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Doctoral dissertation

Assessment of the effects of various carboxylic acids as solvents, characterization and enhancement of mechanical and antibacterial properties of wet-spun chitosan fibers

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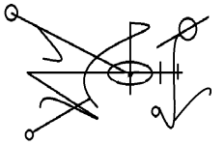
César I. Hernández Vázquez - Assessment of the effects of various carboxylic acids as solvents, characterization and enhancement of mechanical and antibacterial properties of wet-spun chitosan fibers



STRESZCZENIE

W ten rozprawie doktorskiej opisano procedurę wytwarzania włókien chitozanowych metodą przędzenia na mokro, a także oceniono wpływ różnych kwasów karboksylowych, w szczególności kwasu octowego, cytrynowego i mlekowego jako rozpuszczalników w roztworze, na właściwości otrzymanych włókien. Ponadto badania te obejmowały wykorzystanie technik analitycznych takich jak spektroskopia FTIR, spektroskopia UV, SEM oraz testy mechaniczne w celu zidentyfikowania optymalnego rozpuszczalnika, oraz scharakteryzowania włókien. Dodatkowo zbadano możliwość włączenia kwasu ursolowego jako środka przeciwbakteryjnego oraz tripolifosforanu (TPP) jako środka sieciującego, poprawiającego właściwości mechaniczne włókien. Początkowo przygotowano roztwory chitozanu i kwasu najbardziej odpowiedni do procesu przędzenia na mokro. Następnie przygotowano dwa kolejne roztwory chitozanu i kwasu cytrynowego, oraz chitozanu i kwasu mlekowego o stężeniu 7%, a ich właściwości reologiczne również zostały ocenione. Następnie przygotowano włókna chitozanowe z roztworów polimerowych zawierających trzy octowego w dwóch różnych stężeniach polimeru (7% i 8% wagowo), a następnie oceniono lepkość dynamiczną tych roztworów. Spośród tej grupy, stwierdzono, że 7% roztworu chitozanu jest różne rozpuszczalniki, a jako medium koagulacyjne użyto wodorotlenku sodu. Spośród trzech testowanych rozpuszczalników kwas octowy okazał się najbardziej odpowiedni do przygotowania roztworu i produkcji włókien chitozanowych. Wyniki wykazały, że włókna chitozanowe mogą być produkowane z wystarczającą wytrzymałością na rozciąganie i gładką powierzchnią morfologiczną, używając 7% roztworu chitozanu i kwasu octowego. Aby poprawić właściwości przeciwbakteryjne włókien, kwas ursolowy został wprowadzony na powierzchnię włókien na drodze adsorbcji z roztworu, a tripolifosforan (TPP) został użyty jako środek sieciujący, poprawiający ich wytrzymałość mechaniczną. Właściwości włókien były oceniane za pomocą spektroskopii FTIR, spektroslopii UV, testów wytrzymałości na rozciąganie

oraz testów aktywności przeciwbakteryjnej wobec dwóch powszechnie występujących szczepów bakteryjnych: Staphylococcus aureus i Escherichia coli. Wyniki wykazały, że włókna chitozanowe formowane ze stężenia 7% mogą być wytwarzane z wystarczającą wytrzymałością na rozciąganie, gładką powierzchnią morfologiczną, a ich właściwości przeciwbakteryjne mogą być poprawione poprzez użycie kwasu ursolowego jako środka przeciwbakteryjnego, a właściwości wytrzymałościowe poprzez użycie środka sieciującego powierzchnię włókien - TPP.

ABSTRACT

In this PhD dissertation, it is described a procedure in which chitosan fibers were prepared by wet spinning method and the effects of various carboxylic acids, specifically acetic acid, citric acid, and lactic acid as solvents in the dope solution were assessed, as well as their influence on the properties of the resulting fibers, also, this research involves the use of analytical techniques such as FTIR spectroscopy, UV spectroscopy, SEM, and mechanical testing to identify the optimal solvent and characterize the fibers. In addition, the incorporation of Ursolic acid as an antibacterial agent and tripolyphosphate (TPP) as cross-linker to enhance the mechanical properties of the fibers was explored. Initially, dope solutions of chitosan/acetic acid were prepared at two different polymer concentrations (7% and 8%), the dynamic viscosity of the solutions was evaluated. Out of that range, the 7% chitosan solution was determined as the most suitable for wet spinning process; later two more dope solutions were prepared 7% chitosan/citric acid and 7% chitosan/lactic acid and their rheological properties were evaluated as well, after this, it was determined to prepare chitosan fibers with the polymeric solutions containing three different solvents, and as coagulant medium, sodium hydroxide was used. Among the three solvents tested, acetic acid was found to be the most suitable solvent for preparing the dope solution and producing chitosan fibers, the results showed that chitosan fibers can be produced with sufficient tensile strength and smooth morphological surface using 7% chitosan/acetic acid dope solution. To enhance the fibers' antibacterial properties, ursolic acid was incorporated through wet impregnation method, while tripolyphosphate (TPP) was used as a cross-linker to improve their mechanical strength by the same method. The morphology of the fibers was studied by means of SEM and the properties of the fibers were evaluated using FTIR spectroscopy, UV spectroscopy, tensile strength tests, and antibacterial activity tests against two common bacterial strains, Staphylococcus aureus and Escherichia coli. The outcomes proven that 7% chitosan fibers can be produced with adequate tensile

strength, smooth morphological surface and their antibacterial properties can be enhanced by using ursolic acid as an antibacterial agent, as well as the tensile strength properties can be improved by using TPP as cross-linker.

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Chapter 1

Work motivation and aim

1.1 Introduction

Biopolymers are chain-like molecules composed of repeating chemical structures derived from renewable resources and degradable in the environment. The term "biopolymer" alludes to a broad range of materials typically derived from biological sources such as plants, animals, microorganisms, and marine sources [1].

Numerous industries use biopolymers because of their diverse features and uses. Since they are made from renewable resources and may degrade naturally without affecting the environment, they provide sustainable alternatives to traditional polymers made from fossil fuels. As a result of their potential to reduce reliance on petroleum and its derivatives, lower greenhouse gas emissions, and support to a more sustainable and circular economy, its use in industry has been increasing, and they are receiving more researcher's attention.

Humans have primarily utilized biopolymers throughout history as food or in the manufacture of clothes and furnishings. Since the beginning of the Industrial revolution, fossil fuels, such as oil, have been a crucial energy source for practically every commercial product, including plastic, which is widely used. However, fossil

fuels are finite resources, and environmental issues must be considered while using them for both production and energy. We must act sustainably, which means using resources at a rate that allows them to be replenished by our planet's natural cycles [2]. The use of biopolymers in industry began to increase in the 1980s [3], but has experienced significant growth in recent decades as a result of increased environmental consciousness and concern, as well as the demand for sustainable and biodegradable products.

Throughout history, numerous biopolymers have been discovered and developed, including cellulose, starch, polyhydroxyalkanoate (PHA), polylactic acid (PLA), and chitin, among others. These biopolymers are naturally occurring in a variety of biological sources and have been utilized in applications such as food, medicine, cosmetics and agriculture.

Biopolymer production on a large scale has become more feasible as a result of technological advances in production. Developments in enzyme engineering, microbial fermentation, and other biotechnology techniques have enabled the efficient production of biopolymers with specific properties, thereby expanding their potential industrial applications.

Stricter environmental regulations and regulations in many nations have prompted the search for sustainable alternatives to conventional plastics. Biopolymers, which are biodegradable and derived from renewable sources, comply with a number of these regulations and have garnered traction in the industry as a more environmentally friendly option.

Due to this, increased consumer awareness of environmental issues, and demand for more sustainable products has prompted businesses to pursue greener alternatives in their supply chains. Biopolymers are a viable option for manufacturers who wish to meet the needs of environmentally conscious consumers.

Research and development in the field of biopolymers have advanced in specific industries resulting in the creation of novel materials with enhanced properties and a broader range of applications, for instance, in the food industry, biopolymers are used as edible coatings and biodegradable packaging, since they are suitable for human consumption and can reduce plastic pollution in the environment. In addition, due to their biocompatibility and biodegradability, some biopolymers are also utilized in various medical applications. One of the earliest uses of biopolymers in the medical field was their use as sutures. Sutures are sterile filaments used to close and promote the healing of lesions or incisions in biological tissues.

Catgut, which is a suture produced from collagen derived from the intestinal fibers of animals such as ewes or cows, it was one of the first biopolymers used in sutures. Due to safety concerns and the limited availability of animal sources, synthetic or modified biopolymers have gradually supplanted catgut in medical practice. Attributable to advancements in biotechnology and polymer engineering, synthetic biopolymers designed specifically for use in medical sutures has been developed. For instance, polyglycolic acid (PGA) is a synthetic biopolymer widely utilized in absorbable sutures. Because PGA sutures are biodegradable and degrade in the body over time, they are not required to be removed after wound recovery. Another biopolymer used in sutures is polylactic acid (PLA), which is derived from renewable sources such as corn starch. PLA sutures are biodegradable and have been used in a variety of medical procedures, including sutures for plastic, ophthalmic, and gynecologic surgeries.

In addition, biopolymers serve a crucial role in medical drug delivery systems. They are intended to encapsulate and steadily release pharmaceuticals, enabling sustained drug release over extended time periods. This has revolutionized the field of drug delivery, allowing for more precise administration, fewer adverse effects, and

increased patient compliance. Biopolymer-based drug delivery systems are utilized in a variety of medical applications, including the treatment of cancer, chronic disease, and wound healing.

Biopolymers have also been incorporated in tissue engineering and regenerative medicine as scaffolds to support the proliferation of cells and tissues within the body. These scaffolds offer structural support, imitate the natural extracellular matrix, and promote tissue regeneration. Various applications of biopolymer-based tissue engineered include skin grafting, bone restoration, and cartilage regeneration.

Additionally, biopolymers have been incorporated into medical implants and prostheses. They have been utilized in joint replacements, dental implants, and cardiovascular devices due to their biocompatibility and mechanical properties. Chitosan is one of the most well-known and characterized materials applied in tissue engineering. Due to its unique chemical, biological and physical properties chitosan is frequently used as the main component in a variety of biomaterials such as membranes, scaffolds, drug carriers, hydrogels and, lastly, as a component of bio-ink dedicated to medical applications [4].

As a biopolymer, chitosan possesses numerous advantageous physicochemical properties, including solubility, reactivity, adsorption, and crystallinity. Its biological properties consist of biodegradability, antimicrobial activity, cytocompatibility, lack of toxicity, and fungicidal effects. In addition, the compound's anti-cholestemic, antioxidant, macrophage-activating, anti-inflammatory, angiogenesis-stimulating, muco-adhesion, antitumor, granulation, scar-forming, hemostatic, and wound-healing-stimulating properties make it an excellent candidate for numerous biomedical applications.

All of these properties make chitosan so fascinating; however, it has not been thoroughly investigated. Chitosan is currently utilized in a number of industries,

including the textile industry, biotechnology, cosmetics, agriculture, medical sectors, and food industries.

In biotechnology chitosan fibers are utilized in medical products such as bandages, dressings, and sutures because they are antimicrobial, biocompatible, and biodegradable. In tissue engineering, they are used to fabricate three-dimensional scaffolds that can be colonized by cells and promote tissue regeneration.

1.2 Work motivation and aim

The main objective of this PhD dissertation is to advance the understanding about the production of chitosan fibers through wet spinning to achieve superior mechanical and antibacterial properties, as well as this research delves into the effects of different carboxylic acids as solvents in the production process and their impact on the resulting fibers and their properties. This study aims to expand knowledge on how to improve the properties of chitosan fibers, which can lead to the creation of better biomedical materials. Chitosan fibers have demonstrated potential in tissue engineering, but their mechanical properties require enhancement to improve durability and functionality. Additionally, the incorporation of a broader range of agents to enhance antibacterial properties can be beneficial in preventing infections in medical implants and devices. Hence, the research findings can have a significant impact on chitosan and their applications, particularly wet-spun fibers.

This dissertation's significance lies in its potential to contribute to the development of chitosan-based materials with superior properties for various applications. The results can offer valuable insights into selecting appropriate solvents and optimizing wet-spinning conditions to produce chitosan fibers with improved mechanical and antibacterial properties. The study also explores the use of ursolic acid,

which is a relatively understudied antibacterial agent, to enhance the fibers' antibacterial properties. Additionally, this research examines the chitosan cross-linking with tripolyphosphate (TPP) to improve the fibers' mechanical properties. Ultimately, this dissertation's outcomes may significantly impact the current knowledge and understanding of chitosan wet-spun fibers and its applications.

Chapter 2

Concepts

2.1 Types of Biopolymers

Biopolymers are polymers produced from natural sources either chemically synthesized from a biological material or entirely biosynthesized by living organisms. The use of biopolymers from different sources has been investigated for many years for pharmaceutical and biomedical applications. This has resulted in a multitude of healthcare products on the market that use biopolymers in the formulation as a functional excipient or even as an active ingredient [5].

Biopolymers can be divided into two broad groups, namely biodegradable and non-biodegradable biopolymers. Alternatively, biopolymers can be classified on their origin as being either bio-based or fossil fuel-based. The bio-based biopolymers can be produced from plants, animals, or microorganisms [6]. They are classified depending on their origin.

Table 1.Classification of biopolymers

Biodegradable				Non- biodegradable
Bio-based			Fossil-based	Bio-based
Plants	Microorganisms	Animals		
Cellulose and its derivatives[1] (polysaccharide)	PHAS (e.g., P3HB, P4HB, PHBHV, PHBHH,)	Chitin (polysaccharide)	Poly(alkylene dicarboxylates) (e.g., PBA, PBS, PBSA, PBSE, PEA, PES, PESE, PESA, PPF, PPS, PTA, PTMS, PTSE, PTT)	PE (LDPE, HDPE), PP, PVC
Lingin	PHF	Chitosan (polysaccharide)	PGA	PET, PPT
Starch and its derivatives (monosaccharide)	Bacterial cellulose	Hyaluronan (polysaccharide)	PCL	PU
Alginate (polysaccharide)	Hyaluronan (polysaccharide)	Casein (protein)	PVOH	PC
Lipids (triglycerides)	Xanthan (polysaccharide)	Whey (protein)	POE	Poly (ether-esters)
Wheat, corn, pea, potato, soy, potato (protein)	Curdlan (polysaccharide)	Collagen (protein)	Polyanhydrides	Polyamides (PA 11, PA 410, PA 610, PA 1010, PA 1012)
Gums (e.g., cis-1,4-polyisoprene)	Pullulan (polysaccharide)	Albumin (protein)	PPHOS	Polyester amides
Carrageenan	Silk (protein)	Keratin, PFF (protein)		Unsaturated polyesters
PLA (from starch or sugarcane)		Leather (protein)		Epoxy
				Phenolic resins

HDPE, high-density polyethylene; LDPE, low-density polyethylene; P3HB, poly(3-hydroxybutyrate); P4HB, poly(4-hydroxybutyrate); PBA, poly(butylene adipate); PBS, poly(butylene succinate); PBSA, poly(butylene succinate-co-adipate); PBSE, poly(butylene sebacate);PC, polycarbonate; PCL, poly(e-caprolactone); PE, polyethylene; PEA, poly(ethylene adipate); PES, poly(ethylene succinate); PESA,poly(ethylene succinate-co-adipate); PESE, poly(ethylene sebacate); PET, poly(ethylene terephthalate); PFF, poultry feather fiber; PGA,poly(glycolic acid), polyglycolide; PHA, polyhydroxyalkanoate; PHBHHx, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate); PHBHV, poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PHF, polyhydroxy fatty acid; PHH, poly(3-hydroxyhexanoate); PLA, poly(lactic acid), polylactide; POE, poly(ortho ester); PP, polypropylene; PPF, poly(propylene fumarate); PPHOS, polyphosphazenes; PPS, poly(propylene succinate); PTA, poly(tetramethylene adipate); PTMS, poly(tetramethylene succinate); PTSE, poly(tetramethylene sebacate); PTT, poly(trimethylene terephthalate); PU, polyurethane; PVC, poly(vinyl chloride); PVOH, poly(vinyl alcohol).

[1]Acetyl cellulose (ACC) is either biodegradable or non-biodegradable, depending on the degree of acetylation. ACC's with a low acetylation can be degraded, while those with high substitution ratios are non-biodegradable. [7,8]

2.2 Biopolymers production

Biopolymer production has gained increasing interest in recent years due to its potential as a sustainable alternative to traditional petroleum-derived polymers.
Several crucial processes are involved in the production of biopolymers, including the following:

- Feedstock selection
- Extraction/Isolation
- Purification
- Modification (optional)
- Polymerization
- Formulation/Processing
- Final Product

2.2.1 Feedstock selection

The type of biopolymer being generated will determine the feedstock option. For instance, cellulose and starch present in corn, wheat, and potatoes can be utilized as a feedstock to create biopolymers like polylactic acid (PLA) or starch-based polymers. Otherwise collagen, gelatin, and polyhydroxyalkanoates (PHA) can be produced from proteins and lipids derived from either plant or animal sources. Chitin, which is present in shrimp and crab shells as well as in mushrooms, can be utilized as a feedstock to create chitosan.

2.2.2 Extraction/Isolation

Following the selection of the feedstock, it is typically processed to extract or isolate the desired biopolymer. Depending on the feedstock and the biopolymer being manufactured, multiple methods may be employed. For example, glucose or cellulose can be extracted from plants via milling, grinding, or enzymatic treatment in order to obtain the desired polymer. Through processes such as deproteinization and demineralization, chitin can be extracted from the shells of crustaceans. Solvent extraction, precipitation, and hydrolysis are methods that can be used to obtain proteins and lipids.

2.2.3 Purification

Following extraction or isolation, the biopolymer may be purified to eliminate impurities or contaminants. To obtain a highly pure biopolymer, filtration, centrifugation, or chromatography may be utilized. Purification is necessary to guarantee that the biopolymer fulfills the quality and performance requirements of its intended application.

2.2.4 Modification (optional)

Depending on the desired properties of the final biopolymer, additional modification processes may be performed. This may entail chemical or enzymatic reactions to alter its structure, properties, or functionality. For instance, cross-linking, grafting, or combining with other biopolymers or additives can be used to accomplish particular properties such as increased strength, flexibility, or biodegradability.

Modification stages can be tailored to the specific needs of the biopolymer and its intended use.

2.2.5 Polymerization

This process entails joining the individual biopolymer units together to produce a longer polymer chain, can be applied after the biopolymer has been purified or changed. Several techniques can be used to polymerize depending on the type of biopolymer being produced. For instance, in enzymatic polymerization, the monomer units are joined together to form a polymer chain utilizing enzymes as catalysts. In order to start the polymerization reaction, chemical reactions may require catalysts, heat, or other chemical agents.

2.2.6 Formulation/Processing

Depending on the intended application, the biopolymer produced during polymerization can be molded into various forms, such as films, fibers, coatings, or 3D-printed items. Forming the biopolymer into the required product, may involve further processing procedures like casting, extrusion, injection molding, electrospinning or wet spinning. Techniques for formulation and processing are adapted to the unique characteristics, needs, and uses of the biopolymer.

2.2.7 Final Product

To verify its functionality and safety, the biopolymer product is routinely put through quality control testing. This may include tests for properties such as mechanical

strength, biodegradability, thermal stability, or other specific characteristics depending on the intended application.

2.3 Biopolymers in the medical industry

The medical industry employs biopolymers for numerous purposes. A wide range of medical applications can benefit from the biocompatibility, biodegradability, versatility, and sustainability of biopolymers.

2.3.1 Biocompatibility

Biocompatibility is an essential aspect of the medical industry, as it ensures that materials used in medical devices and implants do not cause adverse reactions in the body. It has been determined that biopolymers are biocompatible, making them suitable for medical applications [2].

2.3.2 Biodegradability

Biodegradability is also an important factor, as it ensures that materials used in medical devices and implants do not affect the environment after they have served their purpose. Biopolymers are biodegradable, which means they can be broken down by natural processes, making them an environmentally sustainable choice [7].

2.3.3 Versatility

The adaptability of biopolymers is also a remarkable advantage. They can be used in numerous medical applications, such as drug delivery systems, wound dressings, and tissue engineering [8].

2.3.4 Sustainability

Sustainability is a significant concern in the medical industry, and biopolymers provide a sustainable solution. They are made from renewable resources and can be produced using environmentally responsible methods [9].

2.3.5 Medical applications

Science and technology are indispensable for extending life expectancy. In this regard, a number of innovative approaches, materials and new pieces of machinery have been developed resulting in lower rates of morbidity and mortality.

Due to their biocompatibility, biodegradability, and non-toxicity, biopolymers have garnered plenty of attention in the medical field. There are numerous medical applications for them, including drug delivery systems, tissue engineering, wound therapy, and medical implants [10].

Biopolymers have one of the most promising applications in drug delivery systems. They can be used to encapsulate and deliver pharmaceuticals to specific sites in the body, enabling targeted and sustained drug release [11].

In tissue engineering, biopolymers can be utilized to create scaffolds that support the growth of new tissue. These scaffolds can be created from biopolymers including collagen, chitosan, and hyaluronic acid [12].

Wound healing is another essential application of biopolymers. Biopolymers can be used to create dressings that promote wound healing by offering a moist environment preventing infection [13]. It has been shown that biopolymers such as alginate, chitosan, and collagen promote wound healing.

Biopolymers including polylactic acid (PLA) and polyglycolic acid (PGA), chitosan and silk have been utilized to create biodegradable implants, which main objective is to mimic a body part and are used to replace a damaged organ or structure to sustain normal body function. Some of the most used medical implants consist of heart, bones, eyes, ears, knees, breasts, hips and cardiovascular system implants that can be used to repair or replace damaged tissue [14].

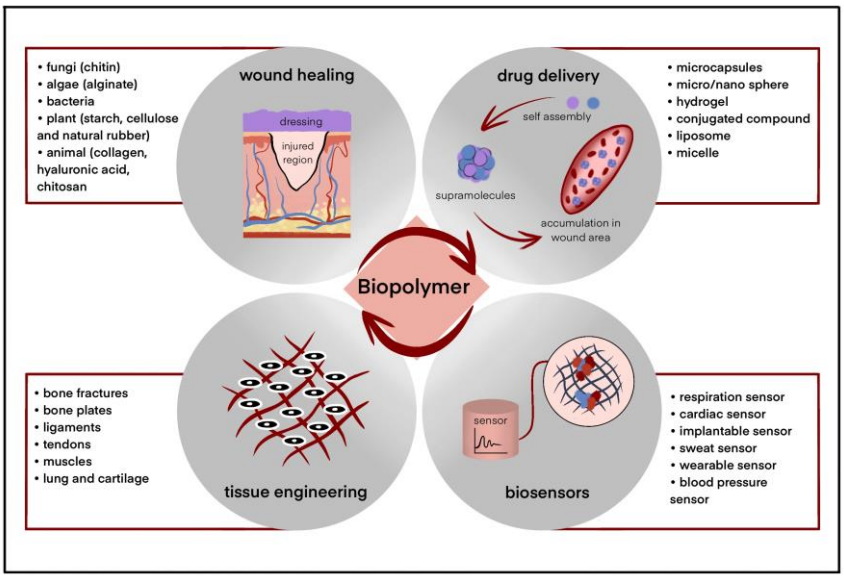

Figure 1. Biopolymer's medical applications

2.4 Chitin and Chitosan

2.4.1 Polysaccharides

Polysaccharides, are high molecular weight carbohydrate polymers composed of long chains of monosaccharide units bound together by glycosidic bonds [6]. Due to the diversity of the repeating units, type of glycosidic linkages, and degree of branching, they exhibit a significant structural variety. Common polysaccharides include cellulose (plant structural integrity), starch (plant energy storage), glycogen (animal energy storage), and chitin (insect exoskeleton or cell walls of fungi).

In living organisms, polysaccharides serve multiple purposes, including energy storage, structural support, cell-cell recognition, lubrication, and biological defense. They are essential biomolecules that play crucial roles in a variety of biological processes and are extremely important in disciplines such as biochemistry, molecular biology, and biotechnology.

2.4.2 Polysaccharides from marine sources

2.4.2.1 Chitin

Chitin is a long-chain polymer of N-acetylglucosamine, which is an amino sugar, and it is synthesized from units of N-acetylglucosamine that form covalent β-1,4 linkages. Chitin is found in the exoskeletons of insects, cell walls of fungi, and shells of crustaceans just to mention few [15]. It is the second most abundant biopolymer on Earth after cellulose. Three polymeric crystal structures of chitin (α, β and γ) are known. α-Chitin is the most crystalline orthorhombic form where the chains are anti-parallel, and can be obtained from the shell of crabs, lobsters and shrimps. β-Chitin has a

monoclinic form where chains are parallel, and is obtained from the pen of squid. γ-Chitin is a mixture of α- and β-chitins. α-Chitin is rather resistant to chemical modifications due to the peptide-like hydrogen bonds between chains, and harsh reaction conditions are required to break it down, whilst β-chitin is less stable, and can be modified under milder conditions. During dissolution or extensive swelling, β-chitin converts to α-chitin [16]. Chitosan is a partially N-deacetylated derivative of chitin and it is the most important derivative of chitin. Their biocompatibility, biodegradability, non-toxicity, antimicrobial, and hydrating properties made them ideal candidates for wound healing, especially for open and deep wound healing. These have the hemostatic effect that accelerates fibroblasts layer formation during the healing. Chitin and chitosan were used with other polymers to make hydrogels, foams, scaffolds, etc. and these copolymers have extensive use in wound dressing applications [17].
Chitin has a wide variety of sources such as:

- Exoskeleton of crustacean (crab, shrimp and crawfish, Oniscus asellus)
- Insect cuticles (Melolontha melolontha)
- Orthoptera species
- Wings of cockroach
- Grasshopper species
- Fungi such as Aspergillus niger, Mucor rouxii, and Rhizopus arrhizus
- Aquatic invertebrates
- Bat guano
- Spiders
- Green algae

2.4.2.2 Chitosan

Chitosan is a biopolymer that is derived from chitin, which is a natural polymer found in the shells of crustaceans such as shrimp, crab, and lobster. Chitosan can also be obtained from fungal sources, where chitin is present in their cell walls. Fungi such as Aspergillus niger, Mucor rouxii, and Rhizopus arrhizus are known to produce chitin as a part of their cell wall structure.

The qualities of chitosan derived from fungi and crustaceans may be comparable, but depending on the particular fungus and the extraction technique utilized, there may be some variations in molecular weight, degree of deacetylation, and other characteristics.

Chitosan is a linear polysaccharide that is composed of randomly distributed β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine units [18]. Chitosan is soluble in acidic solutions due to the presence of amino groups that can be protonated, which results in a positively charged polymer.

The chemistry of chitosan is complex and depends on several factors, including the degree of deacetylation, molecular weight, and pH of the solution. The degree of deacetylation refers to the percentage of N-acetyl-D-glucosamine units that have been converted to D-glucosamine units. This parameter affects the solubility, charge density, and reactivity of chitosan [19]. The molecular weight of chitosan also plays a role in its properties, with higher molecular weight polymers exhibiting greater viscosity and lower solubility, it also may vary different process conditions such as type and strength of acid and alkali solutions, reaction temperature and atmospheric conditions [20]. Finally, the pH of the solution can affect the charge density of chitosan, with lower pH values resulting in higher charge densities due to increased protonation of the amino groups [21].

Chitosan is insoluble in water and alkaline media. This is due to its rigid and compact crystalline structure and strong intra- and intermolecular hydrogen bonding. Chitosan is only soluble in few diluted acid solutions. Chitin/chitosan fibers are characterized by excellent antibacterial properties and wound healing properties. They are additionally hemostatic and fungistatic so that they are widely used as wound care products and for other medical uses. Chitin and chitosan are recommended as suitable functional medical materials because of their excellent properties, for instance, biocompatibility, biodegradability, absorption, ability to form film or fibers, and ability to chelate metal ions [21].

2.4.2.3 Chitin and chitosan production

There are numerous methods for producing chitin and chitosan, which can be roughly categorized into two groups: chemical and biological, but they all involve the same four stages: deproteinization, demineralization, decolorization, and deacetylation. Chitin and chitosan are manufactured commercially via a chemical process, as described in (Fig. 2).

Sometimes, demineralization and deproteinization can occur in reverse order. In certain processes, demineralization precedes deproteinization.

The shells are typically cleansed, pulverized, and then treated with an aqueous 3–5% NaOH solution at 100°C for a few hours in order to decompose the proteins. After neutralization, the shells are treated at room temperature with an aqueous 3–5% HCl solution to remove calcium carbonate and calcium phosphate. A bleaching procedure is used to remove pigments, resulting in chitin that is nearly colorless to off-white and granular. Chitosan is only found in a few species of fungi in nature. Chitosan is

produced commercially by deacetylating chitin with a highly concentrated (40–50%) NaOH solution at high temperature (100–150°C) [22].

Chitin and its derivatives, such as chitosan, are manufactured by approximately 80 companies, primarily in Asia (China, South Korea, India, Thailand, and Japan), North America (USA), and Europe (Iceland, Poland, France).

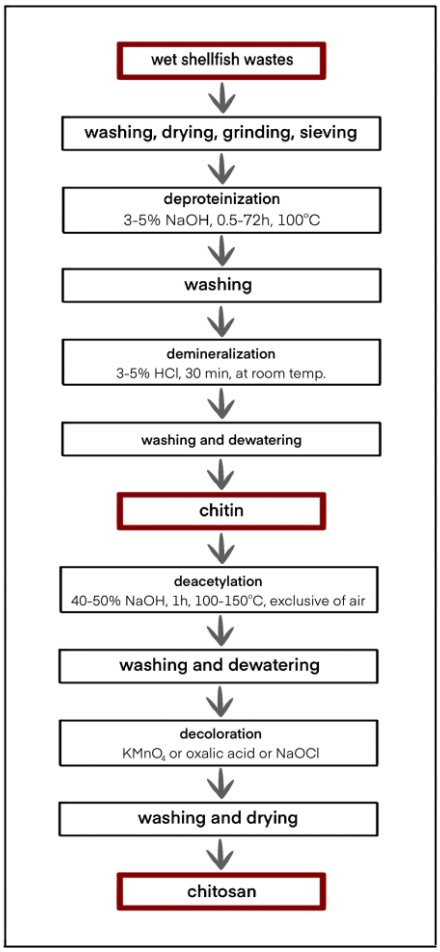

Figure 2. Diagram of chitin and chitosan production

2.4.2.4 Chitin and chitosan properties

The key properties of chitin and chitosan, which are essential for numerous applications, include [21]:

- Semicrystalline structures
- Formation of polyoxysalt
- Capability to form films, fibers, and gels
- Good complex formation with metals
- Capability to bind with negatively charged fats, lipids, cholesterol, metal ions, proteins, and macromolecules
- Non-toxicity
- Biocompatibility
- Biological activity
- Capability to form derivatives
- Biodegradability
- Hemostatic activity
- Wound-healing
- Antibacterial

Chitin and its modified derivatives have wide-ranging applications in the food industry, cosmetics, agriculture, biotechnology, textiles, and medical disciplines. Its rigid and distinct crystalline structure allows it to exist in nature in a variety of polymorphic forms with distinct properties. Due to its biological activities and industrial and medicinal applications, chitosan has a substantial economic impact [23].

2.4.2.5 Chemical structure of chitin and chitosan

With regards to their chemical structures, chitin and chitosan are similar to cellulose, but an acetamide group or amine group replaces the hydroxyl group at the C2 position of the glucosidal ring.

Chitin is a polymer of β-(1→4)-2-acetamido-2-deoxy-D-glucopyranose, while chitosan is a copolymer of β-(1→4)-2-amino-2-deoxy-D-glucopyranose and 2-acetamido2-deoxy-D-glucopyranose, obtained by deacetylation of chitin, (Fig. 3). Because pure chitin with 100% acetylation of the amine groups, and pure chitosan with 100% deacetylation, rarely exist, they are often described as copolymers of N-acetyl-D-glucosamine and D-glucosamine [24].

The amount of acetyl in the polymer determines the difference between chitin and chitosan. The degree of deacetylation (DD) is one of the most crucial structural factors in chitin and chitosan. When the degree of deacetylation of chitin reaches about 50% (depending on the source of polymer), it becomes soluble in an aqueous acidic media and is called chitosan. The deacetylation degree requirement for a typical commercial chitosan is 80–85% or greater. It has a connection with the polysaccharide being transformed into a polyelectrolyte in acidic environments and protonation of the $-NH_2$ group on the C2 position of the D-glucosamine repeat unit.

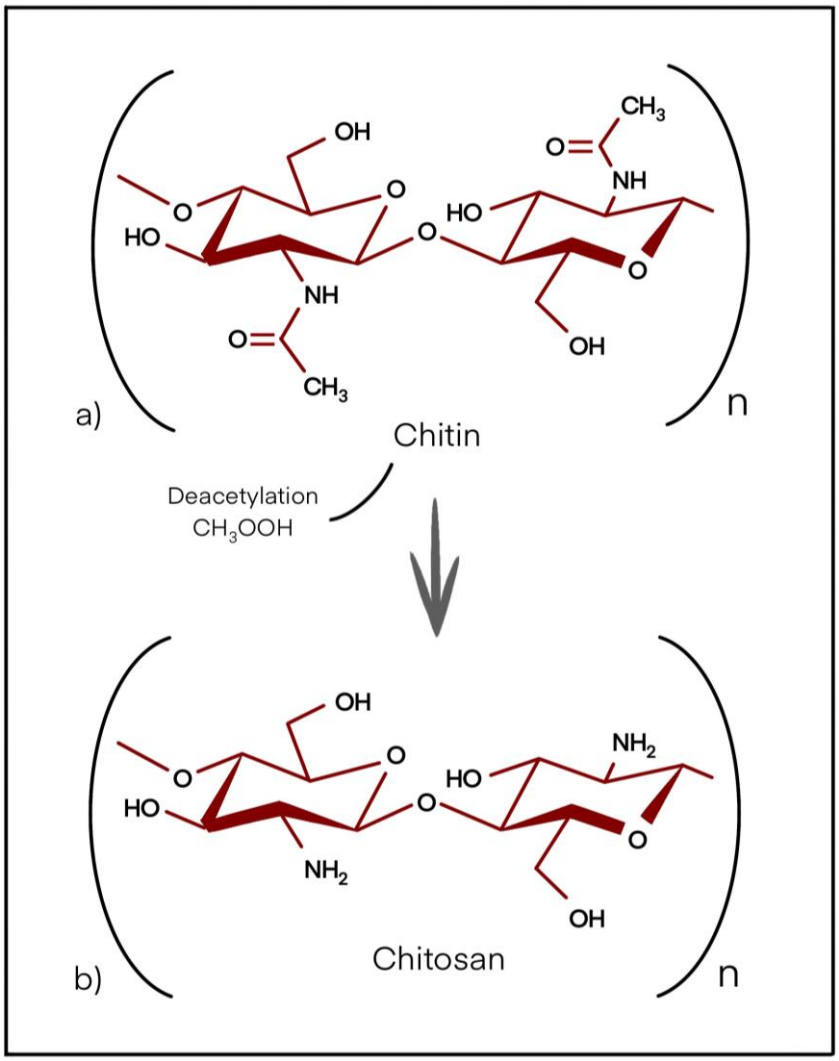

Figure 3. Chemical structure of chitin and chitosan

Chitosan is characterized by having sections in its structure that are both amorphous (non-crystalline) and crystalline. The crystalline sections contain a well-defined arrangement of molecules, in contrast to the amorphous parts, which have a disorganized appearance and do not have a regular repeating pattern. Chitosan's

crystallinity is proportional to its degree of deacetylation. At moderate levels of deacetylation, the minimum degree of crystallinity is reached. Chitosan is often insoluble in organic solvents and aqueous solutions at a pH over 7, this due to its solid crystalline structure. However, because the free amino groups are protonated and the molecule becomes completely soluble below pH 5, it dissolves easily in most diluted organic acids, including formic, lactic, acetic, and citric acids.

2.4.2.6 Chitosan's cross-linking

Chemical cross-linking refers to intermolecular or intramolecular joining of two or more molecules by a covalent bond. The reagents that are used for the purpose are referred to as 'cross-linking reagents' or 'cross-linkers' [25]. This process is utilized in a variety of industries, including the manufacture of polymers, plastics, adhesives and biotechnology. Chemical cross-linking, radiation cross-linking, and physical cross-linking are all viable methods for achieving cross-linking [26].

Chemical cross-linking is the formation of covalent bonds between polymer molecules through the use of chemical agents.

The process of radiation cross-linking involves exposing the polymer to the high-energy radiation, which causes the formation of free radicals. These free radicals then react with the polymer chains, creating new chemical bonds between them.

Physical cross-linking entails the use of physical methods to induce cross-linking, such as heat or pressure.

Cross-linking influences properties such as mechanical strength, chemical stability, aqueous permeability, solubility, and drug release. The low mechanical strength of chitosan-based materials makes it necessary to combine it with other compounds or to improve its properties by cross-linking [4]. Various therapeutic agents,

such as anti-inflammatory agents, antibiotics, anticancer medications, steroids, proteins, amino acids, anti-diabetics, and diuretics, have been investigated for controlled release or drug targeting using cross-linked as well as several types of wound dressings made by chitosan fibers.

Physical and chemical cross-linking agents are the two main categories of cross-linking agents. For chitosan, glutaraldehyde, formaldehyde, tripolyphosphate, and polyaspartic acid sodium salt are the most commonly used as cross-linkers. However, at certain concentrations, glutaraldehyde and formaldehyde are considered toxic and raise health concerns and cause undesirable side effects. To overcome this problem such non-toxic cross-linkers as genipine or TPP are used.

2.4.2.6.1 Tripolyphosphate (TPP) as chitosan's cross-linker

Chitosan's distinctive properties make it suitable for a wide range of applications. Its ability to establish cross-linked networks is an essential aspect of its functionality. Chitosan can be cross-linked using a variety of cross-linkers or cross-linking agents.

Tripolyphosphate (TPP) also sodium triphosphate (STP) or sodium tripolyphosphate (STPP) is an inorganic compound it is the sodium salt of the polyphosphate penta-anion, which is the conjugate base of triphosphoric acid.

Some chitosan cross-linkers commonly used are:

- Glutaraldehyde [27]
- Tricarboxylic acid [28]
- Genipin [29]
- Tripolyphosphate (TPP) [30]
- Epoxy compounds [31]

Pati et al. (2011) prepared Chitosan–tripolyphosphate (TPP) fibers with varying phosphate contents through wet spinning in STPP baths of different pH. TPP bath pH was found to have strong influence on the mechanism of chitosan fiber formation and their physico-chemical properties owing to different degree of ionization [30].

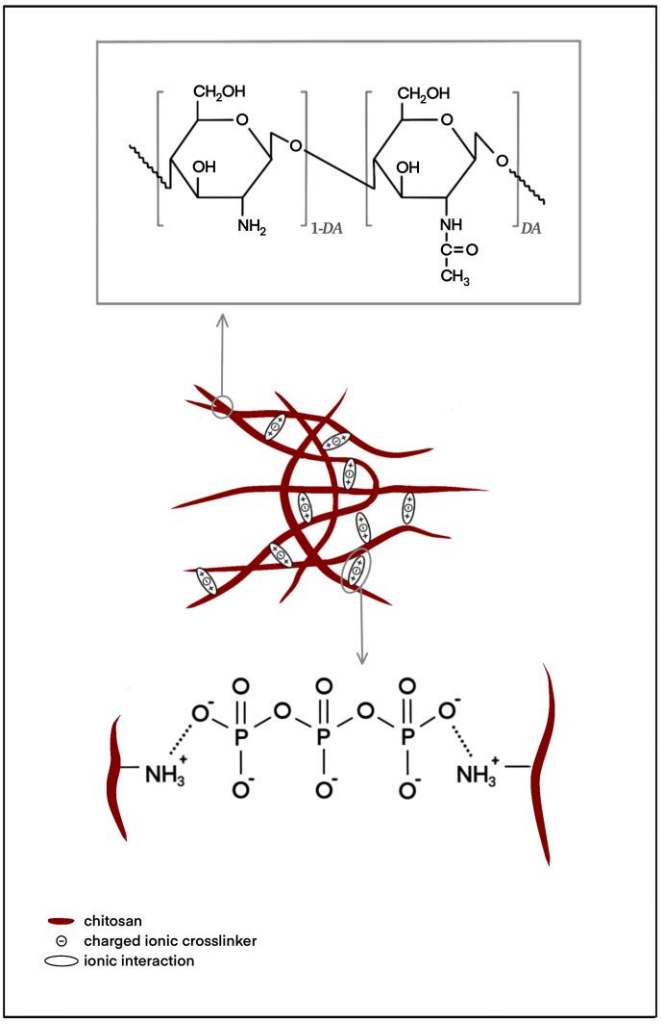

Figure 4. Chitosan's and tripolyphosphate's cross-link reaction

2.5 Wet Spinning

Wet spinning is a fiber spinning technique that involves dissolving a polymer in a suitable solvent, extruding it through the fine orifices of a jet or spinneret immersed into a non-solvent solution termed as coagulation bath, and then stretching the resulting continuous filaments to orient the polymer chains and enhance its mechanical properties by means of take-up rollers. Interest in the use of the wet spinning technique for biomedical and pharmaceutical purposes has increased due to its reproducibility, scalability, and low cost, as well as the fact that it is a "clean technology" [32].

Since it is a gentle process, applying lower temperatures, it has been the preferred method for production of fibers that cannot be melt spun [33]. The process is called wet spinning because the coagulation bath contains a liquid that is miscible with the solvent but not with the polymer, causing the polymer to precipitate and solidify into a fiber.

Wet spinning is a versatile process that can be used to produce a wide range of fibers with different properties and applications. It is widely used in the textile, chemical, and biomedical industries, among others. However, the process requires careful control of the spinning conditions, such as the solvent, coagulation bath, and stretching parameters, to ensure consistent fiber quality and performance.

The diameter of the fibers can be modified by varying the solution flow rate or spinneret whole number. Wet spinning is used to prepare fibers from a variety of polymers, including natural polymers such as alginate, collagen, chitosan, silk, and gelatin and also synthetic polymers such as poly-caprolactone (PCL) and polyethylene glycol (PEG).

2.5.1 Features affecting the wet spinning process

The quality and properties of the resulting fibers are dependent on several variables which impact the wet spinning process. A few of the factors that can significantly affect the wet spinning process are:

- Polymer type
- Polymer Concentration
- Solvent election
- Process Conditions
- Coagulation bath composition
- Fiber Stretching
- Post-treatment

The spinning speeds in wet spinning are lower compared to dry spinning, which has even lower speeds than melt spinning as the process involves filament formation by coagulating in a spin bath. Application of low temperatures and the flexibility of having all spinning and post-spinning operations in a continuous process compensate for the low spinning speeds, and make this method favorable [10].

The main features that have a big influence on the fiber properties during this process are the following:

- Polymer dope solution's viscosity
- Baths' temperature
- Baths' concentration
- Driven rollers' velocity ratio

2.5.2 Polymer type

The type of polymer used in wet spinning is an essential variable. The unique properties of various polymers, such as solubility, viscosity, and coagulation behavior, can influence the spinning process. The choice of polymer depends on the final fiber's desired properties, such as strength, flexibility, and thermal stability.

2.5.3 Polymer Concentration

The polymer concentration in the spinning dope solution can have a significant effect on the wet spinning process. Higher polymer concentrations result in viscosity-increasing spinning dopes, which can impact the performance of the spun fibers, including diameter, uniformity, and coagulation rate. Lessened concentrations may produce fibers with diminished mechanical properties.

2.5.4 Solvent election

The choice of solvent in the wet spinning process is crucial, as it determines the solubility of the polymer and influences the viscosity and coagulation behavior of the spinning dope solution. The solvent must be compatible with the polymer, allowing correct dissolution and extrusion, and helping in precipitation in the coagulation bath. Solvent properties such as polarity, viscosity, and volatility can have an impact on the morphology, strength, and other properties of the fibers.

2.5.5 Process Conditions

Spinning parameters, such as temperature, spinning velocity, and coagulating bath composition, can considerably impact the wet spinning process. In general, higher

spinning temperatures produce quicker coagulation rates and shorter fiber lengths, whereas lower temperatures may result in slower coagulation rates and longer fibers. The spinning rate, which refers to the rate at which the spinning dope solution is extruded, can also influence the diameter and morphology of the fibers.

2.5.6 Coagulation bath composition

The composition of the coagulating bath, where the spun fibers are solidified, is an essential aspect of the wet spinning procedure. The additives in the coagulating solution, such as salts, acids, or other compounds, can influence the rate, morphology, and properties of fibers. The fiber properties can also be affected by the pH, temperature, and concentration of the coagulating bath.

2.5.7 Fiber Stretching

During the spinning process, the stretching or drawing of the wet-spun fibers alters their mechanical properties. Stretching can realign the polymer chains, increase the fiber's tensile strength, and decrease its diameter. To accomplish the desired fiber properties, the stretching conditions, such as the stretching ratio and temperature, can be controlled.

2.5.8 Post-treatment

Post treatment processes such as washing, neutralization, and drying, can also influence the wet spinning procedure. Post-treatment can assist in the elimination of residual solvent or additives, the modification of fiber properties, and the enhancement of the fiber's overall properties.

2.6 Carboxylic acids

Carboxylic acids are a class of organic compounds in which a carbon (C) atom is bonded to an oxygen (O) atom by a double bond and to a hydroxyl group (—OH) by a single bond. A fourth bond links the carbon atom to a hydrogen (H) atom or to some other univalent combining group. The carboxyl (COOH) group is so-named because of the carbonyl group (C=O) and hydroxyl group [34].

Carboxylic acids can be classified into two broad categories based on the presence of an alkyl group: aliphatic and aromatic carboxylic acids. Aliphatic carboxylic acids have an alkyl group attached to the carboxyl group, whereas aromatic carboxylic acids have an aromatic ring attached to the carboxyl group [35].

Some important examples of carboxylic acids include:

- Acetic acid (CH_3COOH)
- Citric acid ($C_3H_6O_3$)
- Lactic acid ($C_6H_8O_7$)

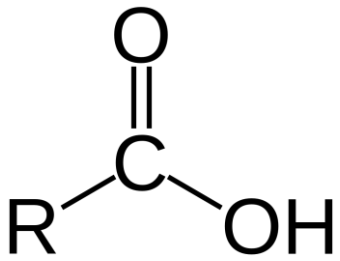

Figure 5. Carboxylic acid structure

2.6.1 Acetic acid

Liquid acetic acid is a hydrophilic (polar) protic solvent, similar to ethanol and water. With a moderate dielectric constant of 6.2, it can dissolve not only polar compounds such as inorganic salts and sugars, but also non-polar compounds such as oils and elements such as sulfur and iodine. Acetic acid is a weak acid that can function as a solvent for certain organic compounds, especially polar or low-molecular-weight compounds. It is utilized as a solvent in the production of cellulose acetate and other plastics, as well as pigments, pharmaceuticals, and photographic chemicals [36]. It has been also commonly used as solvent in chitosan dissolutions [37, 38].

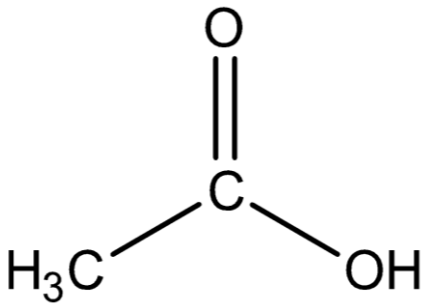

Figure 6. Acetic acid chemical structure

2.6.2 Citric acid

Citric acid, in its anhydrous form, is a tricarboxylic acid that may be found in citrus fruits. Because of the antioxidant characteristics it possesses, citric acid is frequently utilized in medicinal compositions as an excipient. In addition to its role as a preservative, it ensures the consistency of the active components. Additionally, it may be used as an acidulant to adjust the pH of a solution, and it can also work as an anticoagulant by chelating calcium in the blood [39].

Citric acid has been previously studied as chitosan solvent for preparation of fibers by wet spinning [40].

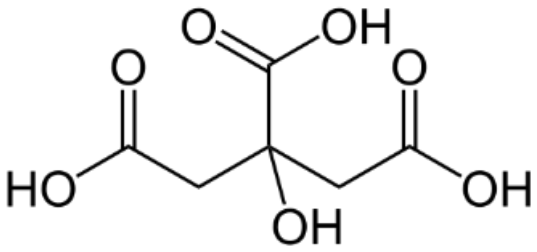

Figure 7. Citric acid chemical structure

2.6.3 Lactic acid

Lactic acid, also known as α-hydroxypropionic acid or 2-hydroxypropanoic acid, is an organic compound belonging to the carboxylic acid family that is present in plant fluids, animal blood and muscle, and soil. It is the most prevalent acidic component in fermented milk products like sour milk, curd, and buttermilk [41].

Lactic acid is an organic compound produced by microorganisms capable of utilizing various carbohydrate sources during fermentation [42].

Due to its prospective applications in biotechnology and medicine, the study of lactic acid has received increased attention in recent years. Probiotics derived from lactic acid bacteria have been shown to have numerous health benefits, including enhanced digestion and immunity. Additionally, lactic acid has been used as a starting material for the production of biodegradable plastics, which have potential packaging applications.

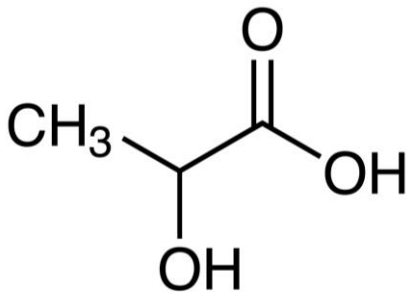

Figure 8. Lactic acid chemical structure

2.7 Oleanolic and Ursolic acid

Oleanolic acid (OA) and its isomer, ursolic acid (UA), are triterpenoid compounds that widely occur in nature in free acid form or as an aglycone precursor for triterpenoid saponins. These acids are based on the structure of isoprene and contain 30 carbon atoms and oxygen. Both compounds are constituents of medicinal herbs and also form an integral part of the human diet.

Ursolic acid is a component of certain traditional medicine herbs and ornamental species and is also found in fruits, such as apples, prunes, cranberries, and blueberries [43]. It is a pentacyclic triterpene acid, is one of the major components of certain traditional medicinal plants and possesses a wide range of biological effects, such as cytotoxic activities, anti-inflammatory, hepatoprotective, anticancer, hypoglycemic, antiprotozoal against Plasmodium falciparum, antioxidant, antibacterial, and prevents abdominal adiposity. The antibacterial properties of pentacyclic triterpenes and their derivatives have been extensively studied [44-46], and the activity of these compounds also resides in their potential to enhance bacterial susceptibility to other compounds, including antibiotics [47]. Bamuamba et al. (2008) demonstrated the potent antimycobacterial activity of ursolic acid present in the extract of leysera gnaphaloides which was comparable with the activity of Oleanolic Acid and fluctuated between 1.25 mg mL^{-1} and 2.5 mg mL^{-1} [48]. Their bacterial growth inhibition and survival, and the spectrum of minimal inhibitory concentration (MIC) values are very broad.

In addition, the antiviral potential of these compounds is of particular interest. OA, UA, and their derivatives inhibit the development of multiple viruses, including HIV [49–52]. The mechanism of their antiviral activity has been partially elucidated, which should permit their therapeutic use in the near future.

The bioactive properties of both compounds may be attributable to the various substructures in A, C, and E rings or other positions.

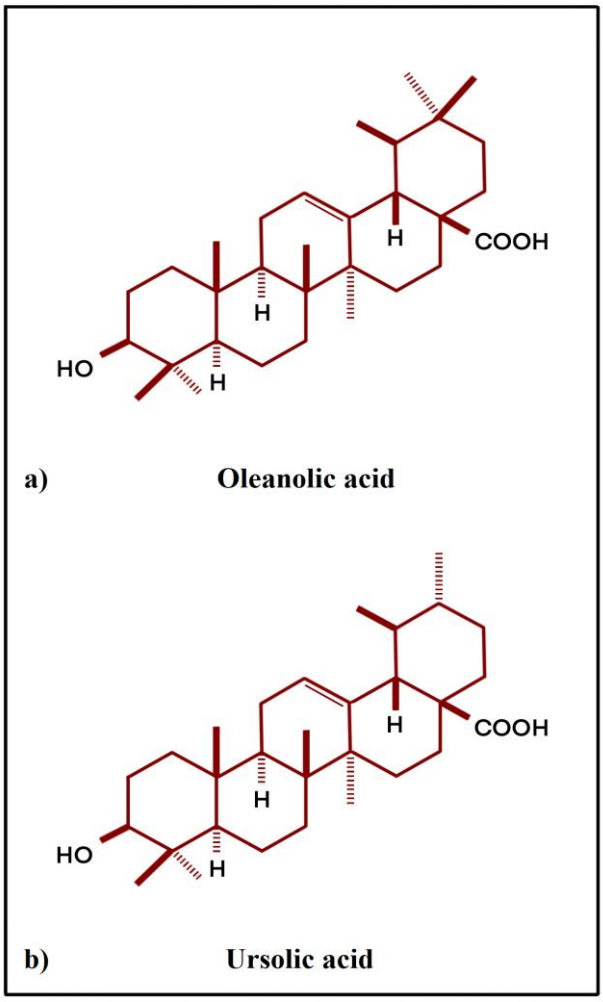

Figure 9. Oleanolic acid and ursolic acid chemical structure

Chapter 3

Experimental Research

3.1 Experimental methods

The experimental and characterization procedures of this doctoral dissertation have been performed in the laboratories of the Institute of Material Science of Textiles and Polymer Composites at the Lodz University of Technology in Łódź, except for the antibacterial activity test which was performed at Łukasiewicz – Łódzki Instytut Technologiczny.

3.1.1 Apparatus, materials and reagents

3.1.1.1 Apparatus

- Analytical balance
- Custom-made wet spinning machine
- FEI Nova NanoSEM 230, field-emission scanning electron microscope (FE-SEM)
- Thermo Scientific 6700 Nicolett FTIR spectrophotometer with ATR head
- UV-Vis from Jasco company, model V-670
- Lyophylizer Christ Alpha 2-4 LSC

- Mechanical stirrer Heidolph RZR 2102 control
- Multifunction meter CX 701
- Rotational rheometer Anton Paar RheolabQC
- Tensile testing machine Instron 5944

3.1.1.2 Materials

- Beakers, capacity 50 ml
- Beakers, capacity 500 ml
- Petri dishes
- Round bottom flasks, capacity 1000 ml
- Watch glasses

3.1.1.3 Reagent

- Chitosan powder commercial product of Sigma-Aldrich; molecular weight 60 kDa; degree of deacetylation (DDA) 96%
- Deionized water (produced at Institute of Material Science of Textiles and Polymer Composites)
- Acetic Acid (CH_3COOH) 99% Pure P.A. commercial product of Poch,
- Lactic acid ($C_6H_8O_7$) 80% Pure P.A. commercial product of Chempur
- Citric acid ($C_3H_6O_2$) 99 % Pure P.A. commercial product of Chempur
- Sodium Hydroxide (NaOH) 99% Pure P.A. commercial product of Poch
- Tripolyphosphate (TPP) ($Na_5P_3O_{10}$) 98% Pure P.A commercial product of Thermo chemicals.
- 2-propanol (C_3H_8O) 99% Pure P.A. commercial product of Eurochem BGD
- Ursolic acid ($C_{30}H_{48}O_3$) ≥ 98% Pure P.A. commercial product of Pol-Aura

3.2 Chitosan dope solution, preliminary trials

This part of this doctoral dissertation, builds upon the findings and analysis presented in my master's degree dissertation [53], on which the objective was to investigate the effect of various parameters on the physico-chemical properties of chitosan fibers, such as polymer concentration, sodium hydroxide concentration in the coagulation bath, stretching and rinse bath concentration. The results obtained from this study provide valuable insights into the wet spinning process and its potential for producing good quality chitosan fibers.

The study revealed that chitosan fibers with favorable properties can be produced by using a polymeric dope solution of 6% concentration of chitosan, a coagulation bath containing sodium hydroxide, a stretching bath with a lower concentration of sodium hydroxide, and a rinse bath containing ethanol. The concentration of sodium hydroxide in the coagulation bath was found to have a significant influence on the physico-chemical properties of chitosan fibers.

The batches prepared with the highest concentration of sodium hydroxide in the coagulation bath showed the best properties. In addition, the study showed that the velocity ratio could influence the morphology of the fibers, smoothness, and adsorption properties, tenacity, and degree of crystallinity. Considering the previous findings, further investigation was conducted for this work and the process is described as follows.

To additionally explore the potential of this method, the next investigation was conducted by testing two different dope solutions; these solutions were prepared with a chitosan concentration of 7 wt% and 8 wt%, and the solvent used was 4% v/v aqueous acetic acid solution. The homogenization process involved mechanical stirring for duration of 6 hours at 225 rpm, at room temperature.

The motivation behind selecting these particular concentration values was to investigate the feasibility of producing chitosan fibers with desirable physicochemical properties at a higher polymer concentration than in my previous research. This study aims to expand upon prior work by exploring the potential benefits of utilizing increased concentrations of chitosan in fiber development.

Table 2. Chitosan 7% and 8% sample nomenclature

Sample ID	Chitosan concentration (wt %)	Solvent
C7AA	7	Acetic Acid
C8AA	8	Acetic Acid

The rheological parameters of each dope solution were measured using the rotational rheometer Anton Paar RheolabQC. This phase was essential for determining if the polymer concentration in the dopes was suitable for the wet spinning process. The rheometer assisted in determining the flow characteristics, viscosity, and elasticity of the dope solutions, which are all crucial factors in wet spinning.

3.3 Rheological measurement of chitosan dope solutions

3.3.1 Rheology

Rheology is the science concerning the deformation and flow of materials under stress and includes elasticity, plasticity, and viscosity.

Rheology in terms of mechanics is the relation between force and deformation in material bodies. The nature of this relation depends on the material of which the body is composed of. The deformation behavior of metals and other solids is represented by a model called the linear or Hookean elastic solid (displaying the property known as elasticity). In fluids, the deformation behavior is represented by a model called the linear or Newton’s viscous fluid (displaying the property known as viscosity) [54].

3.3.2 Viscosity

Viscosity is a measure of the resistance to gradual deformation of a medium and relates to the difference in shear strain-rate (also 'shear velocity' or rate of shear stress") in a flowing medium. Viscosity is due to the friction between neighboring particles that move with different velocities and is dependent on temperature and confining pressure for most media.

Viscosity describes a fluid's internal resistance to flow and can be thought of as the frictional forces acting within a fluid in motion [55].

The terms "dynamic" or "absolute" viscosity are typically used to describe viscosity, which imply that the fluid is in motion. These concepts differ from "kinematic viscosity," which is defined as the dynamic viscosity divided by the fluid's density.

3.3.2.1 Viscosity of Newtonian and non-Newtonian fluids

Fluids are classified as Newtonian or non-Newtonian based on the behavior of their viscosity as a function of shear rate, stress, and deformation history.

3.3.2.2 Newtonian fluids

Sir Isaac Newton (1642 - 1726) defined the flow behavior of fluids with a simple linear relationship between shear stress [mPa] and shear rate [1/s]. Newtonian fluids are named after Sir Isaac Newton. This relationship is now known as Newton's Law of Viscosity, where the proportionality constant η is the viscosity [mPa-s] of the fluid:

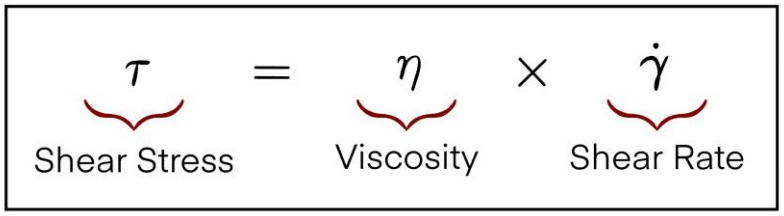

Figure 10. Symbology commonly used in rheology

Among the examples of Newtonian fluids are water, organic solvents, and honey. The only variable affecting the viscosity of these fluids is temperature. Therefore, if we examine a diagram of shear stress versus shear rate (see Figure 11), we observe a linear increase in stress with increasing shear rates, where the slope is determined by the fluid's viscosity. This implies that the viscosity of Newtonian fluids will remain constant (see Figure 12) regardless of the rate at which they are forced to travel through a pipe or channel (for instance, viscosity is independent of shear rate) [56].

Bingham plastics, which are substances that require a minimum amount of stress to flow, are an exception to the rule. These fluids are strictly non-Newtonian, but once the flow begins, they function essentially as Newtonian fluids (such as, shear stress is proportional to shear rate) [57].

Typically, Newtonian fluids consist of small isotropic (symmetric in shape and properties) molecules that are not flow-oriented. However, it is also conceivable for large anisotropic molecules to exhibit Newtonian behavior. Low-concentration protein or polymer solutions, for instance, may exhibit a constant viscosity regardless of shear rate. Some samples may also exhibit Newtonian behavior at low shear rates with a plateau referred to as the zero shear viscosity region [58].

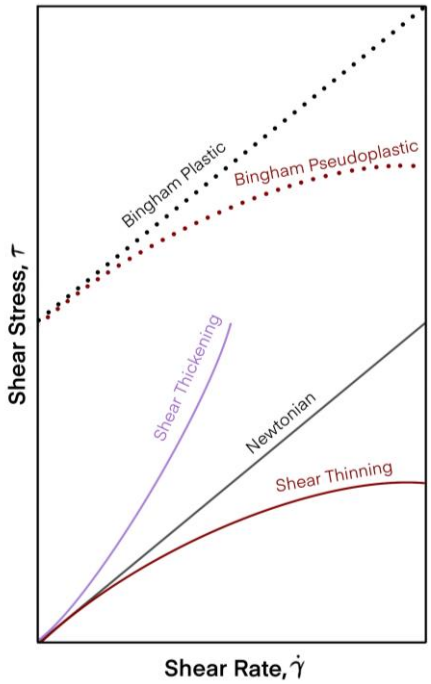

Figure 11. Shear stress as a function of shear rate for several kinds of fluids

3.3.2.3 Non-Newtonian fluids

Non-Newtonian fluids are not bound to Newton's law of viscosity, meaning their viscosity is not constant and can alter under stress. When subjected to force, the viscosity of non-Newtonian fluids can vary from a more liquid state to a more solid one, and this change can be depending on the shear rate or the history of the shear rate. Some non-Newtonian fluids exhibit normal stress-differences or other non-Newtonian behavior, even though their viscosity is shear-independent [56].

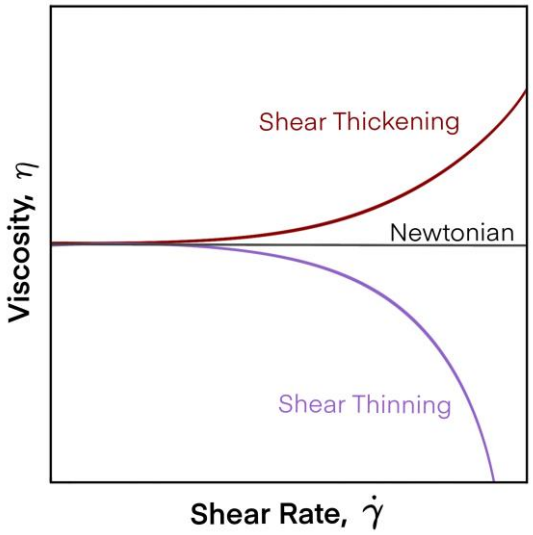

Figure 12. Viscosity of Newtonian, Shear Thinning and Shear Thickening fluids as function of shear rate

Non-Newtonian behavior of fluids can be caused by several factors, all of them related to structural reorganization of the fluid molecules due to flow. In polymer melts and solutions, it is the alignment of the highly anisotropic chains what results in a decreased viscosity. In colloids, it is the segregation of the different phases in the flow that causes a shear thinning behavior.

3.4 Rheological results of samples C7AA and C8AA

The rheological properties of every sample (C7AA and C8AA) were evaluated at five different temperature set points (20ºC, 30ºC, 40ºC, 50ºC, 60ºC), with each sample tested in a clean container separately, the tests were carried out by means of the rotational rheometer Anton Paar RheolabQC. This phase was essential for determining if the polymer concentration in the dope solutions was suitable for the wet spinning process.

The graphs and results are displayed below:

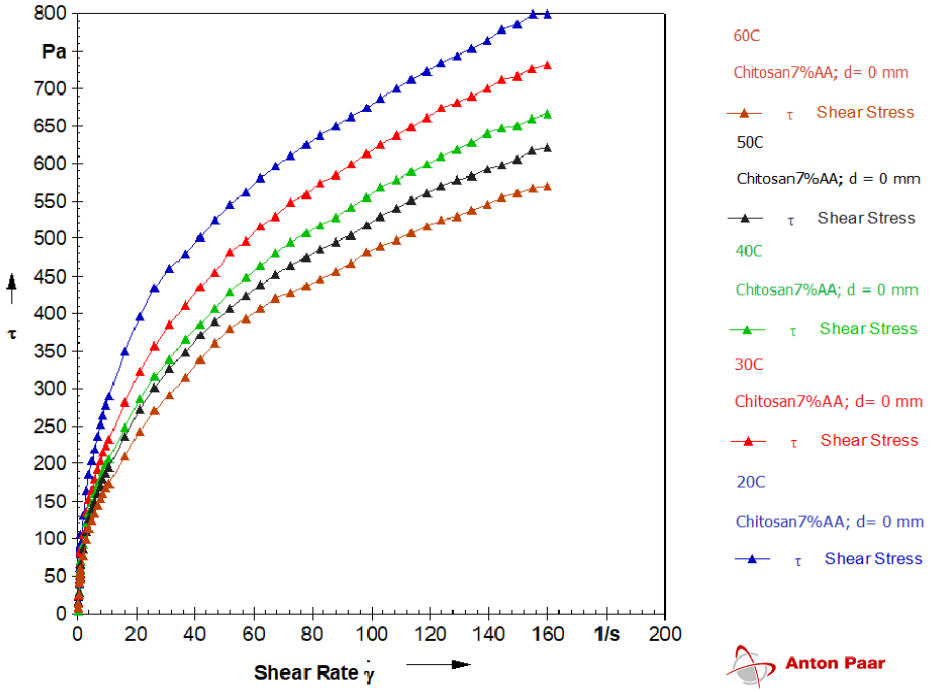

Figure 13.Shear stress graph, C7AA

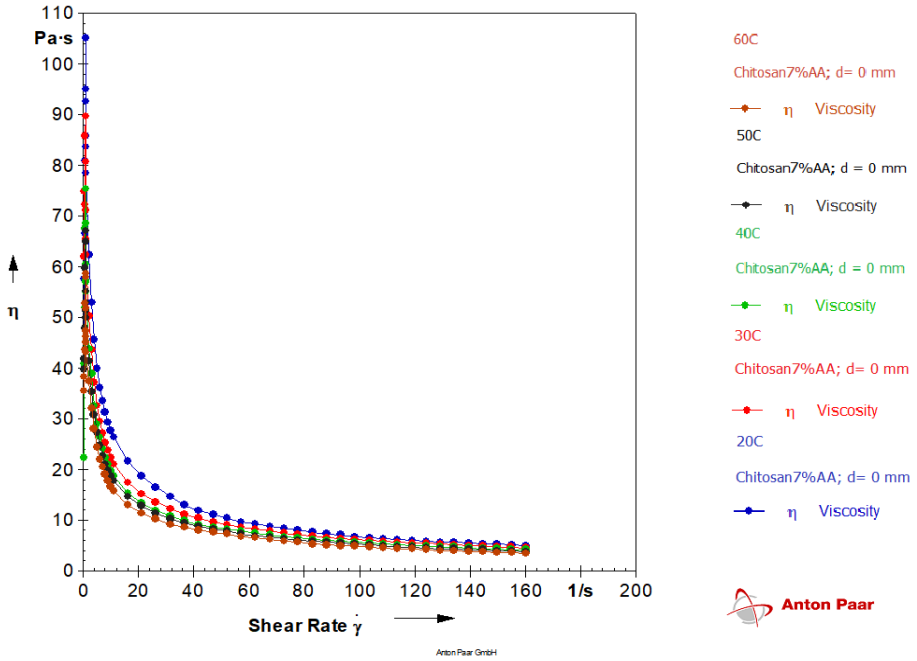

Figure 14. Viscosity graph, C7AA

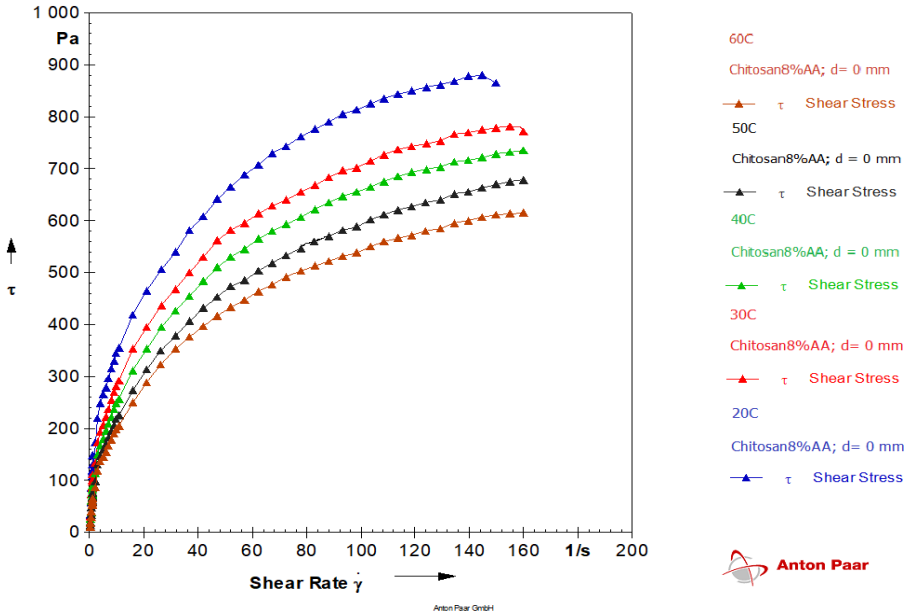

Figure 15. Shear stress graph, C8AA

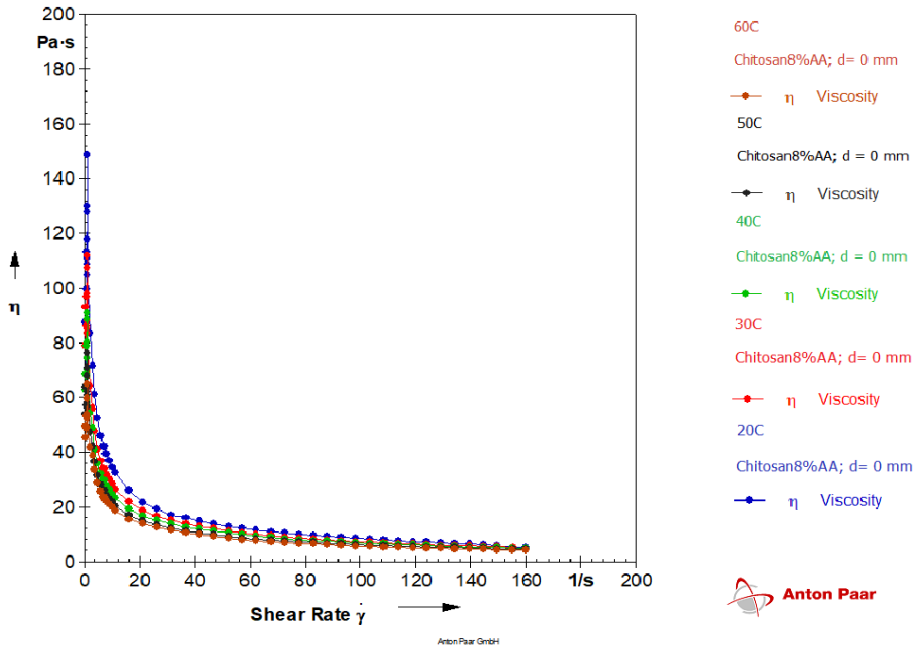

Figure 16. Viscosity graph, C8AA

After the rheological measurements at multiple temperatures were performed, it was observed that sample C7AA exhibited greater stability and uniformity and a typical shear-thinning behavior as the temperature increased, which is advantageous and indicates a positive disposition for spinning process. Conversely, sample C8AA demonstrated a greater level of shear stress and viscosity across various temperature set points, indicating unfavorable suitability for spinning process.

For both samples, as the temperature and shear rate increase, shear stress and viscosity decrease, this behavior is typical of non-Newtonian fluids. Consequently, the sample C7AA was selected for production of chitosan fibers, given its greater stability at different temperatures and favorable shear strength. These observations are significant in the context of fluid mechanics, where viscosity and shear stress are crucial

properties that determine the spinnablility of polymers as well as the final fiber properties.

3.5 Assessment of the effect of different carboxylic acids on chitosan fibers production

Cruz et al., (2016) [40] studied the effect of carboxylic acids, such as, acetic acid , malic acid and lactic acid as solvents in the production of chitosan fibers by wet spinning with polymeric dope solutions at 2.5 wt%. The fibers were characterized using FTIR, swelling degree measurements, SEM, and mechanical tests in terms of tensile strength. The results showed that denser fibers tend to have higher mechanical properties and lesser tendency to swell. The fibers showed good potential for use as sutures.

To broaden Cruz's research, restate some of the findings and implications in a different manner, and explore additional avenues of inquiry that can deepen our understanding of the topic; after determining that sample C7AA showed suitability for the wet spinning process, additional experimentation was deemed necessary to further refine the process. As a result, two more carboxylic acids were identified and picked for individual testing of their rheological behavior in the polymeric dope as solvents.

For this work it was selected to study additionally to acetic acid, the effect of lactic acid and citric acid as solvents in chitosan polymeric dope solutions to produce wet-spun fibers.

The process is described below.

Table 3. Chitosan 7% sample nomenclature

Sample ID	Chitosan concentration (wt %)	Solvent
C7CA	7	Citric Acid
C7LA	7	Lactic Acid

The C7CA and C7LA dope solutions were prepared of chitosan at a concentration of 7 wt%. In order to disperse the solute and create a homogenous solution, two solvents were used: 4% v/v lactic acid aqueous solution and 4% v/v citric acid aqueous solution respectively. The homogenization process involved mechanical stirring for duration of 6 hours at 225 rpm, at room temperature.

3.6 Rheological results of samples C7CA and C7LA

In order to evaluate the rheological properties of samples C7CA and C7LA, every sample was tested at four distinct temperature set points (20ºC, 30ºC, 40ºC, 50ºC). To ensure accurate results, each sample was analyzed separately in a clean container. With the aim of determine the flow characteristics, viscosity, and elasticity of the dope solutions, the Anton Paar RheolabQC rotational rheometer was utilized for these measurements. These rheological parameters were crucial for evaluating the suitability of the polymer solution for the wet spinning process.

The graphs and results are displayed as follows:

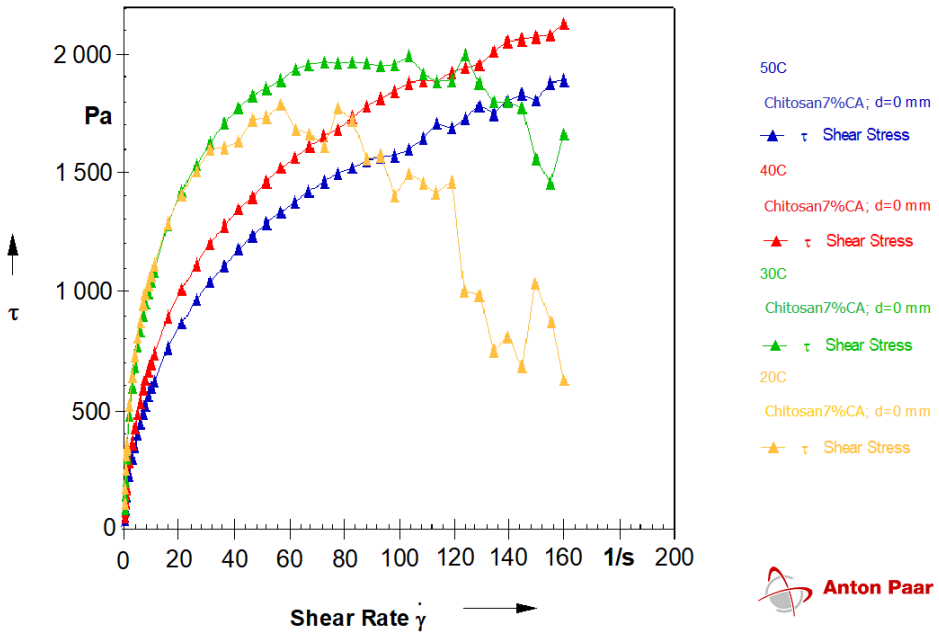

Figure 17. Shear stress graph, C7CA

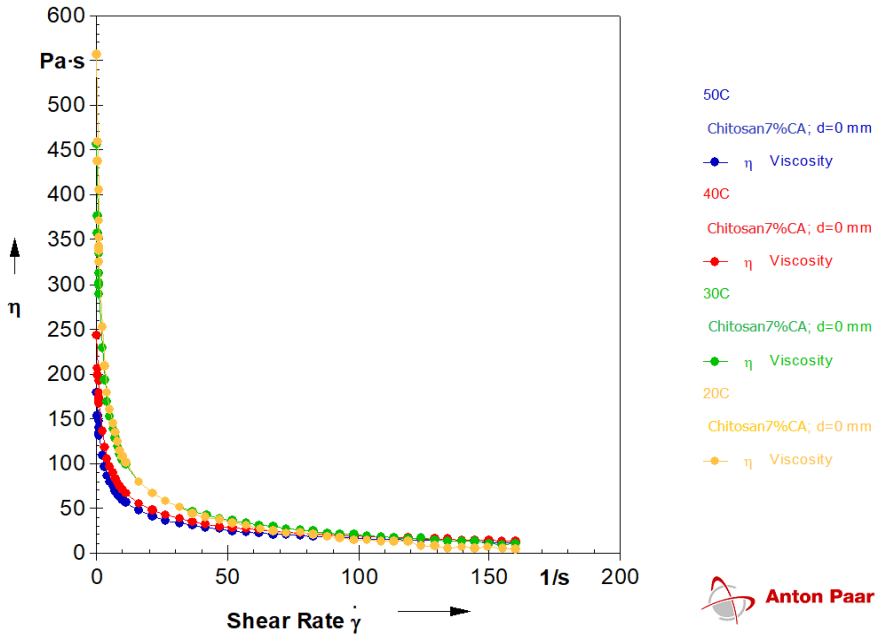

Figure 18. Viscosity graph, C7CA

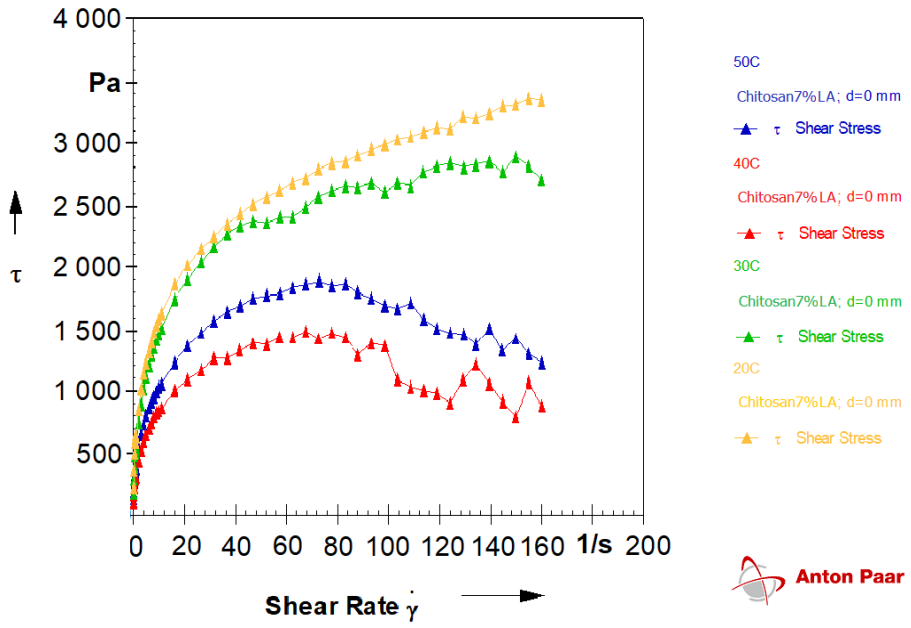

Figure 19. Viscosity graph, C7LA

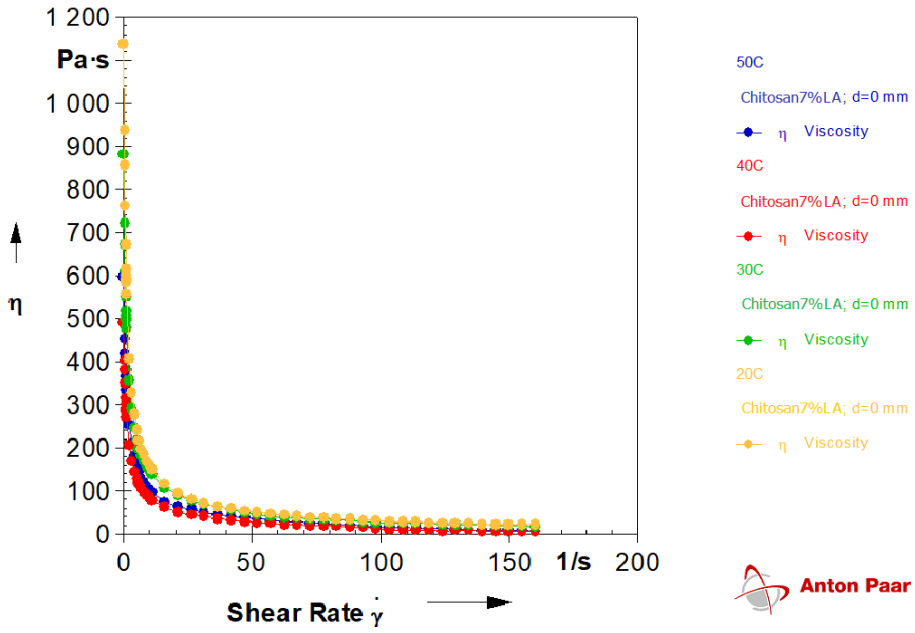

Figure 20. Shear stress graph, C7LA

Regarding the rheological properties of sample C7CA, it was observed that at higher temperatures (specifically, 50°C and 40°C), the dope solution displayed greater stability and exhibited a shear-thinning behavior as the shear rate increased. This behavior is common in many types of polymeric solutions and refers to the fact that as the shear rate or shear stress applied to the solution increases, its viscosity decreases.

The way that the dope behaves can be attributed to the fact that an increase in temperature causes the polymer chains to become more flexible, allowing them to deform more easily under the applied shear stress. This increased flexibility reduces the resistance to flow when the shear stress is applied since the polymer chains tend to align in the direction of the flow; this alignment disrupts the entanglements between the chains, leading to a decrease in viscosity. Additionally, as the temperature increases, the solvent molecules may become more mobile, which can also contribute to a decrease in viscosity. It is also important to note that there is an upper temperature limit beyond which the polymer chains may start to degrade, leading to a decrease in viscosity that is not related to shear-thinning behavior. Therefore, it is essential to carefully control the temperature when studying the rheological behavior of a polymer solution.

The behavior of sample C7LA was found to be quite similar to that of sample C7CA. However, a difference was observed when the stability of both samples was compared at lower temperature set points, specifically at 20°C and 30°C. Sample C7LA exhibited higher stability than sample C7CA, which could be attributed to the difference in acidity between the two acids used in the samples, citric acid in C7CA and lactic acid in C7LA. Solubility is one of the properties affected by the difference in acidity between citric acid and lactic acid. Citric acid has a higher solubility in water compared to lactic acid due to its three carboxyl functional groups, which increase its polarity and enable it to form hydrogen bonds with water molecules more effectively. In contrast, lactic acid only has one carboxyl group, making it less polar and less soluble in water.

3.7 Developing of wet-spun fibers

To develop chitosan fibers using the wet spinning method, a custom-made spinning apparatus with three wet-medium phases and an automated dosing piston was used. The chitosan dope was poured into the dosing piston, which was programmed to inject a consistent volume of dope per minute. The injected dope was passed through a spinneret with 500 orifices, each orifice with a diameter of Φ 0.08 mm, resulting in a final "*yarn*" or "*string"* with 500 continuous filaments.

As the dope solution was injected through the spinneret, it was simultaneously released into the coagulation bath and manually carried to the first set of driven rollers, which helped to initially stretch the fibers. Then the fibers were guided into the stretching bath and subsequently to the second set of driven rollers, which carried them to the rinse bath. Finally, the fibers were collected by the last set of driven rollers and rolled onto bobbins.

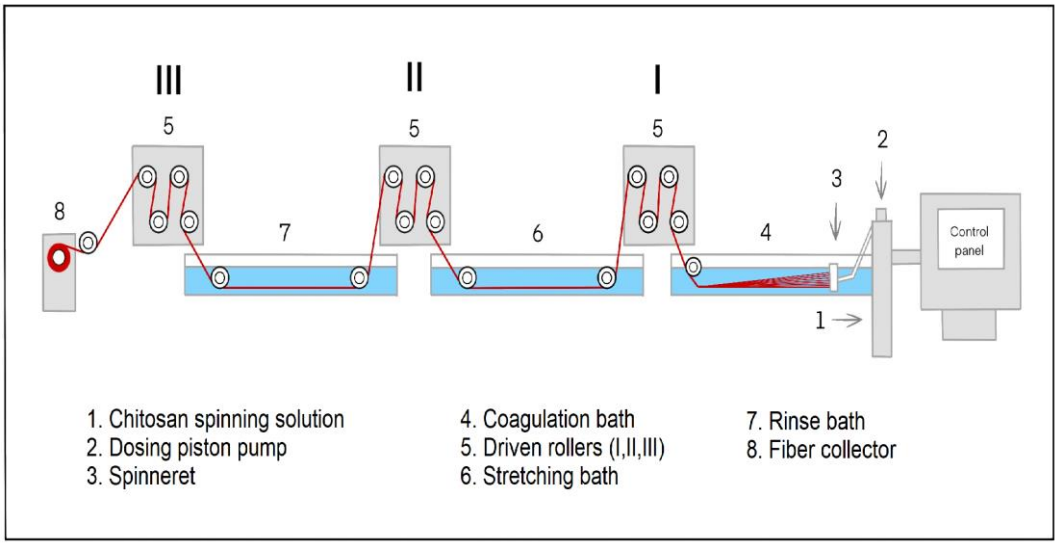

Figure 21. Custom made wet spinning apparatus

3.8 Chitosan fibers batches

Upon analyzing the results of the rheological tests, it was concluded to proceed forward with the wet spinning process to assess the impact of each solvent on the physico-chemical properties of the resulting fibers.

To achieve this, each of the dopes was subjected to a wet spinning process that involved controlling various factors, such as pH, temperature, coagulation bath content, dope feed rate, and draw ratio, to ensure optimal fiber formation.

Based on the findings and analysis presented in my master's degree dissertation, where it was concluded that with a chitosan dope solution of 6wt%, chitosan fibers can be prepared with an average tensile strength of 12.93 cN/tex, and the use of 4%(w/v) solution of sodium hydroxide in the coagulation bath results in greater absorption properties, greater tensile strength and smoother surface of the fibers, compared with lower sodium hydroxide concentrations [53]. It was determined to replicate the wet spinning conditions expecting to obtain similar results.

Table 4. Chitosan fibers batch nomenclature

Sample ID	Chitosan concentration (wt %)	Solvent
C7AA	7	Acetic Acid
C7CA	7	Citric Acid
C7LA	7	Lactic Acid

3.8.1 Chitosan fibers batch C7AA

A dope solution was prepared with a chitosan concentration of 7 wt% and the solvent used was 4% v/v aqueous acetic acid solution. The homogenization process involved mechanical stirring for duration of 6 hours at 225 rpm, at room temperature.

For the coagulation bath 10 000 cm^3 of solution of sodium hydroxide and distillated water were prepared at 4% (w/v). The second bath (stretching bath) was also prepared with 10 000 cm^3 of solution, in this case sodium hydroxide at 0.5% (w/v). And finally for the third bath (rinse bath) it was only used distilled water, also 10 000 cm^3.

The parameters utilized during the wet spinning procedure are detailed in the table below:

Table 5. Spinning process parameters, sample C7AA

Spinning process parameters									
Sample ID	Dope feed rate (cm^3/min)	Chitosan solution temp. (C°)	Coagulation bath		Driven rollers speed I (m/min)	Stretching bath temp. (C°)	Driven rollers speed II (m/min)	Rinse bath temp. (C°)	Driven rollers speed III (m/min)
			Temp (C°)	pH					
C7AA	5.0	21.0	39.8	13.1	2.0	40.0	2.5	20.0	2.6

3.8.2 Chitosan fibers batch C7CA

A dope solution was prepared with a chitosan concentration of 7 wt% and the solvent used was 4% v/v aqueous citric acid solution. The homogenization process involved mechanical stirring for duration of 6 hours at 225 rpm, at room temperature.

For the coagulation bath 10 000 cm^3 of solution of sodium hydroxide and distillated water were prepared at 4% (w/v). The second bath (stretching bath) was also prepared with 10 000 cm^3 of solution, in this case sodium hydroxide at 0.5% w/v). And finally for the third bath (rinse bath) it was only used distilled water, also 10 000 cm^3.

The parameters utilized during the wet spinning procedure are detailed in the table below:

Table 6. Spinning process parameters, sample C7CA

Spinning process parameters									
Sample ID	Dope feed rate (cm^3/min)	Chitosan solution temp. (C°)	Coagulation bath		Driven rollers speed I (m/min)	Stretching bath temp. (C°)	Driven rollers speed II (m/min)	Rinse bath temp. (C°)	Driven rollers speed III (m/min)
			Temp (C°)	pH					
C7CA	5.0	40.2	40.6	13.4	2.0	40.0	2.5	20.0	2.6

Based on the rheological test results, for this sample, it was determined that to achieve greater stability and lower viscosity of the dope, it was necessary to increase the temperature. As a result, the dope was heated to 45 °C. The lower viscosity facilitated the dope feed, making the spinning process more efficient.

3.8.3 Chitosan fibers batch C7LA

A dope solution was prepared with a chitosan concentration of 7 wt% and the solvent used was 4% v/v aqueous lactic acid solution. The homogenization process involved mechanical stirring for duration of 6 hours at 225 rpm, at room temperature.

For the coagulation bath 10 000 cm^3 of solution of sodium hydroxide and distillated water were prepared at 4% (w/v). The second bath (stretching bath) was also prepared with 10 000 cm^3 of solution, in this case sodium hydroxide at 0.5% (w/v). And finally for the third bath (rinse bath) it was only used distilled water, also 10 000 cm^3.

The parameters utilized during the wet spinning procedure are detailed in the table below:

Table 7. Spinning process parameters, sample C7LA

Spinning process parameters									
Sample ID	Dope feed rate (cm^3/min)	Chitosan solution temp. (C°)	Coagulation bath		Driven rollers speed I (m/min)	Stretching bath temp. (C°)	Driven rollers speed II (m/min)	Rinse bath temp. (C°)	Driven rollers speed III (m/min)
			Temp (C°)	pH					
C7LA	5.0	22.3	40.6	13.4	2.0	40.0	2.5	20.0	2.6

Following the spinning process of all samples, the fiber-loaded bobbins were placed inside a Christ Alpha 2-4 LSC freeze dryer and dried at a pressure of 0.50 mbar, a primary drying temperature of −83°C, and secondary drying temperature of 40°C for 24 h.

Chapter 4

Characterization of chitosan fibers

4.1 Scanning Electron Microscope (SEM)

SEM is a powerful instrument for investigating the surfaces of solid objects. As an electron instrument, SEM employs a beam of relatively low-energy electrons that are focused and scanned over the specimen at regular intervals. The electron source and detector are housed in a vacuum chamber, and images are generated by detecting secondary electrons emitted from the specimen surface. SEM is extensively used in many fields, including materials science, biology, and geology, to examine the morphology, composition, and structure of substances. The SEM can generate high-resolution images of surfaces, and it can also be used for elemental analysis, crystallography, and topography. The SEM can accommodate large and unwieldy specimens, and intricate specimen preparation techniques are unnecessary for examination [59]. SEM utilizes a beam of focused electrons of relatively low energy as an electron probe that is scanned in a regular manner over the specimen.

While scanning electron microscopy is indeed a powerful tool for investigating objects at length-scales that are prohibitive using standard optical microscopy techniques, SEMs are equally useful in characterizing the micro- and macro-scale

architectures of transparent, highly reflective, or morphologically complex materials [60].

4.1.1 Sample C7AA

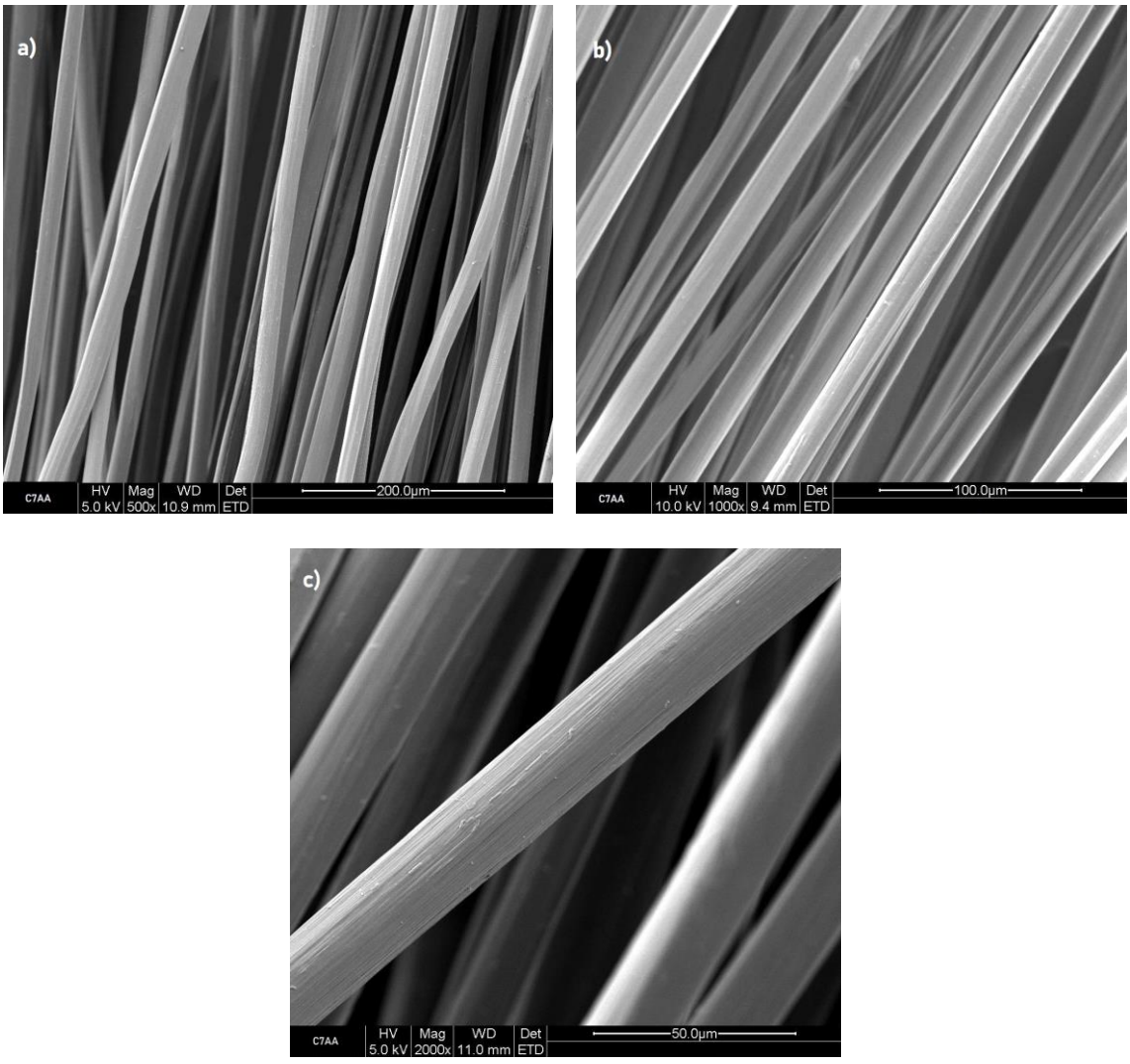

Figure 22. SEM images a) 500x, b) 1000x and c) 2000x

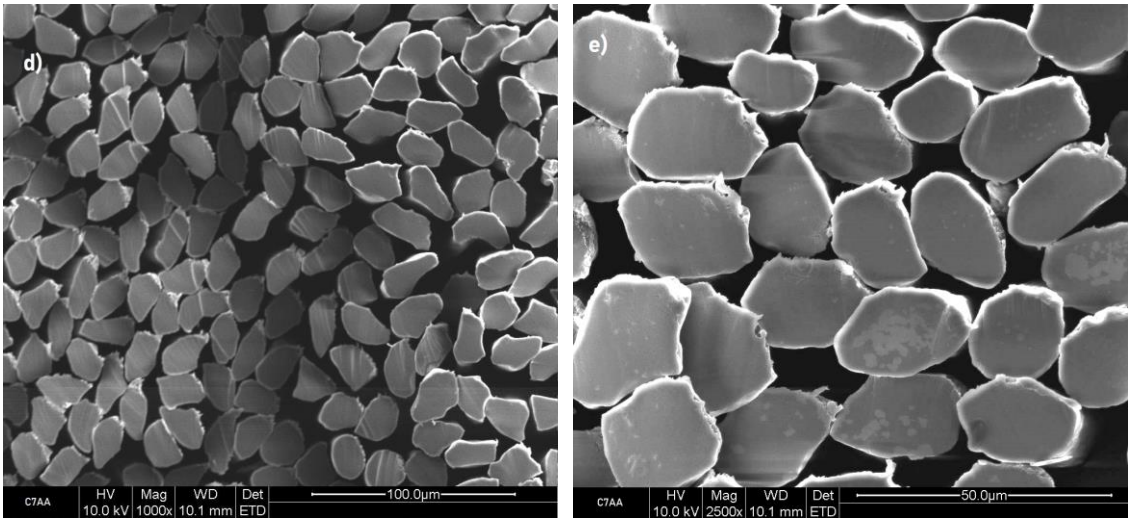

Figure 23. SEM cross-section images, d) 1000x and e) 2500x

Figures 22 and 23, show fibers with smooth surface and partially rounded cross-section morphology. The smooth surface indicates that the spinning process was well-controlled, which can result in fibers with good mechanical properties. The partially rounded cross-sections suggest that the fibers have maintained their shape and structure during the spinning process.

4.1.2 Sample C7CA

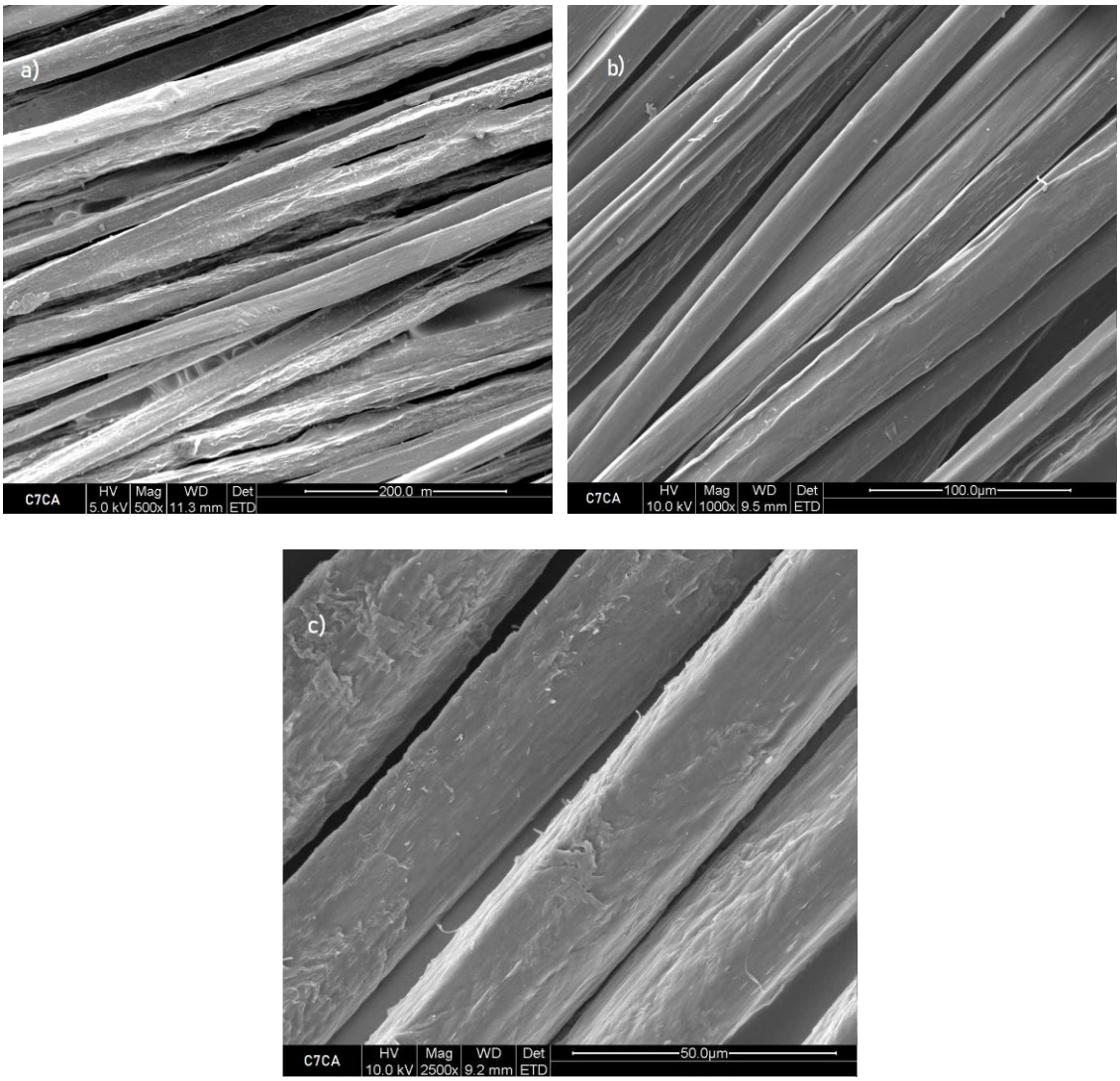

Figure 24. SEM images a) 500x, b) 1000x and c) 2500x

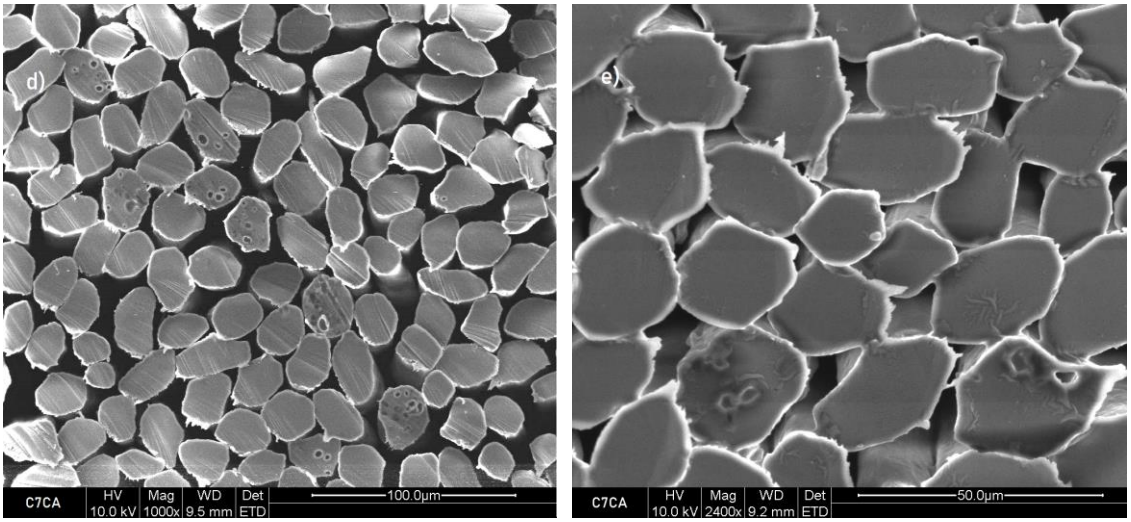

Figure 25. SEM cross-section images, d) 1000x and e) 2400x

In figures 24 and 25 from sample C7CA is shown that fibers with a highly rough surface texture were produced. The roughness may be attributed to incomplete coagulation, which could result from inadequate diffusion of the spinning dope into the coagulation bath. The incomplete coagulation could also have contributed to the irregular shape of the fibers´ cross-sections, which appear to be distorted, not fully rounded and resembles that of a porous sponge. These observations suggest that the coagulation process was not optimal for this sample, and further optimization may be necessary to improve the fiber morphology and properties.

4.1.3 Sample C7LA

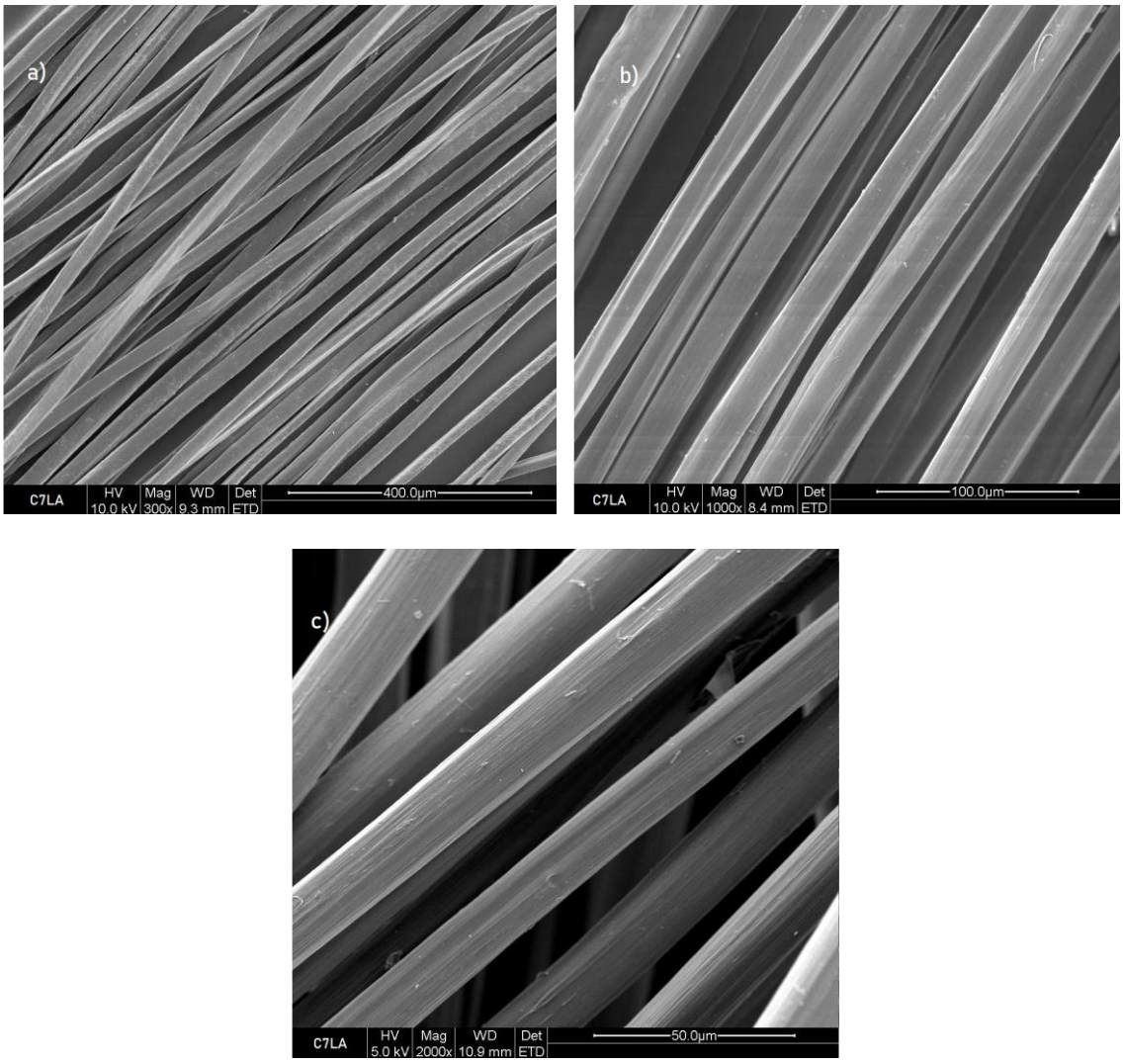

Figure 26. SEM images a) 300x, b) 1000x and c) 2000x

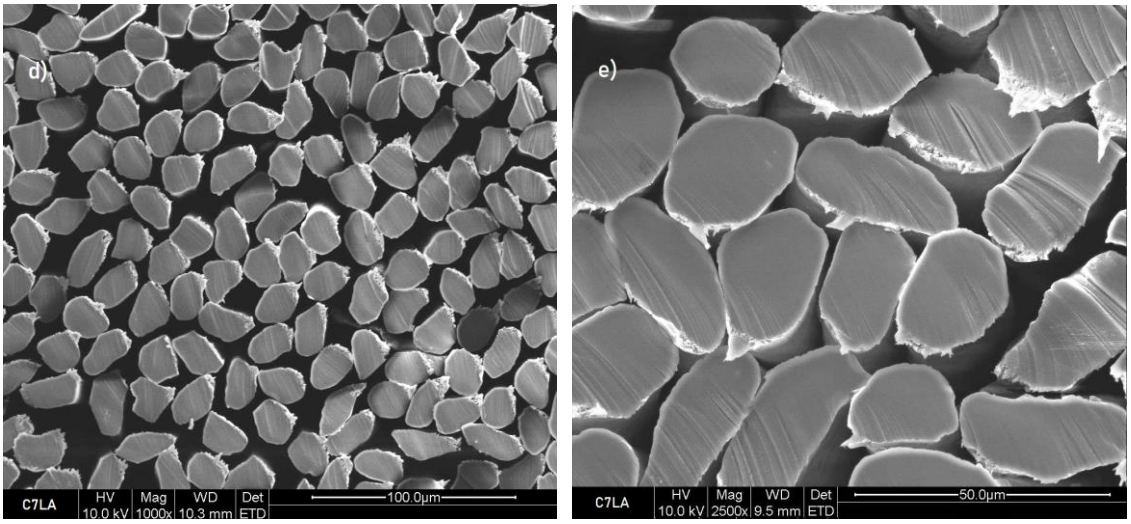

Figure 27. SEM cross-section images d) 1000x and d) 2500x

In figures 26 and 27 can be seen that the fibers in this batch have also smooth and partially rounded cross-section morphology. As mentioned with sample C7AA, the smooth surface indicates that the spinning process was well-controlled, this may lead fibers possess favorable mechanical properties. Furthermore, the partially rounded cross-sections imply that the fibers have preserved their shape and structure during the spinning process.

4.2 Linear density

Linear density in textiles refers to the mass per unit length of a yarn or fiber. It is an important property in determining the quality and performance of textile products. The linear density of a fiber is an expression of its weight per unit length [61]. The linear density of yarn is often given in tex count which is the unit of linear density, equal to the mass in grams of 1000 meters of fiber, or other textile strand that is used in a direct yarn numbering system. The thickness or diameter of a yarn is one of its most fundamental properties, but it is not possible to measure the diameter of a yarn in any direct way. Instead, the linear density of the fiber is used as a measure of its thickness or diameter [62]. The yarn count systems are classified into two main categories: direct count systems and indirect count systems.

4.2.1 Direct count systems

Direct yarn counting system: In this system the yarn number or count is measured as the weight of yarn per unit length. The constant feature of all direct count systems is the length of the yarn, and the higher the yarn count number, the heavier or thicker the yarn. It is a fixed length system and is generally used for jute or silk yarn. The direct system is a universally used system to measure the linear density of the yarns. The concept of yarn count has been introduced to specify a certain numerical value that expresses the coarseness or fineness (diameter) of the yarn and also indicates the relationship between length and weight (the mass per unit length or the length per unit mass) of that yarn [63].

The units of this system are the following:

- Tex - Weight in grams of 1000 meters.
- Denier - weight in grams of 9000 meters.
- Decitex - weight in grams of 10000 meters.

The following formula is used to calculate direct yarn count system:

$$N_d = \frac{W \times l}{L}$$

Where,

N_d = yarn number or count in direct system

W = the weight of the sample (yarn) in units of the system at the official regain

L = length of the sample

l = unit of length of the system

4.2.2 Indirect count system

Indirect yarn counting system: Is a method of determining the yarn number or count by measuring the number of "units of length" per "unit of weight" of yarn. This system is based on a fixed weight and is commonly used for cotton, woolen, worsted, and linen yarn. The higher the yarn count number, the finer or thinner the yarn. This

means that if two yarns have the same weight, the one with a higher yarn count will be longer. The indirect yarn counting system is used to determine the quality of yarn and is an important factor in the textile industry [64].

The units of this system are the following:

- Worsted count Ne w = number of hanks all of them 560 yards long in 1 pound.
- Metric count Nm = number of kilometer lengths per kilogram.

The following formula is used to calculate indirect yarn count system:

$$N_{id} = \frac{L \times w}{l \times W}$$

Where,

N_{id} = yarn number or count in indirect system

W = the weight of the sample (yarn) in units of the system at the official regain

w = unit of weight of the system

L = length of the sample

l = unit of length of the system

4.2.3 Determination of linear density of chitosan fibers

This procedure was performed taking as reference the *ASTMD 1059 – 01 Standard Test Method for Yarn Number Based on Short-Length Specimens* [65]. In order to obtain the linear density of the wet spun fibers, ten "*strings"* of fibers (specimens) were taken from each sample bobbin, measuring 1 meter in length respectively, subsequently the specimens were weighed individually with an analytical balance, (each "s*tring"* of fibers was composed of 500 filaments according to the spinneret used to produce the fibers). The mass in grams of each "s*tring"* of fibers was recorded, and the linear density was calculated using the direct count system formula.

$$\text{Linear density} = \frac{\text{Weight of yarn (g)} \times \text{unit length (m)}}{\text{Length of yarn (m)}}$$

The tex count value was later used to calculate the tensile strength in cN/tex.

4.2.3.1 Linear density results, sample C7AA

Table 8. Linear density results C7AA

Linear density					
Specimen number	**Weight of yarn (g)**	**Unit length (m)**	**Length of yarn (m)**	**Tex count**	**Tex count average**
1	0.138	1000.00	1.00	138	140
2	0.140	1000.00	1.00	140	
3	0.145	1000.00	1.00	145	
4	0.142	1000.00	1.00	142	
5	0.135	1000.00	1.00	135	
6	0.141	1000.00	1.00	141	
7	0.137	1000.00	1.00	137	
8	0.145	1000.00	1.00	145	
9	0.135	1000.00	1.00	135	
10	0.142	1000.00	1.00	142	

4.2.3.2 Linear density results, sample C7CA

Table 9. Linear density results C7CA

Linear density					
Specimen number	**Weight of yarn (g)**	**Unit length (m)**	**Length of yarn (m)**	**Tex count**	**Tex count average**
1	0.124	1000.00	1.00	124	125
2	0.120	1000.00	1.00	120	
3	0.126	1000.00	1.00	126	
4	0.128	1000.00	1.00	128	
5	0.123	1000.00	1.00	123	
6	0.127	1000.00	1.00	127	
7	0.130	1000.00	1.00	130	
8	0.119	1000.00	1.00	119	
9	0.125	1000.00	1.00	125	
10	0.125	1000.00	1.00	125	

4.2.3.3 Linear density results, sample C7LA

Table 10. Linear density results C7LA

Linear density					
Specimen number	**Weight of yarn (g)**	**Unit length (m)**	**Length of yarn (m)**	**Tex count**	**Tex count average**
1	0.140	1000.00	1.00	140	142
2	0.144	1000.00	1.00	144	
3	0.142	1000.00	1.00	142	
4	0.139	1000.00	1.00	139	
5	0.147	1000.00	1.00	147	
6	0.138	1000.00	1.00	138	
7	0.141	1000.00	1.00	141	
8	0.145	1000.00	1.00	145	
9	0.137	1000.00	1.00	137	
10	0.144	1000.00	1.00	144	

Table 11. Linear density average

Sample ID	Tex count average
C7AA	140
C7CA	125
C7LA	142

4.3 Tensile strength test

This test was performed taking as a reference the International Standard *ISO 2062:2009; Textiles — Yarns from packages — Determination of single-end breaking force and elongation at break using constant rate of extension (CRE) tester*.

The standard ISO 2062:2009 outlines procedures for determining the breaking force and elongation at break of textile yarns taken out of packages. In order to calculate the yarn's single-end breaking force and elongation at break, a constant rate of extension (CRE) tester is used. The standard specifies four different methods to carry out the test. This standard's objective is to guarantee that textile yarns can be utilized in a variety of textile applications and meet specific quality requirements [66].

The experiment was carried out in a laboratory with the temperature set at 20 ± 2ºC, and the relative humidity maintained at 65 ± 4% throughout its duration. The length of every specimen was 150.00 mm, and both the testing and calculations were carried out with the assistance of an Instron 5944 Tensile Testing Machine and the software provided by the manufacturer. This apparatus was developed for the purpose of determining the tensile strength of various materials, and the accompanying software assists in the collecting and evaluation of the information that is obtained from the examination. The results obtained from this experiment are reliable and accurate due to the precision of the equipment and the standardized testing conditions.

4.3.1 Tensile strength results, sample C7AA

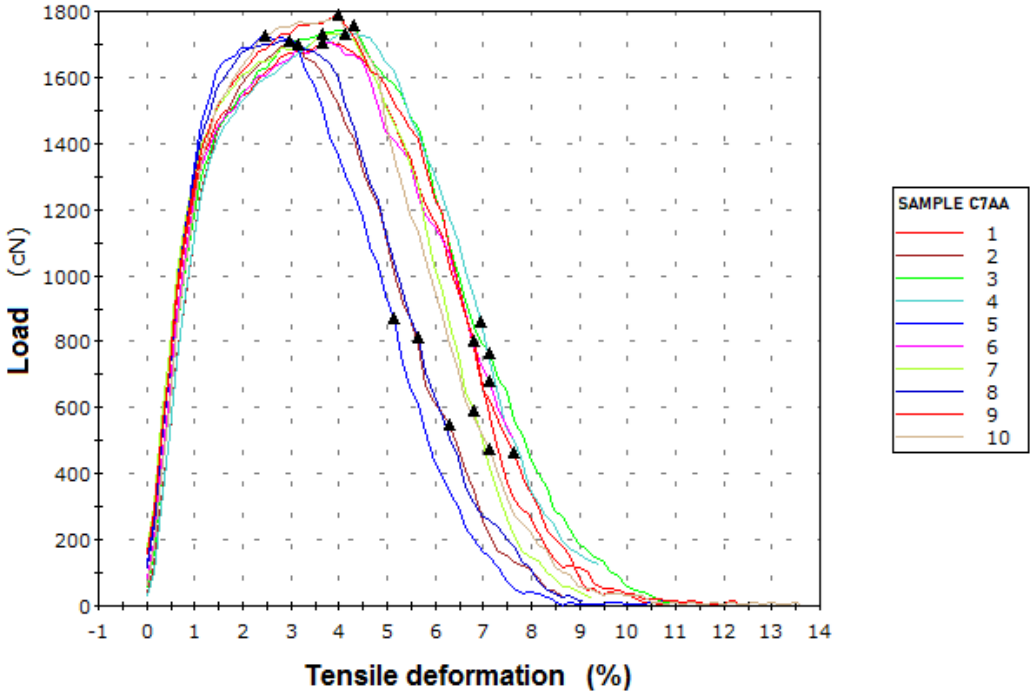

Figure 28. Tensile deformation graphic, sample C7AA

Table 12. Tensile strength results C7AA

Tensile strength		
Number of specimens tested 10	**Specific strength at maximum force (cN/tex) Average**	**Relative elongation at maximum force (%) Average**
	12.39	3.59
Standard deviation	0.24	0.57
Coefficient of variation	1.94	15.93

4.3.2 Tensile strength results, sample C7CA

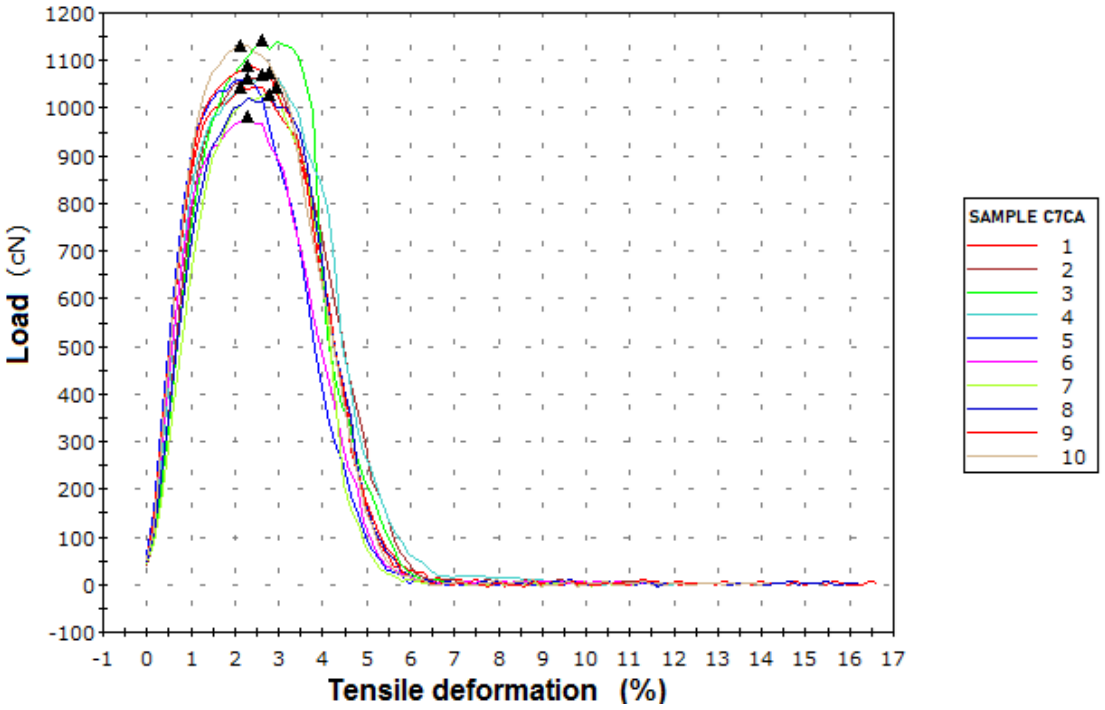

Figure 29. Tensile deformation graphic, sample C7CA

Table 13. Tensile strength results C7CA

Tensile strength		
Number of specimens tested 10	**Specific strength at maximum force (cN/tex) Average**	**Relative elongation at maximum force (%) Average**
	8.70	2.24
Standard deviation	1.54	0.78
Coefficient of variation	5.81	8.16

4.3.3 Tensile strength results, sample C7LA

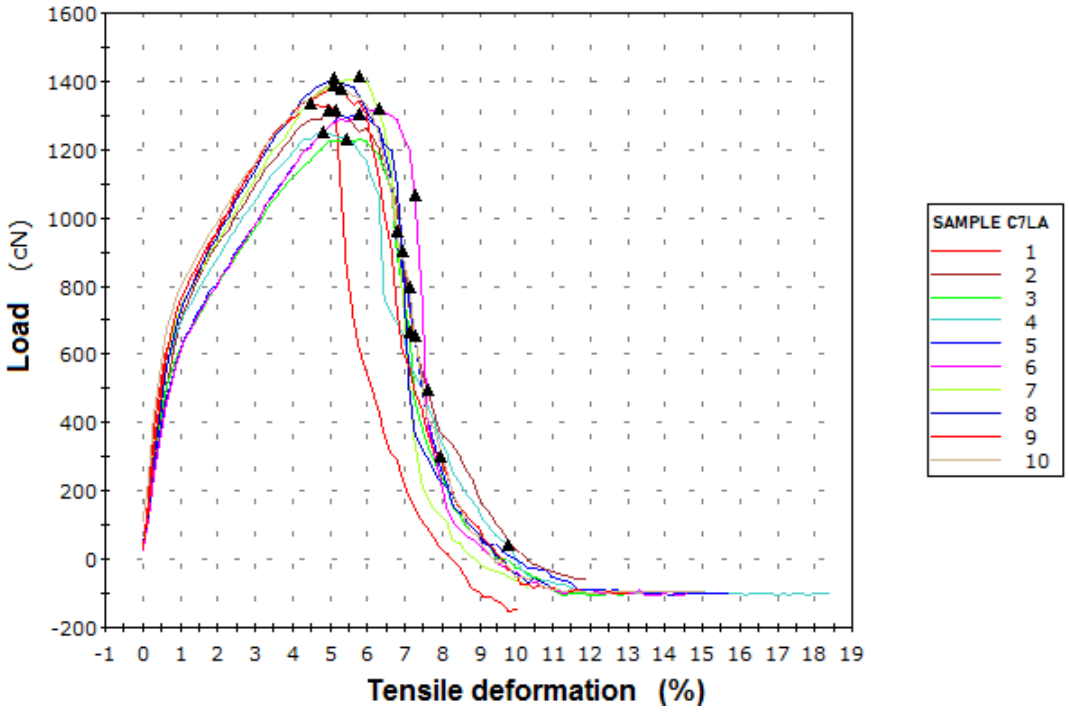

Figure 30. Tensile deformation graphic, sample C7LA

Table 14. Tensile strength results C7LA

Tensile strength		
Number of specimens tested 10	**Specific strength at maximum force (cN/tex) Average**	**Relative elongation at maximum force (%) Average**
	9.40	5.33
Standard deviation	0.45	0.54
Coefficient of variation	4.76	10.19

Table 15. Tensile strength results C7AA, C7CA, C7LA

Sample ID	Specific strength at maximum force (cN/tex) Average	Relative elongation at maximum force (%) Average
C7AA	12.39	3.59
C7CA	8.70	2.24
C7LA	9.40	4.53

According to the obtained results, sample C7AA shown the maximum tensile strength (12.39 cN/tex) among the three samples tested. This indicates that using acetic acid as solvent in the production of chitosan fibers has a positive influence on the resulting fibers mechanical strength. However, the elongation at break for C7AA (3.59%) is significantly less than that of C7LA (4.53%), indicating that C7LA fibers are more ductile and can endure greater strain prior to breaking.

The lower tensile strength observed in sample C7CA, as compared to the other two samples, could be attributed to the rheological behavior of the dope solution. As per the given rheological results, C7CA exhibited a shear-thinning behavior, which means that as the shear rate increased, the viscosity of the solution decreased. This may have led to a reduction in the intermolecular entanglement between the polymer chains, resulting in weaker fiber formation.

4.4 Surface modification of chitosan fibers

After analyzing the outcomes of the wet spun fibers, it was evident that the sample C7AA exhibited the most favorable overall performance. As a result, further investigation was conducted on that sample to enhance the fibers' mechanical and antibacterial properties. To enhance the tensile strength, Pati et al. (2011) [31] research was analyzed, which involved creating chitosan-TTP fibers with varying phosphate contents using STPP baths at different pH levels that affected fiber formation and properties due to ionization, and also Ouyang et al. (2022) investigated the usage of TPP by means of a wet impregnation process [67]. After this, in order to improve the fibers' antibacterial properties, Do Nascimento et al. (2014) research was analyzed, which revealed that ursolic acid shown significant antibacterial activity against six bacterial strains, and the best result was found against S. aureus, with a MIC (minimal inhibitory concentration) value of 32 μg mL^{-1}. Ursolic acid was also effective against E. coli, K. pneumoniae and S. flexneri with a MIC value of 64 μg mL^{-1}. By broadening these studies, it could be possible to improve the C7AA fibers' properties and increase their applicability in a wider spectrum, particularly in the biomedical field, where antibacterial properties are highly desired.

Table 16. Wet impregnation sample nomenclature

Sample ID	Chitosan fibers concentration / dope solvent	Wet impregnation solute
C7AATPP	7wt % / Acetic Acid	Tripolyphosphate (TPP)
C7AAUA	7 wt % / Acetic Acid	Ursolic acid (UA)

4.4.1 Cross-linking of chitosan fibers with tripolyphosphate (TPP) by wet impregnation

A solution of tripolyphosphate was prepared by dissolving TPP in distilled water at a concentration of 1% w/v. In order to get the chitosan fibers ready for the crosslinking process, five separate specimens of chitosan fibers were cut to the same weight of 1.0g each. After that, each specimen was then immersed individually in 100 cm3 of TPP solution at room temperature for varying immersion times of 1, 2, 4, 6, and 8 hours respectively. After each immersion period, the specimens were carefully removed from the TPP solution and rinsed with distilled water to remove any excess TPP solution. This process was repeated for each of the five specimens, and the resulting samples were further characterized and evaluated for their physical, chemical, and mechanical properties by means of FTIR, SEM and dynamometry in order to assess the effectiveness of the wet impregnation method using TPP as a cross-linker.

4.4.1.1 Fourier transform infrared spectroscopy (FTIR)

For structural and compositional analysis, academics and industry have long employed the Fourier transform infrared spectroscopy (FTIR) technique. For the identification and measurement of functional groups, FTIR spectroscopy is a valuable instrument. It is a promising and fast technology that is typically used as the first step in the characterization of materials [68].

In order to characterize and quantitatively analyze polymer blends, determine their compatibility via intermolecular hydrogen bonds, and also look into their degradation processes, Fourier transform infrared spectroscopy (FTIR) is extensively utilized. The physical characteristics of polymer blends are influenced by the molecular chain structures; hence FTIR spectroscopy has been used for blend investigations.

Different polymer blends are capable of displaying various interactions and configurations, depending on the nature of the blending, which can aid in the prediction of their mechanical and physical properties [69].

4.4.1.2 FTIR results, sample C7AATPP

The infrared transmission and reflectance spectra were recorded in the range from 4000 to 600 cm^{-1} with a resolution 4 cm^{-1} and 32 scans.

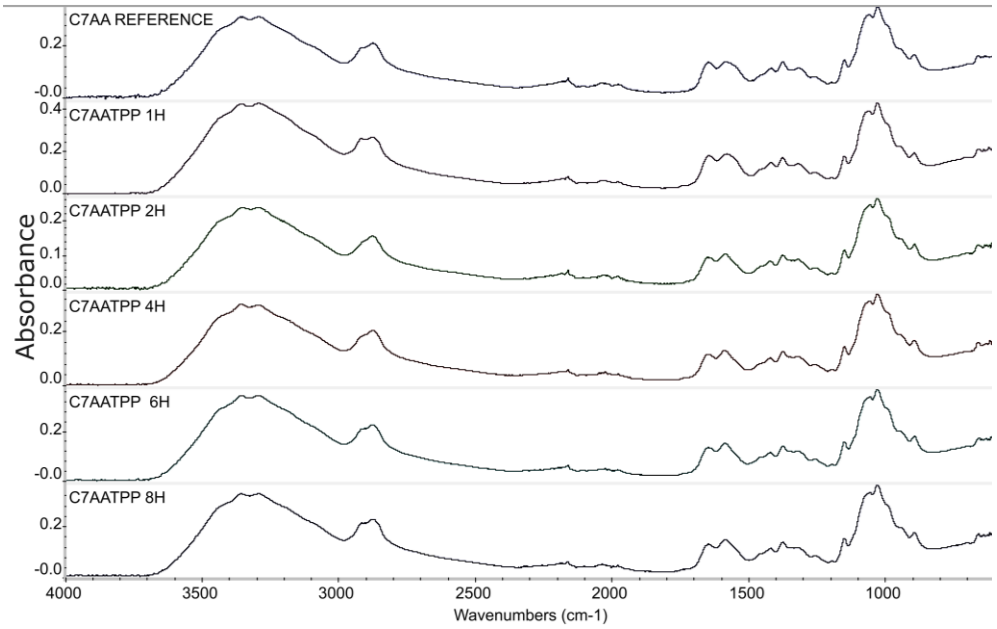

Figure 31. Infrared spectroscopy spectrum graph

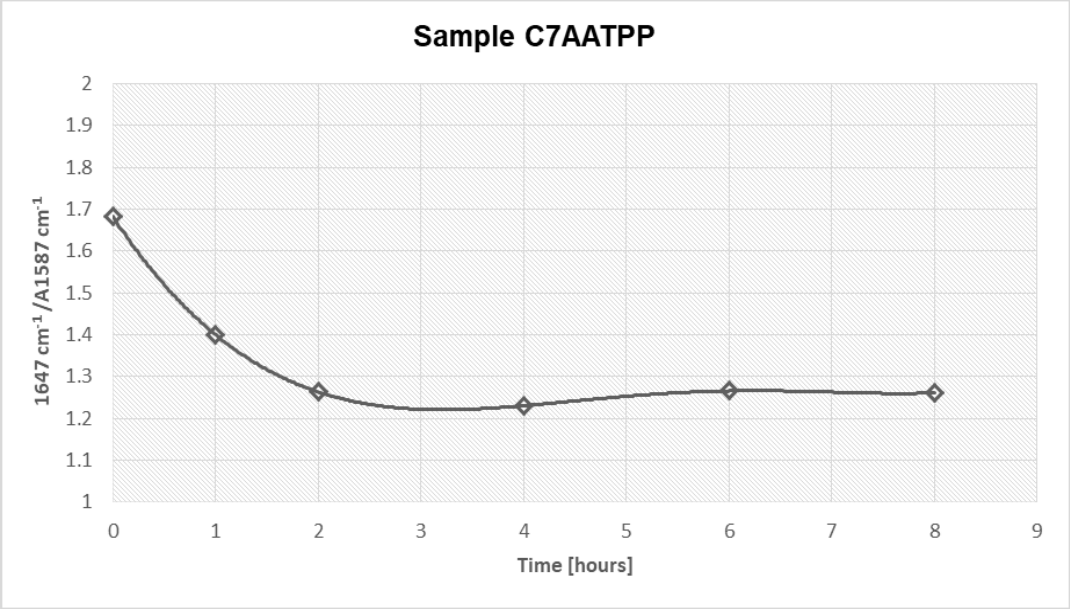

Figure 32. Absorbance area, amide/amine ratio for samples C7AAUA 0, 1, 2,4,6 and 8 hours

Based on the obtained results, the absorbance ratio at 1647 cm^{-1} / 1587 cm^{-1} of samples C7AATPP at different time set points (0, 1, 2, 4, 6, and 8 hours) suggests that the crosslinking reaction between chitosan fibers and TPP ions continued up to 2 hours of immersion, as indicated by the decreasing absorbance ratio. However, after 2 hours of immersion, the absorbance ratio remained relatively constant, indicating that the reaction had reached completion. This suggests that the reaction had utilized most of the available protonated amine groups for cross-linking, and there were no more significant changes in the chemical structure of the sample during the remaining immersion time. Overall, the FTIR results suggest that protonated amine's interaction with TPP ions was successful on the surface of chitosan fibers.

Following, the fiber´s morphology was studied and the mechanical properties were tested to analyze if the cross-linking process improved the fiber´s tensile strength.

4.4.1.3 SEM, sample C7AATPP after 2 hours of immersion

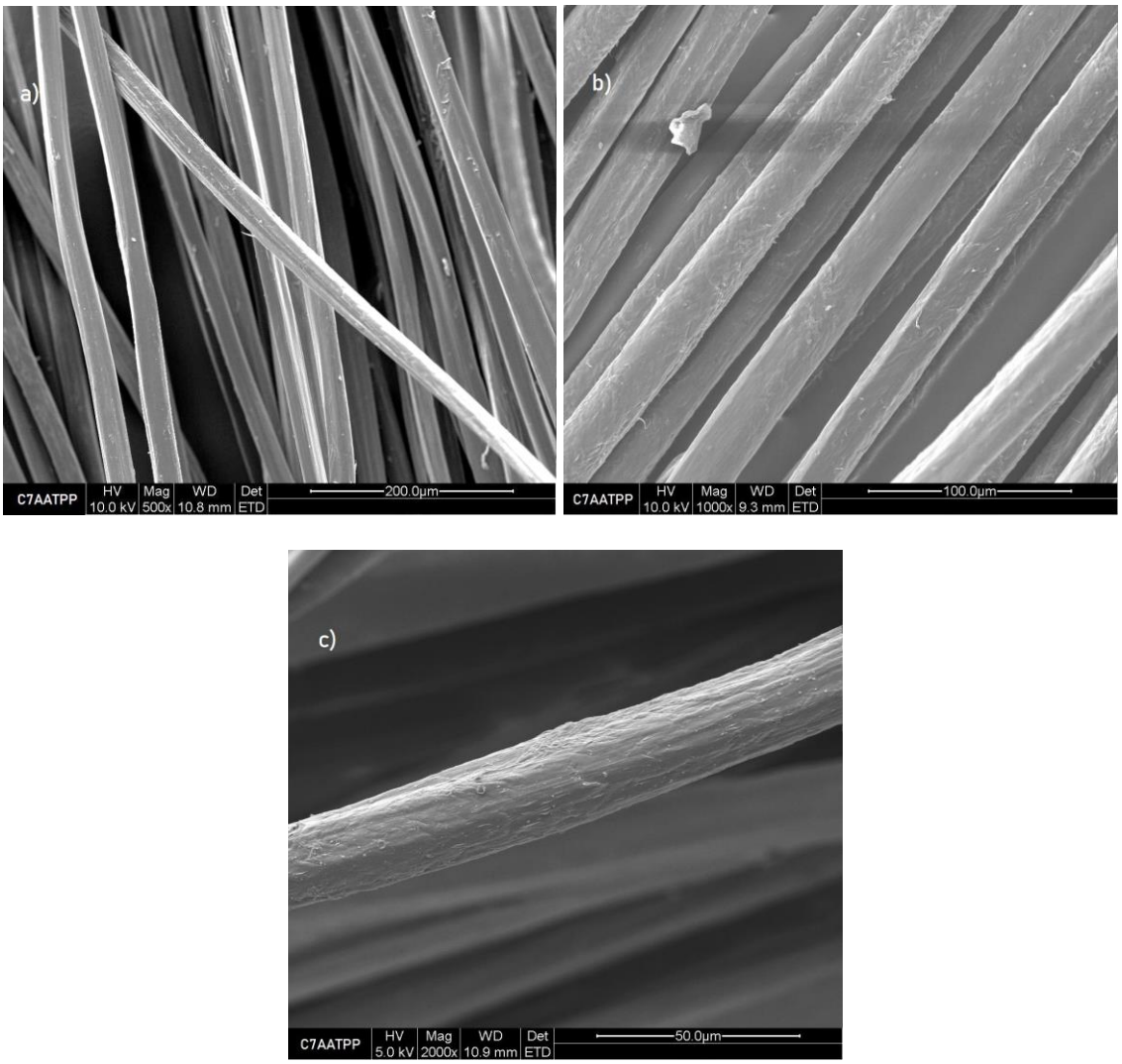

Figure 33. SEM images a) 500x, b) 1000x and c) 2000x

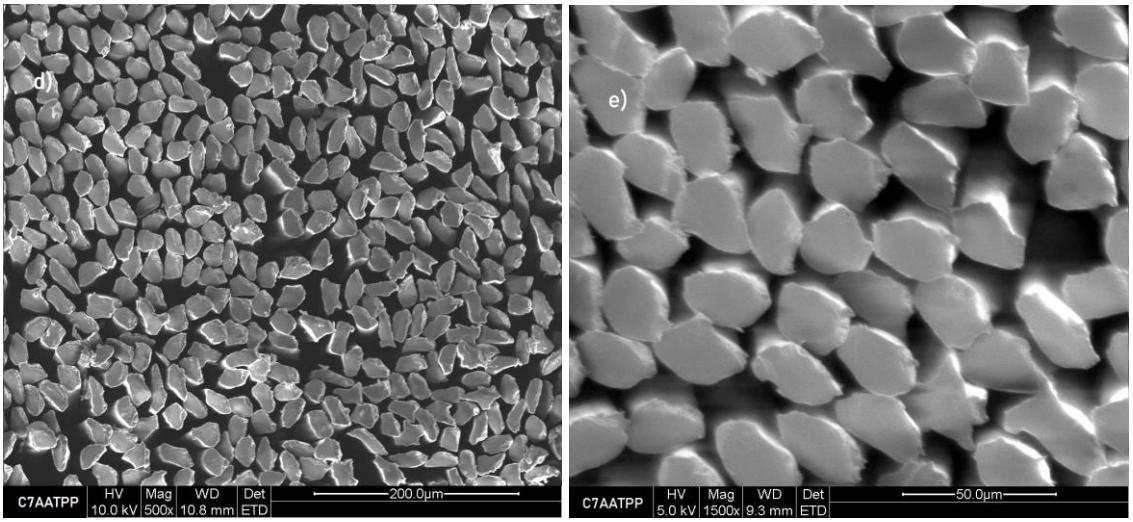

Figure 34. SEM images c) 2000x, cross-section images d) 500x and e) 1500x

The figures 33 and 34 show that C7AATPP has a rougher surface than C7AA (without TPP treatment) see (fig. 22, 23), this suggests that the crosslinking reaction between chitosan and TPP ions might have caused some roughness on the surface of the fibers. It could be due to the formation of new chemical bonds between the chitosan and TPP ions, which can cause some deformation in the surface´s morphology of the fibers.

The images obtained from the samples taken after 4, 6, and 8 hours of immersion, showed a resemblance to the images obtained from the sample after 2 hours of immersion.

4.4.1.4 Linear density results, sample C7AATPP after 2 hours of immersion

Table 17. Linear density results C7AATPP after 2 hours of immersion

Linear density					
Specimen number	**Weight of yarn (g)**	**Unit length (m)**	**Length of yarn (m)**	**Tex count**	**Tex count average**
1	0.143	1000.00	1.00	143	145
2	0.150	1000.00	1.00	150	
3	0.142	1000.00	1.00	142	
4	0.148	1000.00	1.00	148	
5	0.141	1000.00	1.00	141	
6	0.147	1000.00	1.00	147	
7	0.142	1000.00	1.00	142	
8	0.146	1000.00	1.00	146	
9	0.145	1000.00	1.00	145	
10	0.149	1000.00	1.00	149	

4.4.1.5 Tensile strength results, sample C7AATPP after 2 hours of immersion

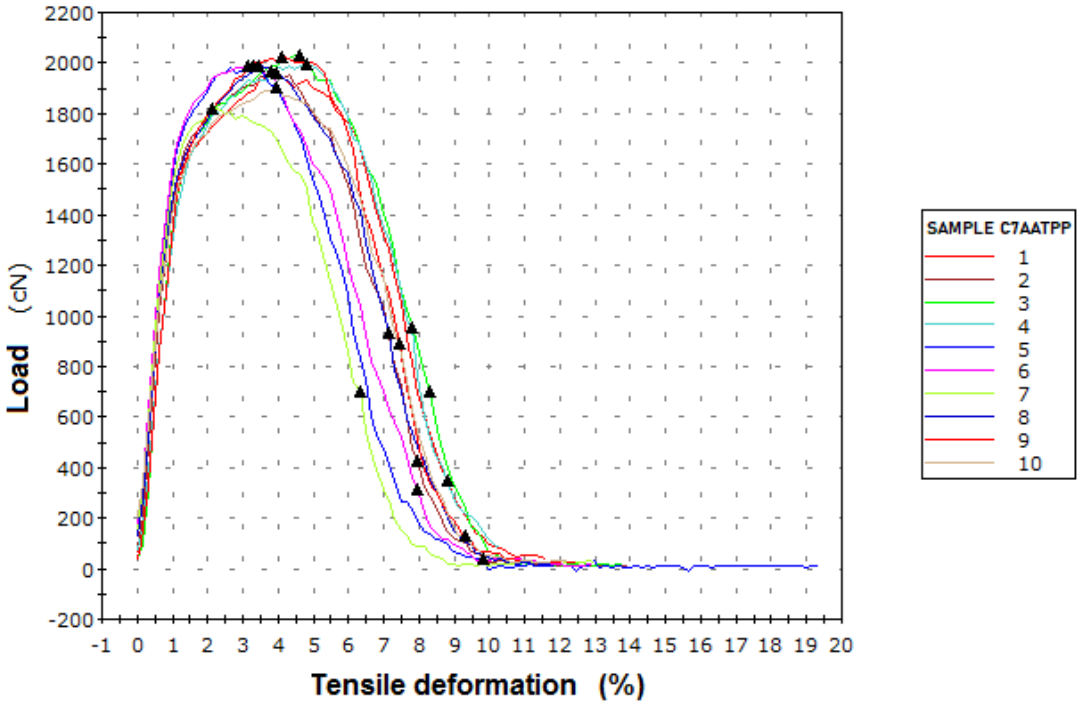

Figure 35. Tensile deformation graphic, sample C7AATPP after 2 hours of immersion

Table 18. Tensile strength results, sample C7AATPP after 2 hours of immersion

Tensile strength		
Number of specimens tested 10	**Specific strength at maximum force (cN/tex) Average**	**Relative elongation at maximum force (%) Average**
	14.25	3.94
Standard deviation	0.56	0.74
Coefficient of variation	2.63	10.89

Table 19. Comparison of results before and after TPP impregnation

Sample ID	Specific strength at maximum force (cN/tex) Average	Relative elongation at maximum force (%) Average	Fiber´s strength improvement (%)	Fiber´s elongation improvement (%)
C7AA	12.39	3.59		
C7AATPP 2 hours	14.25	3.94	15.01	9.75
C7AATPP 4 hours	14.20	3.89	14.60	8.35
C7AATPP 6 hours	14.22	3.87	14.76	7.80
C7AATPP 8 hours	14.17	3.90	14.36	8.63

There was a significant improvement in the strength of the sample C7AATPP after two hours of immersion, compared to the strength of the C7AA sample that did not undergo the wet impregnation treatment. This leads one to assume that the fibers were successfully strengthened by the crosslinking reaction that occurred between chitosan and TPP. The increase in strength can be attributed to the formation of a strong ionic bond between the positively charged amino groups of chitosan and the negatively charged TPP ions, which creates a crosslinked network within the fibers.

Additionally, the increase in relative elongation at maximum force between the two samples suggests that the crosslinking reaction has also made the fibers more ductile.

4.4.2 Chitosan fibers - ursolic acid by wet impregnation

A solution of ursolic was prepared by dissolving ursolic acid in pure 2-propanol at a concentration of 64 μg mL^{-1}. In order to get the chitosan fibers ready for the wet impregnation process, five separate specimens of chitosan fibers were cut to the same weight of 1.0g each. After that, each specimen was then immersed individually in 100 cm^3 of Ursolic acid solution at room temperature for varying immersion times of 1, 2, 4, 6, and 8 hours respectively. After each immersion period, the specimens were carefully removed from the ursolic acid solution and rinsed with an aqueous solution of ethanol and distilled water 4:6 (v/v) to remove any excess of ursolic acid solution and then they were dried at room temperature. This process was repeated for each of the five specimens.

The resulting samples were further characterized and evaluated for their physical, chemical, properties. FTIR and SEM analyses were conducted to investigate the structure and morphology of the chitosan fibers after the wet impregnation. Additionally, an antibacterial activity test was performed to evaluate the effectiveness of the wet impregnation method using ursolic acid as an antibacterial enhancer agent.

4.4.2.1 FTIR results, sample C7AAUA

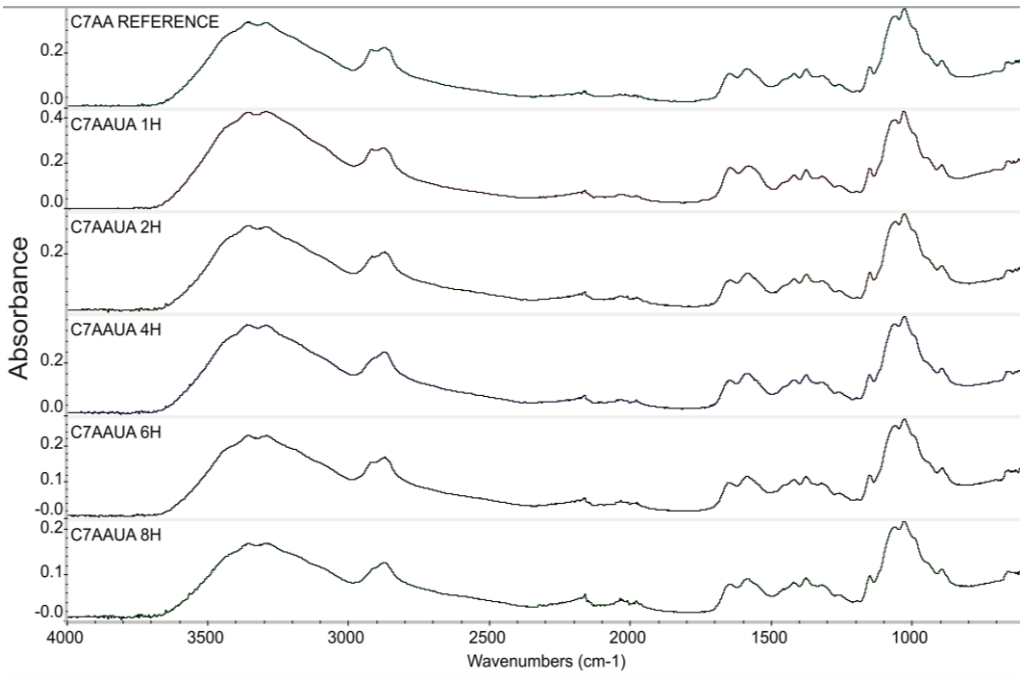

Figure 36. Infrared spectroscopy spectra graph

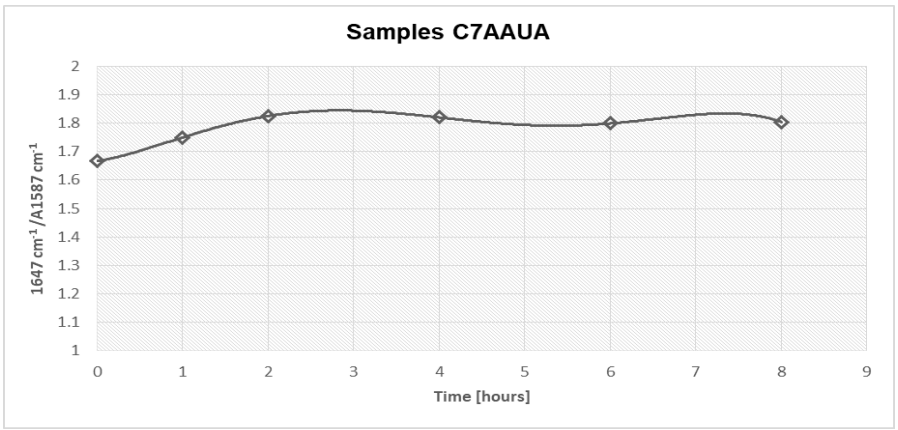

Figure 37. Absorbance area, amide/amine ratio for samples C7AAUA 0, 1, 2, 4, 6 and 8 hours

The FTIR results of the samples C7AAUA immersed in ursolic acid for different time setpoints (0, 1, 2, 4, 6, and 8 hours) showed an increasing absorbance ratio from 1.66 to 1.82 over the course of 2 hours. This suggests that there was an ongoing chemical reaction between chitosan and ursolic acid during this time, resulting in changes to the chemical structure of the sample. However, after 2 hours, the absorbance ratio remained relatively constant, suggesting that the reaction had reached completion and that the chemical structure of the sample remained stable.

The peaks observed at 1647 cm^{-1} and 1587 cm^{-1} are attributed to the amide and amine groups in chitosan, respectively. The appearance of these peaks in the FTIR spectra indicates that the chitosan fibers-ursolic acid adduct was formed through the interaction of the amine groups in chitosan with the carboxylic acid groups in ursolic acid. Following their antibacterial properties were tested to analyze if ursolic acid improved the fiber´s antibacterial activity.

4.4.2.2 SEM, sample C7AAUA after 2 hours of immersion

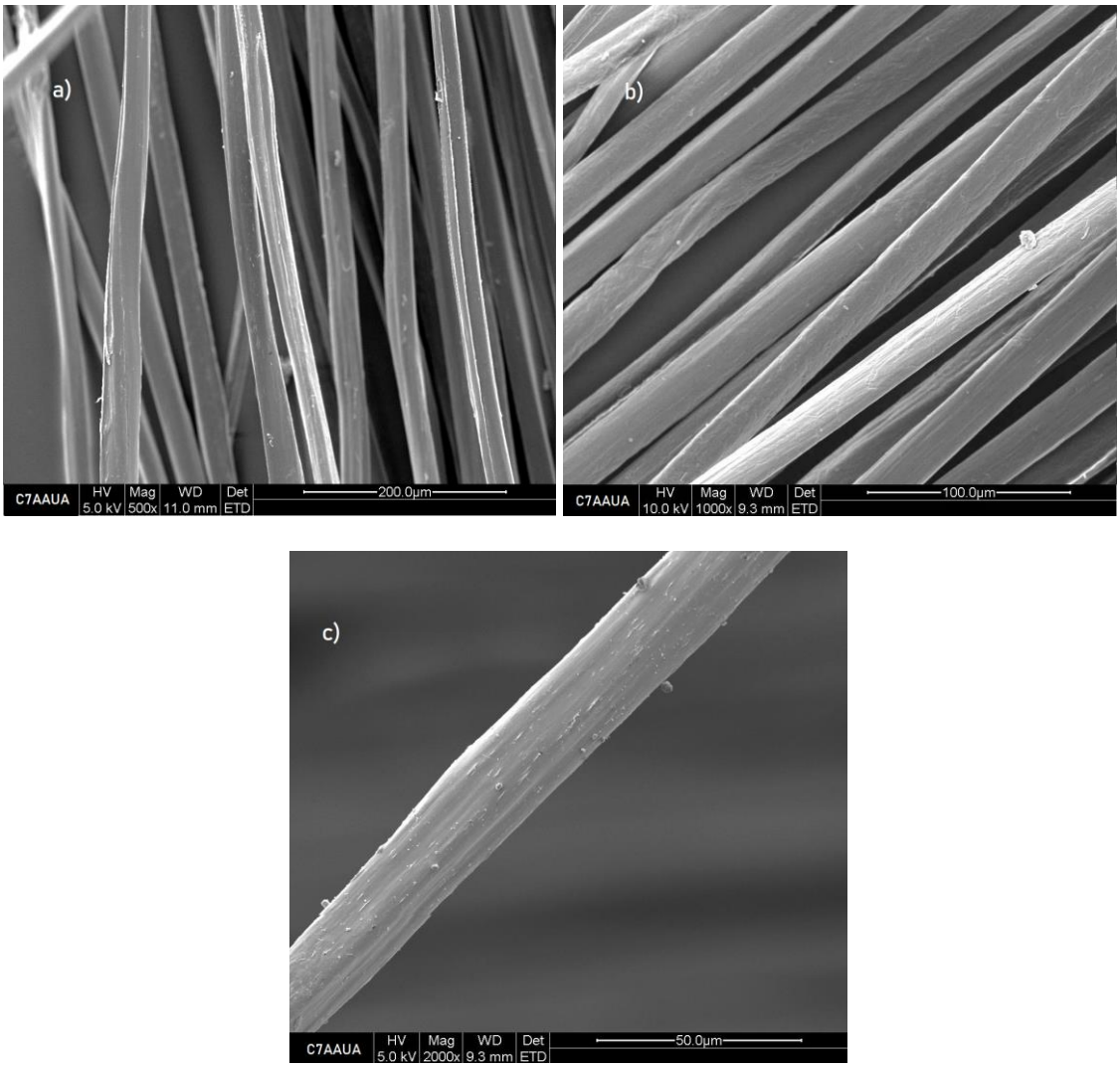

Figure 38. SEM images a) 500x, b) 1000x and c) 2000x

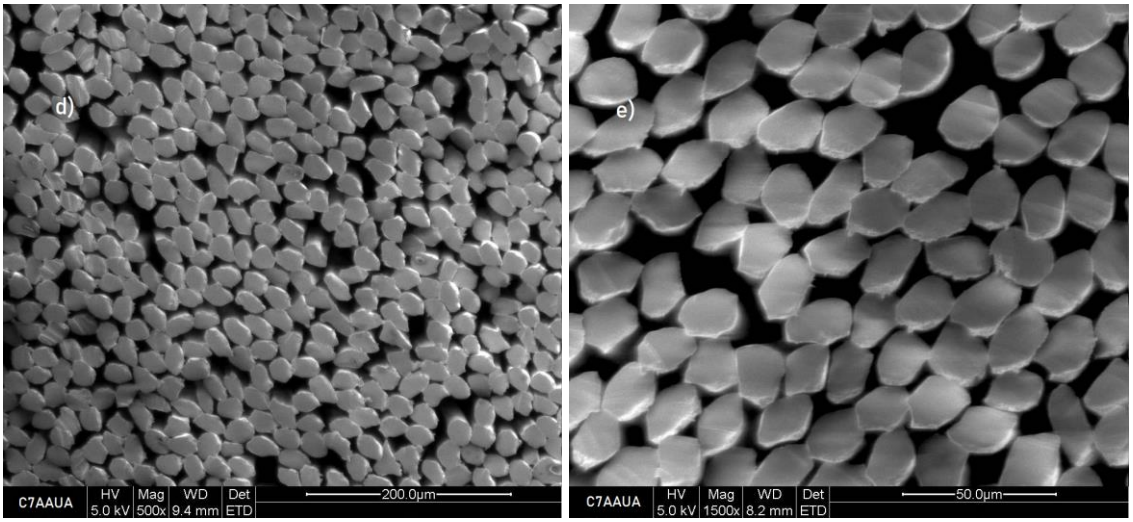

Figure 39. SEM cross-section images d) 500x and e) 1500x

When compared to the surface of sample C7AA (with no ursolic acid treatment), which can be seen in figures 22 and 23, figures 38 and 39 demonstrate that the surface of sample C7AAUA is slightly rougher. This indicates that the impregnation process have successfully modified the surface of the fibers.

The photos that were obtained from the samples that were taken after 4, 6, and 8 hours of immersion exhibited a similarity to the images from the sample that was immersed for only 2 hours.

4.4.2.3 UV spectroscopy

UV spectroscopy or UV-Vis (ultraviolet visible) is a type of optical spectroscopy that examines molecular absorption of light in the visible, ultraviolet, and near-infrared regions of the electromagnetic spectrum. This technique involves studying the molecular absorption in the range of 190 to 800 nm, which includes the ultraviolet region from 190nm to 400nm and the visible region from 400nm to 800nm.When a beam of monochromatic radiation is passed through a uniform solution in a cell, the intensity of the radiation that is transmitted through the solution depends on the thickness and the concentration of the solution [70].

UV spectroscopy is frequently used to provide characterization data for a variety of materials. Inorganic or organic, solid or liquid groups, such as organic molecules and functional groups, can be observed using UV spectroscopy, as can reflectance measurements for coatings, paints, textiles, biochemical analysis, dissolution kinetics, band gap measurements, etc. UV spectroscopy provides values depending on the degree of absorbance or transmittance of a different wavelength of beam light and the various responses of samples [71].

4.4.2.4 UV spectroscopy results, sample C7AAUA

The UV-Vis spectra were obtained by means of UV-Vis from Jasco company, model V-670, the scan range used was from 190 to 400 nm. Resolution 1 nm.

Standard stock solutions containing ursolic acid were prepared in methanol at final concentration of 2.7mg/10mL. After that, serial standard dilutions at three concentrations were analyzed by means UV-Vis, and linearity was verified by regression analysis. Calibration results are presented in figure 40, and table 20.

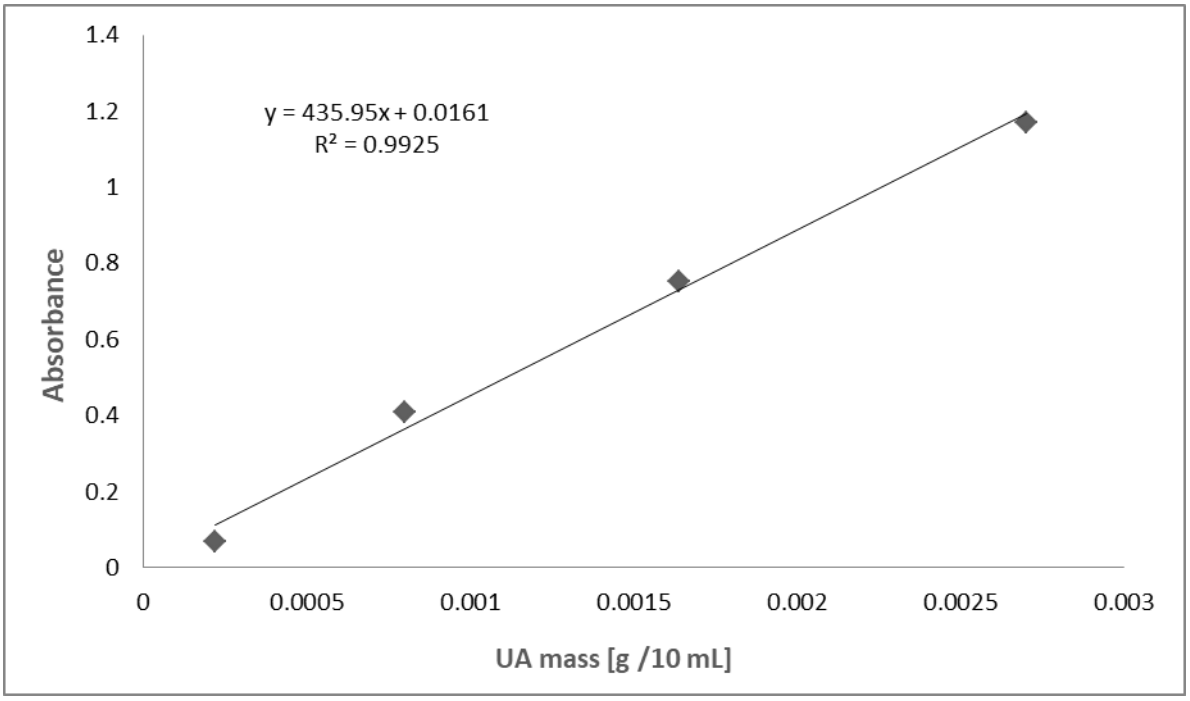

Figure 40. Calibration graph, relationship absorbance/UA concentration 0.0220 mg mL^{-1}, 0.080 mg mL^{-1}, 0.164 mg mL^{-1} and 0.270 mg mL^{-1}

Table 20. Linearity of calibration plots

Linear range (mg/mL^{-1})	**y = ax + b (linear model)**	**Correlation coefficient, R^2**
0.220 – 0.270	y = 435.95x+0.0161	0.9925

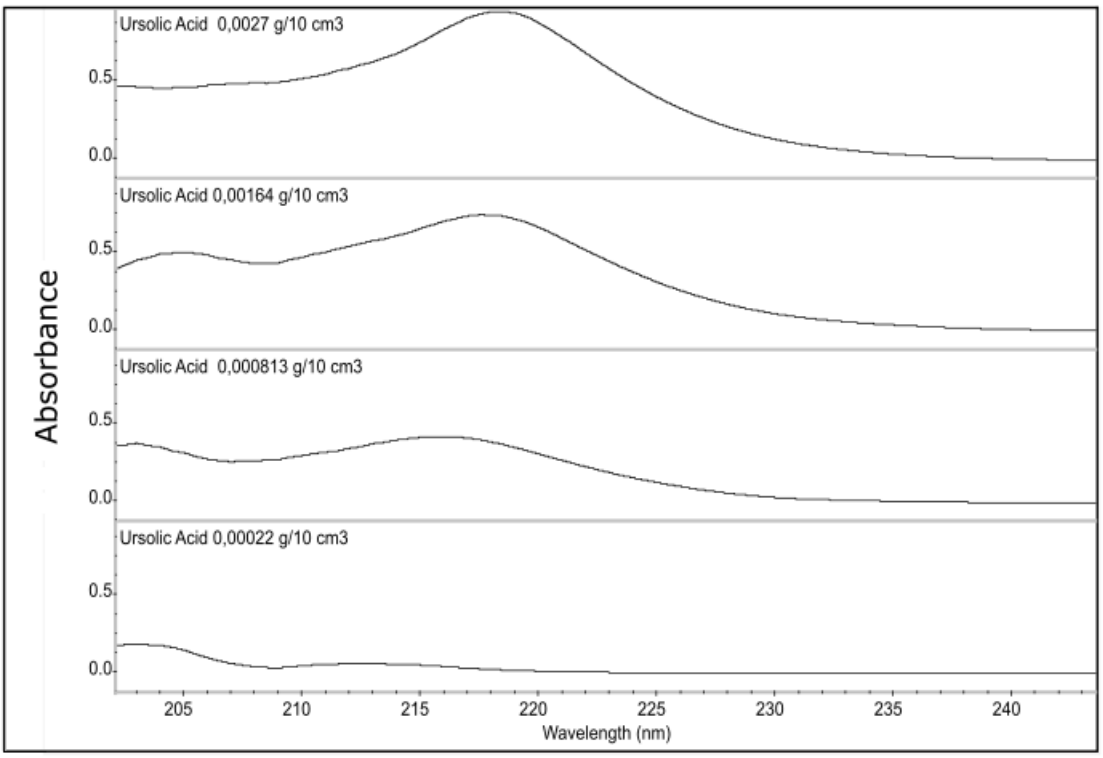

Figure 41. UV Absorption spectra of methanolic solutions of ursolic acid analyzed at different concentrations

The resulting spectra were analyzed at specific wavelengths to detect the distinctive peaks associated with ursolic acid. Previous research studies have reported the UV spectra of ursolic acid, which display absorbance peaks between 210 nm and 220 nm [72,73]. The resulting absorbance values were obtained in ascending order and corresponded to increasing concentrations, with values of 214 nm, 216 nm, 217 nm, and 218 nm.

After conducting the linear analysis, a stock fiber suspension was prepared with methanol at a concentration of 2.3 mg/10mL from the sample C7AAUA that was impregnated with ursolic acid for 2 hours. By adding the fibers into methanol, it was

possible to dissolve the ursolic acid deposited on the surface of the fibers in order to perform the UV-Vis test. This sample was chosen based on the FTIR test results indicating completion of the reaction at that time set point.

UV spectra were performed and the absorbance peak height was appointed at 213 nm and absorbance was 0,056 and based on the linear model, the mass of ursolic acid deposited on the fibers per gram was calculated as follows.

$$y = 435.95x+0.0161$$

$$0.056 = 435.95x+0.0161$$

$$0.056-0.0161 = 435.95x$$

$$mUA_f = x = 0.091 \text{ mg}$$

Where,

mUA_f =Mass of ursolic acid on the surface of the fibers

y= absorbance at UV spectrum

x= concentration [mg/ml]

This process was repeated for all samples C7AAUA that were wet impregnated for 2, 4, 6, and 8 hours. Subsequently, the mass calculation of ursolic acid per gram of chitosan fibers was performed based on the knowledge that 0.091 mg of ursolic acid are present in 2.3 mg of fibers.

Table 21. Mass per gram of ursolic acid in chitosan fibers

C7AAUA	Mass of UA/g in chitosan fibers
2 hours	0.039
4 hours	0.038
6 hours	0.040
8 hours	0.038

4.4.2.5 Antibacterial activity test

This test was performed in accordance to the standard PN-EN ISO 20743:2021 by the Laboratory of Biodegradation and Microbiological Research of the Łódzki Instytut Technologiczny.

PN-EN ISO 20743:2021 is a standard that provides a method for determining the antibacterial activity of textile products through an absorptive method called the plate count method. This standard specifies quantitative test methods to determine the antibacterial activity of all antibacterial textile products including nonwovens.

This standard is applicable to all textile products, including cloth, wadding, thread and material for clothing, bedclothes, home furnishings and miscellaneous goods, regardless of the type of antibacterial agent used (organic, inorganic, natural or man-made) or the method of application (built-in, after-treatment or grafting).

This standard covers three inoculation methods for the determination of antibacterial activity:

a) Absorption method (an evaluation method in which the test bacterial suspension is inoculated directly onto specimens);

b) Transfer method (an evaluation method in which test bacteria are placed on an agar plate and transferred onto specimens);

c) Printing method (an evaluation method in which test bacteria are placed on a filter and printed onto specimens).

The antibacterial activity of the textile product is determined by measuring the reduction in the number of viable bacteria after contact with the textile material.

The plate count method involves placing a suspension of bacteria on a solid nutrient medium and placing the textile sample on the medium. After an appropriate contact time, the number of bacteria that survive on the surface of the textile sample is

determined by counting the colonies that grow on the nutrient medium according to a ten-time dilution method [74].

4.4.2.6 Antibacterial activity results, sample C7AAUA

The antibacterial activity of the samples C7AA (no ursolic acid) and C7AAUA (ursolic acid) was tested against Escherichia coli ATCC 11 229 and Staphylococcus aureus ATCC 6538, the results were later compared.

Table 22. Antibacterial activity results E.coli. C7AA

Antibacterial activity against Escherichia coli ATCC 11 229				
Sample ID	**Incubation time [h]**	**Number of bacteria [CFU*/pr]**	**Value of antibacterial activity "A"**	**Value of growth**
Laboratory control sample	0	$3.7x10^4$		3.37
	24	$8.5x10^7$		
C7AA	0	$3.1x10^4$	0.04	3.40
	24	$7.8x10^7$		

Inoculum concentration: $1.6x10^5$ CFU/ml E. coli

*(CFU – Colony Forming Unit)

Table 23. Antibacterial activity results E.coli. C7AAUA

Antibacterial activity against Escherichia coli ATCC 11 229				
Sample ID	**Incubation time [h]**	**Number of bacteria [CFU*/pr]**	**Value of antibacterial activity "A"**	**Value of growth**
Laboratory control sample	0	$3.7x10^4$		3.37
	24	$8.5x10^7$		
C7AAUA	0	$2.8x10^4$	0.25	3.24
	24	$4.8x10^7$		

Inoculum concentration: $1.6x10^5$ CFU/ml S. aureus

*(CFU – Colony Forming Unit)

Table 24. Antibacterial activity for S. Aureus C7AA

Antibacterial activity against Staphylococcus aureus ATCC 6538				
Sample ID	**Incubation time [h]**	**Number of bacteria [CFU*/pr]**	**Value of antibacterial activity "A"**	**Value of growth**
Laboratory control sample	0	$9.6x10^4$		2.13
	24	$1.2x10^7$		
C7AA	0	$9.6x10^4$	0.32	2.45
	24	$2.7x10^4$		

Inoculum concentration: $2.3x10^5$ CFU/ml S. aureus

*(CFU – Colony Forming Unit)

Table 25. Antibacterial activity against S. Aureus C7AAUA

Antibacterial activity against Staphylococcus aureus ATCC 6538				
Sample ID	**Incubation time [h]**	**Number of bacteria [CFU*/pr]**	**Value of antibacterial activity "A"**	**Value of growth**
Laboratory control sample	0	$9.6x10^4$		2.13
	24	$1.2x10^7$		
C7AAUA	0	$6.7x10^4$	2.93	-0.68
	24	$1.4x10^4$		

Inoculum concentration: $2.3x10^5$ CFU/ml S. aureus

*(CFU – Colony Forming Unit)

Table 26. Criteria for assessing antibacterial activity

Criteria for assessing antibacterial activity (Annex F of the PN-EN ISO 20743:2021 standard)	
Efficacy of antibacterial properties	**Value of antibacterial activity**
low	$A < 2$
significant	$2 \leq A < 3$
strong	$A \geq 3$

Table 27. Comparison of antibacterial activity C7AA vs C7AAUA

Sample ID	Value of antibacterial activity "A"	
	E. coli	**S. Aureus**
C7AA	0.32	0.04
	Non antibacterial	Non antibacterial
C7AAUA	0.25	2.93
	Non antibacterial	**Significant**

According to the results, it can be observed that sample C7AA did not exhibit any noteworthy antibacterial activity against either of the two bacterial strains. The obtained value was below the efficacy threshold of $A < 2$. In contrast, the sample C7AAUA showed a significant and almost strong antibacterial activity with a value of 2.93, indicating that adding ursolic acid improved the antibacterial properties of the fibers. It is important to note that this significant antibacterial activity was only observed against the Gram-positive strain, S. aureus,

The fact that the antibacterial activity of C7AAUA fibers is only observed against S. aureus and not E. coli is likely due to the difference in bacterial cell wall structure between the two organisms. Gram-positive bacteria, such as S. aureus, have a thick peptidoglycan layer in their cell wall that is more susceptible to damage from antibacterial agents, while Gram-negative bacteria, such as E. coli, have a thinner peptidoglycan layer and an additional outer membrane that provides extra protection against external agents. Therefore, it is possible that the antibacterial activity of C7AAUA fibers is not strong enough to overcome the protective mechanisms of E. coli, while it is effective against S. aureus.

Chapter 5

Summary of findings and conclusions

5. Conclusions

The aim of this doctoral dissertation was to investigate the influence of different carboxylic acids as solvents on the production of chitosan fibers using the wet spinning process, and to explore the potential for enhancing their mechanical and antibacterial properties.

The present research involved a comprehensive investigation of chitosan fibers produced through the wet spinning method, the findings of this work are listed as follows.

- The research's results offer valuable insights into the selection of suitable solvents and optimization of spinning conditions for producing chitosan fibers with superior properties.
- After evaluating the three solvents, acetic acid was found to be the most suitable solvent for preparing the dope solution and producing chitosan fibers with a concentration of 7%, resulting in adequate tensile strength and a smooth surface morphology
- Surface modification of chitosan fibers is possible by wet impregnation method with ursolic acid solution.

- Incorporating ursolic acid on the surface of the fibers can significantly enhance their antibacterial properties, particularly against gram-positive strains, specifically Staphylococcus aureus.
- The wet impregnation method can be used to modify the surface of chitosan fibers through a cross-linking reaction with TPP.
- The incorporation of TPP to the surface of chitosan fibers, results in an improvement in their mechanical properties, specifically a 15% increase in tensile strength and a 9.75% increase in relative elongation.
- The fibers showed good potential for their application in wound dressings or scaffolds with enhanced antibacterial and mechanical properties.

The findings of this study represent a valuable contribution to the field of chitosan fibers and their potential applications. Despite this, there are still opportunities for further exploration and experimentation. For example, future work could focus on testing higher concentrations of ursolic acid and determining the optimal conditions for its incorporation into the fibers to maximize their antibacterial properties. Additionally, it would be beneficial to investigate the antibacterial activity of the fibers against other bacterial strains and to assess their biocompatibility and long-term performance in vivo. These potential prospects of investigation would deepen our understanding of the capabilities and limitations of chitosan fibers, and could potentially lead to even more promising applications in the future.

6. References

[1] S. Mohan, O.S. Oluwafemi, N. Kalarikkal, S. Thomas, S.P. Songca, Biopolymers – Application in Nanoscience and Nanotechnology, in: F.K. Parveen (Ed.), Recent Adv. Biopolym., InTech, 2016. https://doi.org/10.5772/62225.

[2] J. Baranwal, B. Barse, A. Fais, G.L. Delogu, A. Kumar, Biopolymer: A Sustainable Material for Food and Medical Applications, Polymers. 14 (2022) 983. https://doi.org/10.3390/polym14050983.

[3] I. Vroman, L. Tighzert, Biodegradable Polymers, Materials. 2 (2009) 307–344. https://doi.org/10.3390/ma2020307.

[4] M. Kołodziejska, K. Jankowska, M. Klak, M. Wszoła, Chitosan as an Underrated Polymer in Modern Tissue Engineering, Nanomaterials. 11 (2021) 3019. https://doi.org/10.3390/nano11113019.

[5] A.M. Smith, S. Moxon, G.A. Morris, Biopolymers as wound healing materials, in: Wound Heal. Biomater, Elsevier, 2016: pp. 261–287. https://doi.org/10.1016/B978-1-78242-456-7.00013-1.

[6] M. Niaounakis, Definitions of Terms and Types of Biopolymers, in: Biopolym. Appl. Trends, Elsevier, 2015: pp. 1–90. https://doi.org/10.1016/B978-0-323-35399-1.00001-6.

[7] A. Samir, F.H. Ashour, A.A.A. Hakim, M. Bassyouni, Recent advances in biodegradable polymers for sustainable applications, Npj Mater. Degrad. 6 (2022) 68. https://doi.org/10.1038/s41529-022-00277-7.

[8] Innovative exploration of additive incorporated biopolymer-based composites | Elsevier Enhanced Reader, (n.d.). https://doi.org/10.1016/j.sciaf.2022.e01359.

[9] A. Das, T. Ringu, S. Ghosh, N. Pramanik, A comprehensive review on recent advances in preparation, physicochemical characterization, and bioengineering

applications of biopolymers, Polym. Bull. Berl. Ger. (2022) 1–66. https://doi.org/10.1007/s00289-022-04443-4.

[10] H. Wang, Biomaterials in Medical Applications, Polymers. 15 (2023) 847. https://doi.org/10.3390/polym15040847.

[11] R. Gheorghita, L. Anchidin-Norocel, R. Filip, M. Dimian, M. Covasa, Applications of Biopolymers for Drugs and Probiotics Delivery, Polymers. 13 (2021) 2729. https://doi.org/10.3390/polym13162729.

[12] P. Zarrintaj, F. Seidi, M. Youssefi Azarfam, M. Khodadadi Yazdi, A. Erfani, M. Barani, N.P.S. Chauhan, N. Rabiee, T. Kuang, J. Kucinska-Lipka, M.R. Saeb, M. Mozafari, Biopolymer-based composites for tissue engineering applications: A basis for future opportunities, Compos. Part B Eng. 258 (2023) 110701. https://doi.org/10.1016/j.compositesb.2023.110701.

[13] I. Gardikiotis, F.-D. Cojocaru, C.-T. Mihai, V. Balan, G. Dodi, Borrowing the Features of Biopolymers for Emerging Wound Healing Dressings: A Review, Int. J. Mol. Sci. 23 (2022) 8778. https://doi.org/10.3390/ijms23158778.

[14] R. Rebelo, M. Fernandes, R. Fangueiro, Biopolymers in Medical Implants: A Brief Review, Procedia Eng. 200 (2017) 236–243. https://doi.org/10.1016/j.proeng.2017.07.034.

[15] D. Elieh-Ali-Komi, M.R. Hamblin, Chitin and Chitosan: Production and Application of Versatile Biomedical Nanomaterials, Int. J. Adv. Res. 4 (2016) 411–427.

[16] M.-K. Jang, B.-G. Kong, Y.-I. Jeong, C.H. Lee, J.-W. Nah, Physicochemical characterization of α-chitin, β-chitin, and γ-chitin separated from natural resources, J. Polym. Sci. Part Polym. Chem. 42 (2004) 3423–3432. https://doi.org/10.1002/pola.20176.

[17] M.C. Biswas, B. Jony, P.K. Nandy, R.A. Chowdhury, S. Halder, D. Kumar, S. Ramakrishna, M. Hassan, M.A. Ahsan, M.E. Hoque, M.A. Imam, Recent Advancement of Biopolymers and Their Potential Biomedical Applications, J. Polym. Environ. 30 (2022) 51–74. https://doi.org/10.1007/s10924-021-02199-y.

[18] M. Rinaudo, Chitin and chitosan: Properties and applications, Prog. Polym. Sci. 31 (2006) 603–632. https://doi.org/10.1016/j.progpolymsci.2006.06.001.

[19] M.R. Kasaai, Various methods for determination of the degree of N-acetylation of chitin and chitosan: a review, J. Agric. Food Chem. 57 (2009) 1667–1676. https://doi.org/10.1021/jf803001m.

[20] H.K. No, S.P. Meyers, Preparation and Characterization of Chitin and Chitosan—A Review, J. Aquat. Food Prod. Technol. 4 (1995) 27–52. https://doi.org/10.1300/J030v04n02_03.

[21] M.N.V. Ravi Kumar, A review of chitin and chitosan applications, React. Funct. Polym. 46 (2000) 1–27. https://doi.org/10.1016/S1381-5148(00)00038-9.

[22] B. Niekraszewicz, A. Niekraszewicz, The structure of alginate, chitin and chitosan fibres, in: Handb. Text. Fibre Struct., Elsevier, 2009: pp. 266–304. https://doi.org/10.1533/9781845697310.2.266.

[23] Chitin and chitosan from the Norway lobster by-products: Antimicrobial and anti-proliferative activities - PubMed, (n.d.). https://pubmed.ncbi.nlm.nih.gov/26920243/ (accessed April 18, 2023).

[24] G.A.F. Roberts, Chitin Chemistry, Macmillan Education UK, London, 1992. https://doi.org/10.1007/978-1-349-11545-7.

[25] B. Arora, R. Tandon, P. Attri, R. Bhatia, Chemical Crosslinking: Role in Protein and Peptide Science, Curr. Protein Pept. Sci. 18 (2017). https://doi.org/10.2174/1389203717666160724202806.

[26] Woźniak A, Biernat M. Methods for crosslinking and stabilization of chitosan structures for potential medical applications. Journal of Bioactive and Compatible Polymers. 2022;37(3):151-167. doi:10.1177/08839115221085738, (n.d.).

[27] M.M. Beppu, R.S. Vieira, C.G. Aimoli, C.C. Santana, Crosslinking of chitosan membranes using glutaraldehyde: Effect on ion permeability and water absorption, J. Membr. Sci. 301 (2007) 126–130. https://doi.org/10.1016/j.memsci.2007.06.015.

[28] Y. Yang, G. Chen, P. Murray, H. Zhang, Porous chitosan by crosslinking with tricarboxylic acid and tuneable release, SN Appl. Sci. 2 (2020) 435. https://doi.org/10.1007/s42452-020-2252-z.

[29] R.A.A. Muzzarelli, M. El Mehtedi, C. Bottegoni, A. Aquili, A. Gigante, Genipin-Crosslinked Chitosan Gels and Scaffolds for Tissue Engineering and Regeneration of Cartilage and Bone, Mar. Drugs. 13 (2015) 7314–7338. https://doi.org/10.3390/md13127068.

[30] Pati, B. Adhikari, S. Dhara, Development of chitosan–tripolyphosphate fibers through pH dependent ionotropic gelation, Carbohydr. Res. 346 (2011) 2582–2588. https://doi.org/10.1016/j.carres.2011.08.028.

[31] S. Jabeen, S. Saeed, A. Kausar, B. Muhammad, S. Gul, D.-M. Farooq, Influence of Chitosan and Epoxy Cross Linking on Physical Properties of Binary Blends, Int. J. Polym. Anal. Charact. 21 (2015). https://doi.org/10.1080/1023666X.2016.1131404.

[32] C.A. Vega-Cázarez, J. López-Cervantes, D.I. Sánchez-Machado, T.J. Madera-Santana, A. Soto-Cota, B. Ramírez-Wong, Preparation and Properties of Chitosan–PVA Fibers Produced by Wet Spinning, J. Polym. Environ. 26 (2018) 946–958. https://doi.org/10.1007/s10924-017-1003-8.

[33] B. Ozipek, H. Karakas, Wet spinning of synthetic polymer fibers, Adv. Filam. YARN Spinn. Text. Polym. (2014). https://doi.org/10.1533/9780857099174.2.174.

[34] Carboxylic acid | Structure, Properties, Formula, Uses, & Facts | Britannica, (2023). https://www.britannica.com/science/carboxylic-acid (accessed January 19, 2023).

[35] M.B. Smith, J. March, March's Advanced Organic Chemistry, John Wiley & Sons, Inc., Hoboken, NJ, USA, 2006. https://doi.org/10.1002/0470084960.

[36] C. Reichardt, Solvents and Solvent Effects in Organic Chemistry. Wiley-VCH, Weinheim/Germany, 2003, 2003.

[37] I. Kuznik, I. Kruppke, C. Cherif, Pure Chitosan-Based Fibers Manufactured by a Wet Spinning Lab-Scale Process Using Ionic Liquids, Polymers. 14 (2022) 477. https://doi.org/10.3390/polym14030477.

[38] G.C. East, Y. Qin, Wet spinning of chitosan and the acetylation of chitosan fibers, J. Appl. Polym. Sci. 50 (1993) 1773–1779. https://doi.org/10.1002/app.1993.070501013.

[39] PubChem, Citric Acid, (n.d.). https://pubchem.ncbi.nlm.nih.gov/compound/311 (accessed January 19, 2023).

[40] R. de C.A.L. Cruz, L.G.M. Diniz, H.M. Lisboa, M.V.L. Fook, Effect of different carboxylic acids as solvent on chitosan fibers production by wet spinning, Matér. Rio Jan. 21 (2016) 525–531. https://doi.org/10.1590/S1517-707620160002.0049.

[41] Lactic acid | chemical compound | Britannica, (n.d.). https://www.britannica.com/science/lactic-acid (accessed January 19, 2023).

[42] E. Abedi, S.M.B. Hashemi, Lactic acid production – producing microorganisms and substrates sources-state of art, Heliyon. 6 (2020) e04974. https://doi.org/10.1016/j.heliyon.2020.e04974.

[43] J. Liobikas, D. Majiene, S. Trumbeckaite, L. Kursvietiene, R. Masteikova, D.M. Kopustinskiene, A. Savickas, J. Bernatoniene, Uncoupling and Antioxidant Effects

of Ursolic Acid in Isolated Rat Heart Mitochondria, J. Nat. Prod. 74 (2011) 1640–1644. https://doi.org/10.1021/np200060p.

[44] M.D.L. e Silva, J.P. David, L.C.R.C. Silva, R.A.F. Santos, J.M. David, L.S. Lima, P.S. Reis, R. Fontana, Bioactive Oleanane, Lupane and Ursane Triterpene Acid Derivatives, Molecules. 17 (2012) 12197–12205. https://doi.org/10.3390/molecules171012197.

[45] K.I. Wolska, A.M. Grudniak, B. Fiecek, A. Kraczkiewicz-Dowjat, A. Kurek, Antibacterial activity of oleanolic and ursolic acids and their derivatives, Cent. Eur. J. Biol. 5 (2010) 543–553. https://doi.org/10.2478/s11535-010-0045-x.

[46] W.R. Cunha, G.X. de Matos, M.G.M. Souza, M.G. Tozatti, M.L. Andrade e Silva, C.H.G. Martins, R. da Silva, A.A. Da Silva Filho, Evaluation of the antibacterial activity of the methylene chloride extract of Miconia ligustroides, isolated triterpene acids, and ursolic acid derivatives, Pharm. Biol. 48 (2010) 166–169. https://doi.org/10.3109/13880200903062648.

[47] P. Do Nascimento, T. Lemos, A. Bizerra, Â. Arriaga, D. Ferreira, G. Santiago, R. Braz-Filho, J. Costa, Antibacterial and Antioxidant Activities of Ursolic Acid and Derivatives, Molecules. 19 (2014) 1317–1327. https://doi.org/10.3390/molecules19011317.

[48] K. Bamuamba, D.W. Gammon, P. Meyers, M.-G. Dijoux-Franca, G. Scott, Anti-mycobacterial activity of five plant species used as traditional medicines in the Western Cape Province (South Africa), J. Ethnopharmacol. 117 (2008) 385–390. https://doi.org/10.1016/j.jep.2008.02.007.

[49] Y. Kashiwada, T. Nagao, A. Hashimoto, Y. Ikeshiro, H. Okabe, L.M. Cosentino, K.H. Lee, Anti-AIDS agents 38. Anti-HIV activity of 3-O-acyl ursolic acid derivatives, J. Nat. Prod. 63 (2000) 1619–1622. https://doi.org/10.1021/np990633v.

[50] C. Ma, N. Nakamura, H. Miyashiro, M. Hattori, K. Shimotohno, Inhibitory effects of constituents from Cynomorium songaricum and related triterpene derivatives on HIV-1 protease, Chem. Pharm. Bull. (Tokyo). 47 (1999) 141–145. https://doi.org/10.1248/cpb.47.141.

[51] A review of natural and modified betulinic, ursolic and echinocystic acid derivatives as potential antitumor and anti-HIV agents - PubMed, (n.d.). https://pubmed.ncbi.nlm.nih.gov/12871156/ (accessed January 20, 2023).

[52] Anti-AIDS agents. 30. Anti-HIV activity of oleanolic acid, pomolic acid, and structurally related triterpenoids - PubMed, (n.d.). https://pubmed.ncbi.nlm.nih.gov/9748372/ (accessed January 20, 2023).

[53] H. Vázquez, C. Israel, Development and characterization of chitosan fibers made by wet spinning method, (2018). https://riunet.upv.es/handle/10251/101102 (accessed December 24, 2022).

[54] F. Eirich, Rheology V2: Theory and Applications, Elsevier, 2012.

[55] H. Hack, Viscosity, in: 2018: pp. 926–928. https://doi.org/10.1007/978-3-319-73568-9_308.

[56] R. Inc, Viscosity of Newtonian and Non-Newtonian Fluids, (n.d.). https://www.rheosense.com/applications/viscosity/newtonian-non-newtonian (accessed April 26, 2022).

[57] R.W. Hanks, On the Flow of Bingham Plastic Slurries in Pipes and Between Parallel Plates, Soc. Pet. Eng. J. 7 (1967) 342–346. https://doi.org/10.2118/1682-PA.

[58] A. Yahia, S. Mantellato, R.J. Flatt, Concrete rheology, in: Sci. Technol. Concr. Admix., Elsevier, 2016: pp. 97–127. https://doi.org/10.1016/B978-0-08-100693-1.00007-2.

[59] A. Abdullah, A. Mohammed, Scanning Electron Microscopy (SEM): A Review, in: Proc. 2018 Int. Conf. Hydraul. Pneum. - HERVEX, Băile Govora, Romania, 2019.

[60] J.C. Weaver, W. Mershon, M. Zadrazil, M. Kooser, D. Kisailus, Wide-field SEM of semiconducting minerals, Mater. Today. 13 (2010) 46–53. https://doi.org/10.1016/S1369-7021(10)70186-3.

[61] Handbook of Nonwoven Filter Media | ScienceDirect, (n.d.). https://www.sciencedirect.com/book/9781856174411/handbook-of-nonwoven-filter-media (accessed April 27, 2022).

[62] Comprehensive Composite Materials II, ScienceDirect. (n.d.). http://www.sciencedirect.com:5070/referencework/9780081005347/comprehensive-composite-materials-ii (accessed April 27, 2023).

[63] Yarn count - by Direct, indirect method, (n.d.). https://www.dspattextile.com/2021/05/yarn-count-by-direct-indirect-method.html (accessed April 29, 2023).

[64] Textile study center, Online Library for Textile Engineers, Yarn Numbering System, Text. Study Cent. (2015). https://textilestudycenter.com/yarn-numbering-system/ (accessed April 29, 2023).

[65] Standard Test Method for Yarn Number Based on Short-Length Specimens, (n.d.). https://www.astm.org/d1059-17.html (accessed February 10, 2023).

[66] ISO 2062:2009(en), Textiles — Yarns from packages — Determination of single-end breaking force and elongation at break using constant rate of extension (CRE) tester, (n.d.).

[67] H. Ouyang, K. Song, J. Du, I. Hussain, B. Tan, A Novel Amine-Impregnated Porous Organic Polymer with Chemisorption Sites for Highly Efficient Co2

Chemical Conversion Under Ambient Conditions, (2022). https://doi.org/10.2139/ssrn.4091643.

[68] M. Shabanian, M. Hajibeygi, A. Raeisi, 2 - FTIR characterization of layered double hydroxides and modified layered double hydroxides, in: S. Thomas, S. Daniel (Eds.), Layer. Double Hydroxide Polym. Nanocomposites, Woodhead Publishing, 2020: pp. 77–101. https://doi.org/10.1016/B978-0-08-101903-0.00002-7.

[69] U. Riaz, S. Ashraf, Characterization of Polymer Blends with FTIR Spectroscopy, in: Charact. Polym. Blends Miscibility Morphol. Interfaces, 2014: pp. 625–678. https://doi.org/10.1002/9783527645602.ch20.

[70] G. Shinde, R.K. Godage, R.S. Jadhav, B. Manoj, B. Aniket, A Review on Advances in UV Spectroscopy, Res. J. Sci. Technol. 12 (2020) 47. https://doi.org/10.5958/2349-2988.2020.00005.4.

[71] D. Patel, D. Panchal, K. Patel, M. Dalwadi, D.U. Upadhyay, A Review on UV Visible Spectroscopy, International Journal of Creative Research Thoughts (IJCRT). 10 (2022).

[72] M. Olszewska, Optimization and validation of an HPLC-UV method for analysis of corosolic, oleanolic, and ursolic acids in plant material: Application to *Prunus serotina* Ehrh, Acta Chromatogr. 20 (2008) 643–659. https://doi.org/10.1556/AChrom.20.2008.4.10.

[73] M.-C. Wei, Y.-C. Yang, Extraction characteristics and kinetic studies of oleanolic and ursolic acids from Hedyotis diffusa under ultrasound-assisted extraction conditions, Sep. Purif. Technol. 130 (2014) 182–192. https://doi.org/10.1016/j.seppur.2014.04.029.

[74] ISO 20743:2021(en), Textiles — Determination of antibacterial activity of textile products, (n.d.). https://www.iso.org/obp/ui/#iso:std:iso:20743:ed-3:v1:en.