

**EFFECT OF ALUMINUM OXIDE ON THE FUNCTIONAL PROPERTIES OF  
CHITOSAN FILMS**

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## **ABSTRACT**

### **EFFECT OF ALUMINUM OXIDE ON THE FUNCTIONAL PROPERTIES OF CHITOSAN FILMS**

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Chitosan is the one of the most abundant naturally occurring polymer in the world and has applications in a lot of different fields, including flexible packaging films. Aluminum oxide in the form of nanoparticle have properties which, when added to a polymer, can improve its properties significantly and increase its applications considerably. This research attempts to develop a biodegradable film with chitosan added with aluminum oxide and study its effect on the functional properties of the chitosan film. The functional properties taken into consideration were tensile strength, % elongation at break, water vapor permeability (WVP), oxygen permeability (OP), antimicrobial and thermal properties. There can be potential novel applications in packaging if these composite films have improved requisite properties.

The oxygen permeability of the films was not affected after adding aluminum oxide and decreased only by 6% after adding 2%  $\text{Al}_2\text{O}_3$ . On other hand, the water vapor permeability values increased significantly ( $p < 0.05$ ) by around 30% after adding 2%  $\text{Al}_2\text{O}_3$ , which is not desired. The tensile strength decreased from 40 MPa to 36 MPa and the % elongation at break reduced significantly ( $p < 0.05$ ) from 98% to 83%. There was no change observed in the thermal properties and antimicrobial properties of the films after the addition of aluminum oxide. These results suggest that the properties of this composite material formed using chitosan with aluminum oxide were not affected in some aspects such as tensile strength and oxygen barrier property; and additional work is required to conclude the hypothesis of generating a composite chitosan film with aluminum oxide as nanoparticles for improved properties.

Dedicated to my parents, Dr. Ashok and Padmavathi Konda,  
and sister, Keerthana Konda

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## **Chapter 1. Introduction**

There has been an increase in the demand for eco-friendly products leading to the development of biodegradable materials in the last few decades (Rendón-Villalobos et al., 2016). These Biodegradable materials are mainly derived from biopolymers including lipids, proteins, polysaccharides, potential alternatives to petroleum based non-biodegradable synthetic polymers. Though the properties of these bio-based polymers limit the applications, compared to that of conventional petroleum-based polymers, the market for bio-based polymers is reported to increase at a growth rate of 15.2% from 2019 to 2024 (Siracusa & Blanco, 2020). Pursuit to find appropriate biodegradable biopolymers having properties akin to those of petroleum-based polymers for packaging applications is still a necessity (Shen, 2009).

A polymer is defined by the International Union of Pure and Applied Chemistry (IUPAC) as a substance made of large molecules that is characterized by the multiple repetitions of one or more species of atoms or group of atoms (constitutional units) linked to each other covalently in amounts sufficient to provide a set of properties that do not vary markedly with the addition or removal of one or a few of the constitutional units (Selke & Culter, 2016).

Polymers can be classified into two groups: synthetic polymers and naturally occurring polymers. polyethylene, polypropylene, nylon are classified as synthetic polymers, mainly from petroleum-based monomers such as ethylene, propylene, etc. (Selke & Culter, 2016). Naturally occurring polymers (biopolymers) such as cellulose, natural rubber, chitin, and DNA are present in biological organisms such as plants, animals, bacteria, human beings, etc. They are usually formed by polysaccharides, polynucleotides and polypeptides (Uragami, 2018). Naturally occurring polymers are being used for centuries for various purposes, cellulose from cotton in the

textile and pharmaceutical industries for food thickening, garments manufacturing, Papyrus for paper and packaging, latex sap (rubber) for waterproofing cement (Lavanya et al., 2011; Saija, 1995; Priyadarshi & Rhim, 2020).

Synthetic plastics are usually derived from petrochemicals derived from petroleum. These plastics came into existence in 1950's and were used in a lot of applications because of their relatively low cost, flexibility, tensile and barrier properties, transparency, moldability, heat sealability just to name a few applications (Priyadarshi & Rhim, 2020). A wide variety of plastics are available with changes in the main chains or the side chains. Polyethylene, polypropylene, polyacrylates and polystyrene are some of the plastics having carbon-carbon backbone bonds. Polyamides, polyesters, polycarbonates and polysulfides are hetero chain polymers which have other elements such as oxygen, nitrogen and sulfur in their backbone, along with carbon and hydrogen.

Composites can be formed to improve properties that cannot be obtained by using only one polymer for example, kevlar when combined with date palm fibers form a composite with excellent mechanical properties and can be used in aerospace, electronics and automotive industry (Muthalagu et al., 2020). Regardless of their advantages and applications in the industry, synthetic plastics of petroleum origin are non-biodegradable and cause serious ecological problems due to the generation and accumulation of compostable waste (Rendón-Villalobos et al., 2016). Only 14.6% of plastic generated is recycled every year, and the rest ends up in landfills or in oceans (Neufeld, 2016).

Polymers from biological origin are known and described as biopolymers or bio-based polymers. They are classified into three different types: natural, synthetic and microbial (Rhim et al., 2013). Naturally occurring polymers are collagen, soy protein, and polysaccharides, cellulose,

chitin, and starch (Rhim et al., 2013). Polylactic acid (PLA), a biomass-based polymer and Polyvinyl alcohol are synthetic biopolymers. Pullulan is reported to be a carbohydrate with microbial properties type biopolymer (Smith, 2005). Biopolymer's biodegradability, availability from renewable resources, environment-friendly properties have increased their applications in packaging, energy, medical, devices etc. (Mohiuddin et al., 2017; Ibrahim et al., 2019).

Cellulose is the most abundant biopolymers on earth. Cellulose is forty to fifty percent of plant tissues (Alberts et al., 2002). The high degree of polymerization and hydrogen bonding made by glucose molecules in cellulose provides strength and rigidity to plants (Basu, 2010). Cellulose melting point ranges between 260 – 270°C (“Cellulose”, 2020), is not soluble in water and is biodegradable in nature. The molecular weight (MW) of cellulose is about between 20,000 – 100,000 and has a density of 1.5 g/cm<sup>3</sup> (Kraemer & Lansig, 2002; “Cellulose”, 2020). It is used for making flexible films and is used in food packaging for its flexibility, strength, availability, relatively low cost and biological degradability.

Chitin is one of the most abundant biopolymer on earth and is present in shells of lobsters, shrimps, crabs, and in cell walls of fungi and yeast (Azuma et al., 2015). Chitosan is deacetylated derivative of chitin. Antimicrobial properties, film-forming capability, viscosity make it a material source in various applications in the field of packaging to substitute petroleum non-biodegradable packaging (Priyadarshi & Rhim, 2020).

Biodegradation is often defined as activities of breakdown, reduction and deterioration to promote the release of carbon dioxide initiated by biological agents such as bacteria, fungi and insects present in the surrounding environment. Various factors, including material, temperature, air quality, pH constitute are contributing and limiting factors in the biodegradation processes.

Biobased plastics and biodegradable plastics are two different things. The process of biodegradation changes the chemical structure of the material (Bandyopadhyay-Ghosh et al., 2015). Biodegradation is a degradation mostly done by biological means such as bacteria and fungi. Biobased plastics are plastics obtained from biomass. Synthetic fossil-based plastics can be bio-degradable and biobased plastics can be non-biodegradable depending on their chemical structure. For example, 100% fossil-based plastics such as PBAT (polybutylene adipate terephthalate) can be biodegradable and 100% biobased plastics such as bio-polyethylene or Green PE can be non-biodegradable.

The marine environment constitutes almost 70% of the world's biodiversity or the Earth's surface is covered with water in form of oceans and seas (Muxika et al., 2017). Chitin is a polysaccharide which is obtained from crustaceans, insects, fungi and is the second most abundant natural polymer after cellulose in the world (Silva et al., 2012). It is a major structural component in the exoskeleton of a lot of marine invertebrates (Khan et al., 2017; Azuma et al., 2015). Chitin is usually extracted from marine shells using chemical methods. The process involves three main steps which are deproteinization of raw material using an alkaline solution (NaOH), followed by demineralization of the obtained product by treatment with an acidic solution (HCl) and last step is the discoloration of the product by another treatment with an alkaline solution containing chlorine for bleaching (NaOCl) (Leceta et al., 2014; Philibert et al., 2017; Bemiller, 1965).

Chitin and its derivative chitosan have many functional properties such biodegradability, non-toxicity, biocompatibility, have good tensile strength (47 MPa), acceptable structural integrity and adsorption properties and they are renewable (Tang et al., 2003; Reddy et al., 2013; Muxika et al., 2017; Elieh-Ali-Komi & Hamblin, 2016). Chitosan and chitin are easy to modify to obtain desirable properties and function for usage in different applications. According to Philibert et al.

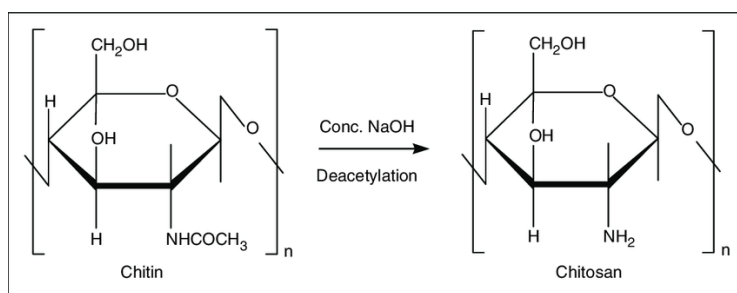
(2017), “The water-insoluble property of chitin is an add-on to all the properties and is probably due to the intra- and intermolecular bonding of hydrogen with -OH and -NH functional groups. These bonds are formed by oxygen and nitrogen atoms of acetamido group, and the degree of N-acetylation has an adverse effect on insolubility and may limit swelling properties of chitin in water as compared to cellulose”. However, chitin has a limited number of applications because of poor solubility in dilute acids. On the other hand, chitosan is a deacetylated form of chitin is readily soluble in dilute acids (Elieh-Ali-Komi & Hamblin, 2016). Chitin can be converted into chitosan through chemical and enzymatic processes. It is described that the chemical method is preferred for large-scale conversion due to the relatively low cost and high production rates (Younes & Rinaudo, 2015). Chitin and its derivatives have applications in food such as dietary food products for the reduction of weight and body (Mhurchu et al., 2004), biomedical as a wound healing, tissue engineering and drug delivery (Dash et al., 2011). It is used in agricultural as bactericide for crops because of its antimicrobial activity and reducing soil-borne diseases (Das et al., 2015), in packaging as edible coatings and film making (thickness < 30µm) for applications in food packaging (van den Broek et al., 2015) and other industries due to their multi-functional properties.

Deacetylated chitin is known as chitosan formed by d-glucosamine and N-acetyl-d glucosamine units, linked by -1,4 glycosidic linkages (Muxika et al., 2017; Narayanaswamy et al., 2016). Chitosan is described as soluble in dilute acids like formic acid, acetic acid, etc. (Yeul & Rayalu, 2013; Kumar, 2000). Deacetylation process involves the removal of acetyl groups from chitin to form chitosan. Chitosan with different degrees of deacetylation is obtained and qualified by the process of N-deacetylation (Priyadarshi & Rhim, 2020).

Chitosan is currently used in various applications including biomedical, agriculture, and packaging. In packaging it is suggested to be used for its film-forming properties, biodegradability,

biocompatibility, low toxicity to humans and antimicrobial properties (Dutta et al., 2009). In medical field, chitosan films are used for wound dressing due to its good tissue regrowth activity and its ability for absorbing extrudates (Alsarra, 2009).

The interaction of food products with water vapor or oxygen lead to some deterioration (Srinivasa et al., 2002). It is also reported that chitosan has mechanical and barrier properties comparable to some commercial polymers such as LDPE and PVDC (Butler et al., 1996). Mean tensile strength of chitosan films made with a plasticizer (glycerol) was reported to be  $22.9 \pm 8.8$  MPa as reported by Butler et al. (1996) which is comparable to LDPE film with a thickness of 34.5 microns and a tensile strength in the range of 10.3 to 18.0 MPa (“Overview of materials for Low-Density Polyethylene (LDPE), Blow Molding Grade”, n.d.). Chitosan film also acts as a good barrier to oxygen with an oxygen permeability coefficient of  $4.7 \times 10^{-5} \pm 3.4 \times 10^{-5}$  cc/m.day.atm (Butler et al., 1996) when compared to low-density polyethylene film with oxygen permeability of 44.756 cc.m/ m<sup>2</sup>/day/Pa (Bastarrachea et al., 2011; Suyatma et al., 2004). Unfortunately, one of the major drawbacks of chitosan is its hydrophilic property with a substantive high values of water vapor transmission rate (Wiles et al., 2000). According to Wiles et al. (2000), WVTR of chitosan films at 84% RH was close to be 984 g/m<sup>2</sup>/day. To overcome the moisture affinity of chitosan, it is suggested to add moisture resistant additives that can improve the water and gases (O<sub>2</sub> and CO<sub>2</sub>) resistance barrier properties.



**Figure 1.1 Deacetylation of chitin to form chitosan**

According to Jambeck et al. (2015), about 448 million tons of plastics were produced in 2015 and around 161 million tons were used as packaging material. The amount of recycled plastics that originated from petroleum in 2015 was 23.5 million, of which 15% was used to produce packaging material (Neufeld, 2016). The remaining 85% plastic was discarded and ended up in landfills and in oceans. The time it takes for the degradation of plastics is unknown and a lot of studies are currently going on to understand the degradation rates for these synthetic polymers (Chamas et al., 2020). Increased use of synthetic polymers day after day is creating more and more of polymeric debris and burdening our environmental ecosystem. It is reported that 270,000 tons of plastic waste is present in oceanic ecosystem, having an adverse negative effect on the life of around 700 marine species (Gall & Thompson, 2015). Several polymers are not biodegradable, as a result, a serious threat is posed to the digestive tract of animals. Eating plastic by animals causes serious health issues, because of slow poisoning, caused by chemicals leaching from plastics as reported earlier (Rustagi et al., 2011).

With the increase awareness of the environmental issues around the world for non or slow biodegradable synthetic plastics used in packaging, a lot of research is being done on the use of biodegradable plastics. Various types of additives are used to improve some properties. Metal oxides are often used to increase mechanical strength and barrier properties against water vapor and oxygen (Garcia et al., 2018). These metal oxides, when used in nano-form exhibit better properties than their bulk counterparts due to small size and increased surface area (Andrievski & Glezer, 2001). These nanomaterials added in the polymeric material are in the form of nanoclays, oxides, or in its elemental form. They can be applied as a coating or be incorporated in the matrix of the polymer. Mostly used metal oxide nanomaterials include titanium oxide, copper oxide, zinc oxide, silver nitrate and aluminum oxide.

Aluminum is the most common metal used in packaging for its good mechanical properties and barrier against water vapor, oxygen and other gases, lightweight, antimicrobial properties, etc. (Anyadike, 2002). Aluminum oxide films are transparent and, when added to packaging material, improves the properties, without changing the transparency of the material.

The main aim of this research was to test the hypothesis that chitosan films with incorporated aluminum oxide in the form of nanomaterial will improve the mechanical and barrier properties of the film as compared to the films without aluminum oxide. Aluminum oxide is added in different concentrations: 0.5%, 1%, 1.5% and 2% in an attempt to identify the optimal quantity needed to improve the properties of the chitosan film. After the development of the films with varying concentrations of aluminum oxide.

### **Objectives**

- Comparing mechanical strength of chitosan films with and without aluminum oxide.
- Evaluating thermal properties of the films with and without aluminum oxide.
- Studying changes in the oxygen permeability of the films after addition of aluminum oxide.
- Determining potential changes in the water vapor permeability of the films after addition of aluminum oxide.



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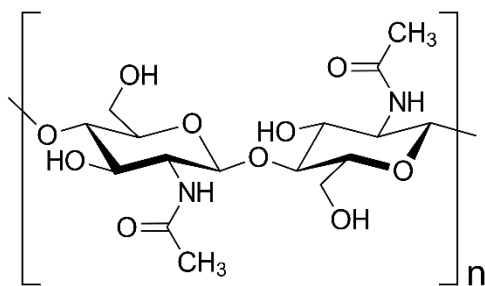
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## **Chapter 2. Literature Review**

### **2.1 Chitin:**

Chitin is the second most abundant natural polymer in the world. It is a nitrogenous polysaccharide found in exoskeleton of crustaceans such as shrimps, lobsters and crabs. It is also found on the cell walls of fungi or mushrooms and the exoskeleton of arthropods. It is easily found in nature and is the by-product of marine animals and (Al-Hassan, 2016). Being produced at a large scale every year, it is available at a low cost from marine resources (Muzzarelli, 1977). Chitin is the main component of organisms' exoskeleton systems. It has a crystalline structure with fibers arranged in an organized manner comprising of unbranched chains of N-acetylglucosamine. They are basically chaining of glucosamine with acetyl groups attached to the different glucosamine molecules (Figure 2.1). The intermolecular hydrogen bonding makes it difficult to dissolve in water (Minke & Blackwell, 1978). The strength and rigidity are due to its crystalline structure which acts as a protection to the organisms that have it (Roberts, 1992). It is insoluble in water, dilute acids and solvents (Muzzarelli, 1977). Concentrated acids such as HCl, H<sub>2</sub>SO<sub>4</sub> can dissolve chitin (Deshpande, 1986).



**Figure 2.1 Chitin molecule**

There are three main types of chitin:  $\alpha$ -chitin,  $\beta$ -chitin and  $\gamma$ -chitin. Chitin obtained from crabs and shrimps fall under the category of  $\alpha$ -chitin and are mostly used in the industry. The polymeric chains are arranged in a non-parallel assembly and have strong hydrogen bonds making them stable (Sikorski et al., 2009).  $\beta$ -chitin is obtained from squid, which is a mollusk and cuttlebone of cuttlefish. It is commercially available in the market similar to  $\alpha$ -chitin, and the polymeric strands are arranged in parallel form. The third type,  $\gamma$ -chitin is found in fungi and has both parallel and non-parallel polysaccharide strands (Kaya et al., 2017). The properties, crystallinity, chain arrangement and purity are affected by the type of chitin and its source (Rinaudo, 2006). Because of poor solubility in solvents and water, chitosan, which is deacetylated form of chitin is used, which is available in various forms such as powders, flakes, solutions, fibers and films and has applications in the field of agriculture, medical, packaging, etc.

## 2.2 Chitosan:

Chitosan is deacetylated form of chitin which is soluble in dilute acids such as acetic acid, formic acid, and nitric acid. The presence of free amine groups in the polymeric chain form an active site for many chemical reactions. The chitosan molecule is basically made up of Carbon (44.11%), Nitrogen (7.97%) and Hydrogen (6.84%) (Tian et al., 2004). The formation of chitosan, from the exoskeleton system of shrimps, crabs, fungi, etc. takes place in four steps involving: 1. deproteinization, 2. demineralization, 3 decolorization and 4. deacetylation.

### 2.2.1 Deproteinization:

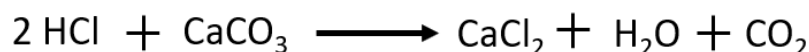
Chitin is present in the shells of crustacean animals along with proteins. These proteins are covalently bonded to the chitin molecule. Some of the proteins can be easily extracted but this is not the case for some of them and needs some treatment to break the bonds (Attwood & Zola,



1967). The proteins are bonded to chitin using aspartyl or histidyl residues which are very stable bonds (Yeul & Rayalu, 2013). The shell obtained from animals is ground and treated with deproteinization reagents such as NaOH, KOH, Na<sub>2</sub>CO<sub>3</sub>, Na<sub>2</sub>S, etc. NaOH is most widely used in a low concentration in the range of 1-10% at temperatures varying from 65°C to 160°C for a period of time depending on the process which can take few minutes or a few days. The use of NaOH can also lead to a certain range of deacetylation and hydrolysis of the chitin reducing its molecular weight (Younes & Rinaudo, 2015). The rate of deproteinization can be controlled by using dilute concentrations of KOH solution (Shahidi & Synowiecki, 1991).

#### 2.2.2 Demineralization:

This step involves the removal of minerals such as calcium carbonate. HCl, H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub>, CH<sub>3</sub>COOH (No & Hur, 1998) or similar acids are used in dilute concentrations at room temperature to decompose calcium carbonate into other calcium salts, soluble in water along with the release of carbon dioxide as shown below with HCl in Figure 2.2 (Younes & Rinaudo, 2015).



**Figure 2.2 Demineralization of calcium carbonate**

Formic acid (HCOOH) at 90% concentrations can also be used for demineralization (Yeul & Rayalu, 2013). The salts produced in this formic acid process can be easily removed by filtration of solid mass and washing it with deionized water. This treatment depends on various parameters such as temperature, particle size, extraction time, acid concentration and solute/solvent ratio. Larger volume of acid solution can also be used as two molecules of HCl are required to convert CaCO<sub>3</sub> to CaCl<sub>2</sub> (Shahidi & Synowiecki, 1991). The treatment with HCl can take from 15 minutes

to two days depending on the preparation method followed. Longer duration of treatment can also result in polymer degradation (Okafor, 1965).

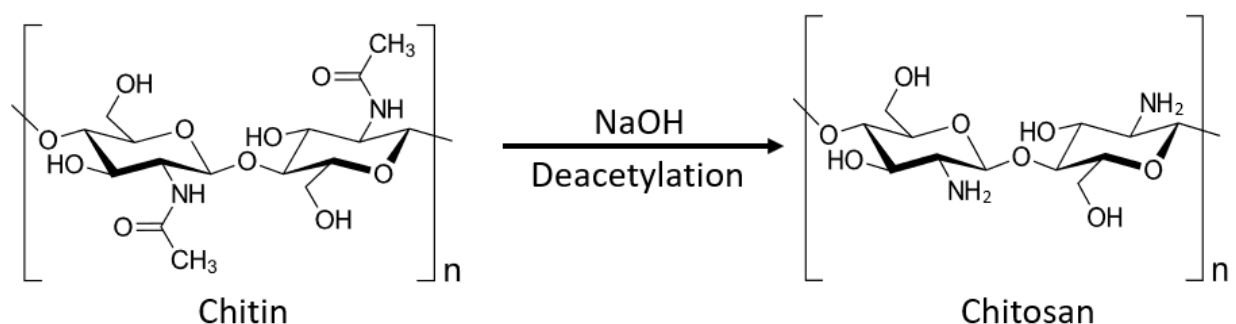
### 2.2.3 Decoloration:

Once all the unwanted proteins and minerals are removed from the chitin by chemical treatments, the color of chitin changes due to the effect of alkalis and acids used. This colorful entities generated needs to be reduced to cream white powder to be accepted commercially. Some pigments present in an organism are chemically bonded to the chitin, which varies from species to species (Yeul & Rayalu, 2013). The chemicals used for decolorization should not alter the properties of the chitin. The process involves extraction by acetone for a few hours at room temperature and then bleaching by using a low concentrated solvent such as sodium hypochlorite in a ratio of 1:10 (solid: solvent) for a few minutes (No et al., 1989). The color of the chitin at the end of the process can vary from cream white to slightly pink. Another process described by Majekodunmi (2016), involves the treatment of deproteinized and demineralized chitin by soaking in a dilute oxalic acid solution for a few minutes to a few hours, which results in decolorization of chitin.

### 2.2.4 Deacetylation:

The last step in the process of conversion of chitin to chitosan is the removal of the acetyl groups from chitin (Figure 2.3). This is achieved by treating the chitin with concentrated potassium or sodium hydroxide solution with a concentration of around 50% for half an hour or more at a temperature of 100°C. The duration of the treatment depends on the number of acetyl groups to be removed from the chitin. The process of deacetylation can be performed in two ways: homogeneous or heterogeneous method. Chitosan produced using homogeneous methods have

better distribution of acetyl groups on the polymeric chain of chitosan (Sannan et al., 1976). And the chitosan produced using heterogeneous method does not have a uniform distribution of acetyl groups on the polymeric chains (Aiba, 1991). The change in the arrangement of the acetyl groups on the polymeric chains alter the physiochemical properties of chitosan and the solubility in aqueous solutions (Younes & Rinaudo, 2015). Along with these properties, the method of preparation can be used to change the parameters such as molecular weight(MW), degree of acetylation and viscosity in solution (Berger et al., 2005 ; Huei & Hwa, 1996).



**Figure 2.3 Deacetylation of Chitin to Chitosan**

## 2.3 Characteristics of Chitosan:

### 2.3.1 Molecular weight (MW):

The molecular weight of chitosan depends on the preparation method and the source of raw material. The temperature, chemicals, shear stress acted on the chitosan during its production can result in a change of the molecular weight. The MW of chitin is around one million Daltons, whereas the MW of chitosan can be over a wide range varying from 100,000 to 1,200,000 Daltons (Li et al., 1992).

### 2.3.2 Color and form:

Chitosan is usually available in off white color and can be powdery or flaky in appearance.

### 2.3.3 Bulk density:

The bulk density of chitin from crustaceans is around 60 to 170 kg/m<sup>3</sup>. The bulk density of chitosan was reported to be between 200 to 380 kg/m<sup>3</sup>, according to Cho et al., (1998).

### 2.3.4 Chemical reactivity:

The presence of hydroxyl (-OH) and amino (-NH<sub>2</sub>) groups on the chitosan monomer makes it very reactive. The reaction of these groups to various chemicals modifies the mechanical and physiochemical properties. The amine functionality of chitosan reacts with alkyl halides and acid chlorides for alkylation and acylation. Reaction on the hydroxyl groups of chitosan results in the formation of ethers or esters, respectively (Yeul & Rayalu, 2013). The presence of these hydroxyl and amino groups in chitosan form hydrogen bonds with the fiber surfaces of the paper. For this reason, chitosan is used as a coating on paper for increasing its strength and barrier properties (Song et al., 2018).

### 2.3.5 Solubility:

Unlike chitin which is insoluble in solvents, acids and bases, chitosan is soluble in dilute acidic solutions such as acetic acid, formic acid and lactic acid (concentration 1-2%). But chitosan does not dissolve easily in inorganic acids. Chitosan degrades before melting and thus needs to be dissolved in a solution for optimization of its properties (Kumar, 2000). Acetic acid is the most commonly used acidic solution (Kamdern et al 2015), though high concentration usage of these acids at a high temperature can depolymerize the chitosan as reported by Roberts & Domszy,

(1982). The properties of chitosan vary according to the ratio of acid to chitosan used. The solubility of chitosan depends on various factors such as the method of preparation used to obtain chitosan, the duration time and the temperature of deacetylation, the particle size and the degree of deacetylation.

#### 2.3.6 Antimicrobial Properties:

Chitosan has been reported as a good antimicrobial material against some organisms including bacteria, fungi, algae and yeasts (Tsai & Su, 1999; Kong et al., 2010; Atay, 2019). The chitosan attaches to the negatively charged surface of the cell resulting in disruption of the cell membrane, which causes the leakage of the intracellular components killing the organism. The factors that affect the antimicrobial property of chitosan are the pH, the physical state of chitosan, the types of microorganisms, the concentrations and the molecular weights of chitosan, the storage time and temperatures (Zivanovic et al., 2004; No et al., 2006); Atay, 2019).

#### 2.3.7 Ion Bonding:

According to various studies, chitosan can easily bond or interact with various metal ions including  $\text{Hg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ , etc. (Priyadarshi & Rhim, 2020). Vold et. (2003) reported that  $\text{Cu}^{2+}$  binds to chitosan better than other metallic ions. The total number of applications of chitosan increase after bonding with metallic nanoparticles (Priyadarshi & Negi, 2017).

#### 2.4 Applications of chitosan:

The properties of chitosan and the ability to bond to various substances has resulted numerous applications of chitosan. Chitosan is used in Medical, cosmetics, water treatment, pulp

and paper making, agriculture, biotechnology, packaging, and food industry (Priyadarshi & Rhim, 2020; Li et al., 1992).

As of today, medical field has the maximum number of applications for chitosan. It is used in tissue engineering, wound healing, cancer diagnosis, dressings, ophthalmology, tumor inhibition, and bone disease treatment (Namita et al., 1988; Sirica & Woodman, 1971; Eliah & Hamblim, 2016). It is used for filtration and removal of metal ions in during water treatment (Tsezos, 1983; Hirano et al., 1980).

In the paper and pulp industry, it is used as a coating to improve the burst strength and folding endurance of paper and paperboards, and to increase the antistatic property of paper when a chitosan layer is applied on a photographic paper (Li et al., 1992). Chitosan is used fin the manufacturing of nail polish, moisturizers, bath lotion, toothpaste, etc. (Bikales et al., 1985; Biagini et al., 1989). Chitosan is used as a coating on leaves and seeds (Hirano, 1999; Pospieszny & Atabekov, 1989) to take advantage of its antimicrobial properties. The coagulating and chelating properties of chitosan has resulted in considerable uses in food applications, and it is also used for removing dyes and solids from orange juice (Seo et al., 1988) and to subtract acid substances from apple juice(Imeri & Knorr, 1988). The bio-based polymer chitosan has a great potential for applications in packaging due to its antimicrobial properties, film-forming capability, mechanical strength and thermal stability (Priyadarshi & Rhim, 2020).

### 2.5 Chitosan-based films:

Chitosan is used in various forms according to the application. They are used as coatings or as films made purely of chitosan or blended with other polymers for improved properties. The

processes used for making these films or coatings are direct casting, extrusion, coating, and layer-by-layer assembly.

#### 2.5.1 Direct Casting:

Solution casting, solvent casting, film casting, or direct casting are different names used in the industry for the same process. It is the most common methods used for producing biodegradable films on a small scale. The process involves the following steps: dissolving chitosan in an acidic solution with pH values less than 6 by stirring to form a viscous solution. The next step is addition of plasticizers, cross likers or other functional compounds for specific properties, followed by stirring to obtain a homogeneous solution, then, filtering the solution to remove any undissolved particles and air bubbles, pouring the solution in petri dishes or any flat surface to obtain desired thickness, shape and size of the film, letting it dry at a controlled temperature and relative humidity for an assumed time and the last step is peeling off the films of the surface and storing them for further investigation (Priyadarshi & Rhim, 2020; Sakurai et al., 1985). Films produced with this method have desired properties and advantages such as low cost, mold simplicity and can be easily made in small batches (Ashter, 2014). Despite all the advantages, there are a few disadvantages associated with it, small changes in the concentration of the constituents during formulation can affect the properties of the film and a lot of care must be taken while making films using the solution casting method and shrinkage of films after drying can be observed (Priyadarshi & Rhim, 2020; Ashter, 2014). This process can be used on a small scale as it takes a long time to prepare the films and is uneconomical (Mujtaba et al., 2019).

### 2.5.2 Extrusion:

Chitosan unlike other polymers such as PE or PP cannot be processed in an extruder and thus cannot be directly used in extrusion. Chitosan is mixed and blended with other materials and extruded to form pellets which are dried. These pellets are converted into films or sheets using an extruder with a cast film flat die or a blown film die (Goskonda & Upadrashta, 1993). The properties of the film with chitosan blended with other polymers formed using the extrusion process showed good mechanical and thermal properties (Martinez-Camacho et al., 2013). In a study done by Wang et al. (2015), chitosan was blended with linearly low density polyethylene (LLDPE) and films were made using blown film extrusion. It was observed that the increasing the content of chitosan decreased the tensile strength and % elongation of the films, whereas the water vapor permeability increased. Extrusion is a good process for obtaining good tensile, thermal and barrier properties as compared to direct casting but the properties can vary if the processing conditions are not controlled and if there is no interaction between the polymer and the additives (Alix et al., 2013; Lago et al. 2014).

### 2.5.3 Coating:

Coating is applied on various food materials such as fruits, vegetables, meat products or is directly applied to the packaging material (El Ghaouth et al., 1991; Khwaldia et al., 2014). The coating is done on a product to protect it from environmental factors such as microbes and improves the gas permeation properties of the product, increasing the shelf life and respiration rate. Chitosan being biodegradable, environmentally friendly, biocompatible and edible can be applied on plant products (Priyadarshi & Rhim, 2020). Coating on seeds increases its antimicrobial



property according to a few studies (Hirano, 1999). Coating can be applied using three different methods: spray coating, dip coating and spread coating.

#### 2.5.4 Spray coating:

Spraying is done using various spraying tools. Along with spray coating another treatment such as heat or gamma irradiation is used for increasing the safety and reduction of the microbial population (Severino et al., 2015; Ban et al., 2015).

#### 2.5.5 Dip Coating:

The product is dipped into a chitosan solution to get a uniform coating all over the surface. This process involves the following steps: (1) preparation of chitosan solution with additives for the desired properties (2) processing the food product to be coated to remove any dirt or microorganisms present by washing, pasteurization, etc. (3) dipping the product in the chitosan solution for a certain time, removing the extra solution (4) drying the food product in a sterile setting followed by packing and storing (Priyadarshi & Rhim, 2020). The coating on the film depends on the surface tension of the surface, dipping time and drying time.

#### 2.5.6 Spread coating:

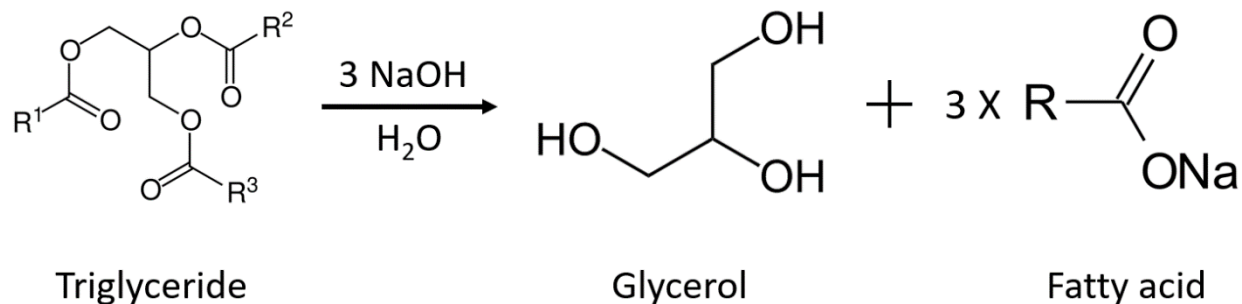
In this process, the food product is coated using a brush to spread the coating uniformly. In some cases, the solution is coated on the packaging material and not on the food product. This process for spread coating involves (1) preparation of chitosan solution with additives for the desired properties (2) processing the food product to be coated (3) spreading the coating uniformly on the food product using a brush (4) drying the coated material in a sterile environment followed by packing and storing (Priyadarshi & Rhim, 2020).

## 2.6 Plasticizers:

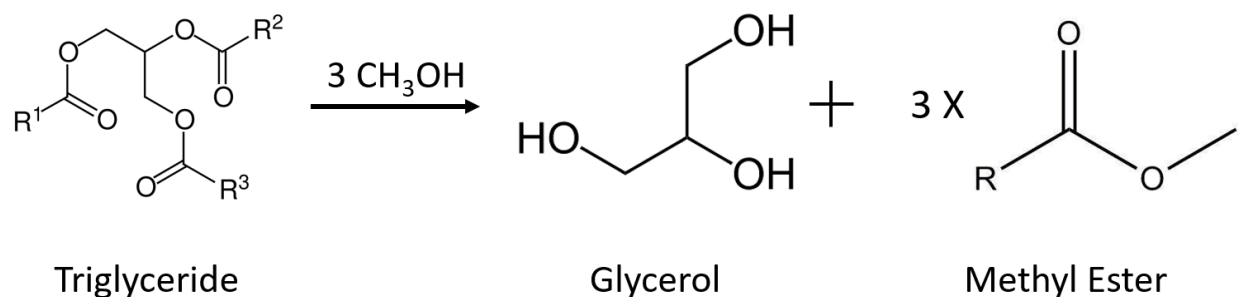
Plasticizers are substances added to a polymer to improve its flexibility and plasticity in the polymer structure by reducing the intermolecular forces of attraction between the molecules and the glass transition temperature of the material thus, enabling the polymeric chains to move freely (Bilck et al., 2015). For improving the mechanical properties, various additives are added which increase the flexibility or plasticity of polymers and help in facilitating polymer processing. Treatment with plasticizers like sugars (glucose and sucrose), polyols (glycerol, sorbitol, and polyethylene glycol) and lipids can improve the mechanical properties of the film (Zhang et al., 2002; Caner et al., 1998; Butler et al., 1996; Bakry et al., 2017).

### 2.6.1 Glycerol:

Glycerol is an odorless, non-toxic, colorless, sweet-tasting, and viscous liquid having desired chemical and physical properties. It is an oleochemical originated from fats and oils derived from vegetables and/or animals (Salimon et al., 2012). Glycerol has a melting point of 18.2 °C and boils at 290 °C. It is denser than water with a density of 1.261 g/cm<sup>3</sup> and is completely miscible in water. The three hydroxyl groups in the structure of glycerol make it reactive to water. Being an oleochemical, it is present in both animal and vegetable sources in the form of triglycerides or esters of glycerol with long chains of carboxylic acids. Figure 2.4 shows the process of hydrolysis using an alkali (sodium hydroxide) as a catalyst resulting in the formation of glycerol and fatty acids. Another process for producing glycerol is by transesterification process as shown in Figure 2.5, which uses methanol as a catalyst to produce glycerol and methyl ester (Pagliaro, 2017).



**Figure 2.4 Hydrolysis of triglyceride with sodium hydroxide**



**Figure 2.5 Transesterification of triglyceride with methanol**

Most of the molecules are connected by hydrogen bonds in glycerol leading to its high boiling point and viscosity. It is a very flexible material having intramolecular and intermolecular hydrogen bonds. For this reason, glycerol is used in polymeric films as a plasticizer which can be added for increasing the mobility and flexibility of the films. It has been reported in various studies that flexible chitosan films can be obtained by the addition of glycerol (Suyatama et al., 2005; Rivero et al., 2015; Debandi et al., 2016). In the study done by Rivero et al. (2015), glycerol was added in the chitosan solution in different concentrations: 0.25, 0.5, 0.75, and 1% of glycerol. According to the results obtained for mechanical strength testing, it was observed that chitosan films with no glycerol had the highest value of tensile strength and this value decreased with the

increase of glycerol added. More flexible films were formed with the addition of 0.75 and 1% glycerol to the chitosan solutions. The values of water vapor permeability showed a significant difference with an increase of the value of permeation with the addition of glycerol to the solution.

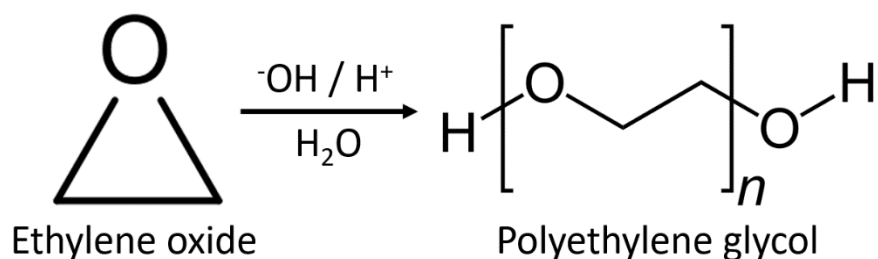
In another study, Tween 20 and glycerol were used as additives (Ziani et al., 2008). The results showed similar changes in the properties of the films as were obtained by Rivero et al. The value of tensile strength with no glycerol was 60 MPa and an elongation at a break of 42%, films with 20% glycerol had a tensile strength of 47 MPa and an elongation at break of 67.3%. The addition of 5% Tween 20 along with 20% glycerol reduced the overall tensile strength to 43.6 MPa and the elongation at break was observed to be 52.5%. According to this observation, both the tensile strength and percent elongation reduced after adding Tween 20, unlike the chitosan films made with only glycerol as an additive. It was also observed that the moisture content of the films increased significantly after adding glycerol which changed from 17.4% for the films with no glycerol to 25.4% for the films with 20% glycerol improving the wettability of the material and the water vapor permeability values also increased after adding glycerol.

The addition of glycerol, gelatin and resinous substance like propolis, which are collected by honeybees from various plant sources, can help in increasing the water vapor barrier properties of the chitosan films (Siripatrawan & Vitchayakitti, 2016; Leceta et al., 2013).

#### 2.6.2 Polyethylene Glycol:

Polyethylene Glycol (PEG) is a synthetic polymer with low toxicity and a semicrystalline property (Dumitriu & Popa, 2013; Yong et al., 2013). It is water-soluble, biocompatible and a flexible material (Sikka & Midha, 2019). It is colorless liquid with an average molecular weight. It can be easily modified for various properties and have applications in biomedical field, food

industry, textile industry, etc. (Zia et al., 2017). PEG 1500 is a type of PEG with molecular weight of 1500 g/mol having a melting point of 48°C, a boiling point of around 250°C and a density of 1.2 g/cm<sup>3</sup> (Polyethylene glycol 1500, n.d.). PEG is produced by reaction of ethylene oxide with water in the presence of any acid or basic catalysts as shown in Figure 2.6. The monomer of PEG: Ethylene oxide, O(CH<sub>2</sub>)<sub>2</sub> has both polar oxygen atom and non-polar (CH<sub>2</sub>)<sub>2</sub> group and is thus soluble in both polar and non-polar solvents (Zia et al., 2017).



**Figure 2.6 Production of Polyethylene glycol**

PEG is used as a plasticizer for improving the flexibility of a material. In a study done by He et al. (2009), the effect of PEG 10,000 on chitosan films was studied, and it was observed that there was no chemical reaction between PEG and chitosan, but there were few interactions due to hydrogen bonds interactions. The crystallinity of the films reduced amount from 28.96% for pure chitosan films to 4.34% for the films with 50% PEG. This change in the crystalline structure of chitosan after increasing the amount of PEG in the film reduced the tensile strength of the material. Similar results were observed by Zhang et al. (2002), PEG with two different molecular weights (PEG 6000, PEG 8000) were added to chitosan in the ratio of 4:1 and 2:1 (chitosan: PEG). The tensile strength of the films was reduced after the amount of PEG increased but there was no significant difference on the values of tensile strength between the two molecular weights PEG used.

### 2.6.3 Nanomaterials:

Materials with dimensions not exceeding 100 nm in at least in one direction are known as nanomaterials. Because of the small size of these particles, the surface area is much larger than that of its bulk counterparts which imply more surface for the reactivity of these materials (Uskoković, 2007). The properties of the bulk counterparts of any material are the average of the quantum forces affecting all the atoms (Berger, 2016). But for nanomaterials, the averaging principle does not work and thus, the properties of a material can differ at nanoscale. The reason for this can be the surface area of nanomaterial which is more than the surface area of its bulk counterpart. This increased surface area in nanomaterials can make it more reactive to chemicals or affect its strength or electrical properties (Berger, 2016). Some nanomaterials have been combined with polymers to improve the mechanical, barrier and thermal properties (Xu et al., 2018). The decrease in the permeability is sometimes attributed to longer and tortuous diffusion path created by the incorporation of nanoparticles (Garcia et al., 2018). According to Duncan (2011), the bonds between nanoparticles and the polymer decrease the interaction of water molecules with the polymer resulting in reduced permeability. The mechanical and barrier properties of the films were improved by Xu et al. (2018) using cellulose nanocrystal and grape pomace extracts as additives. Similarly, a lot of studies have been done with chitosan films embedded with nanoparticles showing improvement in mechanical, antimicrobial, thermal and barrier properties. Some of the nanoparticles used for these improvements included silver, copper, graphene dioxide, sulfur, and lignin nanoparticles (Tripathi et al., 2011; Vimala et al., 2011; Cárdenas et al., 2009; Ahmed et al., 2017; Shankar & Rhim, 2018; Yang et al., 2016). Metal oxide-based nanocomposites were also used for improving the mechanical and barrier properties of polymeric films (Garcia et al., 2018). Some of these metal oxide-based nanoparticles used in food

packaging include aluminum oxide, titanium dioxide, zinc oxide and copper oxide due to their antimicrobial properties (Duncan, 2011).

#### 2.6.3.1 Aluminum oxide ( $\text{Al}_2\text{O}_3$ ):

Metallic aluminum is very reactive in nature and reacts with atmospheric oxygen to form a passivation layer of aluminum oxide on the exposed aluminum. This layer acts as a protective layer and prevents aluminum from further oxidation. It has a white solid appearance with a density of  $3.987 \text{ g/cm}^3$  and a high melting point of  $2072^\circ\text{C}$  when in its nanoparticle form. It is insoluble in water and all solvents.

Aluminum oxide is lightweight, transparent, microwavable and undetected by several metal detector devices. Their properties are stable over a wide range of conditions such as temperatures (Mukherjee et al., 2011).

In a study by Hirvikorpi et al. (2010), aluminum oxide was used as coating on various packaging materials (LDPE, PLA, PET) using Atomic Layer Deposition (ALD) technique. Aluminum oxide coating showed a significant reduction in the transmission rate of oxygen and water vapor. The best improvement of the transmission of oxygen and water vapor rates were obtained on PLA film coated with  $\text{Al}_2\text{O}_3$  layer at 50nm coating thickness as listed in Table 1 (Hirvikorpi et al., 2010).

Aluminum oxide has also been reported to have good antimicrobial activities when used in its nanoparticle form (Mukherjee et al., 2011). They reported that nanoparticles attach to the surface interacting with cell membrane, resulting in the microbe's death (Singh et al., 2019).

The Scanning Electron Microscope (SEM) analysis indicated that even very thin coatings of about 50 nm of Al<sub>2</sub>O<sub>3</sub> provided a good coverage on the surface features of the materials investigated and the barrier properties were significantly improved as listed in Table 1 (Hirvikorpi et al., 2010).

**Table 2.1 Transmission rates of PLA non-coated and coated with 50 nm Al<sub>2</sub>O<sub>3</sub> layer  
(Hirvikorpi et al., 2010)**

	<b>Non-coated PLA</b>	<b>Coated</b>
OTR (cm <sup>3</sup> /m <sup>2</sup> /105Pa/day)	315	32
WVTR (g/m <sup>2</sup> day)	93	3.3

## 2.7 Properties of the chitosan films in packaging:

### 2.7.1 Mechanical Strength:

The mechanical strength of a material is the ability of a material to withstand a load without plastic deformation or failure. Ultimate tensile strength (TS) and percent elongation (%E) at break and young's modulus of a material are used to understand and to predict the mechanical properties of materials. A universal testing machine is usually used for testing mechanical strength, by measuring the load and the elongation of a material. The machine has two crossheads, one being fixed for the length and the second one pulls the sample apart to apply tension. The sizes of the films used are according to the standard followed for testing. The tensile strength ( $\sigma$ ) of a film is calculated by dividing the maximum load or stress applied (F) to the cross-sectional area (A). The cross-sectional area is the product of the thickness and the width of the test sample.



$$\sigma = \frac{F}{A}$$

Percent elongation (%E) is the maximum stretch a film can experience before rupturing or breaking. It is calculated by dividing the change in length (final length ( $L_1$ ) before breaking under stress – initial length ( $L_0$ )) to the initial length ( $L_0$ ) multiplied by 100 for obtaining the units in percentage.

$$\%E = \frac{L_1 - L_0}{L_0} \times 100$$

The tensile strength of chitosan films is in a range of 18 MPa to 68 MPa and the percent elongation varies from 20% to 167% depending on the additives and the method of preparation used (Thakhiew et al., 2013; Patricia Miranda et al., 2004). The tensile strength of the 2% chitosan films reduced from 39.47 MPa to 18.51 MPa and the percent elongation decreases from 37.44% to 22.98% after the addition of Tween 80 in the formulation (Patricia Miranda et al., 2004). The films showed a different behavior for percent elongation after adding glycerol, increasing from 37% to 167% after adding 0.6% glycerol but the tensile strength of the films reduced from 39.47 MPa to 33.69 MPa (Patricia Miranda et al., 2004). The tensile strength of the chitosan films increased from 26.82 MPa to 44.74 MPa after adding zinc oxide nanoparticles, according to research done by Priyadarshi & Negi (2016). In another study done by Thakhiew et al. (2013), the tensile strength of the films increased from 53.9 MPa to 67.5 MPa after adding Galangal extract, and the percent elongation reduced to 19.9% from 31.1%.

### 2.7.2 Thermal properties:

Thermal properties describe the behavior of a material with changes in temperature. This property is important to understand the processing conditions, storing conditions and applications.

#### 2.7.2.1 Thermogravimetric Analysis (TGA):

TGA measures the change in mass and temperature over a period of time change. It is used to understand the thermal stability and phase transition of a material. The result obtained from TGA is a plot of weight change on the Y-axis and temperature on X-axis. For a film, the number of drops in the graph vary depending on its content. A first derivative curve (DTG curve) can be plotted to know the derivative weight change at a particular temperature which can be used to identify the peak maximum of the derivative.

#### 2.7.3 Structural characterization:

##### 2.7.3.1 Scanning Electron Microscopy:

It is a microscopy technique which produces images by scanning the sample surface with high beam of electrons. It is used to gather information about the surface morphology and composition of the sample. The images obtained from SEM analysis are known as micrographs. It is used to understand the distribution of any additives in a sample and the surface roughness of a sample. In a study done by He et al. (2009), micrographs of the film showed irregular holes after addition of PEG, which was speculated to be due to little to no interaction or low compatibility between chitosan and PEG. The surface morphology of the chitosan film with silver oxide nanoparticles displayed agglomerates of silver oxide in SEM analysis, confirming that these nanoparticles were not uniformly distributed in the chitosan matrix (Tripathi et al., 2011).

##### 2.7.3.2 UV Vis Spectroscopy:

It is a quantitative technique used to measure the amount of light absorbed by a material. A beam of light with a wavelength ranging from 180 nm to 1100nm passes through a film. The

sample absorbs this radiation which depends on the concentration and how the sample absorbs the light at a certain wavelength. The transmittance of light depends on the amount of light absorbed by the components present in the film. Transmittance is the amount of light passing through a material. The transmittance will be 100% if there is no absorption.

#### 2.7.4 Antimicrobial properties:

It is the property of a material to inhibit the growth of microorganisms. This property varies according to some properties. Some materials act against fungi which are known as antifungals, whereas some inhibit the growth of bacteria and are known as antibacterial. It is one of the most important properties to evaluate for the material's application in food packaging for preserving the food product packed inside. The shelf life of the product can change according to the nature of packaging used and a package with active ingredients to inhibit the microorganisms will have a longer shelf life (Bari et al., 2007). Active ingredients are chemicals or additives which do not allow the growth of microorganisms. It has been observed that chitosan shows some good antibacterial properties when combined with some additives. In a study by Wang et al. (2017), chitosan was used to analyze the antifungal effect of low molecular weight chitosan on the fungus *Botryosphaeria* sp. W-01. It was observed that the growth of this fungus was inhibited, suggesting that it could be used as a preservative for fruits and vegetables.

#### 2.7.5 Barrier properties:

The barrier property of materials is the ability of a material to control the flow of gases such as oxygen, carbon-di-oxide, and other gases or water vapor. Water Vapor Transmission Rate (WVTR) and Oxygen Transmission Rate (OTR) of chitosan films are used to evaluate these barrier

properties. There are different methods for measuring these values: the gravimetric and the isostatic method.

The gravimetric method uses a glass cup filled with calcium sulfate desiccant to maintain a RH of 0% at room temperature. Then, the cup is wrapped with the film to be tested and sealed properly to make sure no air flows inside the cup from the sides and is known as a permeation cell. This permeation cell is then placed in an environment with desired RH variable from 10 to 100%. The difference in RH between the cell and the surrounding environment will initiate the permeation process. The rate of permeation is determined by the weight gain of the permeation cell over a period of time. This method does not require a lot of investment in technology and capital. There are a few challenges that can arise with gravimetric testing, such as correct sealing, environment chamber to maintain the conditions (temperature and RH) throughout the period of the test. The test is time-consuming and takes about four days for biodegradable films. The salts solutions used can be corrosive and problematic (Mocon, 2017).

Isostatic method, on the other hand, is an automated technique for measuring the water vapor and oxygen transmission rate of films. The working principle for this method involves a test cell divided into two chambers separated by the film to be tested. The inner chamber is filled with a carrier gas (nitrogen) and the outer chamber with a test gas, oxygen or water vapor. Molecules of test gas diffuse through the film to the inner chamber due to differences in concentration and are conveyed to the sensor by the carrier gas. The sensor can either be an infrared sensor or a coulometric sensor. The sensor measures the amount of test gas present in the carrier gas and reports the value as the transmission rate. The test cell is maintained at a certain temperature and relative humidity according to the test conditions. Advantages of using this method are the ease of sample preparation, RH and temperature are easy to control and maintain in the test chamber. This

isostatic method takes less time than the gravimetric method: it takes around a few hours to one day and very little maintenance is required (Mocon, 2017). Although this method has many advantages, it requires a lot of investment in terms of capital and technology, and masking should be done properly for low barrier materials and the size of the samples exposed should be maintained uniform.

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## **REFERENCES**

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### **Chapter 3. Materials and Methods**

#### **3.1 Materials:**

Chitosan (CH) used in this study was from shrimp shell with a deacetylation degree of 75%. The polyethylene glycol solution (PEG) with  $M_w$  of 1400 to 1600 g/mol and poly(vinyl) alcohol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Nano-sized aluminum oxide powder (40 – 50 nm) was obtained from Alfa Aesar (Ward Hill, MA). Glycerol, Anhydrous was obtained from J.T Baker (Phillipsburg, NJ) and Acetic Acid, Glacial from VWR Chemicals (Radnor, PA). Deionized (DI) water was used for all preparations.

#### **3.2 Preparation of the film:**

Solvent casting method was used for producing the films (Priyadarshi & Negi, 2017). An aqueous solution of 1.5% (w/w) chitosan was prepared by dissolving 1.125 g of chitosan powder in 75 g of 1% (v/v) aqueous acetic acid solution. Acetic acid solution was prepared by dissolving 10 ml of acetic acid in 1000 ml of deionized water (DI water). About 75 g of 1.25% (w/v) polyvinyl alcohol solution was prepared by adding 12.5 g of PVOH in 1000 ml of DI water and heated to 88 – 90°C till the water was heated and a transparent solution formed). This polyvinyl solution was added to the chitosan and aqueous acetic acid mixture. The solution was stirred using a magnetic stirrer overnight at room temperature to achieve complete dissolution of chitosan. Appropriate amounts of a 1.5% (w/v) aluminum oxide were prepared by (adding 1.5 g of  $Al_2O_3$  in 100 ml of DI water and stirring using a magnetic stirrer. This aluminum oxide was added to chitosan mixture to reach a final concentration of 0.5%, 1%, 1.5% and 2%. Along with that 0.75 g glycerol and 0.75 g PEG 1500 were added while stirring. The emulsion was left overnight to obtain a uniform visible dispersion of chitosan, aluminum oxide, glycerol and PEG.

This emulsion was then filtered using a fritted glass filter to remove any undissolved particulates and left for another day with stirring to remove air bubbles. The filtered dispersion without air was poured into polystyrene Petri dishes measuring 85 mm in diameter and placed on a leveled surface. The same amount of film of about 25 g was deposited on five 5 Petri dishes to obtain similar film thickness. The filled petri dish was allowed to dry for approximately 48 h at  $23 \pm 2^\circ\text{C}$  and  $50 \pm 2\%$  RH to facilitate the formation of a uniform film. Films without aluminum oxide was also produced and used as a control reference film. Five replicates were made for each type of film.

After drying for 72 hours, the films were peeled off the petri dish casting surface and stored in a desiccator until further evaluation. The desiccator used was a glass desiccator containing  $\geq 98\%$   $\text{CaSO}_4$  and less than  $2\%$   $\text{CoCl}_2$  (Drierite®) desiccant to maintain relative humidity (RH) at  $0\%$ .

### 3.3 Moisture content:

The moisture content of films was determined by measuring weight reduction during oven drying at  $106^\circ\text{C}$  until a constant weight was obtained (oven-dry sample weight), according to the procedure described by Cárdenas et al. (2009). Two replicates were used for each sample type for the moisture determination. The moisture content (MC (%)) was calculated using the equation showed below. The initial weight of each conditioned sample ( $W_i$ ) and the oven-dry weight ( $W_d$ ).

$$MC (\%) = \frac{(W_i - W_d)}{W_d} \times 100$$

### 3.4 Density:

The density ( $\rho$ ) was calculated by dividing the dry weight ( $W_d$ ) of the film in grams with the volume of the film. The dry weight is the weight of a film after oven drying at 106 °C until constant weight. The reported data are in the g/cm<sup>3</sup>.

$$\rho = \frac{W_d}{A \cdot l}$$

The volume of the film was determined by using the average film thickness, width and length of the film. The length and width of the film used were evaluated to be 5 cm  $\pm$  0.2 cm long and 0.635 cm  $\pm$  0.02 cm wide, having an area (A) of 3.175cm<sup>2</sup> and the thickness (l) varied depending on the sample.

### 3.5 Mechanical properties:

The mechanical properties of the composite films were determined by measuring the tensile strength (TS) and percent elongation at break (EB). The films were conditioned and tested at 23  $\pm$  2°C and 50  $\pm$  10 % RH using a laboratory Instron 5565 Universal Testing Machine (Instron, Canton, MA, USA) according to ASTM standard method D882-12 (ASTM, 2012).

Films were cut in rectangular strips 50 mm  $\pm$  2 mm long and 6.35 mm  $\pm$  0.2 mm wide following Shen & Kamdem (2015) protocol. The films were fixed with an initial grip separation of 25 mm with an extension speed of 1 mm/min. This modified specimen size and crosshead speed was used to measure the tensile strength due to the initial small size of the film made on an 85 mm diameter Petri Dish. A microcomputer was used to record the stress-strain curves. Tensile strength (TS), elongation at break (EB) and Young's modulus were calculated. Four replicates of each test sample were run.



### 3.6 Thermogravimetric Analysis (TGA):

TGA was performed using TGA 2950 equipped with Universal Analysis Software Package (TA Instruments, New Castle, DE) to evaluate the thermal stability of the films prepared. Samples weighing 5-7 mg were used for testing and the temperature ranged from 25°C to 650°C at 10°C/min heating rate under a nitrogen flow rate of 40 ml/min. Weight losses of samples were measured as a function of temperature. TGA (weight loss/change/reduction as a function of temperature) and derivative thermogravimetry (DTG) curves were recorded. All the measurements were conducted in duplicate.

### 3.7 Ultraviolet- Visible Spectroscopy (UV-Vis Spectroscopy):

The ultraviolet, visible spectroscopy analysis was performed to measure the absorption of light through the film. Shimadzu UV 1800 UV-Vis Spectrophotometer (Kyoto, Japan) was used for this test. The film samples were placed in a spring-loaded sample holder and the air was used as a reference. The absorption was measured from 190 nm to 900 nm at an interval of 1 nm and at medium speed.

### 3.8 Antimicrobial property:

A Gram-negative bacteria: *Salmonella enterica* (*S. enterica*) and a Gram-positive bacteria: *Listeria innocua* (*L. innocua*) were used for evaluating the antimicrobial properties of the films. Agar diffusion method was used following the method described by Pelissari et al. (2009).

The agar dilution test was used to assess the density of the bacteria in a broth. Broth was made by diluting 1 to  $1 \times 10^{-9}$  g/ml which consist in adding 1 ml of fresh broth into 9 ml of sterile 0.9% NaCl solution. The solution was mixed completely and shaken, followed by serial dilutions and 1

ml of each solution was sub-cultured on agar plates and dispersed. The settled plates were stored in an incubator for 24 hours. The number of colony-forming units was counted on the agar plate. The films were cut into 4.95 mm diameter discs with a die punch. The samples were placed on Brain-heart infusion (BHI) agar petri dish plates for *L. Innocua* and Nutrient agar petri dish plates for *S. enterica* and stored in an incubation chamber at 37°C for 24 hours. The diameter of the inhibition zone was measured using a caliper to the nearest 0.01 mm. The inhibition zone is defined as the zone on the plate around the film with no visual growth of bacteria. The area of the inhibition zone was calculated, and the area of the film was subtracted to determine the area of inhibition. Triplicates of the samples were tested for each type of film. The antimicrobial activity of the film was calculated as a percentage by dividing the inhibited surface area with the total potential surface of the inoculated petri dish. 0% Inhibition indicates that there is no inhibition observed and 100% corresponds to complete inhibition of the microbes on the petri dish.

$$\text{Antimicrobial activity (\%)} = \frac{\text{Inhibited surface area}}{\text{Total potential surface}} \times 100$$

### 3.9 Water Vapor Permeability (WVP):

Water vapor transmission rate was measured using Mocon PERMATRAN-W Model 3/33 (Modern Controls Inc., Minneapolis, MN). All the tests were performed at 37.8°C and 50% RH according to the method used by Struller et al., (2014). The WVTR results were obtained in units of g (water vapor)/m<sup>2</sup>.day. The WVTR results were obtained in units of g of water vapor/per unit surface in m<sup>2</sup> per day (g(water vapor)/m<sup>2</sup>.day) as recommended in the ASTM standard method F1249-90 (ASTM, 1990). Two replicates of each sample set were tested. Chitosan film with no aluminum oxide was used as a reference for these test samples modified with the addition of different amounts of aluminum oxide.

### 3.10 Oxygen Permeability (OP):

Oxygen transmission rate was measured using Mocon OX-TRAN Model 2/22 (Modern Controls Inc., Minneapolis, MN) operated using standard method ASTM D3985-05 (ASTM, 2010). The instrument has a coulometric detector to measure the amount of oxygen transmitted through the film. All the tests were performed at 23°C in a dry environment (0% RH) according to the method used by Akter et al. (2014). Four replicates of each sample were tested, conditioned for 2 hours and purged with nitrogen for 10 minutes before exposing it to 100% oxygen flow. The OTR results were obtained in units of cc (O<sub>2</sub>)/m<sup>2</sup>.day. The values were then converted to oxygen permeation by dividing the OTR by the thickness. Chitosan film with no aluminum oxide was used as a reference to the samples made with different amounts of aluminum oxide.

### 3.11 Scanning Electron Microscopy (SEM):

The morphology of the film surface and cross-section was investigated using Scanning Electron Microscope (SEM) model JSM-6610 (JOEL USA, Peapody, MA). The samples were cut and mounted on aluminum stubs using double-sided carbon tape. The samples were mounted on edge for evaluating the cross-section of the film surface. The films were coated with Iridium and analyzed at 12kV under X500 magnification for the surface or the film and X1000 magnification for cross-section.

### 3.12 Statistical Analysis:

All the results were presented as average  $\pm$  standard deviation for each test. One-tailed student's t-test was used for all the statistical analyses. The significant difference between the values compared using Duncan's multiple range test with criteria  $p < 0.05$ .

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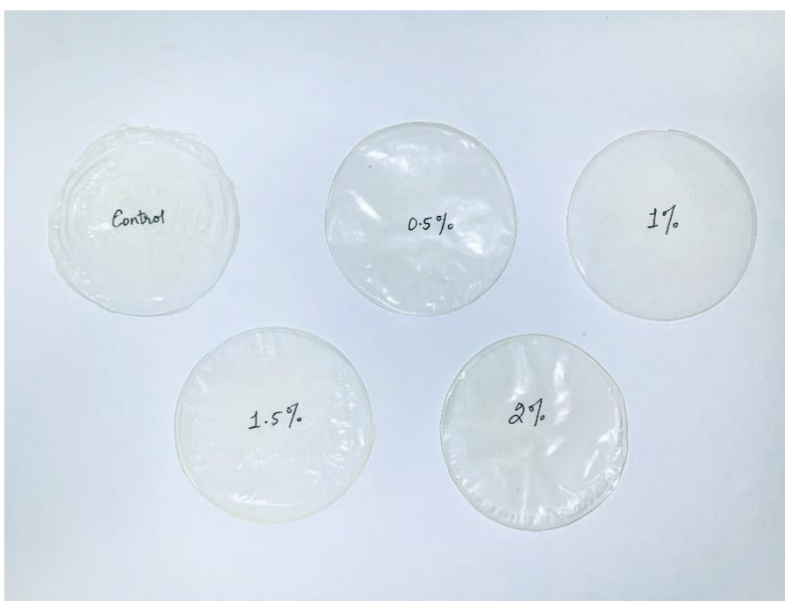
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## **Chapter 4. Results and Discussions**

### **4.1 Film Formulation:**

All the films are made with the same lot of raw materials to keep consistency in materials nature across all the films. The films formed were smooth, homogeneous and flexible. Some of the films had an orange peel texture, which might have occurred due to problems in drying or the surface tension of the solution. The solution of chitosan was transparent. The transparency of the chitosan solution decreased with an increase in the amount of aluminum oxide. But all the films looked similar to each other, after casting and drying appearing to be transparent. The color of the films changed with the storage time from colorless to films with a very slight tint of yellow color.



**Figure 4.1 Chitosan films with different concentrations of aluminum oxide**

#### 4.2 Physical properties of the film:

The thickness of all the films was in the range of 0.071 mm to 0.086 mm. There was no significant difference ( $p < 0.05$ ) between thickness of the films except the film with 0.5%  $\text{Al}_2\text{O}_3$  which was significantly different from other films. The **Table 4.1** lists the values of thickness, moisture content and density of all the films with different amounts of aluminum oxide added to it.

**Table 4.1 Thickness (mm), Moisture content (%) and Density (g/cm<sup>3</sup>) of Chitosan films prepared with Aluminum oxide ( $\text{Al}_2\text{O}_3$ )**

Sample	Thickness (mm)	Moisture Content (%)	Density (g/cm <sup>3</sup> )
Control	0.082 ± 0.009 <sup>a</sup>	23.59	0.955
0.5% $\text{Al}_2\text{O}_3$	0.071 ± 0.006 <sup>b</sup>	22.58	0.961
1% $\text{Al}_2\text{O}_3$	0.086 ± 0.009 <sup>a</sup>	22.51	0.921
1.5% $\text{Al}_2\text{O}_3$	0.080 ± 0.007 <sup>a</sup>	22.16	0.959
2% $\text{Al}_2\text{O}_3$	0.082 ± 0.005 <sup>a</sup>	24.42	0.927

Data correspond to the average and standard deviation of 4 replicates for thickness and 2 replicates for MC% and density.

The values with different superscript letters in the columns are significantly different from each other ( $p < 0.05$ )

Density of a film is the indicator of how the components are packed inside the film. The density of all the films were in a range of 0.921 to 0.961 g/cm<sup>3</sup>. These values were lower than the values reported by Singh et al. (2015). The density of the films with aluminum oxide should be higher than the density of control films due to the addition of a highly dense compound, aluminum

oxide but it is not observed. This may be due to the amount of aluminum oxide added to the films is very insignificant to show a difference.

The moisture content of the films was in a range of 22.16 to 24.42 %. The values of percent moisture content obtained were similar to those reported by Wiles et al. (2000).

#### 4.3 Thermal properties of the films:

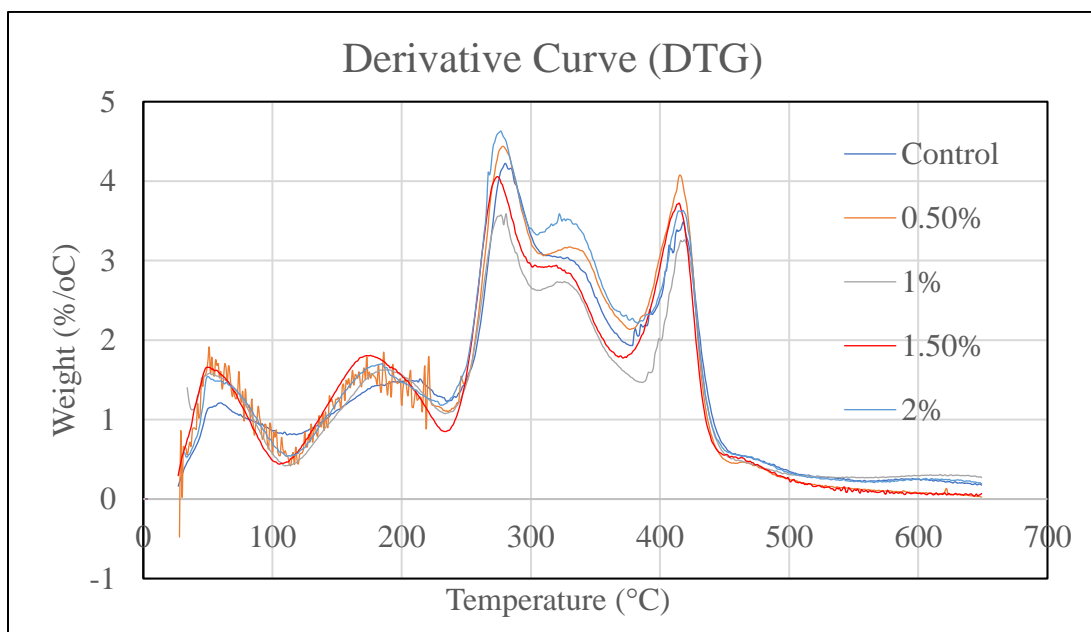
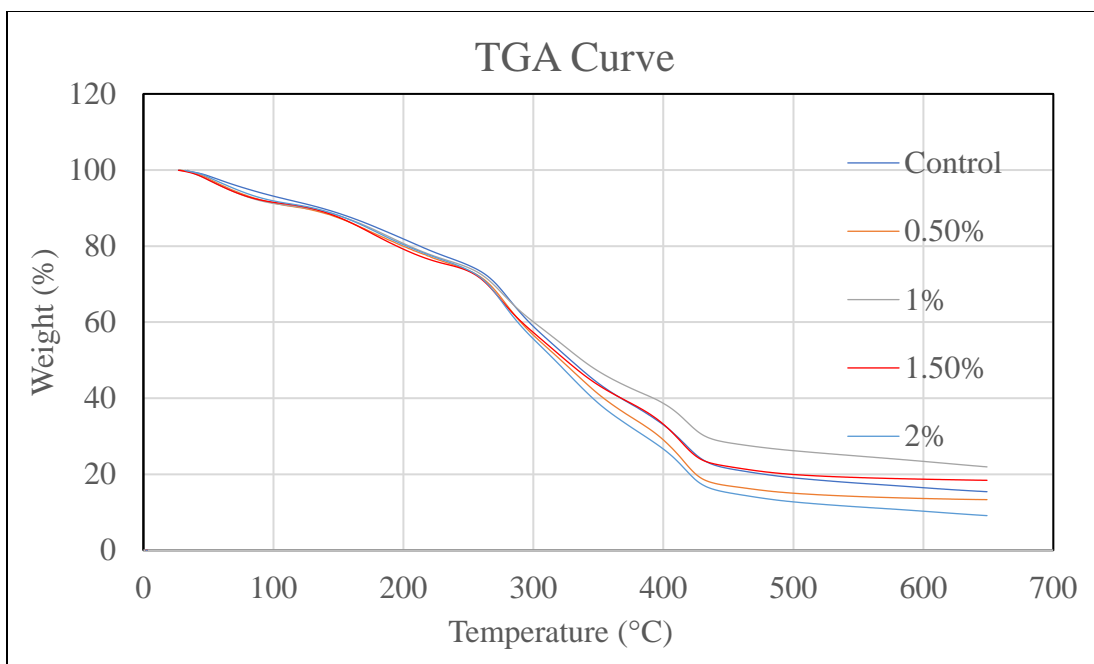
TGA analysis was done to evaluate the thermal stability of the chitosan films after adding aluminum oxide. TGA curves and their derivative curves (DTG) are shown in the *Figure 4.2*. The **Table 4.2** shows the maximum decomposition temperature ( $T_{d \max}$ ) and the residue left at the end of the test in percentage. The maximum decomposition values were obtained from the maximum temperature of the peaks on the DTG curve.

Five stages of weight loss were observed in all the films which were clearly visible in the DTG curve, as shown in **Figure 4.2**.

**Table 4.2 TGA and DTG parameters of the film**

Sample	$T_{d \max}$ (°C)					Residue (%)
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	
Control	51	180	276	319	414	15.4
0.5% $Al_2O_3$	47	182	274	326	411	13.32
1% $Al_2O_3$	47	182	277	322	412	21.93
1.5% $Al_2O_3$	47	172	271	316	411	18.14
2% $Al_2O_3$	46	181	273	325	414	9.12





(b)

**Figure 4.2 Results of TGA (a) and DTG (b) curves of chitosan films with different concentrations of aluminum oxide**

The first weight loss was observed at a temperature range between 20 and 120°C with  $T_{d\max}$  of about 47°C, which corresponds to the evaporation of water, volatile compounds, and other impurities present in the film (Chakravartula et al., 2019). There was a weight loss of about  $2.5 \pm 2\%$  in this region.

The second thermal degradation was seen between 120 and 210°C, corresponding to the degradation of glycerol (Luo et al., 2013; Basiak et al., 2018). The  $T_{d\max}$  was in the range of 172 to 182°C, with a weight loss of about  $17 \pm 2\%$ .

The third stage of thermal degradation was at a temperature ranging from 210 to 300°C with a  $T_{d\max}$  ranging from 271 to 277°C. The weight loss in this stage was around  $40 \pm 10\%$ . This weight loss corresponds to the degradation of chitosan (Tripathi et al., 2011; Cárdenas et al., 2009).

The fourth thermal degradation was between 300 and 370°C corresponding to degradation of PVA (Gomma et al., 2018; Reguieg et al., 2020). The peak of this region was in the temperature range of 316 to 326°C with a weight loss of  $16 \pm 2\%$ .

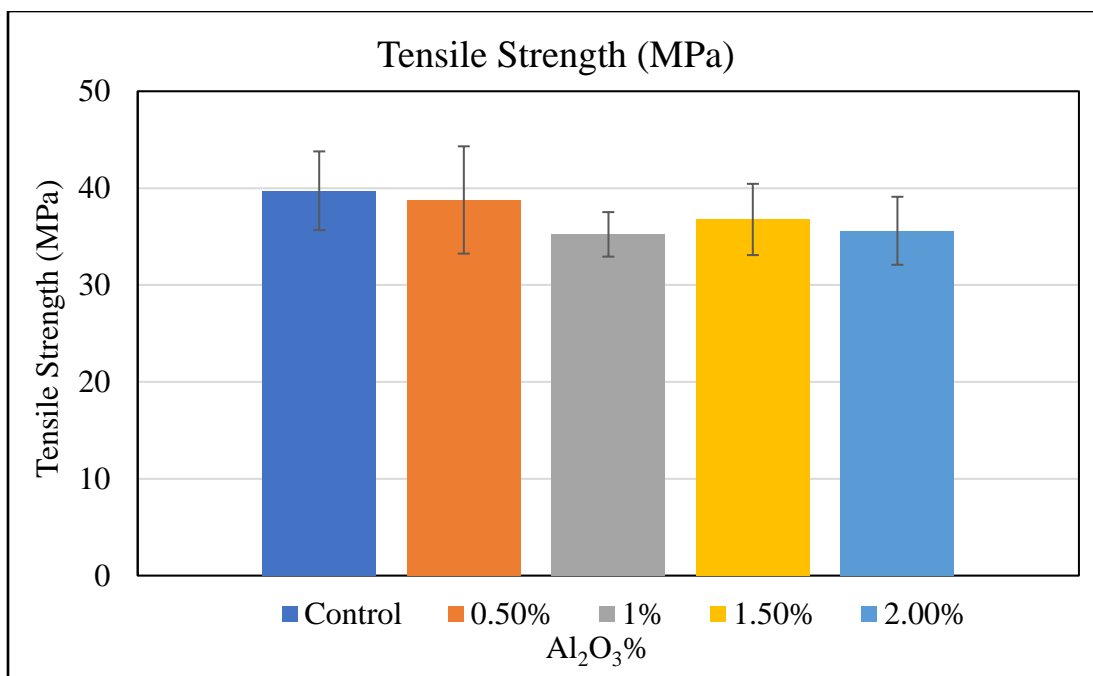
The final stage of degradation was between 370 and 450°C with a  $T_{d\max}$  between 411 and 414°C. The weight loss corresponding to this stage was  $12 \pm 3\%$ . PEG was the component degraded at this temperature according to the literature (Dorigato et al., 2019; Qian et al., 2017).

According to the residue left at the end of the test, 1%  $\text{Al}_2\text{O}_3$  has the maximum amount of residue which is 21.93% and the film with 2%  $\text{Al}_2\text{O}_3$  has the least amount of residue left corresponding to 9.12%. Ideally, the amount of residue should be the most in the film with 2%  $\text{Al}_2\text{O}_3$ , though it is not expected to be significantly more. The amount of residue should be lowest in the film with no aluminum oxide, control film, but it is not reflecting in that manner, which might be because of the amount of sample used for this test, the amount of aluminum oxide used

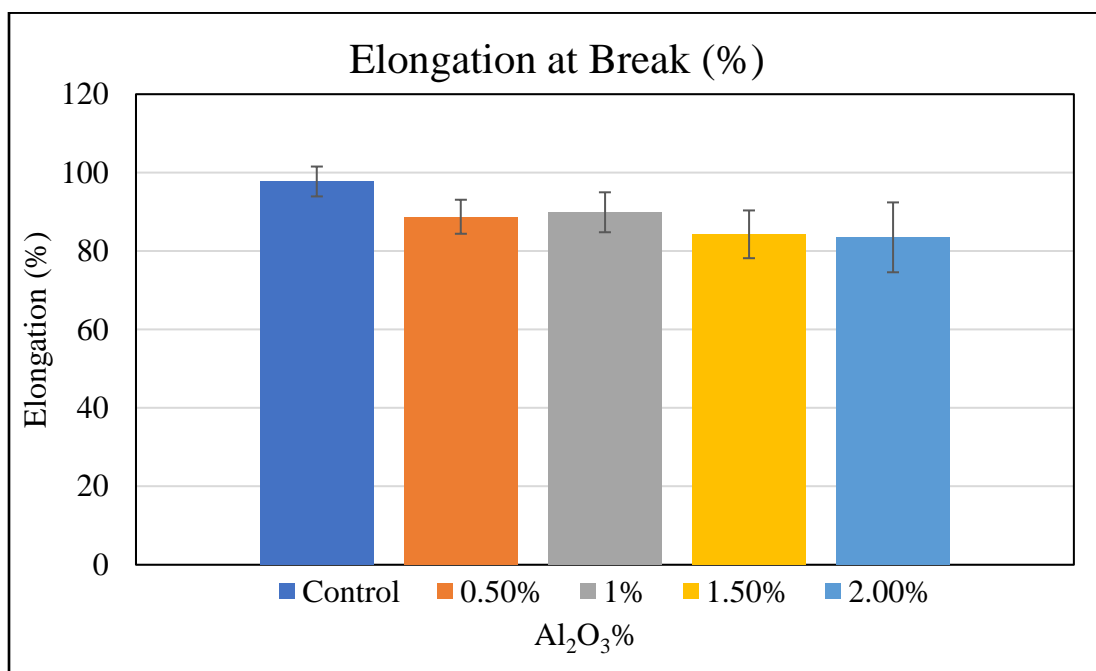
and distribution of aluminum oxide in the film. Even the process of film formation, solution casting, slight changes in the formulation or storage conditions can have an adverse effect on the properties of the film (Priyadarshi & Rhim, 2020).

#### 4.4 Mechanical Properties:

According to the results, the addition of aluminum oxide in the considered concentration range to the chitosan films affected the mechanical properties of the film. The tensile strength of the chitosan films slightly decreased when the aluminum oxide concentration increased from 0.5% to 2% but the change was not significant ( $P>0.05$ ). The tensile strength of the control film without any aluminum oxide was measured to be  $39.73 \pm 4.06$  MPa, which decreased to  $35.59 \pm 3.5$  MPa after adding 2%  $\text{Al}_2\text{O}_3$  nanoparticles. It was observed that the percent elongation at break (%EB) significantly reduced ( $p<0.05$ ) on the addition of  $\text{Al}_2\text{O}_3$  nanoparticles. The control film extended almost double its original length, 24.43 mm or  $97.74 \pm 3.8\%$  whereas, the film with 2%  $\text{Al}_2\text{O}_3$  showed an extension of 20.87 mm or  $83.49 \pm 8.9\%$ .



(a)



(b)

**Figure 4.3 Tensile strength (a) and % elongation (b) of chitosan films with different concentrations of aluminum oxide**

The decrease in the tensile strength can be due to the presence of Al<sub>2</sub>O<sub>3</sub> nanoparticles in the matrix of the film. There might be no crosslinking or interaction between the Al<sub>2</sub>O<sub>3</sub> nanoparticles and chitosan, thus the interaction between the additive and chitosan (polymer-nanoparticle) might be weaker than the interaction between chitosan strands (polymer-polymer) in the polymer matrix.

This interaction may have created structural discontinuities decreasing the flexibility. Values of tensile strength obtained were similar to the results reported by Shen & Kamdem (2015) and Martins et al., (2012), but they differed from the values measured by Butler et al. (1996) and (Priyadarshi & Rhim, 2020). This difference can be due to several conditions such as chitosan source, percent of deacetylation and composition, different plasticizers or additives used, thickness, preparation and storage conditions.

**Table 4.3 Tensile strength and Elongation at Break of the chitosan films**

Sample	TS (MPa)	EB (%)
Control	39.73 ± 4.06 <sup>a</sup>	97.74 ± 3.8 <sup>a</sup>
0.5% Al <sub>2</sub> O <sub>3</sub>	38.78 ± 5.54 <sup>a</sup>	88.74 ± 4.34 <sup>b</sup>
1% Al <sub>2</sub> O <sub>3</sub>	35.23 ± 2.30 <sup>a</sup>	89.88 ± 5.09 <sup>b</sup>
1.5% Al <sub>2</sub> O <sub>3</sub>	36.77 ± 3.68 <sup>a</sup>	84.26 ± 6.09 <sup>b</sup>
2% Al <sub>2</sub> O <sub>3</sub>	35.59 ± 3.5 <sup>a</sup>	83.49 ± 8.9 <sup>bc</sup>

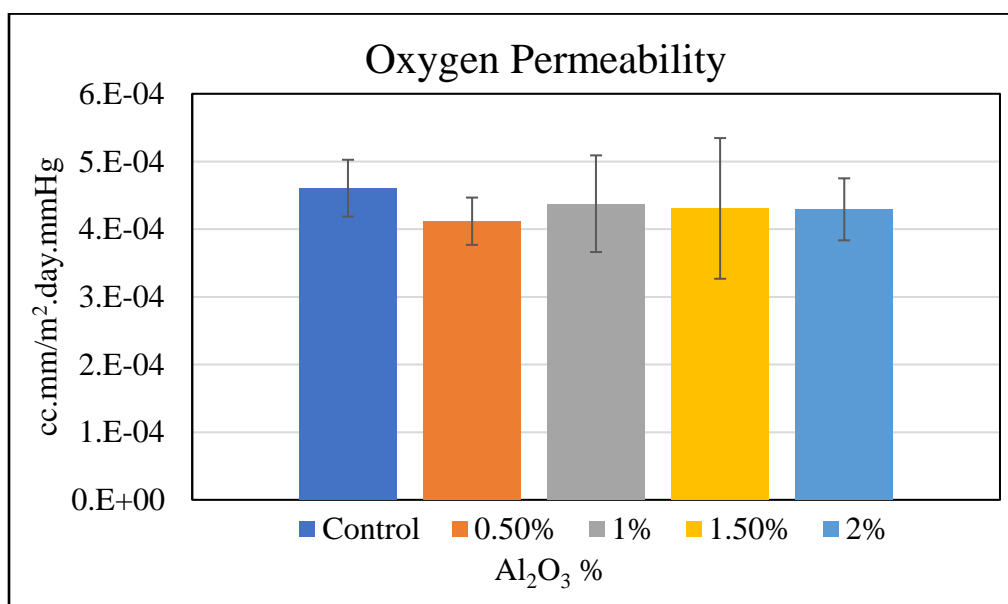
Data corresponds to the average and standard deviation of 4 replicates

The values with different superscript letters in the columns are significantly different from each other (p<0.05)

#### 4.5 Oxygen Permeability:

Oxidation affects the quality of a product packed inside a flexible packaging film, let it be a food product or any metal object. It is very important to understand the oxygen barrier properties of packaging material to estimate the shelf-life of a product or to understand applications of that material. Aluminum oxide has been used as a coating to improve the oxygen barrier properties of flexible packaging materials.

In this study, the oxygen barrier properties were improved after the addition of  $\text{Al}_2\text{O}_3$  nanoparticles, but the change was not significant ( $p < 0.05$ ). The control film had an OP of  $4.61 \pm 4.20 \times 10^{-4} \text{ cc.mm/m}^2\text{.day.mmHg}$  and OP of the films with 2%  $\text{Al}_2\text{O}_3$  nanoparticles was  $4.29 \pm 4.58 \times 10^{-4} \text{ cc.mm/m}^2\text{.day.mmHg}$ . The OP of films decreased for all the films after adding the  $\text{Al}_2\text{O}_3$  nanoparticles, but the least OP was observed in the film with 0.5%  $\text{Al}_2\text{O}_3$  nanoparticles which was  $4.21 \times 10^{-4} \text{ cc.mm/m}^2\text{.day.mmHg}$ . This suggests that 0.5% is the optimum content that can be added to get the best barrier properties against oxygen.



**Figure 4.4 Oxygen permeability of films with different concentrations of aluminum oxide**

The antioxidant property of aluminum oxide can be evidently seen with a decrease in OP values. Similar trends were observed in a study done by Struller et al. (2014) using  $\text{Al}_2\text{O}_3$  as a coating on BOPP films and another research done by Johansson et al. (2017) who used aluminum oxide as an atomic layer deposited (ALD) coating on LDPE coated paper.

Even though the test was done carefully controlling all the parameters, there can be a few errors that could have manipulated the data such as a non-uniform thickness of the films, problems with masking, the diameter of mask, RH and temperature inside the machine.

**Table 4.4 Oxygen Permeability and Water Vapor Permeability of the chitosan films**

Sample	OP (cc.mm/m <sup>2</sup> .day.mmHg)	WVP (g.mm/m <sup>2</sup> .day.mmHg)
Control	4.61E-04 ± 4.20E-05 <sup>a</sup>	1.36 ± 0.19 <sup>a</sup>
0.5% $\text{Al}_2\text{O}_3$	4.12 E-04 ± 3.49E-05 <sup>a</sup>	1.26 ± 0.08 <sup>ab</sup>
1% $\text{Al}_2\text{O}_3$	4.38 E-04 ± 7.14E-05 <sup>a</sup>	1.47 ± 0.15 <sup>c</sup>
1.5% $\text{Al}_2\text{O}_3$	4.31 E-04 ± 1.04E-05 <sup>a</sup>	1.64 ± 0.08 <sup>d</sup>
2% $\text{Al}_2\text{O}_3$	4.29 E-04 ± 4.58E-05 <sup>a</sup>	1.78 ± 0.17 <sup>de</sup>

Data corresponds to the average and standard deviation of 4 replicates

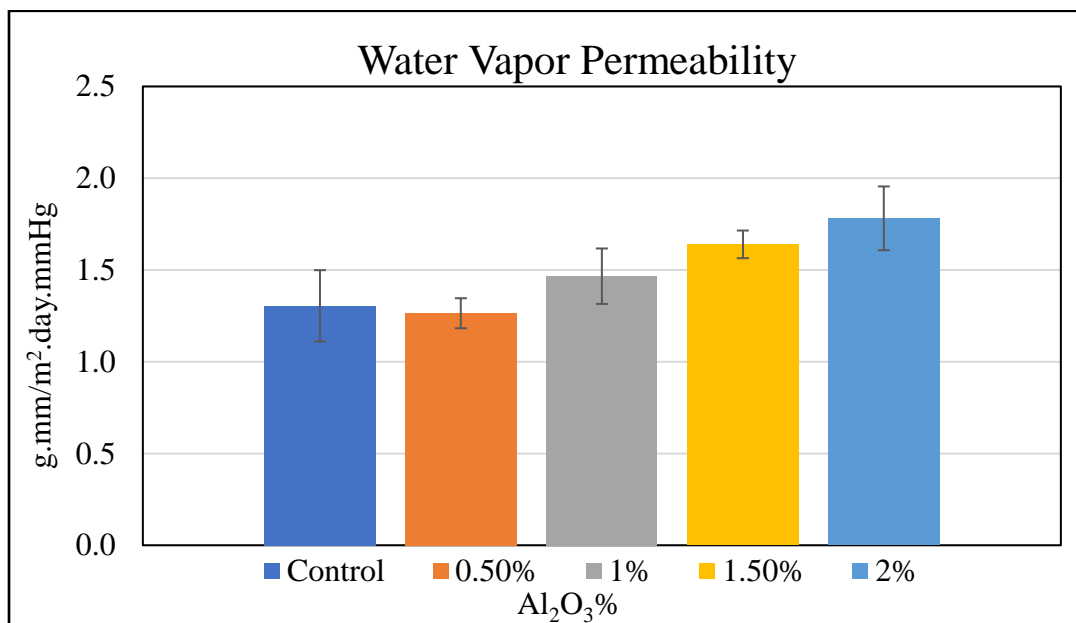
The values with different superscript letters in the columns are significantly different from each other (p<0.05)

#### 4.6 Water Vapor Permeability (WVP):

Water vapor permeability is an important parameter to understand the properties of a material. A lot of applications depend on the water vapor permeability. Different products need to be stored at a different RH for maintaining its shelf life and if the wrong material is chosen, the safety of the product can be compromised. The WVP of the chitosan films significantly increased

( $p < 0.05$ ) after the addition of  $\text{Al}_2\text{O}_3$  nanoparticles, which means that the water vapor barrier properties decreased.

The WVP of control film was found to be  $1.36 \pm 0.19$  g.mm/m<sup>2</sup>.day.mmHg and that of the film with 2%  $\text{Al}_2\text{O}_3$  nanoparticles was  $1.78 \pm 0.17$  g.mm/m<sup>2</sup>.day.mmHg. But, for the film with 0.5%  $\text{Al}_2\text{O}_3$  nanoparticles the WVP measured was less than the control film being  $1.26 \pm 0.08$  g.mm/m<sup>2</sup>.day.mmHg which is similar to the results obtained for OP. This proves that adding 0.5% additive to the chitosan film is optimum and displays the best barrier properties.



**Figure 4.5 Water Vapor permeability of films with different concentrations of aluminum oxide**

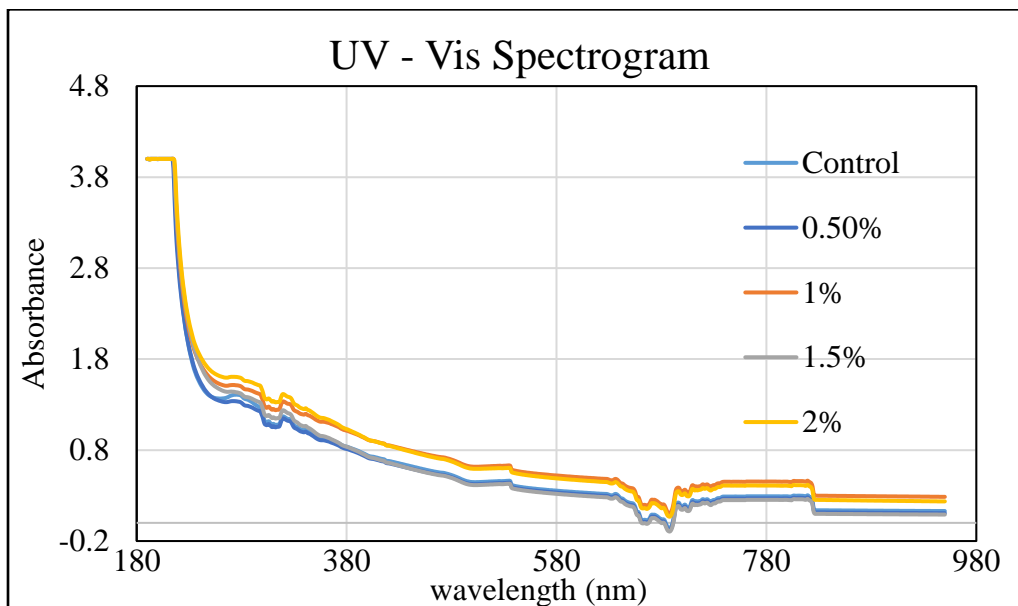
The increase in the WVP values might be due to the presence of  $\text{Al}_2\text{O}_3$  nanoparticles which are not linked to the polymer but just present in the matrix. The presence of acids in the films makes it soluble in water or at least reactive to water (Sakai et al., 2001). Interaction of the films



with water vapor during the test can result in expansion of these films, increasing the void spaces within the films and thus, increasing the permeability.

#### 4.7 UV-Vis Spectroscopy:

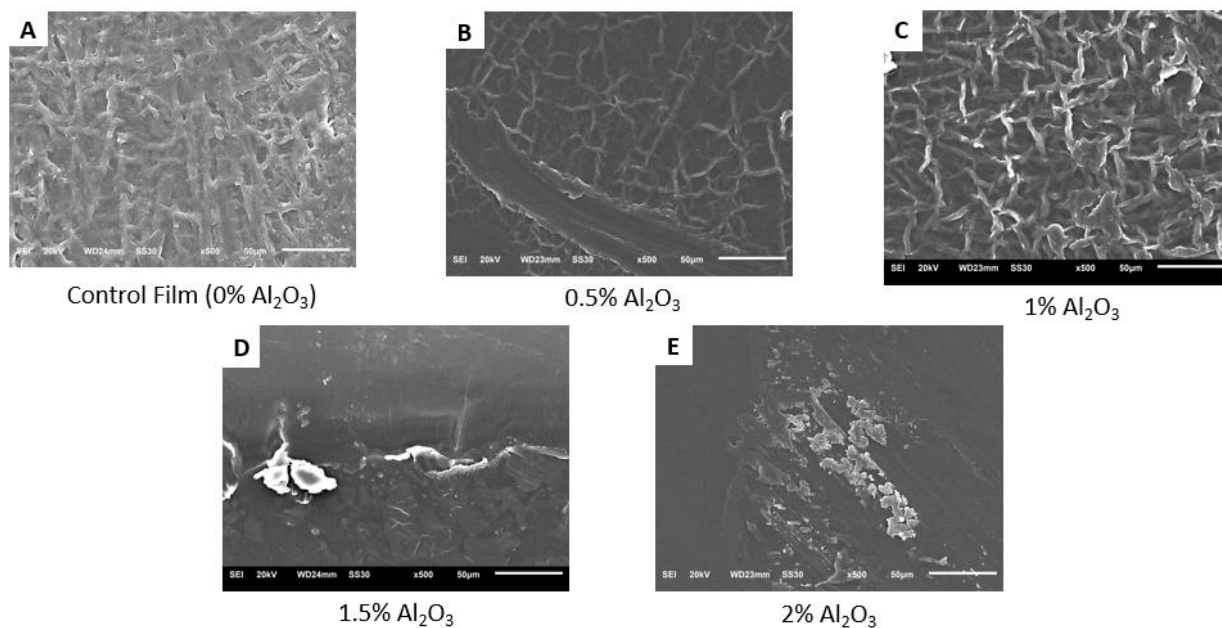
According to the spectrograph produced from the UV-vis spectroscopy, there was not a major difference between the color of the films after adding aluminum oxide. Aluminum oxide nanoparticles were white in color, but the amount added was too less to change the color of the films. The absorbance value of aluminum oxide was found out to be between the range of 200 and 300 nm according to the literature (Al-Jawad et al., 2015; Prashanth et al., 2015). Films with 2% aluminum oxide had the highest absorbance value of 1.605 at 273 nm confirming the presence of aluminum oxide in the maximum amount. The absorbance value of control film at 273 nm was observed to be 1.408.



**Figure 4.6 Absorbance of chitosan films with aluminum oxide at wavelength between the range of 200 to 900 nm**

#### 4.8 Scanning Electron Microscopy (SEM):

The SEM analysis was done at a magnification of 500x to observe the surface morphology of the film after adding aluminum oxide in different concentrations. According to the observation after looking at the images shown in Figure 4.7, films with no aluminum oxide (control film), 0.5%  $\text{Al}_2\text{O}_3$  and 1%  $\text{Al}_2\text{O}_3$  had uneven and rough surface formed during drying of the film due to changes in viscosity and the fluctuating storage conditions. The films with 1.5%  $\text{Al}_2\text{O}_3$  and 2%  $\text{Al}_2\text{O}_3$  had white patches in the film which could be the aluminum oxide nanoparticles but was not confirmed. The low concentration and the small size of aluminum oxide made it difficult to locate it at this magnification.



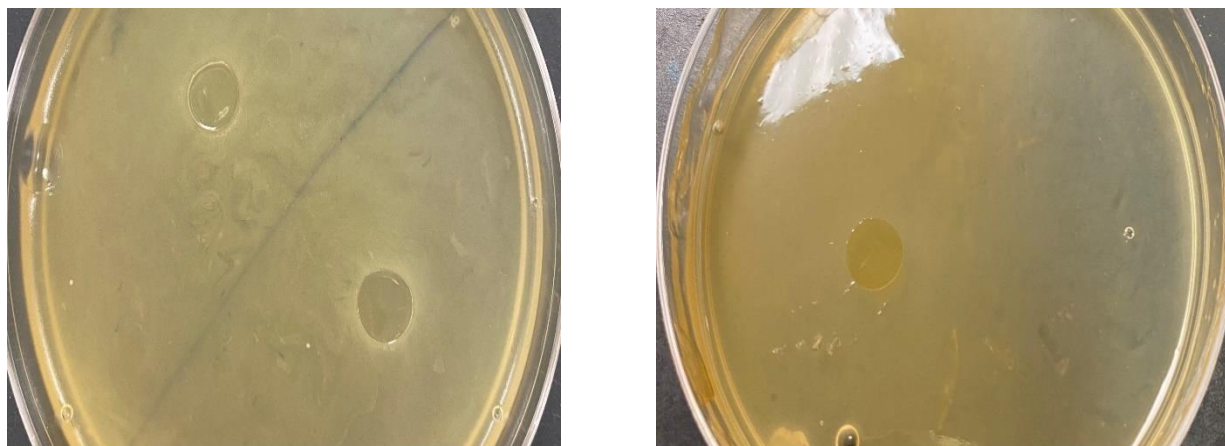
**Figure 4.7 Surface microstructure of chitosan films with aluminum oxide. Images captured through SEM analysis at a magnification of 500x**

To overcome this problem, Iridium was used as a coating and a machine with higher magnification was used for the analysis at an electron range of 2kV. At this setting, the surface of

the films could be visible properly (images not shown) but to confirm the presence of aluminum oxide nanoparticles, backscatter and EDX was needed to confirm its presence which was possible at an electron range of 4-5 kV. But at this setting, the sample was observed to crack and melt and EDX could not be done.

#### 4.9 Antimicrobial Properties:

The films did not show any antimicrobial activity against Both the gram-positive *L. innocua* and the gram-negative *S. enterica*. There was no inhibition zone observed around the films in both of the bacteria. This could be due to the amount of aluminum oxide added to the films which was too less to show any antimicrobial activity.



**Figure 4.8 Petri dishes with chitosan films incorporated with 2% Al<sub>2</sub>O<sub>3</sub> showing no inhibition zone**

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## **REFERENCES**

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## **Chapter 5. Conclusion**

This is the first report that shows the effect of aluminum oxide nanoparticles incorporated in the matrix of the chitosan films, made using the solution casting method. Aluminum oxide offers good barrier properties against water vapor and oxygen, high mechanical strength and antimicrobial properties. All these properties are stable over an increased range of temperature increasing their application in various fields.

In this study though, there was no change observed in the oxygen permeability and thermal properties of the film after adding aluminum oxide. All the constituents present in the film except aluminum oxide were clearly visible with distinctive peaks from the TGA analysis. The tensile strength, percent elongation and water vapor barrier properties of the films reduced with increase in the amount of aluminum oxide. Also, this study indicates that the addition of 0.5-2% aluminum oxide to polymer does not show any antimicrobial properties against two different bacteria studied. Results obtained from different analyses indicate that chitosan film incorporated with studied concentrations of aluminum oxide is not helping in improving the mechanical properties, water vapor barrier properties, and antimicrobial properties. Further experimentation with different concentrations of aluminum oxide could be pursued before concluding that aluminum oxide doped chitosan does not help in improving the desired properties of the polymer.

## **Chapter 6. Recommendations**

Though the results did not show any noticeable improvement in the properties of the films prepared, further work and evaluation is definitely warranted to see the improvement in the properties of the film on doping with the metal oxide. Polymeric solution with metal oxide is required to be stirred with high-speed stirrers for longer periods, as SEM studies indicate that metal oxide did not get distributed uniformly in the solution. The solution should be poured in petri dishes in such a way that biopolymers with metal oxide having uniform thickness are prepared. So, Intense care needs to be taken while adding the constituents to the polymeric solutions, as it is observed that slight variation in the amounts of additives is altering the properties significantly, it may be because of non-uniform distribution, which requires to be studied in depth. The solution casted in petri dishes should be stored in an environment with highly controlled temperature and relative humidity.

Slight improvements (not significant) in the barrier properties were noticed in the films with 0.5%  $\text{Al}_2\text{O}_3$ . More replicates at this concentration of aluminum oxide and films with concentrations of 0.1%, 0.2%, 0.3% and 0.4%  $\text{Al}_2\text{O}_3$  can be formulated to evaluate whether any improvement in the barrier properties is there at these concentrations, as preliminary results indicate.

Surface morphology of aluminum oxide nanoparticles were not studied in this study which can be done in the future to see the average size and shape of aluminum oxide nanoparticles. Finally, other metal oxide nanoparticles such as titanium oxide, copper oxide, silver nitrate, etc. can also be used to see changes in the properties of the chitosan film.