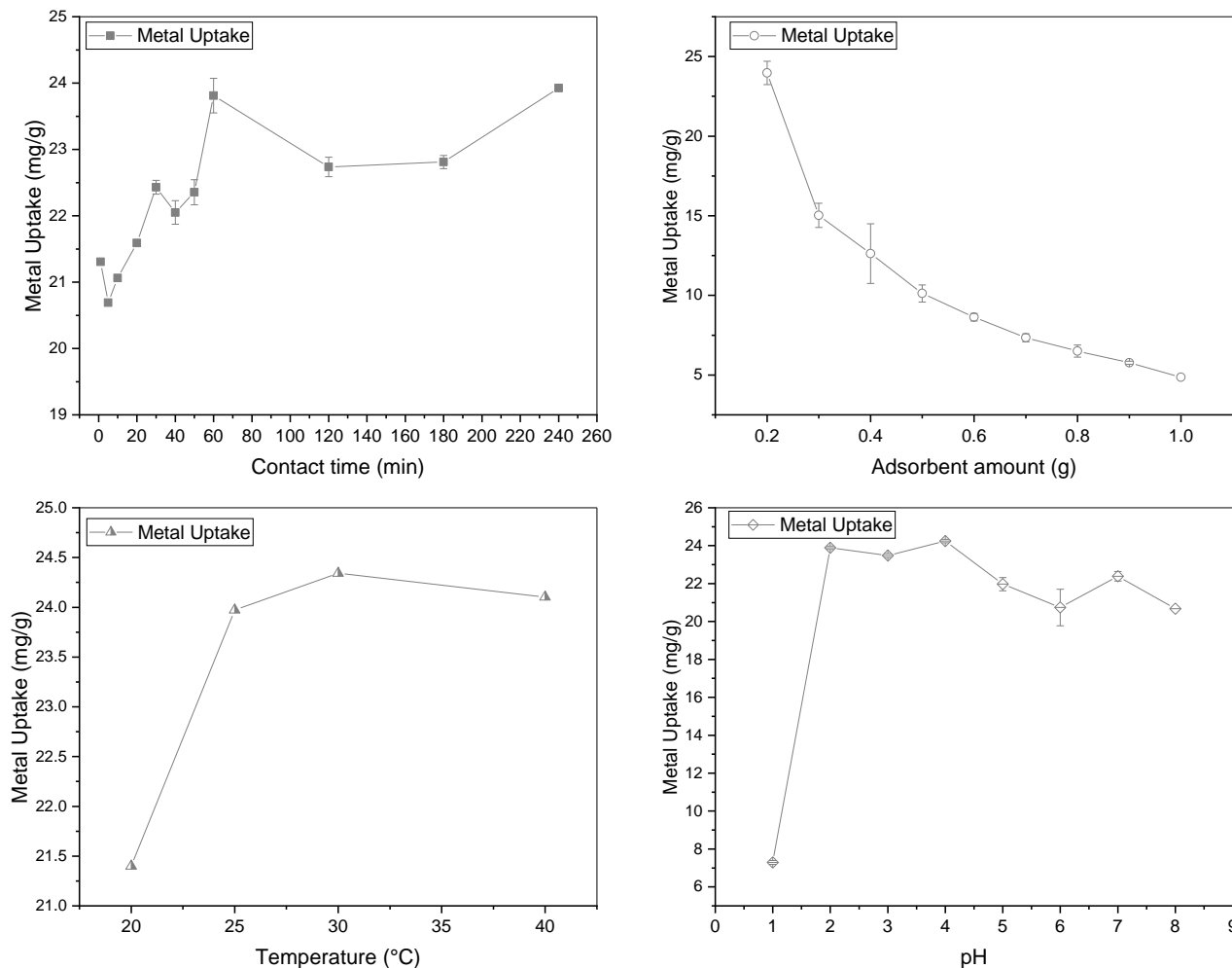


**Figure 3.2.** SEM images for a) Crab Shell 100X 100 μm. b) Crab Shell 15000X, 3 μm

## **Batch Experiments**

### **High concentration 100 mg/L**

Optimization of the product was done according to the process previously detailed. Adsorbent amount, contact time, pH, and temperature effect were evaluated. As observed in Figure 3.3 under constant conditions of pH, initial concentration, contact time, and temperature, increased addition of adsorbent will decrease adsorption capacity of heavy metal ions due to steady state saturation kinetics. With respect to contact time, longer times allow increasing adsorption, in which the removal efficiency varies from 98.66% after one hour to 99.32% after four hours; the adsorption capacity changes from 25.5 to 26.5 mg/g. With respect to pH, higher adsorption is achieved for pH between 3 and 4. These phenomena are due to the species of lead that are formed at that pH which allows precipitation at the surface of the crab shell followed by removal of lead. In addition, the acidic pH helps in the ionization of calcium carbonate, favoring formation of different complexations between heavy metal and calcium carbonate ions. Finally, temperatures ~ 30 °C will favor the heavy metal uptake by crab shell.



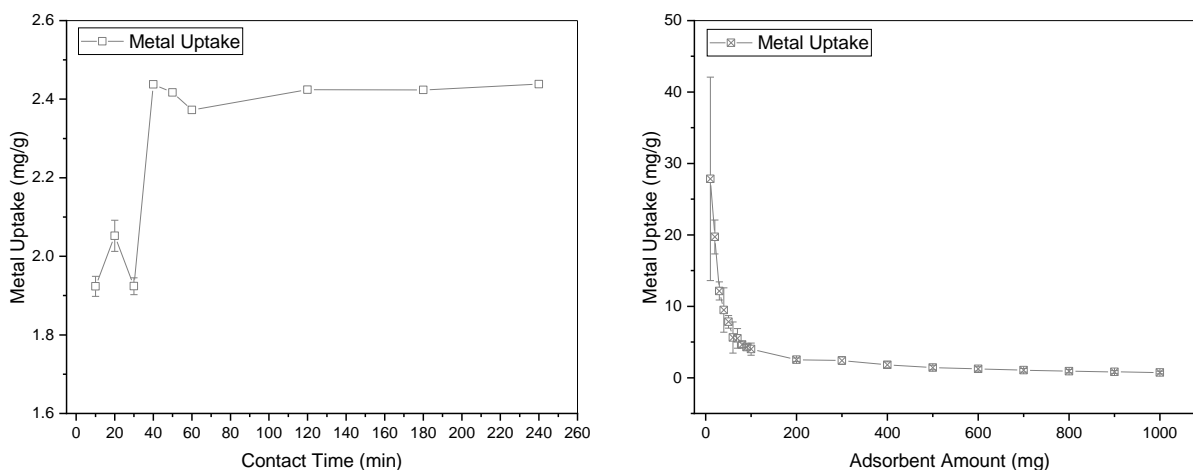
**Figure 3.3.** Effect of different adsorption parameter in adsorption capacity of lead by crab shell with 100 mg/L of lead as starting concentration. a) Adsorbent amount b) Contact time, c) pH, d) Temperature.

### Low con concentrations 10 mg/L

Common concentrations for lead in raw drinking waters are in the range of 10 mg/L. However, most adsorbents fail to remove heavy metals at this concentration. Consequently, it was decided to evaluate the performance of heavy metal ions at concentrations starting at 10 mg/L; the target was to reach at least 0.01 mg/L. Even when crab shell was proven to remove large amounts of lead, it reached a plateau ~ 0.2 mg/L. As seen in **Figure 3.4**, similar conditions for high concentrations were evaluated. As a result, at low concentrations, maximum metal uptake

corresponded to 42 mg/g at 10 mg of material; indeed, pH 5 is ideal and at ~ 60 min of residence time.

The conditions were evaluated at pH obtained from optimization at high concentrations. Since pH changes speciation of lead in water, it is logical to assume that the optimum pH for high concentrations would also be optimum at low concentrations.



**Figure 3.4.** Effect of different adsorption parameter in adsorption capacity of lead by crab shell with 10 mg/L of lead as starting concentration. a) Contact time, b) Adsorbent amount

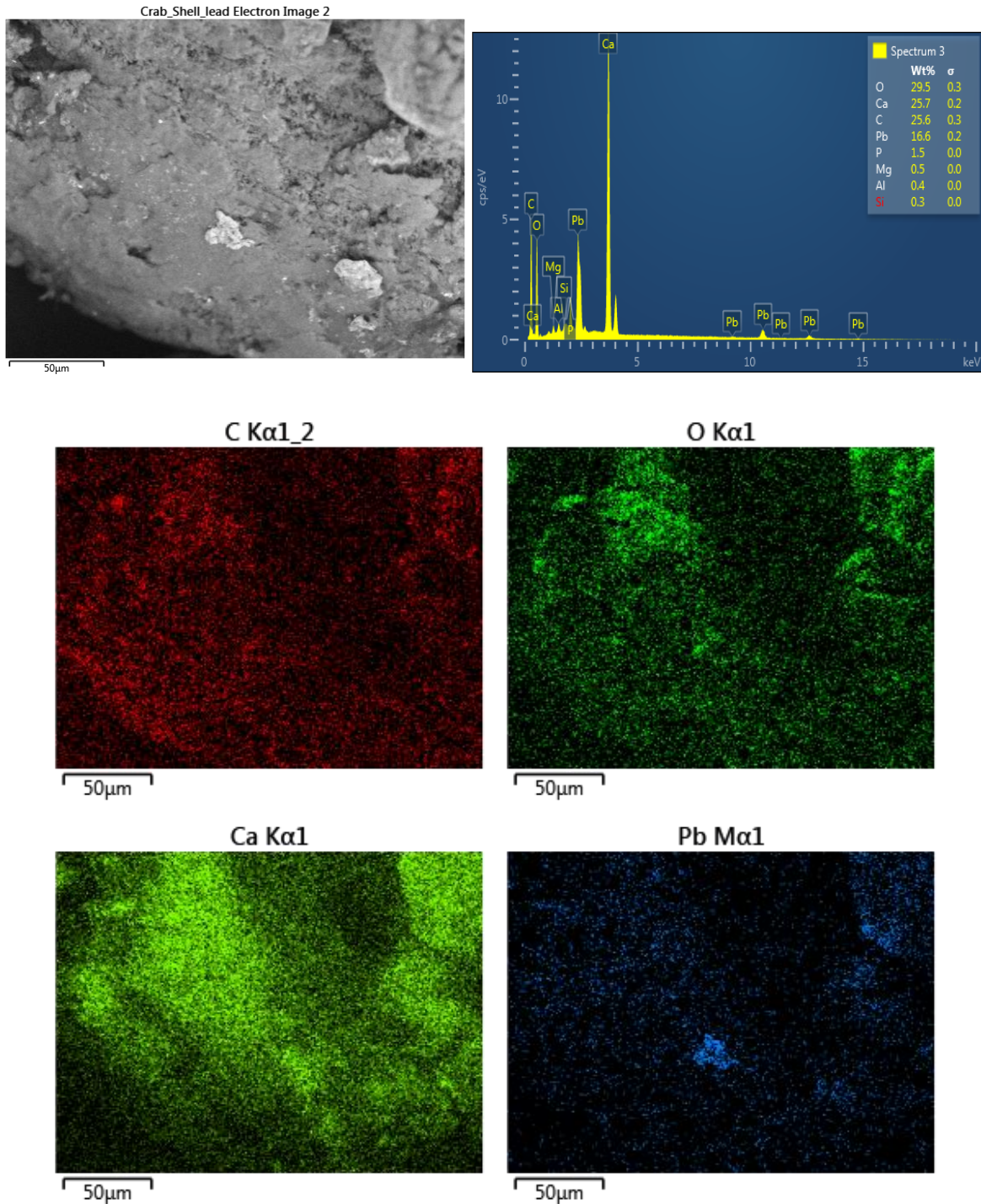
### Energy-dispersive X-ray spectroscopy (EDS)

EDS Analysis were used to estimate the final concentration of different elements in the crab shell surface. As seen in

Figure 3.5, in the spectra the presence of lead can be observed in the surface. Additionally, it is important to consider the peaks associated to calcium since this element is abundant in crab shell composition and plays a key role in the adsorption process.

The calcium in the surface is mainly due to the calcium carbonate and in the case of the adsorption by crab shell; the main mechanism for removal of heavy metal is a cation exchange between the calcium and the metal ions. At the end, the expected is a precipitation of (Pb, Ca) CO<sub>3</sub>

compounds in the surface that facilitates the removal. This has been the case not only for lead, but for other heavy metal ions removed by calcium enriched adsorbents [141].

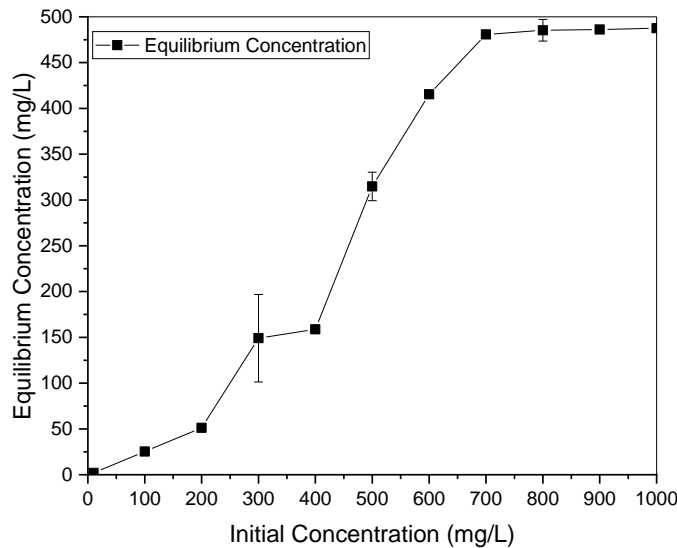


**Figure 3.5.**EDS spectra and X-Ray mapping for crab shell biosorbents after lead adsorption.



### Adsorption isotherm

Once optimum results for adsorption of lead were obtained, an adsorption isotherm was evaluated at optimum pH, residence time, and adsorbent levels. The adsorption isotherm is shown in Figure 3.6. Using the adsorption isotherm, experimental data were fitted according to a Langmuir and Freundlich isotherm (eq 5). The maximum adsorption capacity using the first model was 384.615 mg/g reported in Table 3.1. In this case, the system does not fit 100% to the Langmuir isotherm, this allows to assume a monolayer adsorption at least in one part of the process, then multilayer adsorption will take place as later confirmed with the BET studies.



**Figure 3.6.** Adsorption Isotherm.

**Table 3.1.** Langmuir isotherm linear model parameters.

Regression Parameter	Langmuir Isotherm	Freundlich Isotherm
$1/Q_{Max}b$	0.316	N/A
$Q_{Max}$ (mg/g)	384.615	N/A
$b$	0.008	N/A
$\ln K_f$	N/A	0.890
$K_f$	N/A	7.757
$n$	N/A	1.555

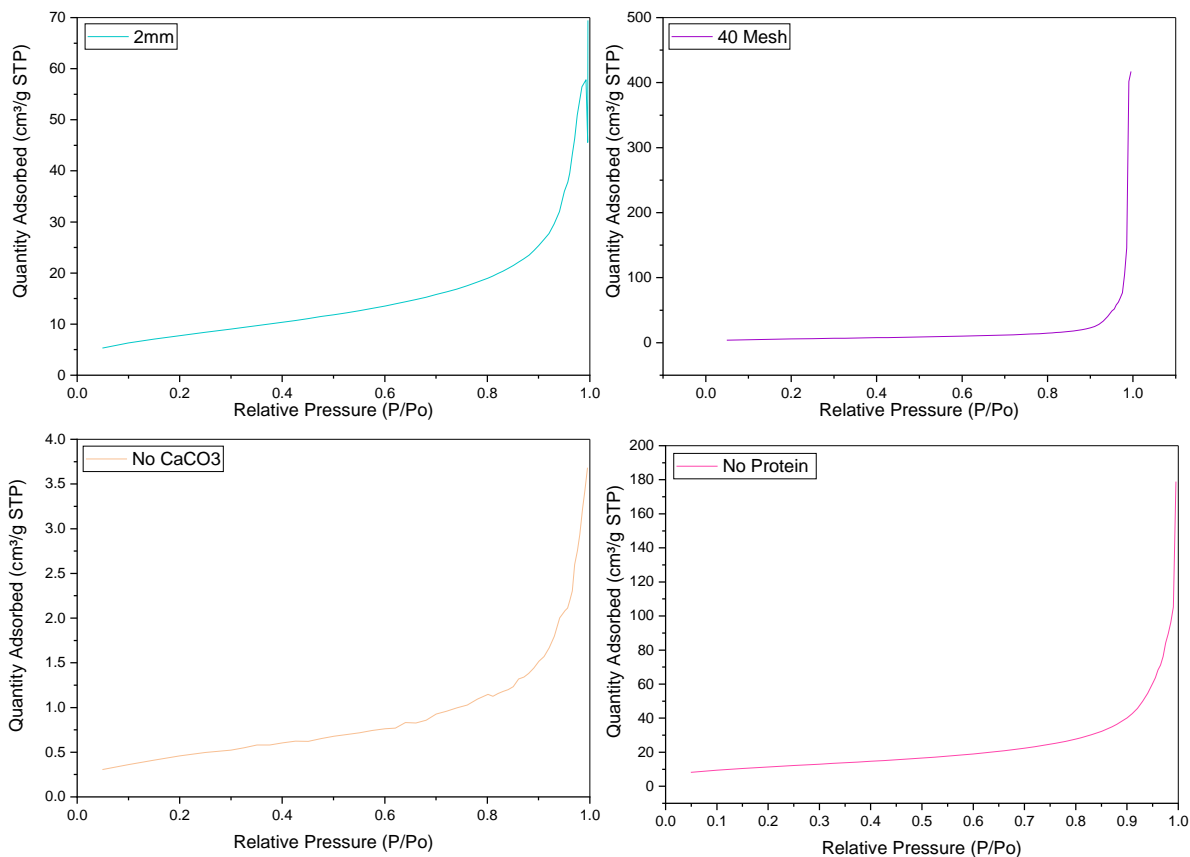
### **BET Surface Area**

BET analysis was performed with the objective to determine surface area values for the materials along with the type of adsorption that our material. As a result, we can have an idea of the porous texture of the solid. In Table 3.2 results associated to the total surface area can be observed for crab shell and crab shell after alkali treatment (No protein) and acid treatment (acid). In relation with the results obtained by ICP-MS, a higher adsorption is obtained by crab shell once the protein is removed. This phenomena takes place, since after protein removal, the calcium carbonate is more exposed, offering a higher interaction with the heavy metal ions.

**Table 3.2.**BET Surface area results.

<b>Material characteristics</b>	<b>Surface Area (m<sup>2</sup> /g)</b>
Crab Shell 40mesh	28.73
Crab Shell No Protein (No Protein)	49.12
Crab Shell No Mineral (No CaCO <sub>3</sub> )	2.18

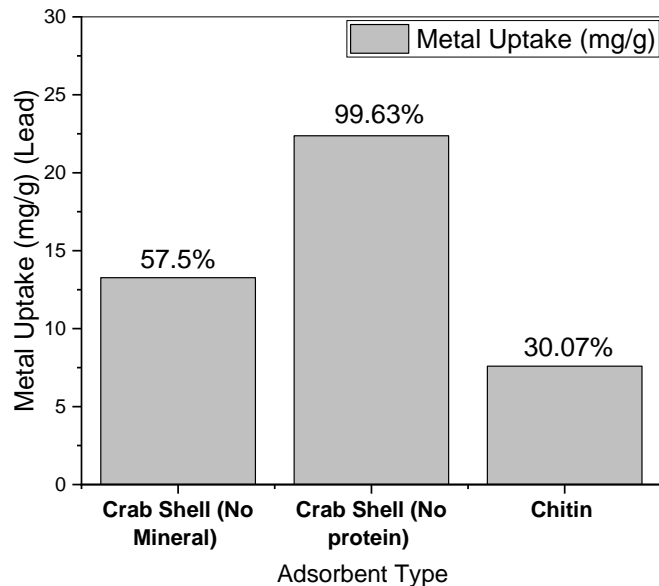
On the other hand, an analysis of the relative pressure vs adsorption gives us an idea of the type of adsorption. As seen in Figure 3.7, the behavior of crab shell adsorption is more similar to type II adsorption isotherm. In this case, at low pressure there is the formation of a monolayer, while at high pressure a multilayer adsorption takes place. As a result, we could infer a macro porous structure within the crab shell [142].



**Figure 3.7.** Relative pressure vs Quantity adsorbed for a) Crab shell 2mm, b) Crab shell 40 mesh, c) Crab shell no CaCO<sub>3</sub>, d) Crab shell No protein

### Additional studies

Looking for possible explanations for the behavior observed, three additional tests were performed (Figure 3.8). In these tests, calcium carbonate and protein were removed from the matrix of the crab shell. The products were evaluated once again to determine performance. It was found that a higher adsorption was obtained when protein was removed from the crab shell. Therefore, it can be presumed that calcium carbonate plays a key role in the adsorption process of the heavy metal ions through formation of different compounds between the heavy metal ions and the ion species formed by the dissociation of calcium carbonate [79]. The mechanism behind removal is a synergetic effect between ion exchange followed by precipitation of lead-carbonate compounds and complexation with chitin.



**Figure 3.8.** Lead adsorption by partially modified crab shell.

In addition, chitin plays a key role in the adsorption process by the complexation of heavy metal ions; however, its efficiency was linked to the radius and the size of the molecule. Bigger contaminants such as lead, for example, would be more likely to interact with chitin.[70].

### 3.4. Conclusions

Crab shell might be a suitable option for heavy metal ion removal. Optimal conditions for lead removal include 1-hour residence time, pH 4, and temperature of 30 °C, and 50 mg of adsorbent. Calcium Carbonate appears to be the key component of adsorption by crab shell.

### Acknowledgments

We are grateful to the NC Biotechnology Center (Grant No. 571068) whose generous support made parts of this work possible. This work was performed in part at the Analytical Instrumentation Facility (AIF) at North Carolina State University, which is supported by the State of North Carolina and the National Science Foundation (award number ECCS-1542015). The AIF is a member of the North Carolina Research Triangle Nanotechnology Network (RTNN), a site in

the National Nanotechnology Coordinated Infrastructure (NNCI). This work was also performed in part at the Environmental and Agricultural Testing Service Laboratory (EATS), Department of Crop and Soil Sciences, at North Carolina State University and Mass Spectrometry CORE Laboratory, Chemical Research Instrumentation Teaching & Core Labs (CRiTCL, at University of North Carolina Chapel Hill.

## **4. DESIGN OF EXPERIMENTS FOR MARINE EXOSKELETON-BASED BIOSORPTION OF CADMIUM, CHROMIUM AND ZINC**

### **4.1.Introduction**

Today, heavy metal ion removal from water represents a big challenge in water treatment. Different methods are used for water remediation being adsorption the most used one due to its easy operation, low energy consumption, and easy implementation [143]. However, materials used for adsorption are often expensive when treating large amounts of water. High costs of adsorption are associated with material cost, which can vary according to availability and chemical or physical treatments needed to increase adsorption capacity. Among the materials used in adsorption, one finds zeolites, ion exchange resins, and activated carbon. In spite of its relatively good performance, activated carbon often is an expensive and a unaffordable material for heavy metal ion removal due to a high energy consumption and low yield in its production. All this makes activated carbon along with other materials expensive materials for treating large amounts of water.

In looking for efficient, low cost option to remediate heavy metal ions from water, research has focused on biological derivatives for adsorption. Then, the process when contaminants are removed from water using either living or dead organism is known as biosorption. This process considers a physico-chemical operation, which includes the following mechanisms: adsorption, ion exchange, and precipitation. In general, adsorption can be classified according to the type of phenomenon occurring during the removal of a contaminant. Chemi-sorption accounts for high energy in which a monolayer is in effect and interactions are mainly relegated to ionic and covalent bonding. On the other hand, in physi-sorption process, no electron exchange is observed and

interactions are mainly limited to electrostatic forces, hydrogen bonding, Van der Waals, or dipole-dipole interactions (London Dispersion Forces) [124].

In the case of biosorption, both mechanisms might be observed simultaneously depending on the type of components of the organic matter used in the process. As an example, we have crustacean shells. Commonly dismissed by the seafood industry as a valueless byproduct, crab shells have been proven to be able to efficiently remove heavy metal ions such as lead [79], [80],[103], zinc [81],[88], cadmium [81], copper (II) [103] [81], chromium [81], cobalt (II) [86], and arsenic (V) [104] [84] [4].

In this work, crab shells are used as part of a careful experimental design for assessing the efficiency of removal of zinc, cadmium, and chromium.

## **4.2. Materials and Methods**

### **4.2.1. Materials**

Crab shells were purchased from a seafood processing company. Sodium Hydroxide, hydrochloric acid, and lead nitrate were used for pH adjustment and heavy metal stock solution preparation.

### **4.2.2. Methods**

**Shell cleaning and particle size reduction** Crab and shrimp shells were washed and cleaned using deionized water and oven dried at 100 °C for 4 h. Particle size reduction was performed using a Wiley mill until 40 mesh particle size was achieved.

**Product performance** for the tests related to product performance, metal uptake and removal efficiency were calculated using the following equations:

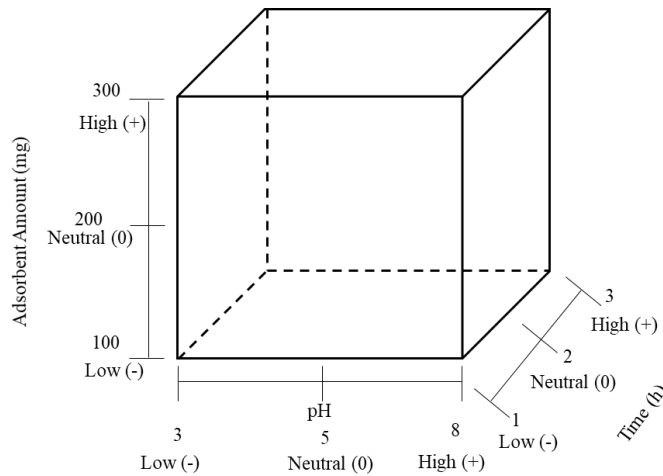
$$q_e = \frac{V \times (C_0 - C_e)}{W} \quad (3)$$

$$\%Removal = \frac{C_0 - C_e}{C_0} \times 100\% \quad (4)$$

Where  $q_e$  is the metal uptake by the material (mg/g),  $V$  is the volume of the solution (mL),  $W$  is the weight of the adsorbent (g),  $C_0$  is the concentration of metal ions before adsorption (mg/mL) and  $C_e$  is the concentration of metal ions after adsorption (mg/mL).

**Preliminary Design:** Preliminary experiments were done in order to determine the parameters with higher impact on adsorption capacity and removal efficiency by the crab shell of the heavy metal ions studied. In addition, a validation with other heavy metal ion removals was performed in order to have a basis of comparison with the experimental data obtained.

**Experimental Design:** After preliminary design and validation was performed, a factorial 33 design of experiments was developed using JMP®. Figure 4.1 shows the parameters and evaluated levels for each heavy metal ion.



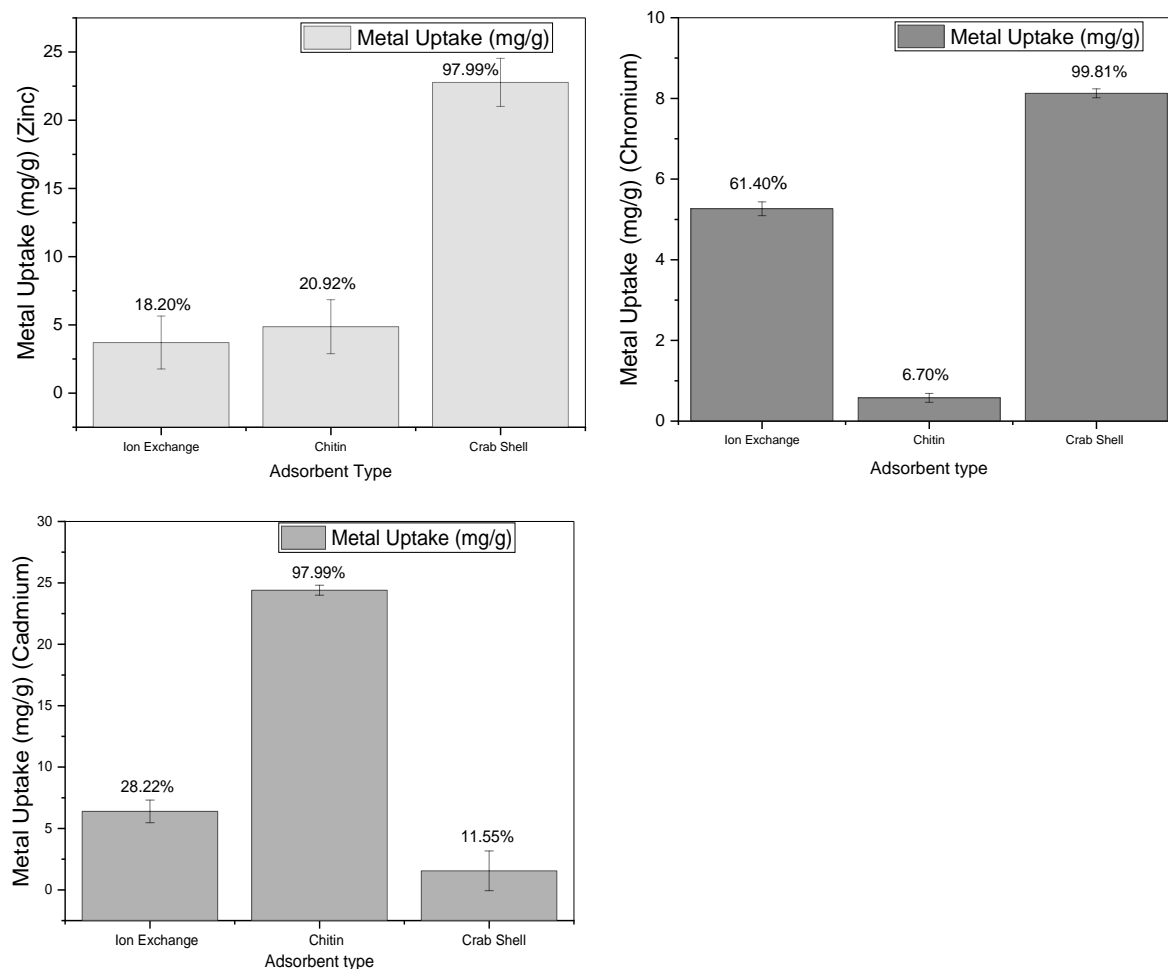
**Figure 4.1.** Factors and levels used in the factorial design.

### 4.3. Results and discussion

Crab shell, chitin, and a commercially available ion exchange resin were in contact with different heavy metals whose results are shown in **Figure 4.2**. In this case, chromium, zinc, and cadmium were tested. Results showed crab shell as a competitive option with commercially



available products. For zinc, crab shell achieved a 97.99% of removal efficiency with an adsorption capacity ~ 23 mg/g. compared to other adsorbents. Similar results were obtained for chromium; however, in the case of cadmium; chitin had a better performance compared to crab shell and ion exchange.



**Figure 4.2.** Performance evaluation of ion exchange resin, crab shell and chitin for a) Zinc b) Cadmium and c) Chromium.

### Experimental Design

Results for average removal efficiency of zinc, cadmium and chromium by crab shell can be seen in Table 4.1. The results show a higher efficiency for cadmium removal. For cadmium, the highest removal obtained was 99.73% under pH 8, 100 mg of adsorbent, and 3 hours of

residence. On the other hand, the zinc results show a 99.96% removal under pH 8, 300 mg of adsorbent and 3 hours of residence time. For chromium, the highest removal achieved was 83.68% under pH 3, 300 mg of crab shell, and 3 hours of residence time.

In the case of these 3 heavy metals, a long residence time is needed compared to lead (see chapter 3).

**Table 4.1.** Design matrix and the results for removal efficiency of the 33 full factorial design for each heavy metal.

Run	pH	Adsorbent amount (mg)	Time (h)	Average Removal Efficiency (%) Zn <sup>+2</sup>	Average Removal Efficiency (%) Cd <sup>+2</sup>	Average Removal Efficiency (%) Cr <sup>+3</sup>
1	+	+	-	99.88%	99.30%	83.62%
2	-	-	+	72.93%	97.06%	52.54%
3	0	0	0	93.21%	99.57%	75.46%
4	-	+	-	92.08%	98.52%	83.38%
5	0	0	-	70.71%	98.74%	75.78%
6	-	-	-	47.28%	66.44%	51.50%
7	+	-	-	70.52%	99.74%	52.11%
8	+	+	+	99.96%	99.22%	83.59%
9	+	0	0	98.59%	99.79%	75.94%
10	-	+	+	83.76%	99.21%	83.68%
11	+	-	+	99.65%	99.73%	52.51%
12	0	0	0	92.64%	99.41%	75.93%
13	0	-	0	68.29%	98.36%	54.20%

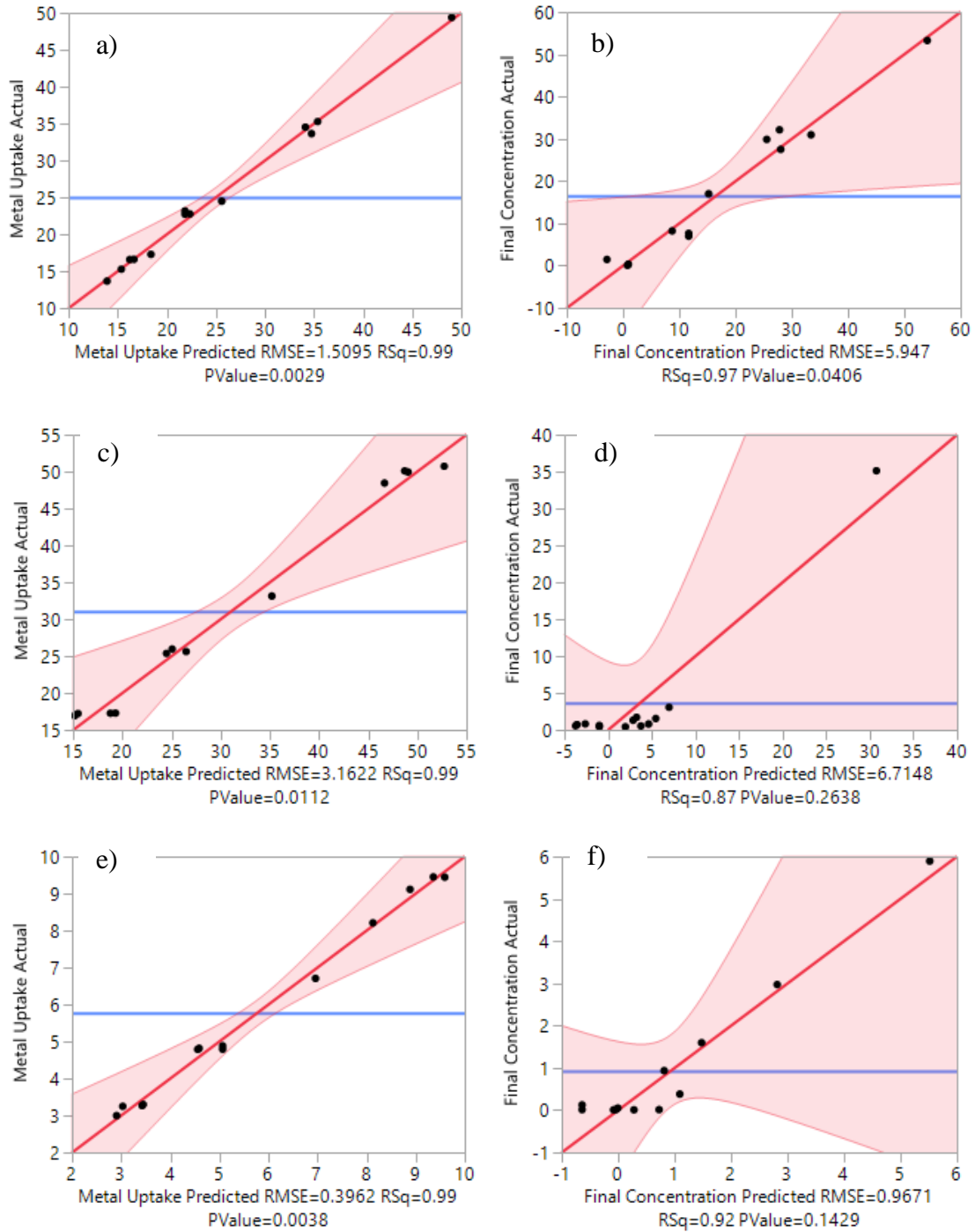
The results were analyzed used JMP and the main effects and interactions for each heavy metal ion were determined related to the final concentration and metal uptake. A multivariable analysis was done for each heavy metal ion. The significant factors in the regression model can be determined by performing an ANOVA [144]. As shown in Table 4.2, for zinc, adsorbent level, pH, time, and the interaction of adsorbent amount\*time and pH\*adsorbent amount have a higher significance with P values < 0.05. On the other hand, the cadmium-only adsorbent amount is

shown to be significant. Additionally for chromium, there are two parameters that showed significance: adsorbent amount and pH. From the P-value, it appears that the main effect of each factor and interaction effects are statistically significant because P values < 0.05

Significant values are highlighted in red.

**Table 4.2.** Effect of different parameters in final response for the heavy metal ions studied.

Source	Zinc		Cadmium		Chromium	
	LogWort h	P Value	LogWort h	P Value	LogWort h	P Value
<b>Adsorbent Amount(100,300)</b>	3.4730	<b>0.0003</b>	3.0040	<b>0.0010</b>	3.5080	<b>0.0003</b>
<b>pH(3,8)</b>	2.2010	<b>0.0063</b>	0.9830	0.1040	0.7400	0.1819
<b>Adsorbent Amount*Time</b>	2.1840	<b>0.0066</b>	0.7490	0.1781	1.3580	0.0439
<b>Time(1,3)</b>	2.1070	<b>0.0078</b>	0.7400	0.1821	1.4290	<b>0.0373</b>
<b>pH*Adsorbent Amount</b>	1.8400	<b>0.0145</b>	0.8960	0.1270	0.4640	0.3436
<b>Adsorbent Amount*Adsorbent Amount</b>	1.1160	0.0765	1.2620	0.0547	1.3020	<b>0.0499</b>
<b>Time*Time</b>	0.6110	0.2450	0.0460	0.8988	0.1470	0.7131
<b>pH*Time</b>	0.3560	0.4410	0.7330	0.1848	0.7150	0.1928
<b>pH*pH</b>	0.2880	0.5155	0.6580	0.2197	0.4810	0.3302



**Figure 4.3.** Prediction plot for metal uptake and final concentration a) metal uptake for zinc, b) final concentration for zinc, c) metal uptake for cadmium, d) final concentration for cadmium, e) metal uptake for chromium, f) final concentration for chromium.

Following the main effects, a model fitting was used to determine how the data obtained deviated from the prediction established by the multivariable study. In this case, a better fit was obtained for metal uptake. On the other hand, for final concentration, higher deviations are seen, especially for chromium and cadmium.

According to ionic radius theory of adsorption by crab shells [81], the favorability for the crab shell adsorption is given by the following order  $Cr > Cd > Zn$ ; however, under the experimental conditions, the order is  $Cd > Zn > Cr$ . As the ability to exchange ions in the presence of calcium carbonate also plays a key role in the capture of these heavy metal ions, we suggest that under these pH, cadmium is more likely to form such complexes, precipitate, and become easier to remove.

#### **4.4. Conclusions**

In the case of the experimental design, better sorption conditions were in effect for cadmium because the removal efficiency was the highest. According to the adsorption order, experimental conditions favored zinc down to chromium. It is important to highlight that as much as the parameters for adsorption of the heavy metal ions were determined to be as close as possible to an optimum, pH were not at exact values to obtain maximum removal for some of the metals. Additionally, it can be stated that crab shell continues to be a great tool for heavy metal ion removal obtained at very low cost.

#### **Acknowledgments**

We are grateful to the NC Biotechnology Center (Grant No. 571068) whose generous support made parts of this work possible. This work was performed in part at the Analytical Instrumentation Facility (AIF) at North Carolina State University, which is supported by the State of North Carolina and the National Science Foundation (award number ECCS-1542015). The AIF

is a member of the North Carolina Research Triangle Nanotechnology Network (RTNN), a site in the National Nanotechnology Coordinated Infrastructure (NNCI). This work was also performed in part at the Environmental and Agricultural Testing Service Laboratory (EATS), Department of Crop and Soil Sciences, at North Carolina State University and Mass Spectrometry CORE Laboratory, Chemical Research Instrumentation Teaching & Core Labs (CRiTCL, at University of North Carolina Chapel Hill.

## **5. CRUSTACEAN SHELL – BASED NANOFIBERS PRODUCTION AND APPLICATIONS**

### **5.1.Introduction**

Nowadays, nanofibers have been the focus of multiple research efforts. Due to its incredible properties, nanofibers production from natural and synthetic materials have become a trending topic in research. In general, nanofibers are able to improve mechanical properties when included in a composite, have attractive optical properties, and higher surface area than common fibres.

In recent years, nanofibers have enhanced the scope for multiple applications such as tissue engineering [145], paper electronics [146], packaging [147], etc., and they have been seen as a potential replacement for plastics due their versatility for production from natural polymers. Due to their abundance in nature, cellulose and chitin/chitosan are among the most common materials used in the nanofiber production.[148]

Among natural polymers, chitin and chitosan have been the target of growing interest. Chitin and chitosan are cellulose analogues with the classic motif of glucopyranose units bonded by  $\beta$ -1-4 linkages. In the case of chitin, N-acetyl units are attached in C2 position while chitosan contents amine groups in the C2 position that is the deacetylated form of chitin. Although chitin is a semi-crystalline biopolymer with very interesting morphology, most of it is discarded in landfill [148]. Because the main source is non-edible crustacean shells, considered seafood waste, most of its value is not realized.

It is estimated that ~ 8 million tonnes per year of crustaceans are produced by the seafood industry. As the demand for shrimp and crabmeat increases, higher production is needed, which therefore demands a more efficient management of the shells because it can be maximized for

revenue and avoid waste disposal [1]. A boost in the production of chitin and chitosan can help not only in the production of biodegradable products, but also in the reduction of solid waste that commonly goes to landfill. However, even though chitin and chitosan have extraordinary properties, low yields and considerable chemical treatment lead to expensive chitin/chitosan production. According to [24], chitin price via chemical modification can go up to 15,000 Euros/ton (~17,000 dollars/ton)

In the preparation of chitin nanofibers, one of the key elements is chitin extraction. Common methods include chemical extraction by using an alkali and acid treatments [14]; enzymatic treatments [16], and non-chemical treatments that use hot water and carbonic acid [15]. However, these methods have a direct impact on molecular weight and degree of acetylation [149], which affects to a great extent nanofiber formation.

Chitin nanofibers are commonly produced by either electrospinning or mechanical treatment. Via electrospinning, chitin has some disadvantages because gamma radiation is needed in order to partially depolymerize the material because it has a low solubility in most common solvents [23]. The common deacetylation degree is ~ 8% for chitin in electrospinning [150] for which typical solvents for electrospinning include mixtures of N, N-dimethylacetamide (DMAC) with lithium chloride (LiCl), 1,1,1,3,3,3-hexa-fluoro-2-propanol (HFIP), a mixture of sodium hydroxide with urea, and different mixtures of ionic liquids [23].

On the other hand, in mechanical grinding a slurry of isolated chitin (~ 1%) [148] and water is passed through either a Matsuko grinder, a dynamic high pressure homogenizing or high-pressure water jet system [23]. However, chitin drying following extraction creates hydrogen bonding among hydroxyl groups, acetamide, and amino groups, that also display strong dipole



measurements on the surface [151]; as a result, it is necessary to induce electrostatic repulsion because interfibrillar hydrogen bonding makes it difficult to disperse in solution [23]

As part of this work, we produced crustacean shell-based nanofibers by a simple mechanical grinding by taking advantage of the multiple components of the matrix to avoid increased viscosity from hydrogen bonding and other issues associated for chitin nanofibers production.

## **5.2. Materials and Methods**

### **5.2.1. Materials**

Crab shells were purchased from a seafood processing company, sodium hydroxide pellets, hydrochloric acid 6 N and acetic acid glacial (98%) were purchased from VWR. A super mass collider grinder, Matsuko MKCA 6-5 was used with MKGA-C stone for the nanofibrillation process.

### **5.2.2. Methods**

**Shell cleaning and particle size reduction** Crab shells were washed and cleaned using deionized water and then oven dried at 60 °C for 4 h. Particle size reduction was performed using a Wiley mill until 40 mesh particle size was achieved.

**Components extraction** In the fibrillation process, crab shell samples containing different levels of the main components were analyzed. Components were removed using alkali and acid treatments. For chitin (Ch) extraction, starting from the 40-mesh crab shell, protein was removed using sodium hydroxide 1 M at 65 °C for 2 hours. Next, calcium carbonate and other minerals were removed by an acid treatment with hydrochloric acid 1.5 M at room temperature for 5 hours. Non-protein crab shell (NPCS) and Non-Calcium Carbonate Crab shell (NCCS) were produced removing only protein and minerals, respectively, by alkali and acid treatments mentioned earlier.

**Crustacean shell-based slurry preparation and grinding** The materials prepared by the chemical treatment and raw 40-mesh crab shell were made into a slurry with tap water with a solid content of 3%. For chitin, a 1% solution was used [49], in which pH of the solution was kept at 3 using acetic acid [148]. Once the solutions were prepared, the materials were ground using the Matsuko at 2000 rpm with a variable gap width. Finally, the slurry was freeze-dried and characterized.

### **Characterization**

**Fourier transform infrared spectroscopy (FTIR).** The analysis of functional groups present in the composite material was accomplished using UATR in a PerkinElmer Frontier IR single range spectrometer. The range used for the analysis is 4000-650 cm<sup>-1</sup> at a resolution of 4 cm<sup>-1</sup> and 32 scans.

**X-Ray Diffraction (XRD)** Rigaku Smart Lab X-Ray diffractometer was used for XRD measurements of powdered samples. The experiments are carried out at  $2\theta=5-40^\circ$  [14]. Crystallinity was determined by the area under the curve of the peaks associated to each of the components.

**Scanning electron microscopy (SEM)** Morphological analysis of the samples was done by SEM using the Field Emission Scanning Electron Microscope – FEI Verios 460L. For sample analysis, the samples were covered with a 7 nm layer of gold. Conditions used include voltage between 1.00-2.00 kV and a 13 pA current was used. For higher magnification samples, a 500 V bias was used to reduce sample charging.

**X-ray photoelectron spectroscopy (XPS)** The XPS study was carried out using Kratos Axis Ultra DLD X-ray Photoelectron Spectrometer with a graphite monochromator and Al K $\alpha$  radiation.

### **Crustacean Based Nanofibers Applications**

Once the crustacean shell-based nanofibers were produced, two main applications were considered: crustacean shell-based nanofibers and crustacean shell co-grinding with cellulosic pulps. For crustacean shell-based nanofibers, Non-Protein Crab Shell (NPCS), Non-Calcium Carbonate Crab Shell (NCCS), and Chitin (Ch) were evaluated as blood clotting materials. For that matter, a hemostatic assay and confocal microscopy were employed.

**Hemostatic Assay, Rapid Clot test** 50  $\mu$ l fibrin clots (2.5 mg/ml fibrinogen) were generated in the presence or absence of 0.25  $\mu$ /ml thrombin in tubes. Clotting was visually observed over 10 mins.

**Confocal microscopy** 50  $\mu$ l fibrin clots (2.5 mg/mL fibrinogen) were formed in the presence or absence of 0.25  $\mu$ /mL thrombin with Alexa 488 labeled fibrinogen for visualization. Clots were formed with 10% total volume NCCS, NPCS and Ch particles. Clots were polymerized for 3 hours prior to imaging.

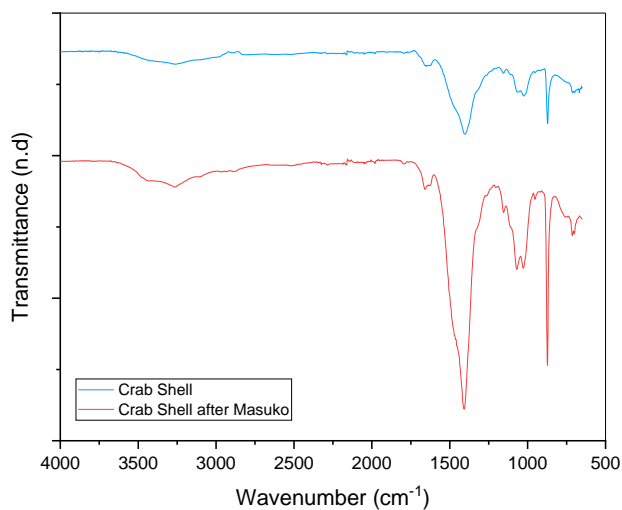
**Crustacean shell co-grinding with cellulosic pulp** A slurry was prepared at 3% total solid contents with different ratios of Crab Shell: Cellulose (10:90, 20:80, 30:70). The mixtures were passed through the Matsuko grinder at 2000 rpm and variable gap width. Final slurry was freeze-dried and characterized.

### **5.3.Results and discussion**

Crustacean shell-based nanofibers were produced using the Matsuko grinder where the final slurry was freeze dried; finally the powder obtained was characterized.

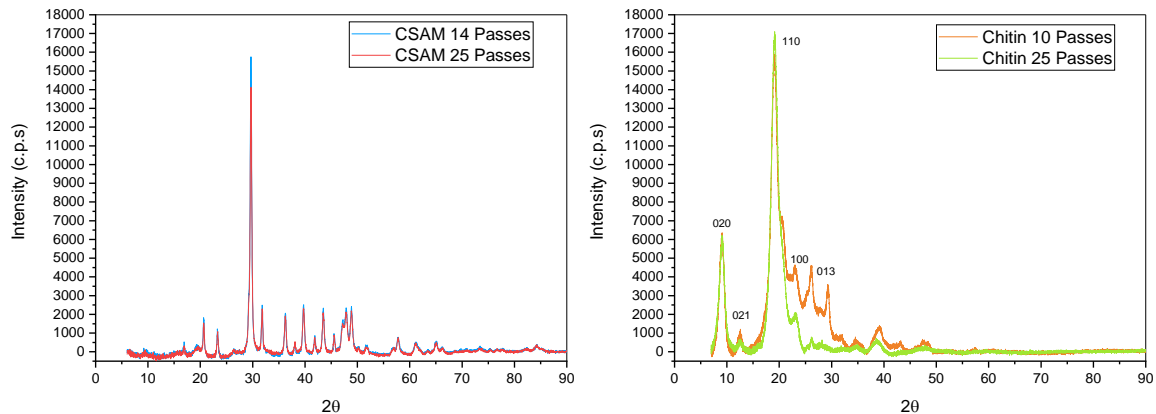
**FTIR** via ATR was performed for the samples before and after the mechanical treatment (see Figure 5.1). The main goal of this technique is to identify key functional groups and determine to which extent the mechanical treatment or the particle size reduction might be inducing chemical

changes as indicated by the infra-red absorption. For minerals, it was known that a particle size reduction can shift to lower wavenumbers [152]. Consistent with this theory, in the samples containing minerals, previously identified as calcite, the peaks associated with the presence of calcium carbonate showed an increase in intensity but not considerable shifts were observed. Regarding the peaks associated with chitin, no differences were observed. The explanation is that grinding affects absorbance of individual particles and the overall structure [153]. In crab shell, peaks at 3262, 1635, 1402, 1052 and 872 are observed. The peaks are attributed to the NH stretching, -N-H-CO stretching, NH<sub>2</sub> groups in protein, and calcium carbonate [95]. [137].



**Figure 5.1.** FTIR Crab shell and crab shell after Matsuko.

**XRD** X-Ray Diffraction on the samples was performed to determine possible effects of the mechanical grinding on the crystallinity of the species, in this case, calcium carbonate and chitin. As seen in **Figure 5.2** in chitin slight changes can be observed when the number of passes and the energy increases. In general, chitin is a linear semi-crystalline polymer with diffraction peaks at  $2\theta \sim 9.24^\circ, 12.90^\circ, 19.18^\circ, 23.36^\circ$  and  $26.14^\circ$ ; from the planes (020), (101), (110), (130), and (013), respectively [154][155]. In crab shell, the main peaks correspond to minerals which are present in a higher quantity; in this case, calcium carbonate in the form of calcite.



**Figure 5.2.** X-Ray diffraction patterns for crab shell and chitin after masuko at different passes.

Regarding the effect of the energy/ number of passes applied in the samples, for crab shells, a higher peak intensity was observed when less passes were used to grind the samples. As a conclusion, a lower degree of crystallinity was observed due to less order and reduced repetition of molecular packing, specifically for calcium carbonate.

On the other hand, for chitin, peak intensity is also higher when a lower number of passes was used in some areas. For example, for chitin, at 25 passes, some of the peak intensities after 20~30 tended to decrease and almost disappeared, while a slight increase in the intensity of the peaks corresponding to the planes (020) and (110) was observed. In this case, for chitin we cannot conclude a steady effect on crystallinity because broad differences were not appreciated. Other research has reported on the effect of ball mill grinding in chitin. When dry, it can be expected to have a decrease in the crystallinity, however, in our case, when water is used in grinding, it acts as a plasticizer partially restoring crystallinity to the structure [155], [156].

### **SEM (Scanning Electron Microscopy)**

Scanning electron microscopy pictures were taken for chitin, non-protein crab shell, and raw crab shell after Matsuko grinding. In order to analyze the effect of the mechanical treatments, samples with different number of passes were observed. Measurements for particle size

estimations were done using Image J software. In average, for chitin, fiber diameter was  $0.178 \mu\text{m}$ , for non-protein crab shell was  $0.120 \mu\text{m}$ , and for pure crab shell  $0.081 \mu\text{m}$ .

At this stage, we can witness formation of nanofibers; however, for only chitin the formation of bundles and the formation of strong hydrogen bonding makes harder the analysis of the sample after drying. On the other hand, the presence of other components might also help in grinding by lowering the of the suspension and avoiding to some degree the extent of hydrogen bonding making them easier to grind. The results for SEM can be seen in Figure 5.4

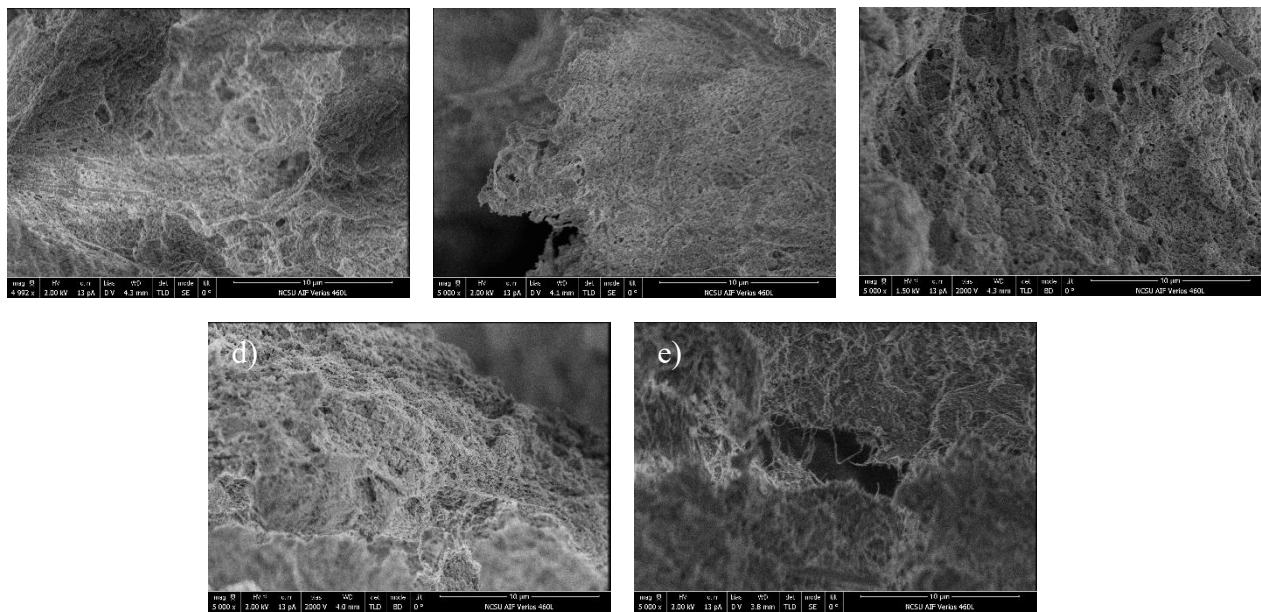


Figure 5.5 and Figure 5.3.

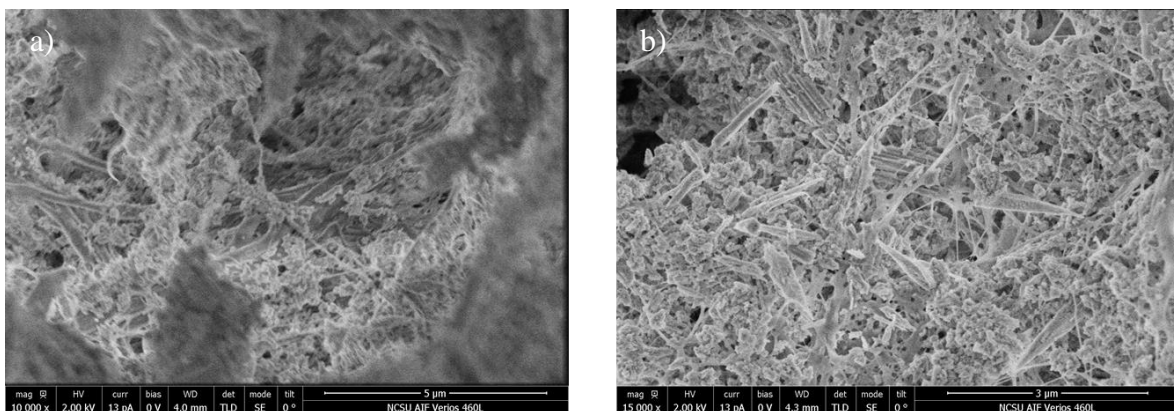
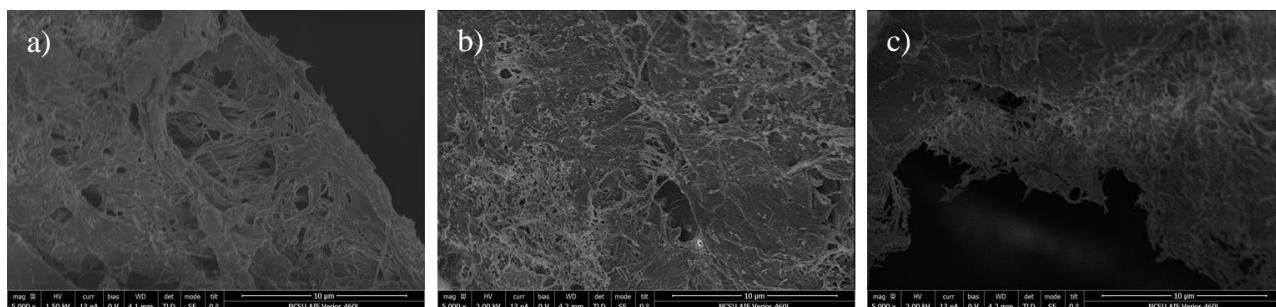
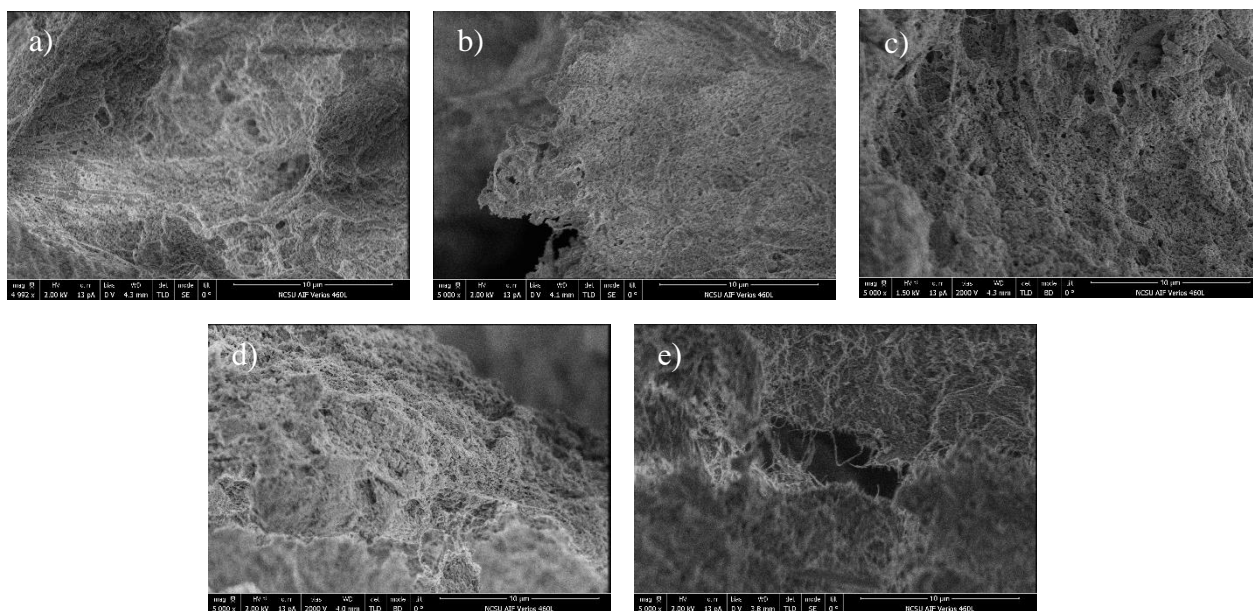


Figure 5.3. SEM images for pure crab shell after Matsuko grinding a) 14 passes, b) 25 passes,



**Figure 5.4.** SEM images for Chitin after masuko grinding a) 5 passes, b) 10 passes, c) 25 passes.



**Figure 5.5.** SEM images for non-protein crab shell after Matsuko grinding a) 5 passes, b) 10 passes, c) 15 passes, d) 20 passes, e) 25 passes.

### X-ray photoelectron spectroscopy (XPS)

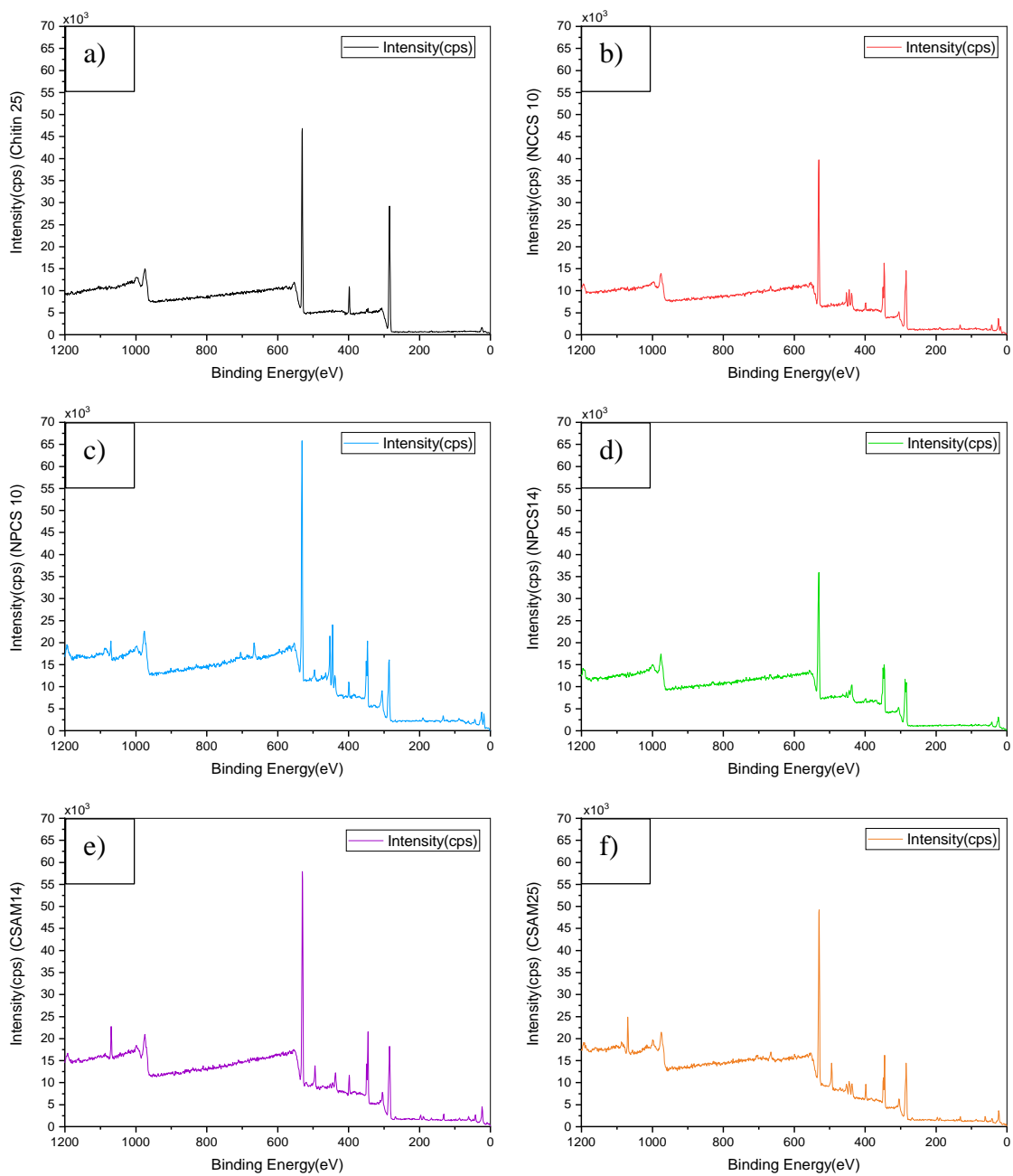
XPS analysis were done with the objective of determining the type of functional groups available in the surface in the material after grinding. **Figure 5.6** shows the survey spectra for the analyzed samples. The peaks corresponding to O 1s, C1s and O auger are observed in all samples. For crab shell samples, calcium peaks can be appreciated more clearly than in non-protein crab shell. Also, crab shell samples show peaks related to sodium and chlorite, which indicated the presence of salts used in the cleaning process prior grinding.

In addition to the survey spectra, spectra for nitrogen, oxygen and calcium were taken. The spectra can be found in the annexes and a summary of those results is described in **Table 5.1**. The results are obtained by determining the ratio of the areas under the curve for each element analyzed. In the case of chitin, because calcium is almost zero, the ratio C/C<sub>a</sub> is high. For crab shell samples the differences are mainly due to the C/N ratio because it decreased with a higher number of passes; however, the difference is not as significant as the other samples.

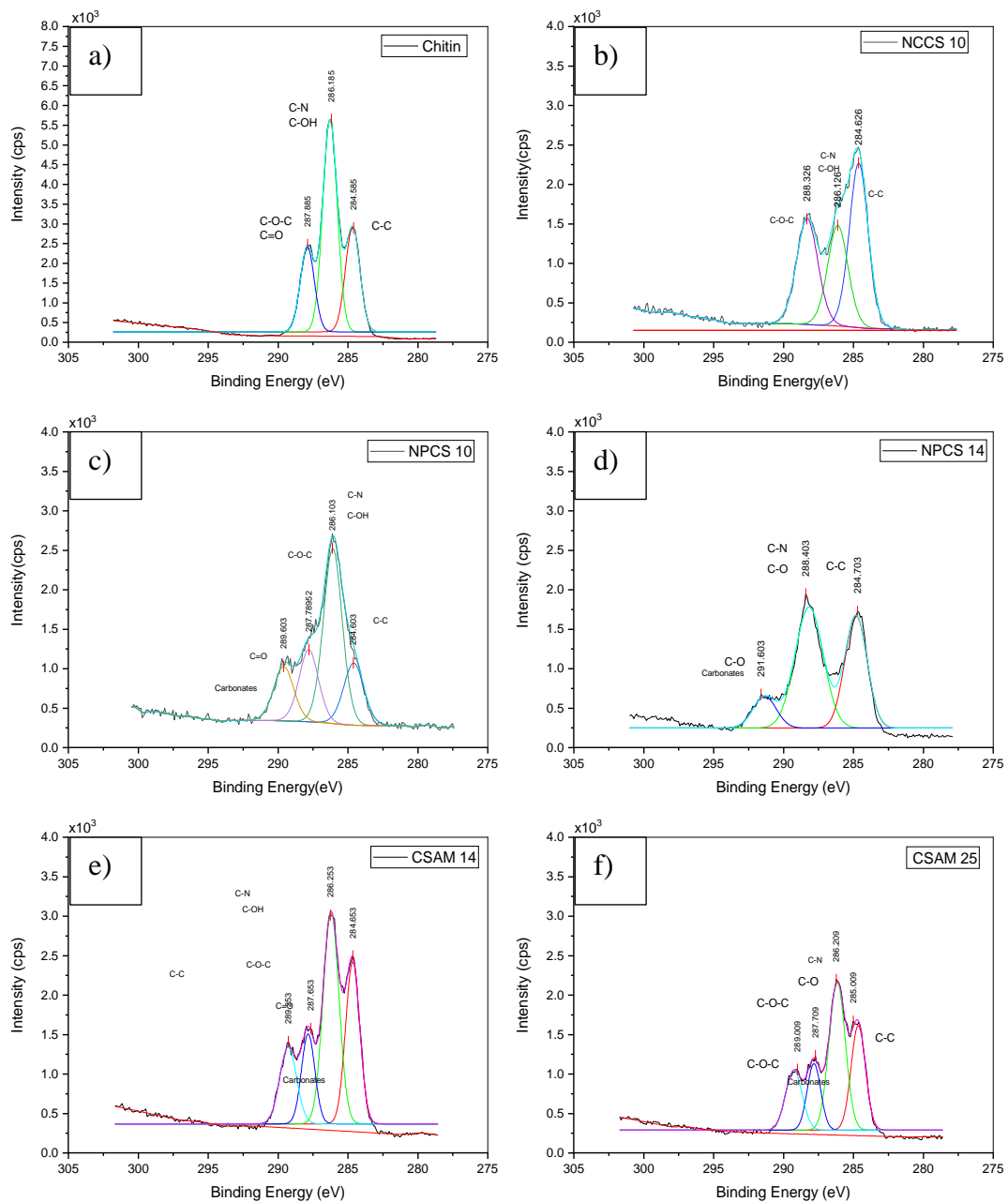
**Table 5.1.** XPS results comparison

<b>Ratio /Sample</b>	<b>Chitin 25</b>	<b>NPCS 14</b>	<b>CSAM 14</b>	<b>CSAM 25</b>
<b>C/O</b>	2.58	1.54	1.42	1.29
<b>C/N</b>	12.45	38.05	12.64	11.46
<b>C/Ca</b>	400	7.36	8.35	8.70
<b>O/N</b>	4.82	24.75	8.88	8.82





**Figure 5.6.** Survey spectra from XPS results for a) Chitin, b) Non-calcium carbonate Crab shell 10 passes, c) Non-protein Crab shell 10 passes d) Non-protein Crab shell 14 passes, e) Crab shell 14 passes, f) Crab Shell 25 Passes.



**Figure 5.7.** XPS Carbon spectra for a) Chitin, b) Non-calcium carbonate Crab shell 10 passes, c) Non-protein Crab shell 10 passes d) Non-protein Crab shell 14 passes, e) Crab shell 14 passes, f) Crab Shell 25 Passes.

## Crustacean Based Nanofibers Potential Applications

### Hemostatic Assay: Rapid Clot test

Inducing blood clotting has been a widely studied, because producing materials that can stop bleeding quickly is highly desirable. Today, the materials used for this purpose might vary from inorganic to organic compounds and synthetic or natural polymers [157].

Blood clotting has two hemostatic pathways: a protein based system and a platelet based system. In our case, we have decided to focus on the protein-based system. This system ends in the conversion of fibrinogen to fibrin, leading to the formation of thrombi [158]. In order to have blood clotting via a protein-based system, the ampholytic mucoproteins negative charges are bonded with positively charged groups in the material [159]. For that matter, chitin and chitosan have been studied as a suitable option due to the cationic nature provided by the amine group on the surface [157]. In general, it has been established that not all forms of chitin or chitosan are suitable for blood clotting, because hemostatic properties of these biopolymers are dependent on the degree of acetylation, molecular weight, and crystallinity [157].

On the other hand, calcium has been proven to induce a lower time in blood clot initiation, after which the presence of calcium in blood increases the coagulation index [160] which has been associated with a higher clot strength [161].

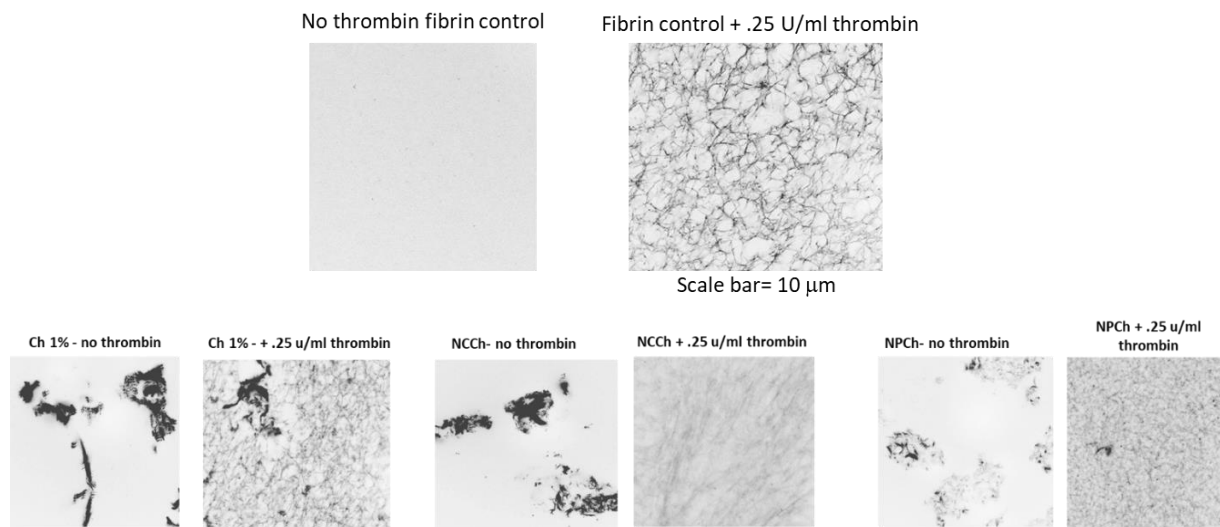
Thus, pure chitin, non-protein crab shell, and non-calcium carbonate crab shell were used in hemostatic tests to determine the efficiency of these materials for blood clotting. In the case of chitin particles, it did not appear that it induced clotting within time period in the absence of thrombin. In addition, their presence did not appear to hinder fibrin clot formation in the presence of thrombin. As seen in **Table 5.2**, pure chitin seems to be the most efficient of the material since it forms a clot faster than the other materials in the presence of thrombin.

**Table 5.2.** Hemostatic Essay Results

<b>Condition</b>	<b>Time to clotting (seconds)</b>
Control:PPP, no thrombin	No clotting observed
Control:PPP, + thrombin	36 s
Ch 1%: PPP, no thrombin	No clotting observed
Ch 1%: PPP, + thrombin	15 s
NCCS:PPP, no thrombin	No clotting observed
NCCS:PPP, + thrombin	30 s
NPCS: PPP, no thrombin	No clotting observed
NPCS:PPP, + thrombin	35 s

### **Confocal microscopy**

From the tests with confocal microscopy, it can be seen that pure chitin nanoparticles are able to induce some blood clotting. As seen on Figure 5.8. For the sample containing calcium carbonate, the clotting induced seems almost none since not network formation is observed. Finally, for non-protein crab shell, more network formation is observed that is mainly due to the presence of the calcium carbonate in the matrix. In addition to what was stated before, possible formation of calcium salts might be contributing to clotting. Also, when in solution, calcium ions are able to interact with negative charges on the proteins present in the blood [162].



**Figure 5.8.** Confocal microscopy for blood clotting analysis for a) Chitin, b) Non-Calcium carbonate crab shell and c) Non-protein crab shell

### **Crab Shell co-grinding with cellulosic pulp**

One of the major issues of cellulose grinding is the strong hydrogen bonding formed between cellulose chains that is favored by a wet state. This problem limits nanocellulose dispersability creating a restriction for some applications. Trying to solve this problem, some pre-treatments are applied to the pulp aiming to limit the hydrogen bonds and repulsive charges [163]. Some of those treatments included enzyme treatment, acetylation, pulp refining, acid and alkali treatments and TEMPO<sup>3</sup> mediated oxidation [163].

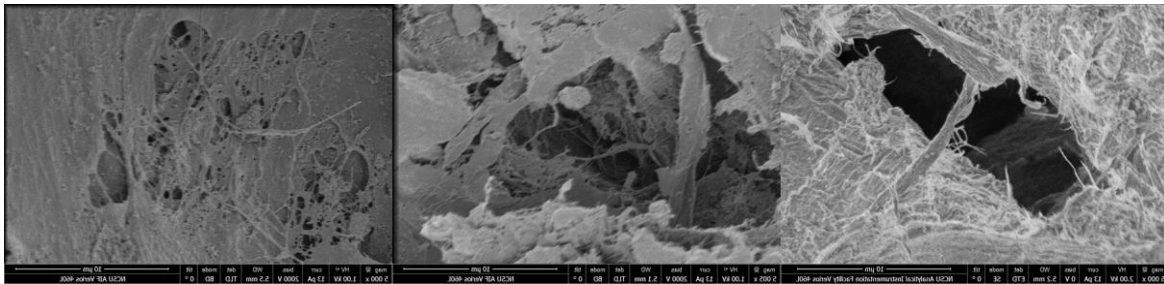
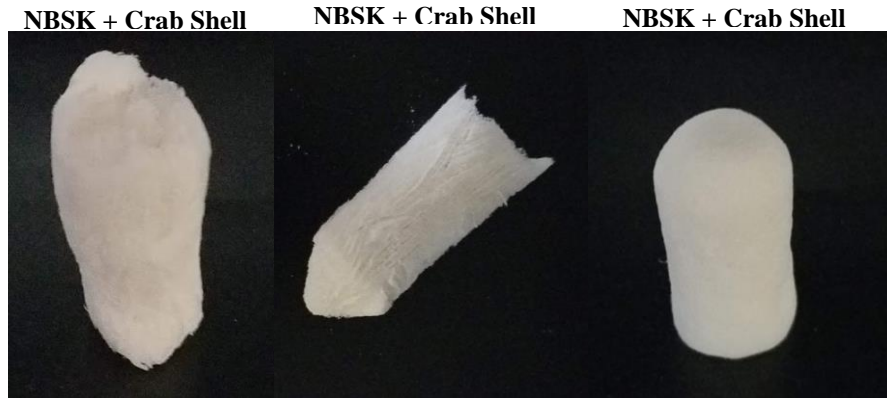
At an industrial scale, several companies use calcium carbonate as a filler to avoid increased viscosity due to the formation of hydrogen bonding. Thus, in this study, a mixture of crab shells (CS) with Northern Bleached Softwood Kraft pulp (NBSK) was mechanically reduced by using a Matsuko grinder. For that purpose, a suspension at 3% solid content was prepared with different NBSK : CS ratios whose suspensions were freeze-dried.

<sup>3</sup> TEMPO: 2,2,6,6-Tetramethylpiperidin-1-yl)oxyl

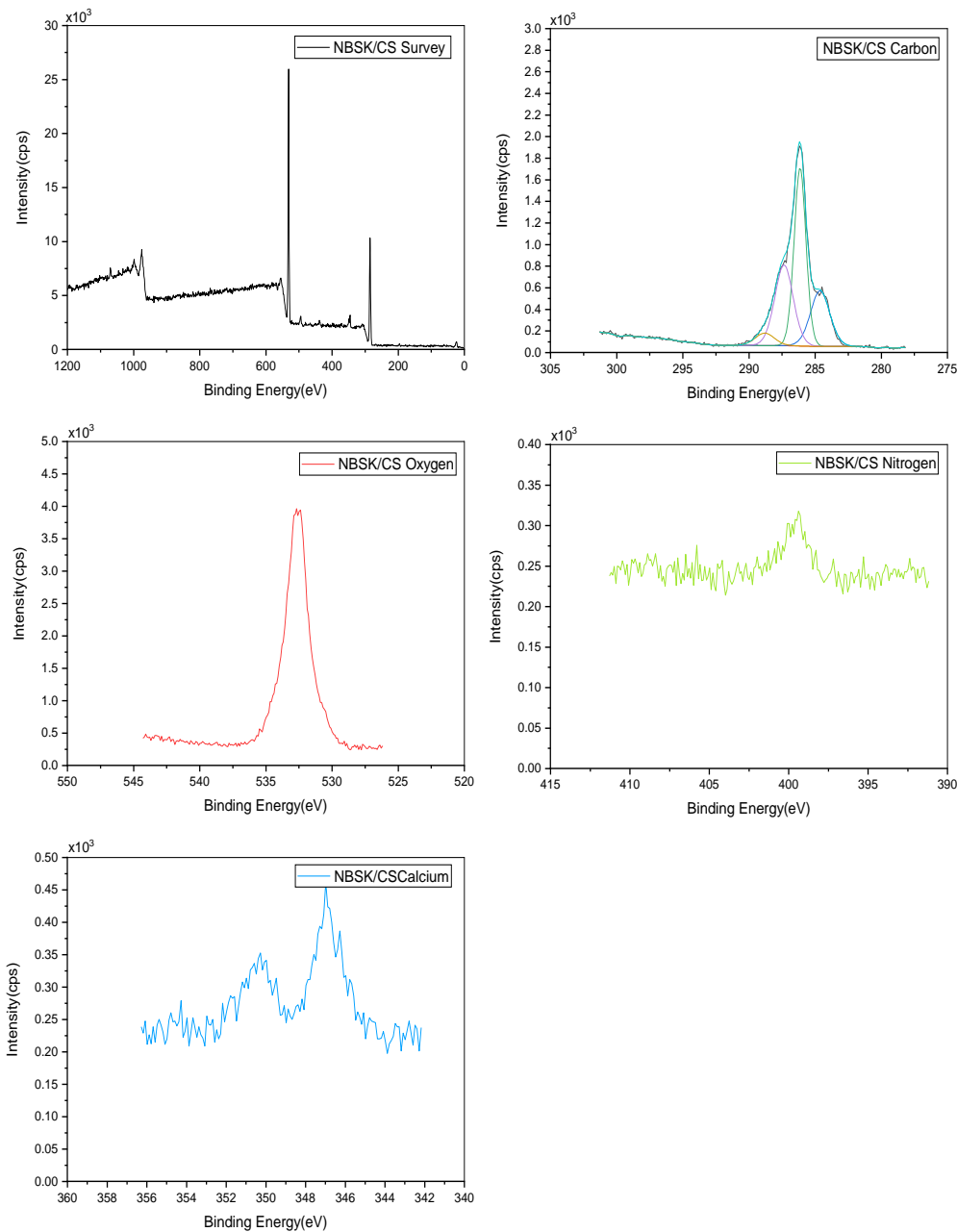
It is well known of the ability of nanocellulose to form foam materials. However, one major drawback is the avoidance of structural collapse during solvent removal thus limiting scale up of this technology [164]. Some studies have used a delayed calcium induced gelation, which by virtue of multivalent ion-induced aggregation of cellulose nanofibers, foam formation and an increased strength will be favored [165].

Preliminary results in this study showed formation of rigid and compression resistant structures seen in Figure 5.9. The samples were observed under SEM to show a gamut of fiber diameters. This means that not all cellulose fibers are yet on the nanoscale dimension, but some of them can be observed after reductions in particle size.

Regarding XPS spectra, traces of nitrogen and calcium can be seen on the surface. Peaks are not as intense as pure crab shell samples, but the presence of the two elements is an indication of chitin/protein and calcium carbonate present on the surface. On the other hand, in the carbon spectra, peaks associated with carbonates show very low intensity; this is mainly to a predominance of cellulose in the mixture in which C-O peaks are more sharp and thin.



**Figure 5.9.** Cellulose- Crab shell foams formed by material co-grinding.



**Figure 5.10.** XPS Spectra for NBSK/CS 70:30.

## 5.4. Conclusions

In conclusion, crustacean shell-based nanofibers can be produced by mechanical treatment of crab shells effected by Matsuko grinding, a suitable option for this purpose. Out of the characterization performed in this study, no major differences in the crystallinity or other surface



properties of the material can be observed, but more rigorous tests should be performed in the future.

Regarding applications, the matrix of chitin-calcium carbonate or even chitin-protein can open up a wide range of applications in which the sample time remains an affordable option for existing products.

### **Acknowledgments**

We are grateful to the NC Biotechnology Center (Grant No. 571068) whose generous support made parts of this work possible. This work was performed in part at the Analytical Instrumentation Facility (AIF) at North Carolina State University, which is supported by the State of North Carolina and the National Science Foundation (award number ECCS-1542015). The AIF is a member of the North Carolina Research Triangle Nanotechnology Network (RTNN), a site in the National Nanotechnology Coordinated Infrastructure (NNCI). This work was also performed in part at Chapel Hill Analytical and Nanofabrication Laboratory, CHANL, a member of the North Carolina Research Triangle Nanotechnology Network, RTNN, which is supported by the National Science Foundation, Grant ECCS-1542015, as part of the National Nanotechnology Coordinated Infrastructure, NNCI. Confocal Microscopy and rapid clot test were performed at Advanced Wound Healing Lab by Kimberly Nellenbach.

## 6. LIGNIN MODIFICATIONS AND PERSPECTIVES TOWARDS APPLICATIONS OF PHENOLIC FOAMS: A REVIEW<sup>4</sup>

### 6.1. Introduction

Foams are solids with void spaces known as cells [166]. The formation of void spaces in the solid are possible because of the dispersion of gas bubbles (discontinuous phase) in the matrix (continuous phase). The diffusion of gas in the solid phase is a key process in foam manufacturing to achieve a foam structure. Gas diffusion in the continuous phase affects the structural parameters, such as the density, cell type (open or closed), and cell size distribution, which influence the final characteristics and properties of the foams [167]–[169]

In addition to structural parameters, the material used in foam manufacturing and the processing conditions contribute to not only the structure and final characteristics, but also determine the foam applications. In foams, polymers are widely used because of different advantageous properties. Low density of a polymer results in weight reduction, which allows for lighter foams. Low heat transfer makes polymeric foams a suitable option for insulation purposes. Also, polymers allow for the manufacturing of flexible and soft foams, which provide comfort when used in furniture [170] (Aseeva et al. 2004).

Foams can be classified depending on the material used for manufacturing, such as polyurethane, polystyrene, poly (vinyl chloride), phenol-formaldehyde, etc. [170]. Alternatively, they can be classified into flexible or rigid foams according to the final properties, which can define the final product applications. Flexible foams are commonly used in furnishing, aircraft,

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<sup>4</sup> The material in this appendix has been published as:  
Carolina Londono-Zuluaga, Jing Du, Hou-Min Chang, Hasan Jameel, Ronalds W. Gonzalez. Lignin Modifications and Perspectives towards Applications of Phenolic Foams: A Review. *Bioresources* 13 (4) (2018) 9158–9179

bicycles, automobiles, clothing, packaging, electronics, and other areas. Rigid foams are widely used as a thermoset material, which makes them highly valuable for insulation applications [170].

Today, the insulation market growth is driven by regulatory schemes, along with consumer awareness of energy efficiency and preservation. These trends are expected to contribute to a market that was worth 52.30 billion USD in 2017 and is expected to keep growing at a compound annual growth rate of 8.6% from 2018 to 2025 [171]. These factors represent an opportunity and challenge to improve foam thermal properties for insulation materials.

Despite the properties and multiple applications of polymeric foams as insulation, concerns about sustainable raw materials and energy efficient buildings have led to research into feedstocks from renewable resources that can perform as well as synthetic polymer-based materials or even better. As a renewable and abundant resource, lignin has attracted enormous research attention as a potential precursor for both thermosetting and thermoplastic materials. Lignin is one of the three major components in vascular plants [171] and the most abundant aromatic bio-polymer [172]. In the United States and Canada, the pulp and paper industry produces approximately 31 million tons of lignin per year [173]. However, most of the lignin generated is burned in boilers to recover inorganic pulping chemicals and generate energy, which results in limited lignin availability (approximately 2%) for market products [174].

In addition to the availability, the thermal stability that lignin possesses and its diverse functional groups, such as hydroxyl and methoxyl groups, indicate the potential compatibility in polymer blending and other composites [175]–[177]. The production of vanillin [178], [179], carbon fibers [180], and concrete admixtures [181] from lignin have been reported in the literature. Also, lignin has been shown to work experimentally in phenol manufacturing [182], adhesives [183], [184] foams, animal feed [185] (Knudsen 1997), emulsifying agents [186], and heavy metal

sequesters [187], [188]. Many reviews are available on the utilization of lignin in different areas, such as lignin-based adhesives, polyurethane foams, and carbon fibers. However, few reviews were found concerning the application of lignin in phenolic foams. The only work related to the topic is by Obaid et al. (2016), which is a book on lignin-based foaming materials. However, the authors did not do a comprehensive review on the topic [189].

To understand the effect of lignin in phenolic foams, it is important to characterize foams according to their properties and structural parameters. Density refers to the polymer fraction in a matrix. A higher density is an indication that the amount of polymer is higher than the gas fraction in the matrix. If the gas phase is predominant in the foaming process, a low fraction of polymer is present in the foam. As a result, a low density and light foam is obtained. The density directly correlates to the mechanical properties. A low density leads to inferior mechanical properties and vice versa [168], [190]. Standard values for the density and mechanical properties of various foam types are given in Table 6.1.

Besides density, cell type is directly related to several properties, including energy absorption, insulation, sound absorption, and water retention. Because open-cell foams allow air to flux easily, it makes this type of foam suitable for gas exchange, fluid retention, absorption, sound deadening, and other similar applications [190], [191]. In contrast, closed-cell foams are desired for their energy absorption, sound absorption, insulation, and improved mechanical properties. Because the cells show strong resistance to air and moisture, the polymer phase dominates the matrix and provides an elastic material resistance to external disturbances [151].

**Table 6.1.** ASTM standards for rigid phenolic foams for insulation purposes [192]

Property	Units	Type I	Type II	Type III1	Type III2	Type III3	Type III4
Density	kg/m <sup>3</sup>	N/A	32	32	60	80	120
Compressive Resistance	kPa	108	124	124	207	345	517
Tensile Strength	Pa	7180	N/A	N/A	N/A	N/A	N/A
Water absorption (by volume)	%	3	3	3	3	3	3
Apparent thermal conductivity (foam core) at 24°C	W/mK	0.025	0.021	0.026	0.032	0.033	0.035

Type I: Roof Insulation board, Type II: Sheathing or Rigid panel; Type III: Pipe insulate

The cell size distribution has a direct impact on the mass and heat transfer. As is shown in Fig. 1, the thickness of the wall is determined by the distribution of the void spaces in the matrix, which allows or prevents the circulation of heat or mass through the material. These properties are especially important in certain areas, including insulation, packaging materials, and medical devices [190]

This review mainly focused on the utilization of lignin in rigid phenolic foams. Lignin use in phenolic foams often follows one of two approaches: 1) direct use in the synthesis process without any preliminary chemical modification and 2) chemical modification of the lignin structure before use in foam preparations.

The aim of this work was to provide a brief overview of rigid lignin-based phenolic foams and the various applications that could potentially benefit from lignin incorporation. Additionally, this review highlights the importance of bio-based products on real market trends.

## 6.2. Phenolic Foams

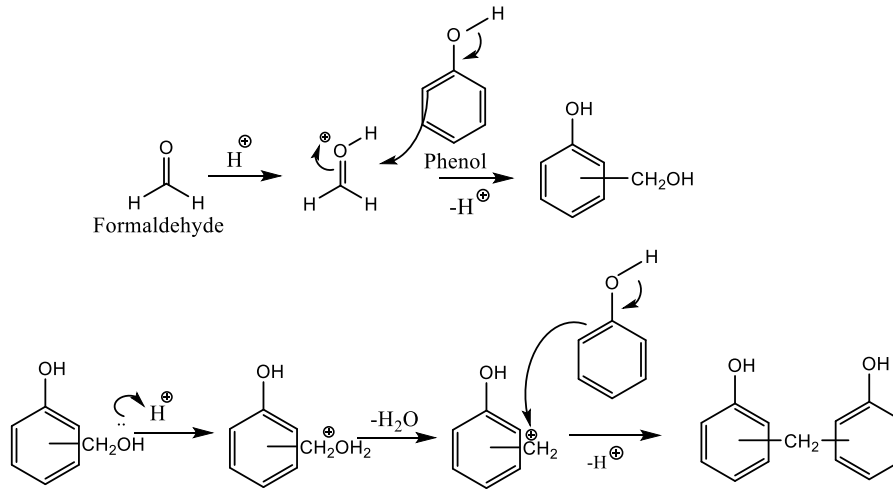
Phenolic compounds are widely used in different products like adhesives and foams. In general, polystyrene and urethane foams are preferred because of their density and thermal conductivity. However, the generation of toxic gases during combustion has led to phenolic foams

potentially replacing these foams. Unlike other foams, the properties of low flammability and outstanding fire and chemical resistance make phenolic foams highly suitable for insulation purposes [193]–[195].

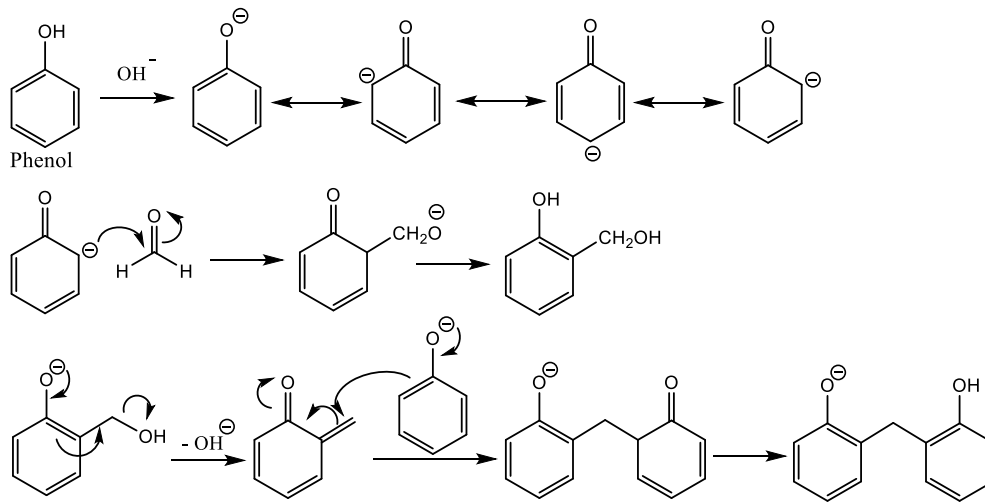
### **6.2.1. Phenol-formaldehyde Synthesis**

Phenol-formaldehyde (PF) resins can be synthesized in either an acidic or alkaline medium Figure 6.1 [196]. In addition to their use as adhesives, PF resins are the basic component of low-density PF foams that have a low thermal conductivity and high service temperature [189] Synthesis begins when phenol and formaldehyde are mixed in a solution at a low pressure and high temperature. During this reaction, the double-bonded carbonyl group in formaldehyde facilitates crosslinking with phenol in an acid- or base-catalyzed reaction. The pathway of this reaction depends on the formaldehyde concentration and process conditions.

Acid-catalysed reactions:



Alkaline-catalysed reactions:



**Figure 6.1** Reaction pathway for acid and based catalyzed phenol-formaldehyde resins

### 6.2.2. Preparation of Phenol-formaldehyde Foams

In Phenol Formaldehyde (PF) foams, each component plays a key role in the foaming reaction. Basic components include a PF resole, blowing agent surfactant, and curing agent. PF resoles provide the solid matrix for gas diffusion and cellular structure formation. Their use in foams is limited by the solid content. The optimum resole solid content in foam preparations is 80%. Before using PF resoles, the excess water is evaporated [197], [198].

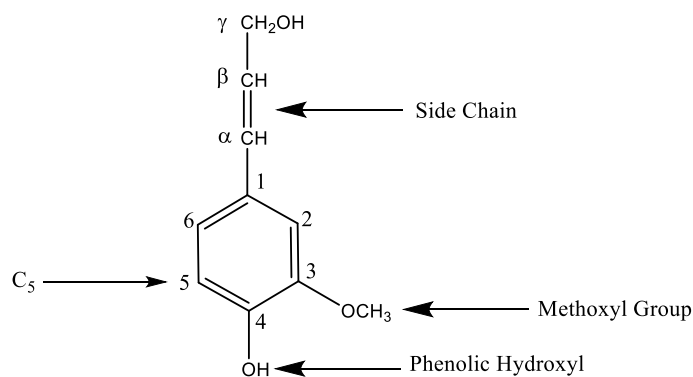
In contrast, a blowing agent is added to assist foam formation. The blowing agent is commonly introduced to the formulation in a liquid state and has a relatively low boiling point. Expansion of the blowing agent can produce a cellular structure during the foaming process because of its evaporation. During the preparation of phenolic foams, blowing agents can be used alone or in combination with a curing agent depending on the desired final density of the foam. For phenolic foam production, N-pentane is commonly used [199], [200]. In combination with resoles and blowing agents, surfactants are used to modify the characteristics of the polymer matrix during the foam formation process. They are used to emulsify the liquid components, regulate cell size, and stabilize the cell structure to prevent collapse and surface defects. In phenolic foam production, Tween 40 (polyoxyethylene (20) sorbitan monopalmitate) and Tween 80 (polyoxyethylene (20) sorbitan monooleate) are the most commonly used surfactants [201],[202]. Finally, the phenolic foam forming reaction is catalyzed with a curing agent, such as an acid like sulfuric or hydrochloric acid [203]. Part of the acid works to neutralize the sodium hydroxide used in the resoles preparation process and the rest functions as a catalyst to lower the activation energy for the crosslinking reactions of PF resoles [201].

### **6.3.Lignin modification**

Lignin can be described as a complex, aromatic and highly branched heterogeneous polymer [204] [205] composed of multiple functional groups, such as hydroxyl and methoxyl groups, along with a phenolic backbone. A generic representation of a monomeric building block within lignin can be seen in Figure 6.2. Variations on lignin monomer are due to methoxylation of the hydroxylcinnamyl alcohol in the C3 and C5 positions; resulting in the formation of monolignols that originate the guaiacyl (G), syringyl (S) and p-hydroxyphenyl (H) units [205].



Within unmodified lignin of hardwood species, the C5 position is substituted with a second methoxyl group. In addition, the phenol-propane units are often linked to each other by means of various covalent bonds originating at the  $\alpha$  and  $\beta$  positions. Some other branch points within lignin involve the 4 position [206] i.e. the phenolic hydroxyl; such connections within the lignin polymer greatly decrease the number of phenolic –OH groups that might be theoretically anticipated. The functional groups indicated in the figure are the most important groups when lignin modifications need to take place [175]–[177].



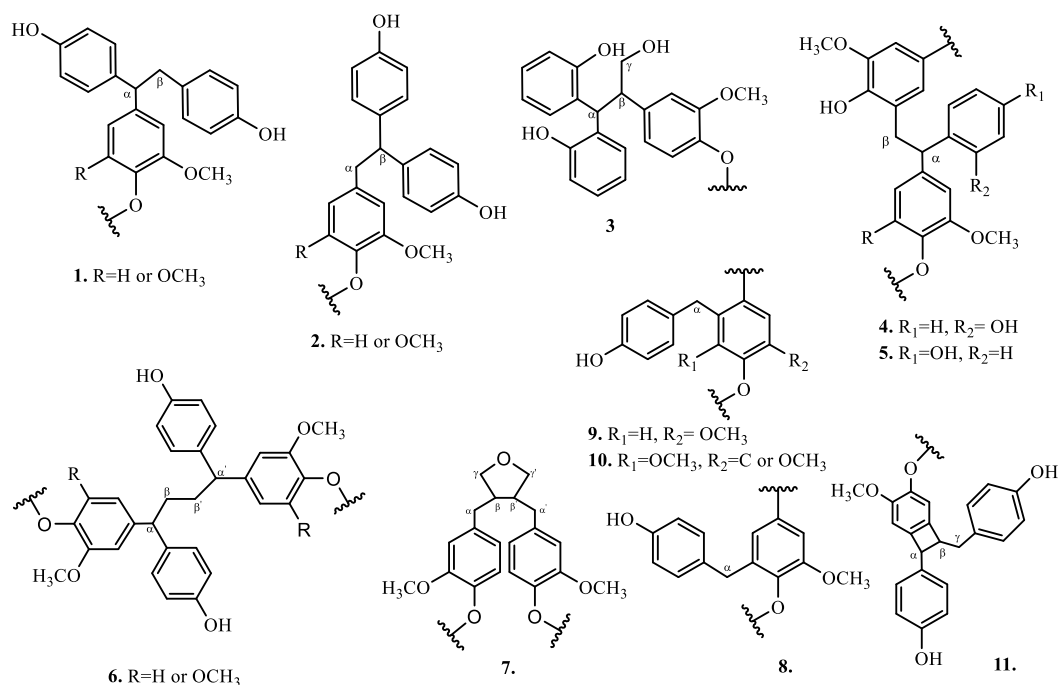
**Figure 6.2.** Lignin monomer

Two factors that limit the further utilization of lignin in phenolic foams include the low reactivity and high molecular weight. Compared with phenol, lignin possesses fewer reactive sites for formaldehyde, which leads to fewer crosslinks formed in the foams, thus lowering the mechanical properties of the products. The reactivity can be increased either by introducing new reactive groups or by creating reactive sites on the original structure. The high molecular weight of lignin makes it difficult to reach an 80% solid content. Popular modification methods to improve the utilization of phenolic compounds include phenolation, hydroxymethylation, demethylation, and depolymerized hydrolysis lignin [177], [207].

### 6.3.1. Phenolation

One of the most promising methods for lignin modification is phenolation. This method is an option for improving the reactivity of lignin by increasing the amount of phenolic hydroxyl groups and decreasing the molecular weight [208]. Different methods have been proposed for lignin phenolation. In phenolation reactions, a phenol is attached to the lignin structure. The first step is the protonation of the benzyl hydroxyl group, which is followed by the dehydration of  $\alpha$ -carbon. Phenol undergoes electrophilic aromatic substitution with the carbonium ion, which allows for the formation of phenol-lignin condensation products. Fragmentation takes place after the incorporation of an ortho- or para-phenyl compound to substitute the  $\alpha$ -hydroxyl groups in the propyl side chains [209]. Some side reactions can also occur depending on the reaction (processing) conditions [206], [210].

Newer studies have proposed that lignin substructures ( $\beta$ -O-4',  $\beta$ -5'/ $\alpha$ -O-4',  $\beta$ - $\beta$ ',  $\alpha$ -carbonyl, etc.) react, which increases the amount of phenolic OH present in the structure. In addition to considering ortho and para positions for lignin phenolation, Jiang et al. (2018) suggested the presence of more substructures (Figure 6.3) because of the elimination of formaldehyde from  $\gamma$ -carbon.



**Figure 6.3.** Plausible substructures present in phenolated lignin; adapted from Jiang *et al.* (2018)

In addition to the effect of the reaction conditions, the phenolation of lignin can be affected by the lignin isolation process, which is found on the natural form of lignin [211]. Different types of lignin can be used for phenolation. The effect of phenolation on lignins (hardwood, softwood, and annual plants) and pulping methods (organosolv, kraft, soda, sulfite, and hydrolysis) have been studied [212]. Phenolation has also been studied with other lignin types, such as ammonium lignosulfonates; the conditions that must be employed to achieve phenolation of this lignin type without compromising the mechanical properties include a high temperature (120 °C), long reaction time (160 min), and low lignosulfonate content (30%) [200].

As well as increasing the number of reactive sites with the presence of a higher number of p-hydroxyphenyl units, it is known that phenolation can decrease the molecular weight and polydispersity of lignin, which are more pronounced at higher temperatures and longer reaction times. This phenomenon is a result of lignin fragmentation during the phenolation process [200]. These properties make lignin attractive for different applications. In foams, lignin phenolation has

potential value, but the main restriction of lignin use in foams is the gelation of resol during evaporation to an 80% solid content. However, controlled phenolation processing conditions can lead to low molecular weight lignin, which decreases the viscosity of the resol and avoids resol gelation [200].

Phenolation is performed to improve the lignin reactivity towards formaldehyde by attaching phenol to the lignin structure, as well as by lowering the molecular weight by cleavage of ether bonds, such as  $\beta$ -O-4. Phenolation can be performed either in an acidic medium or under alkaline conditions. The synthesis of phenolated lignin under alkaline conditions has been reported by [213]. In their work, four types of technical lignins were phenolated, and their structure and functional groups were compared. According to their report, no major differences in the  $\beta$ -O-4 and  $\beta$ - $\beta$  amounts were seen, while the H-unit content increased after phenolation. However, it was not clearly established whether the phenol residues were covalently bonded or if the residual phenol was physically attached to the lignin.

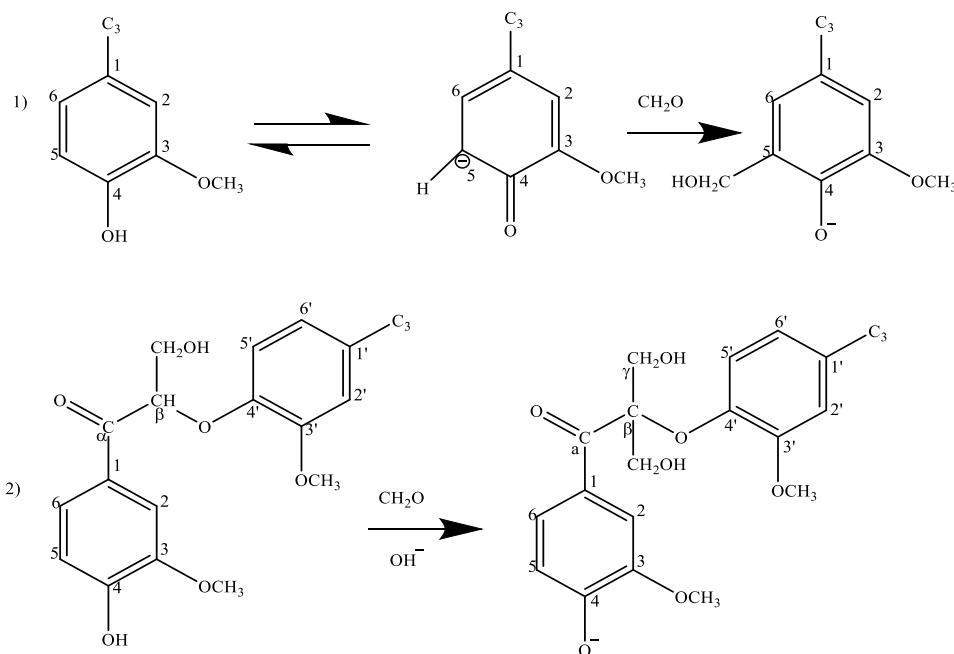
### **6.3.2. Hydroxymethylation**

The objective of hydroxymethylation is to increase the reactive sites by introducing hydroxymethyl (-CH<sub>2</sub>OH) groups to lignin macromolecules. The reaction takes place following electrophilic aromatic substitution (Sen et al. 2015). Hydroxymethylation can be carried out by mixing lignin with formaldehyde at a specific ratio and reacting at 75 °C to 90 °C in an alkaline medium [214]–[216]. The reaction pathway for the introduction of hydroxymethyl groups to the lignin structure is shown in Figure 6.4.

During the characterization of hydroxymethylated lignin, ether bonds are also cleaved, which leads to a lower molecular weight and higher flexibility. Because of less blocking of the C5

position, softwood is a better starting material for hydroxymethylation compared with hardwood [217].

Despite showing potential phenol replacement and similar properties to phenolic products, hydroxymethylation has not been used on an industrial scale. The main restriction of hydroxymethylation for lignin modification is the occurrence of side reactions in which formaldehyde reacts with itself and aliphatic methylol groups are introduced in lignin side chains [218]. New laws concerning formaldehyde emissions from wood products have limited the use of hydroxymethylation in adhesives and resins, which have major applications in wood products [219]. Consequently, the application of this method in phenolic foams has also been limited because the potential uses include building insulation and the standards for free formaldehyde emissions are rigorously enforced.



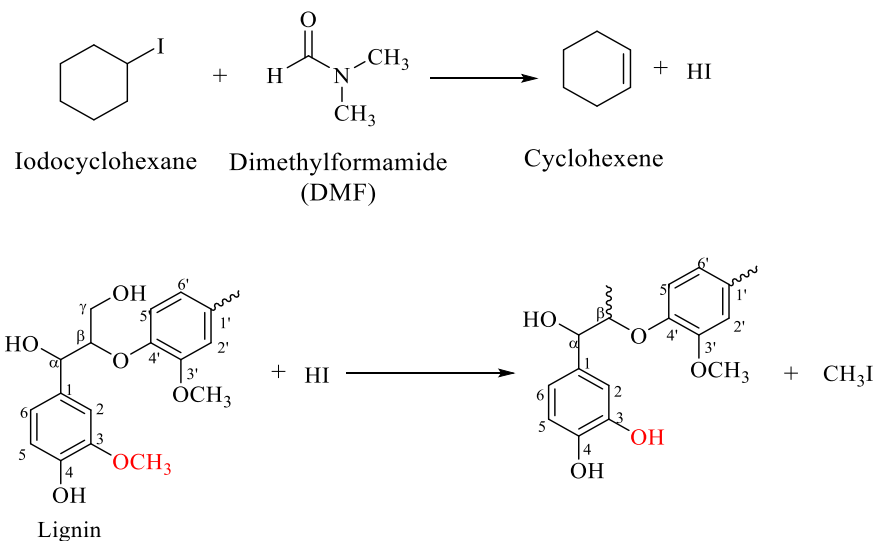
**Figure 6.4.**Reaction pathway of hydroxymethylation of lignin. Adapted from Benar et al 1999 [214]

### 6.3.3. Demethylation

Demethylation converts methoxyl groups into hydroxyl groups. In raw lignin, the ortho positions are blocked by methoxyl groups, which are not available to react with formaldehyde. Demethylation improves the reactivity by making reactive sites more available [218]. Lignin demethylation can be performed by biological means or under acidic or alkaline conditions. The primary products include vanillin, syringaldehyde, and other aromatic aldehydes, with yields ranging from 5% to 10% at an industrial scale [220], [221].

Because side reactions may occur frequently, the demethylation yield is low. Different options to make this process more cost-effective have been studied. Some of these options include sodium periodate [222], iodocyclohexane with dimethylformamide Figure 6.5 [223], and Lewis acid catalyzed demethylation [224]. For less energy input, enzymatic oxidation is a well-established method. Oxidized lignin production using the white-rot fungus *Bjerkandera adusta* has been studied with reported aldehydic monomer yields of approximately 40% [225]. Despite the low yield, demethylation can increase the amount of hydroxyl groups by 28% [224], which leads to an improved reactivity towards formaldehyde.

Similar to other modification methods, demethylation is highly dependent upon the lignin type. In this case, the presence of guaiacyl and syringyl propane units is essential to achieve lignin demethylation because these two lignin precursors contain methoxyl groups in their structure. The products obtained can be used as oxidized phenols, chelating material, or controlled-release matrices. The applications may be related to different processes, depending on the functional groups. Because of its low yield at an industrial scale, demethylation is not commonly used.

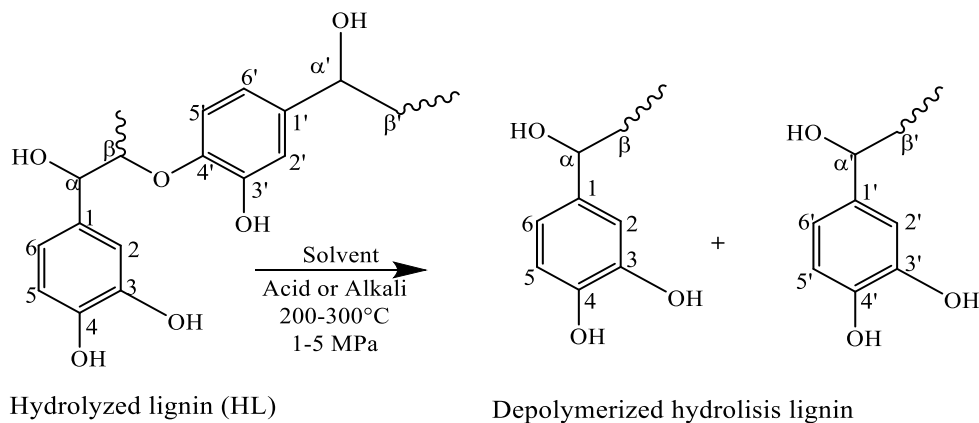


**Figure 6.5.** Chemical pathway for lignin demethylation.

#### 6.3.4. Depolymerized Hydrolysis Lignin

Hydrolysis is a process in which water is used to break down molecules. For lignin, hydrolysis can be done using either an acidic or alkaline medium [226]. Hydrolytic treatments yield lower molecular weight structures by cleaving lignin bonds [227].

In an acidic medium, lignin is reacted with a strong acid and catalyst, which demethylates the phenyl-propane unit and results in ortho substitution by hydroxyl groups. Hydrolyzed lignin is placed under a high temperature to cleave the ether bonds of the phenyl-propane units present in the lignin structure, which results in more reactive monomeric units. In contrast, an alkaline medium uses -OH groups as a primary catalytic agent, which is commonly used in pulping reactions for breaking lignin apart [228]. Hydroxyl groups present in the medium will attack different lignin bonds and yield different ether bonds that can be easily cleaved by other treatments [227]. A general pathway of lignin hydrolysis is shown in Figure 6.6.



**Figure 6.6.** Depolymerized hydrolysis lignin pathway

After either an acidic or alkaline treatment, lignin is prone to degradation, which yields new phenolic hydroxyl-containing units with low molecular weights. In an alkaline medium, lignin degradation products can be useful in condensation reactions that result in phenolic products. Even though a large number of phenolic compounds are produced in acidic media, this process is usually performed in non-aqueous media, and the product must be separated from an organic solvent before it can be used [229].

The potential applications of this modification for use in phenolic foams rely on the fact that low molecular weight lignin can avoid gelation stages during solvent and water evaporation to obtain an 80% solid content for foam formation. Likewise, by avoiding gel formation, a higher amount of lignin can be included in the material without compromising the mechanical properties.

### 6.3.5. Comparison of different modification methods

Lignin structural modification is done with the intention of achieving a higher phenol substitution in the foam formula, as well as better mechanical and thermal properties. There is no clear choice when choosing a modification method. The modification method, as well as the processing parameters, depend on the lignin type and characteristics.



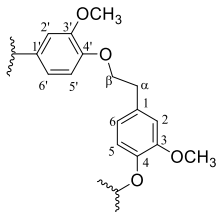
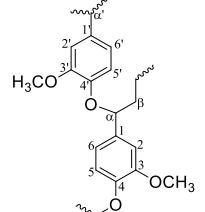
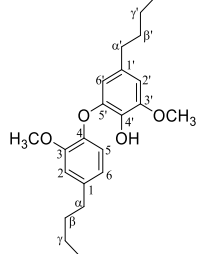
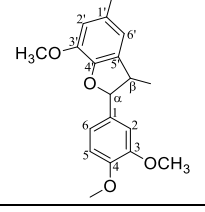
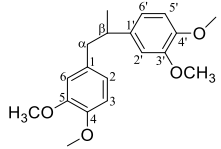
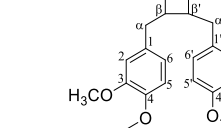
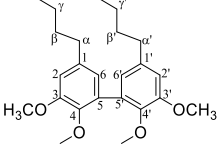
Phenolation has been shown to be one of the most promising modification methods. It works to increase the reactivity by introducing reactive sites and lowers the molecular weight and polydispersity. Theoretically, phenolation works for all types of lignin with different amounts of incorporated phenol. The degree of phenolation depends on the lignin type, functional groups, and lignin structure, as well as the phenolation process. Podschun et al. (2015) investigated the structure-function relationships and proposed a correlation between the degree of phenolation and aliphatic hydroxyl groups. However, the relationship might have been arbitrary because the degree of phenolation also correlated with the amount of other reactive sites, such as  $\beta$ -O-4 and carbonyl groups [212].

Table 6.2 shows the common linkages in softwood and hardwood lignin [230]–[232]. The given numbers were calculated based on the original lignin in wheat straw, corncob, bagasse, agricultural residues, wood, switchgrass, corn stover, straw, and hybrid poplar without any chemical or physical pretreatment. The functional groups and linkages of the available technical lignins vary with the pretreatment process. For example, depending on the processing conditions,  $\beta$ -O-4 linkages in softwood lignin decreased to 5% to 7% after kraft pulping [233], [234]. The phenolation efficiency for organosolv lignin is generally higher than kraft lignin, as is shown by the amount of incorporated p-hydroxyl in Figure 6.4.

Unlike phenolation, which works for almost all kinds of lignin, hydroxymethylation is restricted because it requires unblocked C3 and C5 positions on the original lignin structure. As is listed in Table 6.2, the number of activated sites (free C3 and C5 positions) in softwood lignin is higher than in hardwood and non-woody lignin. Therefore, hydroxymethylation works better for softwood lignins than other lignin types. The effects of hydroxymethylation on different lignin types are summarized in Table 6.3. Also, the efficiency of hydroxymethylation is limited because

of unwanted reactions on the side chain of lignin (Figure 6.4) and the occurrence of undesirable Cannizzarro reactions of the formaldehyde [216], [235], [236].

**Table 6.2.** Common linkages in softwood and hardwood lignin [230]–[232]

Linkage type	Percent of total linkages		
	Hardwood	Softwood	
$\beta$ -O-4		60-62	45 -50
$\alpha$ -O-4		6 - 8	6 - 8
4-O-5		6 - 9	4 - 7
$\beta$ -5		3 - 11	9 - 12
$\beta$ -1		1 - 7	1 - 9
$\beta$ - $\beta$	$\alpha$ 	3 - 12	2 - 6
5-5		3 - 9	20 - 25

Another disadvantage compared with phenolation is that hydroxymethylation barely changes the lignin structure. As is shown in Figure 6.4, the main reactions that occur during hydroxymethylation are the incorporation of hydroxymethyl groups onto the open C5 positions and lignin side chains. Hydroxymethylation shows no remarkable modification to the molecular weight and molecular weight distribution of the original lignin. When substituting phenol with lignin in phenolic foams, one of the biggest challenges is the difficulty in reaching an 80% solid content because of the higher molecular weight. Thus, although hydroxymethylation improves the reactivity of lignin, it is not as sufficient for modifying the lignin structure for better foam processing. Hydroxymethylation may be more suitable for lignins with lower molecular weights or it could be combined with solvent fractionation for better utilization in phenolic foam production.

**Table 6.3.** Functional groups of various lignin samples and of their hydroxymethylated derivatives [216] [237] [238]

Samples	Resource	OH total groups	Ar-OH groups	Incorporated -OH	S/G ratio
Wheat straw lignin	Non-woody	1.06	0.93		0.82
Wheat straw lignin-H		1.16	0.98	0.10	0.97
Sarkanda grass lignin	Non-woody	1.05	0.91		0.82
Sarkanda grass lignin-H		1.14	0.95	0.09	0.85
Kraft lignin	Softwood	1.30	0.58		0.37
Kraft lignin-H		1.65	0.57	0.35	0.46

-OH groups: per C9 groups based on proton nuclear magnetic resonance (<sup>1</sup>H NMR); [216], [217], [238]

Although demethylation improves the reactivity of lignin towards formaldehyde, it does not help with modifying the lignin structure and molecular weight. For lignin demethylation, the

disadvantages mainly lie in the expensive reagents and catalysts, low yields, and the residual heterogeneous structure of the demethylated lignin. Because of these disadvantages, only a limited body of work is available on the utilization of demethylated lignin in foaming products.

Lignin hydrolysis can be conducted under either acidic or alkaline conditions, as well as in a solvent system with a catalyst [239]–[241]. Matsushita et al. (2008) studied the acid hydrolytic degradation of lignin for the synthesis of ionic exchange resins. Because of the cleavage of various linkages, such as  $\beta$ -ether and  $\alpha$ -ether bonds, the molecular weight of lignin decreases and the hydroxyl groups increase after acid hydrolytic degradation [239]. The main disadvantages of hydrolysis in an acidic medium lie in the unfavorable re-polymerization of the degraded lignin products, which generates repolymerized lignin with a higher molecular weight, and the challenge of waste treatment because of the use of acid. The hydrolysis of lignin under alkaline conditions has also been investigated. Similar to acid hydrolytic degradation, there is competition between de-polymerization and re-polymerization [240]. In general, the cost of the hydrolytic degradation of lignin is higher than for phenolation and demethylation because of the severity of the reaction conditions (higher temperature or pressure) and the need for expensive catalysts in some cases. Therefore, this method may not be economically viable.

The modification described in the section above has encouraging results for improving some properties of phenolic foams; however, the amount of lignin used to replace phenol in the mixture is high and it is still a challenge to achieve the total replacement of phenol. As was demonstrated in the current review for the choice between the direct use and indirect use of lignin, there is not a remarkable improvement in the substitution rate. New methods are required to obtain a higher amount of lignin in the matrix.

**Table 6.4.** Functional groups and activated sites in various technical lignins. Adapted by the authors from El Mansouri et al. 2006 [229]

Resource		UV-spectroscopy	1H NMR		Activated sites/C9	
		OH phenolic	OH phenolic	OH aliphatic	UV-spectroscopy	Mannich reactivity
Kraft lignin	Softwood	4.50	4.10	10.09	0.32	0.40
Soda-Anthraquinone lignin	Softwood	4.40	4.50	3.10	0.36	0.36
Organosolv lignin	Non-woody	2.66	3.33	3.50	0.16	0.21
Ethanol process lignin	Hardwood	2.30	2.65	4.73	0.23	0.28
Lignosulfonate	Softwood	2.00	NA	NA	0.23	0.24

## 6.4. Utilization of lignin in phenolic foams

### 6.4.1. Direct Utilization of Lignin in Phenolic Foams

The direct use of lignin in phenolic foams without previous modification has not been adequately studied. Because lignin has a low reactivity, the substitution rate of phenol in foams is low and an increase in the lignin amount affects the mechanical performance of the foams. Additionally, a high molecular weight of raw lignin leads to the gelation of the resol during evaporation when a higher substitution of phenol is desired. When the amount of lignin is increased, the mechanical properties, such as the flexural and compressive strengths, are negatively affected. However, in contrast to the mechanical properties, the thermal performance of foams is improved with the addition of lignin [203].

As was discussed in the Lignin Modification section, the biological source of lignin (hardwood, softwood, or annual plants) has an impact on the final properties of the products it is used to generate. Different lignin types have been studied for application in phenolic foams. For example, lignin from steam-exploded corn stalk has been used for lignin foams as a concentrated

liquor from alkali extraction. The addition of lignin in foams has resulted in a higher apparent density and compression strength compared with that of conventional phenolic foams. However, studies have shown that increased substitution rates do not result in a major improvement in the thermal conductivity and fire-retardant properties. In related works, the achieved substitution rate has been up to 30%. For the morphological properties, lignin-based phenolic foams have shown similarities in pore size and distribution with phenolic foams [242].

Lignin derivatives have also been used in phenolic foams. For example, lignin nanoparticles have been used as a filler and achieved an 8.5% substitution rate for optimum mechanical properties. Although the substitution rate is comparatively low, one of the benefits of lignin nanoparticles is the reduction in the blowing agent amount without affecting the mechanical performance [199]. Table 6.5 shows some properties of phenolic foams using different reinforcements, including lignin nanoparticles.

**Table 6.5.** Compressive mechanical properties and apparent density of several reinforced phenolic foams. Adapted by the authors from: [200]

Foam	Reinforcement (wt.%)	$\rho$ (kg/m <sup>3</sup> ) <sup>a</sup>	E (MPa) <sup>b</sup>		$\sigma_c$ (MPa) <sup>c</sup>		
Phenolic	Unreinforced	0	120	14.7	(100)	0.474	(100)
	Lignin nanoparticles	8.5	-	18.9	(128)	0.824	(174)
	Wood flour	1.5	-	19.1	(130)	0.731	(154)

a Apparent density, b Compressive strength (strain 10%), c Compressive modulus

#### 6.4.2. Modified Lignin in Phenolic Foams

To further increase the lignin substitution rate in foams while maintaining acceptable mechanical and thermal properties, research has focused on modifying the lignin structure [207]. However, most of the research has been done with adhesives and only a few works relate to phenolic foams [243], [244]. In general, the works done with modified lignin phenolic foams have shown improved thermal properties. Conventional phenolic foams have a degradation temperature

of approximately 260 °C, while modified lignin phenolic foams have a degradation temperature of 400 °C or higher [194], [228], [245]. More information on the flammability, thermal conductivity, and fire and smoke stability needs to be obtained. Despite the importance of thermal conductivity in phenolic foams for insulation applications, only one study has discussed this property. For lignin-modified phenolic foams, the reported thermal conductivity is 0.04 W/m·K [228]. For reference, the thermal conductivity values range from 0.019 W/m·K to 0.046 W/m·K for typical insulants [246].

The mechanical performances of foams are related to the processing conditions, such as the phenol to formaldehyde ratio, temperature, reaction time, etc., and the foam properties, such as the density. Even though a broad range of variables and parameters can influence these properties, a reference value of approximately 0.18 MPa [247] has been established for the compressive strength. As can be seen in

Table 6.6, the compressive strength of modified-lignin phenolic foams ranges from 0.09 MPa to 10 MPa. For the elastic modulus of lignin phenolic foams, values ranging from 2.16 MPa to 12.8 MPa have been reported [194], [201], [202], [228], [245]. Even when it is a wide range, these values are comparable to standard values for phenolic foams (

Table 6.6). A deeper study needs to be done into the mechanical properties of lignin phenolic foams.

Of the properties discussed above, density deserves more attention. One of the advantages of conventional phenolic foams is their lightweight structure. Density values for modified lignin foams range from 28 kg/m<sup>3</sup> to 66 kg/m<sup>3</sup>, which falls in the range of standard density values for conventional foams (25 kg/m<sup>3</sup> to 60 kg/m<sup>3</sup>) (BS EN 131662010). However, as can be seen in

Table 6.6, the density for some modified processes is high. For these cases, the presence of lignin increases the resin molecular weight, which makes the attainment of an 80% solid content for foam formation harder to achieve by hindering the evaporation of the blowing agent during the foaming process [228]).



**Table 6.6.** Lignin modifications for use in phenolic foams and lignin-modified phenolic foams properties.

Modification	Type of Lignin (Source)	Substitution Rate	Property					Reference
			Thermal Decomposition	Compression Strength	Elastic Modulus	Density	Thermal Conductivity	
<b>Phenolic foam (standard)</b>	None	0%	NR	0.10 MPa - 0.12 MPa	NR	32 kg/m <sup>3</sup> - 120 kg/m <sup>3</sup>	0.021 W/m·K - 0.045 W/m·K	[192]
<b>Phenolation</b>	Lignosulfonate	20%	443 °C	0.15 MPa	NR	28.55 kg/m <sup>3</sup>	NR	[194]
	Liquefied lignocellulosic biomass	NR	NR	0.09 MPa - 0.21 MPa (10% Strain)	3.4 MPa - 12.8 MPa	32 kg/m <sup>3</sup> - 66 kg/m <sup>3</sup>	NR	[201]
<b>Hydroxymethylat ion</b>	Organosolv (Sugarcane Bagasse)	25%	300 °C - 400°C	NR	NR	450 kg/m <sup>3</sup>	NR	[245]
	Organosolv (Sugarcane Bagasse)	25%	NR	10 MPa (ASTM-D-1621,2016 )	NR	250 kg/m <sup>3</sup> - 450 kg/m <sup>3</sup>	NR	[248]
<b>Oxidation</b>	Lignosulfonate	30%	NR	0.15 MPa	NR	50 kg/m <sup>3</sup> - 55 kg/m <sup>3</sup>	NR	[202]
<b>Depolymerized Hydrolysis</b>	Hydrolysis lignin (Hardwood)	30% - 50%	450 °C	0.15 MPa - 0.41 MPa (10% Strain)	2.16 MPa - 7.56 MPa	40 kg/m <sup>3</sup> - 108 kg/m <sup>3</sup>	0.04 W/m·K - 0.033 W/m·K	[228]

In addition to the importance of having a full characterization of the final product, a full description of lignin is important to understand the advantages and disadvantages of each modification method. As was previously mentioned, the effectiveness of the lignin modification methods is dependent on the biological source and processing conditions. Additionally, the lignin molecular weight can have a large impact on the final properties of the foam. Modification needs to be done not only to increase the reactivity, but also to lower the molecular weight of the structure to make the foaming process more cost effective by decreasing the evaporation time to achieve an 80% solid content and increasing the amount of lignin in the material.

The results in

Table 6.6 show the potential of phenolation and depolymerized hydrolysis lignin. These two processes can reduce the lignin molecular weight and increase the reactivity on the lignin molecule by making more sites that are reactive and available for formaldehyde to attach and crosslink. In contrast, hydroxymethylation can improve the reactivity with formaldehyde, but it also increases the molecular weight. This leads to high-density foams, which are undesirable for final product application because the formed foams are more brittle, which leads to a poor mechanical performance.

Finally, additional work to characterize better lignin-phenolic foams is important to have a better idea of the potential applications. For example, it is also important to consider corrosion testing. One of the biggest problems of conventional phenolic foams is that they can accelerate iron and steel corrosion in low pH environments, which is a big issue if the application of pipe insulation is considered for example [247]. Additionally, the potential presence of residual formaldehyde in foams and water absorption need to be evaluated. All of this is necessary to better understand the potential uses and scale up process of this product.

## 6.5. Conclusions

Of the lignin modifications studied, phenolation is the most common modification for incorporating lignin into phenolic compounds and results in a remarkable improvement in the thermal properties. However, the degree of phenolation of lignin is as an important issue because the effectiveness of this process is affected by the lignin type and process. This is because the reactive sites available vary for different lignin types.

For lignin-based phenolic foams, little work has been published, which opens the opportunity to investigate higher substitution rates and acceptable mechanical properties. This review summarized the reported works and proposed modification methods for lignin-based phenolic foams. While depolymerized lignin-based phenolic foams with a substitution rate of up to 50% have been successfully prepared in the literature, this method still faces a wide range of challenges ahead, such as the commercial availability of depolymerized lignin. Furthermore, the results presented herein are still controversial and have not performed scale-up testing to verify the processability and stability. Therefore, the development of lignin-based phenolic foams with a well-defined structure, acceptable mechanical properties, and thermal properties is still a great challenge. In contrast, various types and characteristics of lignin may lead to various formulations and processes to obtain optimum properties, and there is no existing literature concerning this. Therefore, the optimum foaming process, including the blowing agent levels, catalyst loading, and curing temperature, needs to be further investigated.

## 7. FUTURE WORK

From single nanoparticles to film/ barrier coatings, polysaccharides nanofibers have demonstrated a high permeation flux performance [249], Nano-micro meter scale pores in membrane production [250] and adsorption of heavy metals based on ionic interaction of charged metal ions and negatively charged matrix [251] serving on the purposes of water purification. Additionally to that, nanofiber membranes have been use for osmotic process, water-oil separation, bactericidal effect and membrane distillation [252]. Yet when the its performance it is not at its best, the advantage of nanofibers is the flexibility in chemical modification for the introduction of desired functional groups[249].

Based on the results obtained both for crab shell in heavy metal ion removal and crab shell-based nanofibers, future work can be focused on the use of nanoscale crab shell for water remediation purposes. A higher surface area and small pore size provided by the grinding of the material can improve the results obtained in chapters 3 and 4.

One major issue for heavy metal ion removal is the removal of arsenic from water, then potential research goals can be oriented to modification/ functionalization of nanofibers for this purpose. At the same time, taking advantage of intrinsic properties of the material, might also contribute to the removal of other contaminants as dyes and other heavy metal ions.

Due water scarcity becoming one major issue for humanity, international organizations, environmental agencies and governments have encouraged and supported multiple programs for the development of new technologies towards water remediation. Then, the future of this area is still broad and not completely settle.

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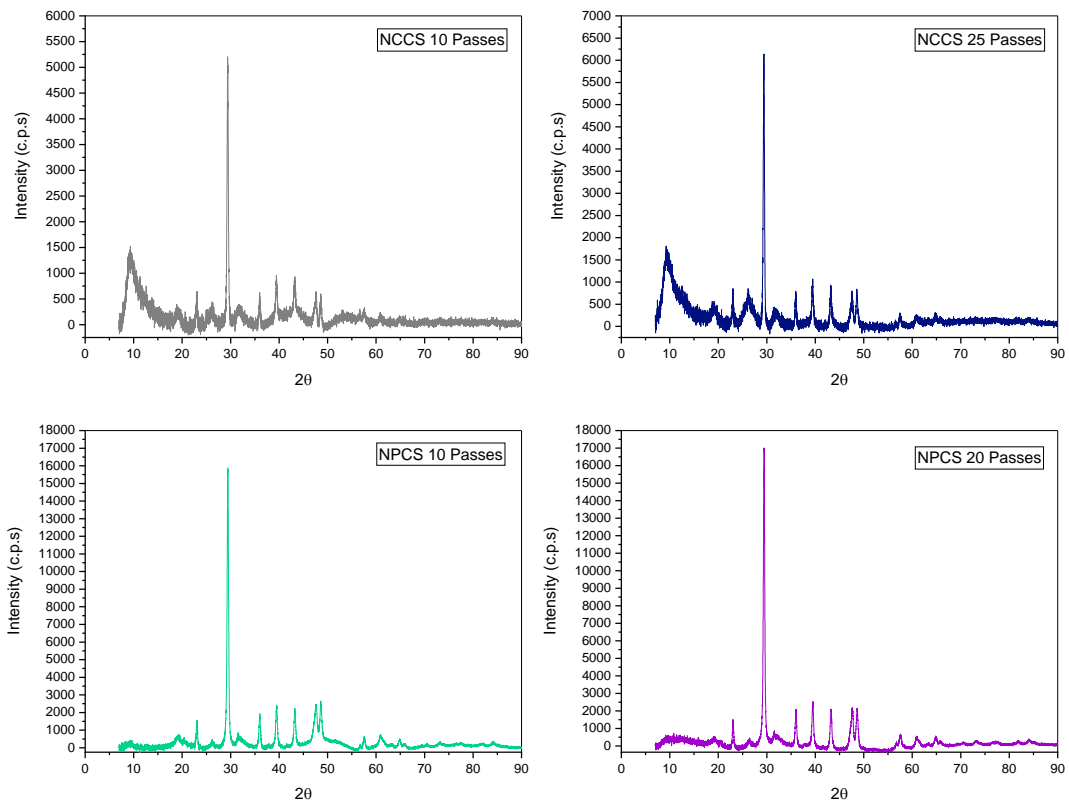
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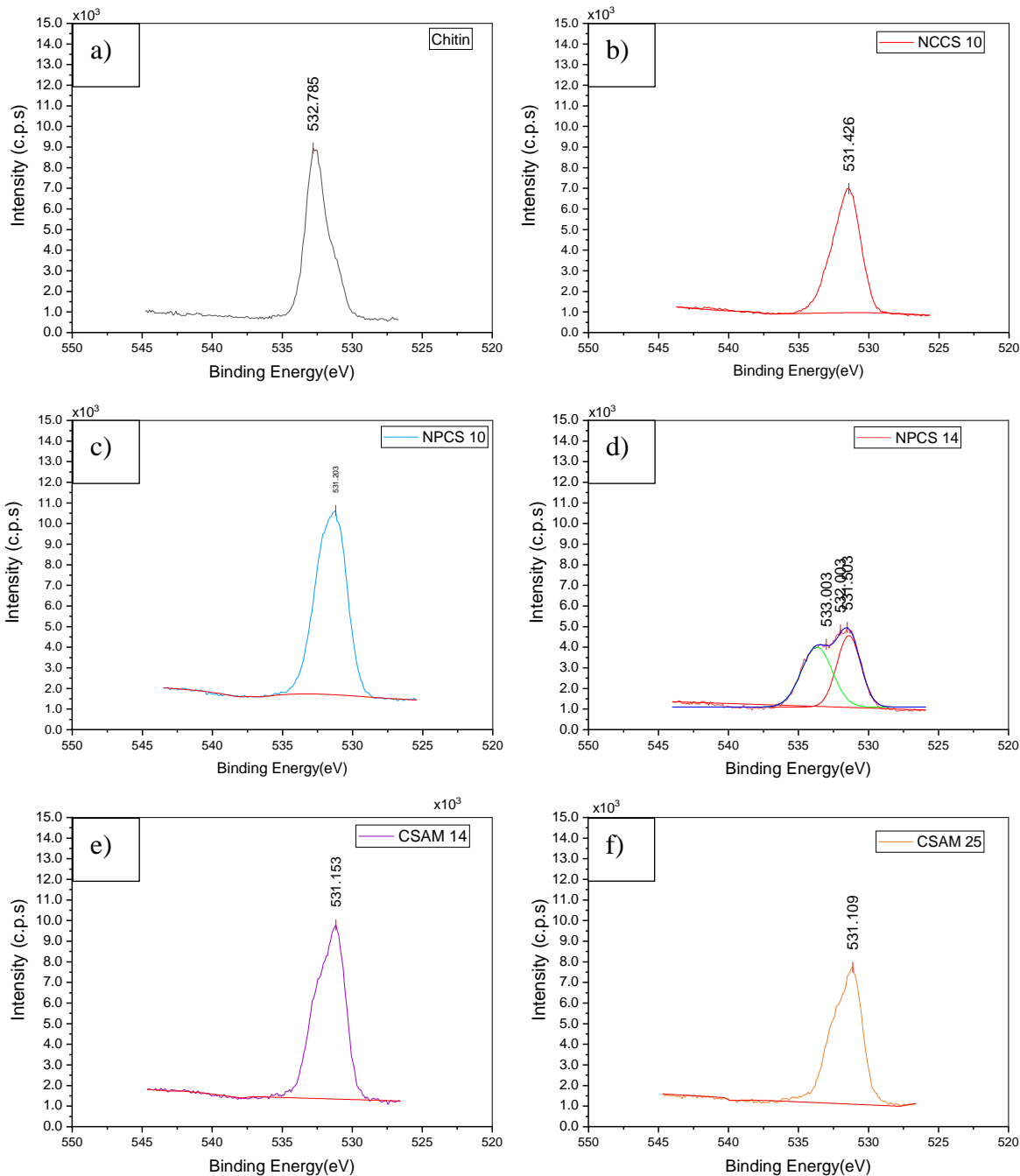


## APPENDICES

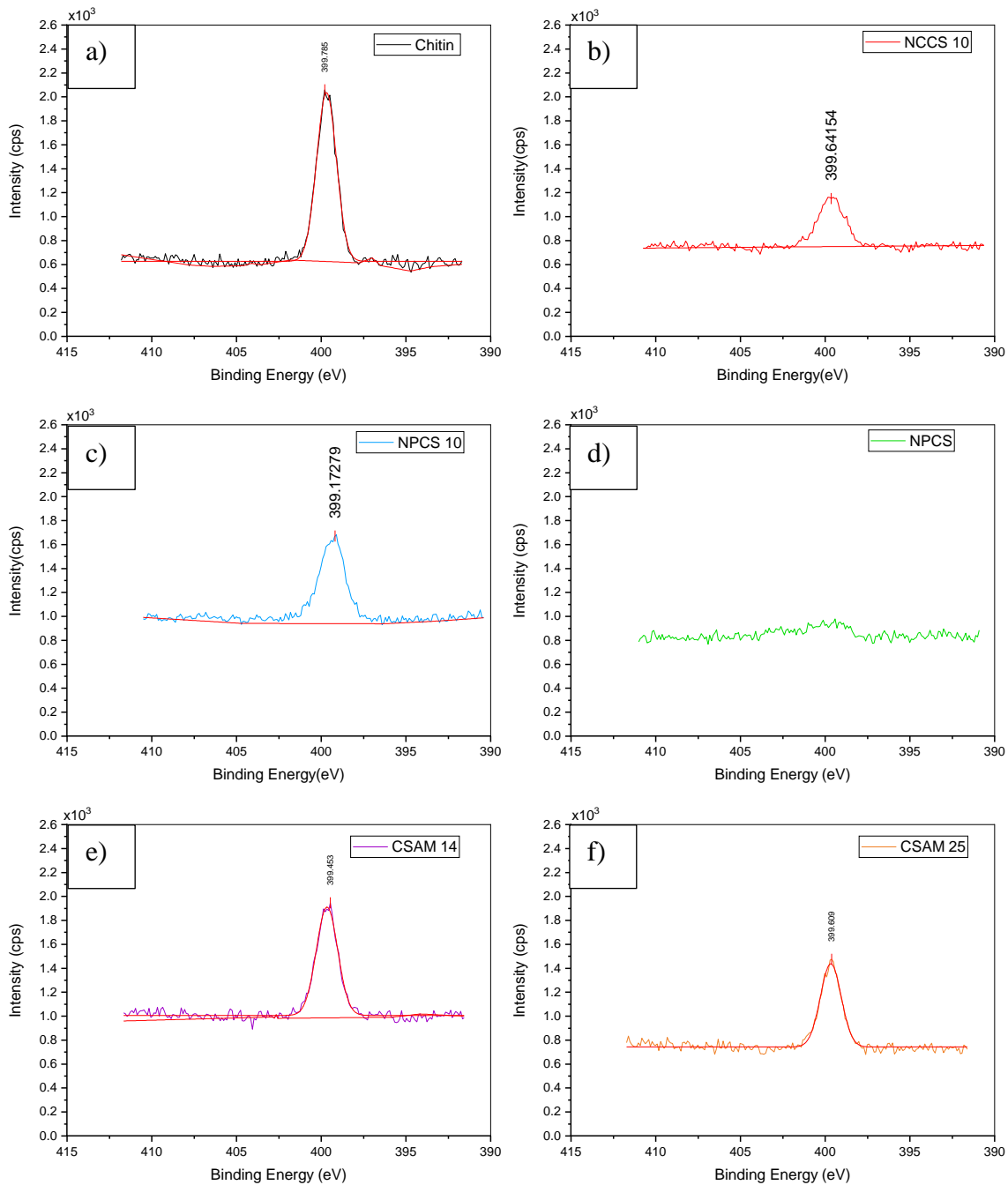
## APPENDIX A: XRD and XPS for crustacean shell-based nanofibers



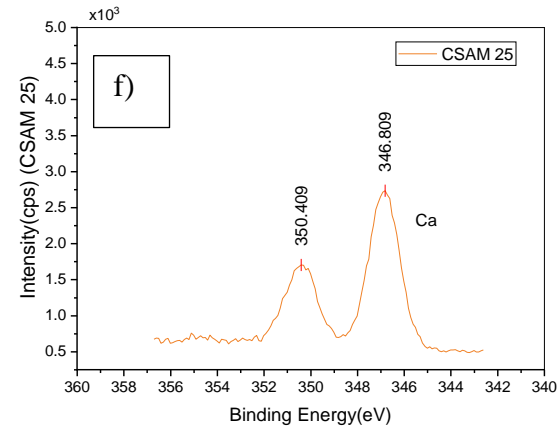
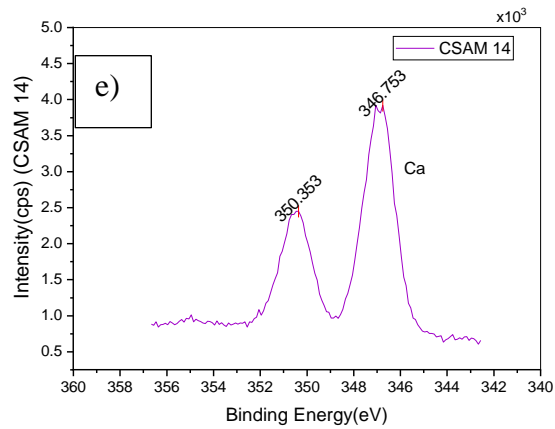
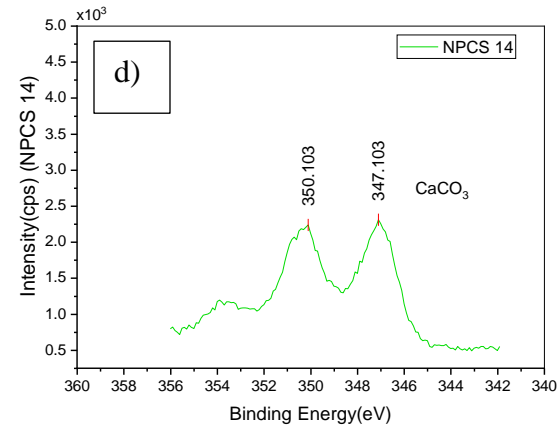
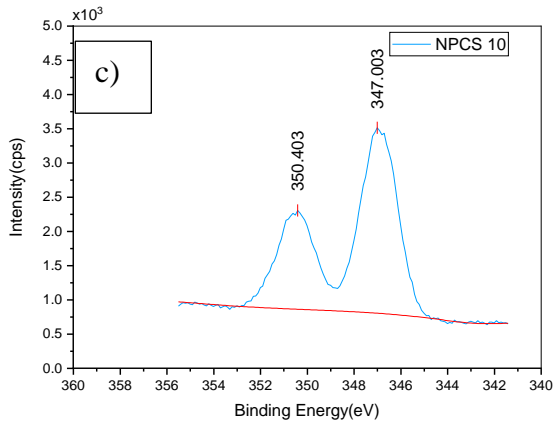
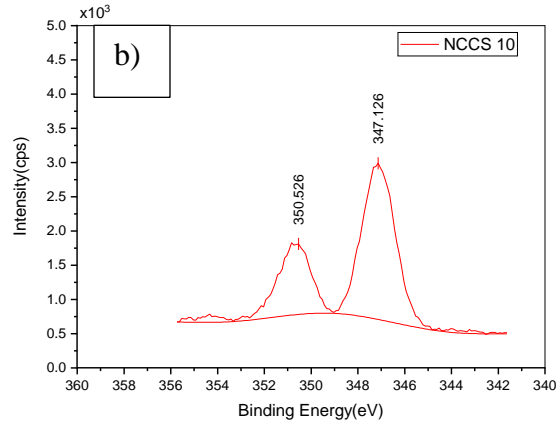
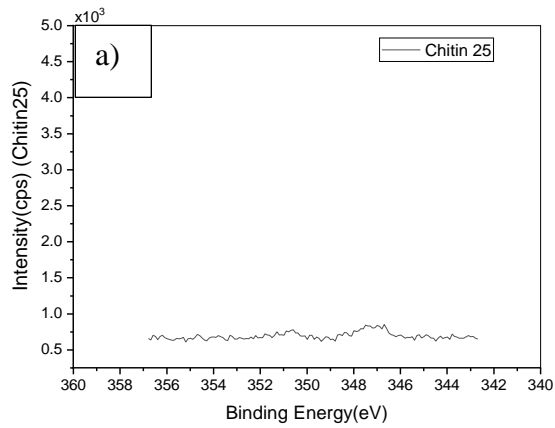
XRD data for NCCS 10 passes, NCCS 25 Passes, NPCS 10 Passes and NPCS 20 Passes



XPS Oxygen spectra for a) Chitin, b) Non-calcium carbonate Crab shell 10 passes, c) Non-protein Crab shell 10 passes d) Non-protein Crab shell 14 passes, e) Crab shell 14 passes, f) Crab Shell 25 Passes



XPS Nitrogen spectra for a) Chitin, b) Non-calcium carbonate Crab shell 10 passes, c) Non-protein Crab shell 10 passes d) Non-protein Crab shell 14 passes, e) Crab shell 14 passes, f) Crab Shell 25 Passes



XPS Calcium spectra for a) Chitin, b) Non-calcium carbonate Crab shell 10 passes, c) Non-protein Crab shell 10 passes d) Non-protein Crab shell 14 passes, e) Crab shell 14 passes, f) Crab Shell 25 Passes

**APPENDIX B: Zeta potential measurements for crustacean shell-based nanofibers at different pH values**

