DEVELOPMENT OF NOVEL ZEIN-CELLULOSE NANOCOMPOSITE FILMS

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ABSTRACT OF THE THESIS

Preparation of Novel Zein - Cellulose Nanocomposite Films

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Zein is a hydrophobic biopolymer, which naturally forms biodegradable films. These films are rigid, brittle, and lack good mechanical and barrier properties for packaging applications. This study aims at improving the mechanical and barrier properties of zein films by the development of zein-cellulose nanocomposites. A ‘nanocomposite’ is a mixture of polymer matrix, which forms the continuous phase and the filler (having at least one dimension less than 100nm), which forms the dispersed phase.

Zein-cellulose nanocomposites have zein as the matrix and cellulose as the filler. Nano-sized cellulose was prepared using wet-media milling machine and mass ratios of 1%, 3%, 5% and 10% of both, naked (not stabilized) nano-cellulose and GA - stabilized nano-cellulose were used in the film formulation. Tributyl citrate (TBC) was used as the plasticizer. The films were prepared using solvent-cast technique and characterized to test their mechanical and barrier properties. The controls were pure zein films and films with only plasticizer.

Texture Analysis showed that filler loading of 5% was most effective in increasing the
elongation-at-break to about 16 to 20 times of the controls. However, the tensile strength did not change. Water vapor permeability of the nanocomposites was minimum at 1% by wt. of naked cellulose (~4 times less than control) or at 5% by wt. of stabilized cellulose (~6 times less than control). Water absorption results showed that the rate of water uptake decreased as the filler loading increased. DSC and FTIR suggested that there is no interaction between the zein and the nano-cellulose in the films. The AFM results showed that the films’ surface is flat, but the thickness of the films increased with filler loading.

Therefore, incorporation of nano-cellulose improved the flexibility and water barrier properties of the films. Gum Arabic, which was used as a stabilizer for nano-cellulose, seemed to have aided the even distribution of cellulose in the zein matrix. Thus, these films can be a good starting point to further study the arrangement of cellulose within the zein matrix and test its potential applications on food systems and as edible films.
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1. INTRODUCTION

1.1 Zein: Biodegradable Polymer

1.1.1 Overview

Zein belongs to the class of proteins known as prolamine, which occur in most of the cereals. Commercial production of zein began in 1939 from corn gluten meal (contains 62-74% protein on dry basis), a coproduct of corn wet mills. “Freeman Industries, USA and Showa Sangyo Corp., Japan” are the only two companies which currently produce zein commercially. Its price depends on its purity, because of its high price and manufacturing cost its utilization is limited [1].

1.1.2 Zein Structure

Zein is made of different peptide chains linked by disulfide bonds. Based on the difference in molecular size, solubility and charge of the peptide chains, the protein is classified into four classes: \( \alpha \), \( \beta \), \( \gamma \) and \( \delta \) [2]. \( \alpha \)-zein is the major zein protein; it represents ~80% of total prolamine present in corn and is soluble in 60 - 95% aqueous ethanol [2]. It is made of 2 proteins Z19 and Z22, with molecular weights of 19kDa and 22kDa, respectively. \( \beta \)-zein makes up about 10% of the total zein present in corn and other 2 fractions are present in minor quantities [2]. Zein is a hydrophobic protein having an average hydrophobicity of 1.365 J/mol [3, 4]. The hydrophobicity is due to the presence of high proportion of non-polar amino acid residues. \( \alpha \)-zein is more hydrophobic when compared to other fractions [4]. The structural model of \( \alpha \)-zein is reported in many
publications but the crystal structure of the protein is still unavailable in Protein Data Bank \[5\]. \(\alpha\)-zein is generally known to have helix secondary structure and an asymmetric globular folding contour. The structure of zein changes when dissolved in different solvents based on the concentration of the zein solution. The structure also depends on pH and temperature of the solvent \[5, 6\].

### 1.1.3 Solubility of Zein

Zein’s defining characteristic is its insolubility in water, which is due to its amino acid composition. It is largely made of non-polar amino acids such as leucine, proline, alanine, glycine, valine and others, due to which the solvent selected for zein must contain both ionic and non-ionic polar groups as well as nonpolar groups. Mixture of solvents or pure solvents can be used \[1\]. Aqueous ethanol solutions (60-95%) are widely used to dissolve zein. Other solvents commonly used with zein are ketones, amide solvents, in high concentration of salts, esters and glycols. The presence of the following groups in the solvent is important: -OH, -NH\(_2\), -CONH\(_2\), or -COOH \[1\].

As mentioned above, zein exhibits different solution behaviors. Argos et al. (1982) proposed that zein in methanol were seen as antiparallel helices clustered within a distorted cylinder \[7, 8\]. Matsushima and coworkers \[9\] proposed a prism 13 x 1.2 x 3 nm\(^3\) as a model with 9-10 helical elements aligned in antiparallel fashion in 70% (v/v) aqueous ethanol solution at 2.0 – 40.0 mg of \(\alpha\)-zein/ml of solvent, after taking an SAXS measurement \[8\]. Later Momany et al. (2006) supported the same model \[10\]. Li et al. (2011) observed scaling behavior of \(\alpha\)-zein in acetic acid solutions; they observed two distinct scaling regions under identical critical concentrations \[5\]. Studies have also shown that zein has altered secondary and tertiary structures in acetic acid or 60% - 95% ethanol
60-100% acetic acid solutions can be used to dissolve α-zein. Acetic acid was chosen as a solvent in this research. It has been used as a good solvent for zein during isolation of zein from corn extract and has been shown to produce smoother and featureless zein films when compared to zein films cast from aqueous ethanol solutions. Shi et al. (2011) also showed that zein solutions with acetic acid were less yellow and had lower viscosity when compared to zein solutions with aqueous ethanol. The plasticization effect of carboxylic acid is known to be the reason for the lower viscosity of acetic acid-zein solutions.

1.1.4 Applications of Zein

Zein has been commercially available for 60 years now. Before petroleum, zein was extensively used in adhesives, binders, films, etc. Currently, zein has a variety of industrial and consumer applications due to the need to switch to biodegradable alternatives. Zein is used as an ingredient in coatings, fibers, biodegradable films and adhesives. It is also used in controlled released drugs and a few health applications. Zein has been classified as GRAS (Generally recognized as safe) by FDA and is suitable for use in the food industry. In the food industry it has been widely used as a barrier for oxygen, moisture, etc. for various products like nuts, candies, etc.

1.1.5 Film forming properties of Zein

In early 1960s zein was extensively used in fibers and coating industry. Later, petroleum-based products became more popular because of its low cost, lightness, flexibility in thermal and mechanical properties, thermo-seal ability, etc. One major disadvantage of such polymers is that they are not biodegradable. Owing to the recent interests in
biodegradable packaging, biopolymers such as starch, cellulose, zein, etc. are being extensively studied to replace conventional polymers. The investigation of such biopolymers plays along with the trend of novel material development. Keywords such as “functional”, “environment-friendly”, “green”, “sustainable”, etc. could be used for such developments.

Zein has a natural ability to form films. The zein films are “tough, glossy, hydrophobic, greaseproof and are resistant to microbial attacks” [1]. Zein films are formed by the development of hydrophobic interactions, hydrogen bonds and limited disulfide bonds between zein chains [15]. The problems with zein films are that they are brittle, and although hydrophobic, zein films are shown to have high water vapor permeability and low tensile strength when compared to commodity polymers [16]. To successfully use zein films as replacement for plastic packaging, its properties need to be improved. To achieve improved properties, chemical modification of zein itself or incorporation of plasticizers are the common approaches used. However, the latter is more convenient and economical and hence is a popular approach.

Plasticizers are low molecular mass organic compounds, which are added to soften rigid polymers. The addition of plasticizers to biopolymers is known to decrease the intermolecular forces between the polymer chains and thus they improve the flexibility and decrease brittleness in the polymer film. The low molecular size allows the plasticizer to occupy intermolecular spaces between the polymer chains, disrupting the hydrogen bonds between them. These molecules also change the 3D molecular arrangement of the polymer, reducing the energy required for molecular motion. As a result, free volume is increased and, hence, chain flexibility is observed [17]. The affect of
plasticizers is often a function of composition, size and shape of the plasticizers \[17\]. In the past, several molecules/compounds such as sugars, PEG, and fatty acids have been used as plasticizers for zein films. Ghanbarzadeh et al. (2007) used sugars such as glucose, fructose and galactose to achieve plasticizing effects \[15, 18\]. Scramin et al. (2011) used oleic acid to improve the properties of zein films. His results showed that lower amounts of plasticizer improved the mechanical properties of the films. However the Young’s modulus decreased with the increasing content of the plasticizer \[19\]. In another study, Santosa et al. (1999) used oleic acid and linoleic acid to plasticize zein sheets and were able to develop zein films which had high clarity, low Young’s modulus, high elongation and toughness but low tensile strength. The water absorption by the zein sheets was also lowered due to the incorporation of the plasticizers but all this was due to a re-plasticization process, which was time consuming \[20\].

Plasticizers are divided into water-soluble and water-insoluble \[17\]. The film formation and its properties are highly affected by the type and properties of the plasticizers. Hydrophilic plasticizers such as glycerol, sorbitol, propylene glycol, and polyethylene glycol dissolve in the aqueous medium and high concentrations can contribute to an increase in water permeability \[15\]. These plasticizers increase the zein chain mobility and hence the free volume, this in turn increases the coefficient of diffusion, facilitating moisture penetration \[19\]. Therefore, hydrophobic plasticizers have to be included in filmogenic formulations to reduce this behavior. They are known to reduce the micro-voids in the film, which in turn reduces the water uptake. The incorporation of a hydrophobic plasticizer could be enhanced by optimizing the stirring rate of the polymer-plasticizer solution; otherwise it may lead to phase separation and will not improve the
properties of the polymer film \[17\].

Hydrophobic plasticizers are used for the production of biodegradable materials mainly in the pharmaceutical industry and less used in film preparations, mainly because of its low toxicity and low migration \[21\]. Citrate esters, which are derived from citric acid, are examples of hydrophobic plasticizers. Citrate esters are non-toxic and are used in plastics for medical application, personal care, and food contact applications. They also present a fast biodegradability \[21\]. Labrecque et al. (1997) used tributyl citrate, acetyl-tributyl citrate, triethyl citrate and acetyl-triethyl citrate hydrophobic plasticizers in the production of films extruded through poly-vinyl alcohol (PVA). The resulting films were extremely flexible with elongations reach >600%. However, as the addition of plasticizers increased, the tensile strength decreased by 80%. This also reduced the glass transition temperature of the resulting films \[21\].

In this research, tributyl citrate (TBC) was chosen as the plasticizer for zein films. Figure 1 represents the structure of TBC.

![Figure 1. Structure of Tributyl Citrate (TBC)](image-url)
Although plasticizers improve the zein films’ properties, the production of zein films, which is close to conventional synthetic polymer films, is still challenging. Therefore, a new strategy has to be developed to produce the zein films so as to increase its usage in the food industry.

1.2. Nanocomposites

1.2.1. Background Information

Nanotechnology is defined as creation and subsequent utilization of structures with at least one dimension less than 100nm\textsuperscript{[19]}. Nanotechnology could be one of the methods to further improve the properties of zein films for food packaging purposes. Nanotechnology methods have helped food packaging industry in a number of ways. It has helped in improving package material properties, in establishing nano-encapsulation techniques to aid in control release of antimicrobials, nutraceuticals, etc.

“Nanocomposites are hybrid nanostructured materials with improved mechanical, thermal and gas barrier properties“ \textsuperscript{[22]}. ‘Nanocomposite’ refers to composite materials with low concentrations of nanoparticles. The use of nanocomposites made from biopolymers for food packaging not only protects the food but are also environment friendly, since it reduces the requirement to use conventional plastics as packaging materials.

In general, polymer composites are made up of mixtures of polymers, which form the continuous phase and a discontinuous phase or fillers. Fillers are usually in the form of fibers, platelets or particles. Any form could be used in the film formulation. Several polymer-filler combinations have been used to obtain composites with enhanced
mechanical and thermal properties. Composites in which the filler has at least one dimension less than 100 nm are called nanocomposites. Nanocomposites exhibit increased barrier and mechanical properties over their conventional composites \[16\]. Nanosized montmorillonite clay is a classic example of filler used to improve properties of nylon. Other examples include kaolinite clay, cellulose nanofibers, etc. A good interaction between the polymer matrix and the filler is highly desired and has always been a challenge. Good interaction between the polymer and the filler improves the properties of the packaging film. Therefore, it is important to know the nature of interaction between the polymer and the filler, the knowledge of which will help tuning the properties of the nanocomposites as desired. In general, it is said smaller particle size and good dispersion of the filler in the matrix will help achieve better properties \[19\]. Increased interest in sustainable packaging has led to the use of biopolymers. One such biopolymer would be zein, which is used in the food industry as a coating agent. The use of biopolymers in packaging applications is limited, since they have poor mechanical and barrier properties. Thus, nanocomposite technology has proven to be a promising option for biopolymer – based packaging materials, which results in improved mechanical and barrier properties \[16\]. The use of fillers and plasticizers, which are environment friendly and biodegradable, can further help achieve sustainable packaging goals. Nanocomposites can withstand the stress during thermal processing, transportation and storage \[16\]. There are two main techniques used to produce nanocomposites \[23\]:

- Water/solvent evaporation by solvent-casting; and
- Extrusion (also called the melting compound technique, using freeze-dried nanoparticles)
The first technique is the most widely used. Extrusion of nanocomposites is not commonly used in a laboratory set-up but scale up from lab to industry would be easy and realistic using the second method. Electrospinning could be a third method to produce nanocomposite films/fibers.

Zein nanocomposite films contain zein as the continuous polymer phase and filler as the dispersed phase. Choosing the right filler, which limits the water barrier properties and improves the mechanical properties, is important. Nanofillers are known to introduce a tortuous path for moisture or gas molecules to pass through, thereby reducing the gas/moisture permeability, as represented in figure 2 below. As tortuosity is included in the polymer matrix, higher barrier properties are achieved. Tortuosity is given by the equation below,

$$\frac{P}{p} = \frac{1}{1 + \left( \frac{L}{2W} \right) \Phi}$$

Where, according to figure 2, P and p represent the gas/moisture permeability of the nanocomposite and the pure polymer film respectively, the term $\tau = 1 + \left( \frac{L}{2W} \right) \Phi$ is called the tortuosity factor. L and W are the length and thickness of the filler layers respectively, and, $\Phi$ is the volume fraction of the filler. Therefore, the greater the tortuosity factor lesser the gas/moisture permeability. $(L/W)$ is also known as the aspect ratio of the barrier, therefore a higher aspect ratio gives a greater barrier to moisture and gas $^{[24]}$. Figure 2 represents layered silicate/platelets as the geometry of the filler. Aspect ratio and degree of dispersion of the bio-based filler are important factors to achieve greater barrier properties.
Nanofillers are also known to improve mechanical properties and thermal properties of the composite films [25, 26]. The improvement in mechanical properties, particularly the tensile strength is achieved when there is a good interaction between the nanofillers and the polymer in nanocomposites as seen in past research [25, 26]. The interaction results in the formation of a fibrilar network, which increases the tensile strength of the nanocomposite films. The interaction between the filler and the polymer in the nanocomposites has also increased the glass transition temperature of the films as shown in some past research work [25, 27], and therefore greater thermal stability of the films is achieved. Glass transition temperature ($T_g$) is the temperature at which the polymer goes from glass state to rubber state. DSC is often used to measure the $T_g$ of materials and defines $T_g$ as the change in heat flow as the material goes from being glass-like to rubber-like state. Since this transition is an endothermic transition it is known as a second order transition i.e. it requires ‘heat’ to undergo transition from one state to another. Therefore DSC results often represent $T_g$ as a step change rather than a peak. Higher $T_g$ indicates
resistance to process temperatures and therefore greater thermal stability. Therefore, pure zein film has a fixed $T_g$ value, which changes once the filler is added due to change in material properties based on whether the filler interacts with zein or not. If there is an interaction between zein and filler then the $T_g$ value may increase or decrease, where increased $T_g$ value indicates greater thermal stability.

Cellulose was chosen as the bio-based filler in this research. Microcrystalline Cellulose (or MCC) was used as raw material to develop nano-sized cellulose particles by a wet-media milling method.

1.2.2. Cellulose: Overview

Lignocellulosic biomass (includes agricultural crops and residues, animal and municipal wastes, etc.) is the largest source of cellulose in the world. $2 \times 10^{11}$ tons of lignocellulosic residues are produced every year $^{[28]}$.

Applications of lignocellulosic biomass include:

- Pulp and paper making
- Source for cellulose for textiles, composites and paper
- Production of ethanol and other sugars by fermentation
- Converted to C, H$_2$ and O$_2$ to produce various chemicals, enzymes and proteins

Three main chemical components in the biomass are Cellulose, Hemicellulose and Lignin. Cellulose is the most abundant natural biopolymer on earth. It is renewable, biodegradable and non-toxic. Natural cellulose fibers are commonly referred to as ‘Ultimates’, e.g. Cotton and kapok $^{[28]}$. Retting process is used for cellulose extraction i.e. separation of cellulose fiber from non-cellulosic substances in lignocellulosic
byproducts. Retting is brought about by bacteria and fungi, mechanical and chemical methods.

Cellulose is a natural polymer, which is both abundant and renewable $^{[23]}$. It is a homopolysaccharide composed of β-D-glucopyranose units linked by β-(1,4)-glycosidic bonds. They are naturally present in the form of Microfibrils, which are linked together to form cellulose fibers. As shown in figure 3, they are long ‘thread-like’ molecules laterally stabilized by hydrogen bonds $^{[23]}$. Figure 4 shows the cellulose structure along with its smallest repetitive unit – Cellobiose, which is a single unit in a microfibril.

Infrared spectroscopy and X-Ray Diffraction studies have shown that cellulose is mainly made of crystalline regions with amorphous regions interspersed throughout the molecule$^{[23]}$.

![Figure 3. Scheme of cellulose in cell wall and its organization - Siqueira et al. (2010)](image)
1.2.3. Hydrolysis of Cellulose fibers

As mentioned earlier, cellulose can be separated from its lignocellulosic biomass by retting process. The resulting cellulose fibers can be further broken down by hydrolysis. There are three main methods used to hydrolyze cellulose fibers, they are: enzymatic cleavage, chemical and mechanical methods.

**Enzymatic:** The use of cellulose hydrolyzing enzymes called cellulases can break the polymer into smaller units. Depending on where the enzymes attack on the cellulose molecule, cellulases are classified into three types:

- **Endoglucanases** - randomly cleaves internal bonds of cellulose chains
- **Cellobiohydrolases** – attacks chain ends
- **β-glucosidases** – active on oligosaccharides and cellobiose units releasing glucose monomers

Figure 5, highlights the cleavage sites for the three types of cellulases described above.

Enzymatic hydrolysis depends on various factors like the substrate’s physical properties, enzyme synergy, mass transfer and the intrinsic kinetics \(^{29}\). Thus, enzymes bring about enzymatic hydrolysis. These enzymes act as catalysts to break the glycosidic bonds of cellulose. Cellulases from strains of *Trichoderma spp.* are often used \(^{28}\).
Figure 5. Cleavage sites of different cellulases during enzyme hydrolysis.

Chemical: Use of alkalis, mild acids and enzymes break down or separate the cellulose fibers. Sodium hydroxide is the most commonly used chemical for extraction of cellulose fiber. Acids (sulfuric acid and oxalic acid) in combination with detergents are also used \([28, 30]\).

Mechanical: Extraction of cellulose fibers by mechanical methods includes milling methods, Tilby process, steam explosion, and sonication. Tilby process, for example, is used to extract cellulose from wheat straw and rind of sugarcane for various applications in the manufacture of textiles, paper, composites, etc \([28]\). Two or more of the above hydrolysis methods are often combined to produce nano-sized cellulose.

1.2.4. Wet-Media Milling Process: Mechanical Method

Du Pont introduced wet-media mill commercially in 1948, primarily used by the paint and lacquer industries \([31]\). Recently, media milling has been a popular method to prepare
nanoparticles in the drug industry \textsuperscript{[31]}. Wet-media mills are used to break down the compound (poorly soluble in water) into smaller nano-meter sized particles. A suspension of the substance in water forms the water-based fluid phase. Figure 6 shows the schematic diagram of using wet-media milling technology \textsuperscript{[32]}; the milling chamber contains the crude slurry of the poorly water-soluble compound with/without the stabilizer. Once the motor activates the impeller the media and the compounds in it, along with the beads (Zirconium) and the chamber walls bring significant shearing forces resulting in particle size reduction from hundreds of microns to nanometer size \textsuperscript{[33]}. The particle size reduction is a function of processing time, mill speed, and mill power. It is shown that the mean residence time for most crystalline compounds is 30 to 120 min \textsuperscript{[33]}. 

![Figure 6. A schematic diagram of the wet-media milling technology; not shown in the picture are the milling beads inside of the milling chamber - Liversidge et al. (2003) ](image)

Yeh et al. (2010) used media milling to reduce the particle size of MCC; they achieved this by combining cellulase enzyme into the MCC suspension. The aim of the work was to study the effect of particle size on the rate of enzymatic hydrolysis of cellulose \textsuperscript{[34]}. 
Liimatainen et al. (2011) used media milling to produce cellulose fiber of length <50 µm and diameter ranging from few hundreds of nanometer to 1 µm, from Kraft wood pulp. The result was seen after only 5 min of milling.\textsuperscript{35}

An important aspect of particle size reduction is that smaller the particle sizes greater the agglomeration/aggregation. The increased surface area of the nanoparticles creates a positive gain in free energy and therefore tends to aggregate. Addition of stabilizer reduces the free energy associated with the particles and acts as an energy barrier to prevent agglomeration.\textsuperscript{33} Commonly used stabilizers include gum acacia, starch, lecithin, polysorbates, etc. Charged stabilizers (e.g. sodium lauryl sulfate) and neutral polymers are often combined to prevent agglomeration.\textsuperscript{33} In this research, Gum Arabic or GA and starch were chosen as stabilizers. Cellulose solutions with starch and GA was tested separately to choose the best stabilizer for this research.

GA is an exudate from mostly \textit{Acacia senegal} trees. It is a complex polysaccharide obtained as calcium, magnesium, and potassium salt. The backbone is made of 1,3-linked β-D-galactopyranosyl units. The branched polysaccharide has a side chain of 1,3- linked β-D-galactopyranosyl units, which is linked to the main chain by 1,6-linkages of galactopyranose units.\textsuperscript{36} GA is known to contain traces of lipid content, which may be attached to the branched chain. These lipids are known to provide the emulsifying ability for GA.\textsuperscript{37} GA has been used as a stabilizer, thickening agent, and emulsifier commercially. Modified starch has been used as stabilizer and emulsifying agent in industries as well. The non-polar character of the chemical groups present in modified starches gives most of its emulsifying or stabilizing ability.\textsuperscript{38}
Therefore, this study aims at producing nano-sized cellulose by milling Avicel™ PH-101/MCC using media-mill technology. The media-mill that was used in the research is shown in figure 7, it is called Wet Mills MiniSeries (Netzsch Premier Technologies, LLC., PA, US). The resulting nano-sized cellulose will be used to make ‘Nanocomposites’.

![Figure 7: MiniSeries Wet Mill from Netzsch premier Technologies, LLC](image)

1.2.5. Cellulose: nanofiller for Zein Nanocomposites

Various fillers have been evaluated in the past to improve the properties of zein nanocomposites. Silica based nanofillers were used to make zein nanocomposites which drastically improved its properties [29]. Luecha et al. (2010) used MMT clay to improve the properties of zein nanocomposites, they found that the thermal resistance of zein
improved as the MMT content increased however, the mechanical and barrier properties of zein had a non-linear relationship with MMT concentration. The improvement of mechanical properties, especially the tensile strength, was due to the interaction of zein and MMT, 5% concentration of filler was concluded to be the critical concentration in the study. They also stated that the improvement of barrier properties depends on the nanocomposite structure (i.e. if the structure is intercalated, exfoliated or both) as well as the orientation of the filler in the matrix of zein [25].

100% bio-based films are obtained by using all materials including filler that are biodegradable. These bio-based fillers have excellent mechanical strength, flexibility, lightness and sometimes are also edible. Oliviero et al. (2011) studied the supramolecular structure of zein-lignin bionanocomposites. They found that zein with alkaline lignin at low concentrations showed relevant protein structural changes, where strong H-bonds are formed between functional groups of lignin and amino acids of zein, which causes secondary structure of zein to break down [39].

Cellulose has been used as nano-reinforcements to produce nanocomposites. Those nanocomposites have had good tensile strength. Microfibrils and whiskers are the most commonly used types to form composites. Microfibrils are fibers, which are polymer chains stabilized by hydrogen bonds. They have lengths in micrometer range and diameter in nanometer range. The crystalline parts of microfibrils can be isolated by various treatments to form whiskers (also known as nanocrystals, nanorods, and cellulose microcrystals).

MCC also commercially known as Avicel is closely related to cellulose whiskers. Avicel was first prepared by alpha – cellulose as disclosed by the patent of Battista et al. (1961).
It is available in both powder and gel forms and widely used in food preparations to replace dry or fat based ingredients. It adds no taste, calories, and smell to food.

MCC is obtained by acid hydrolysis leaving the less accessible crystalline regions as fine crystals. Depending on the source and the method of treatment, the degree of polymerization is about 140-400 \cite{27}. Enzyme hydrolysis of cellulose has certain disadvantages; it occurs at a low rate and gives a minimum yield due to lignin present in the lignocellulosic biomass. Therefore, acid hydrolysis is more advantageous; it leads to solubilization of cellulose and formation of products with low degree of polymerization.

Acid hydrolysis occurs at a faster rate and acids are much cheaper than enzymes. Dilute acids are desirable since concentrated acids give glucose as the end product \cite{40}.

Azeredo et al. (2009) used cellulose nanofibers (CNF) to improve tensile properties, water vapor permeability and glass transition temperatures of mango puree films, the results showed that as the CNF concentration increased, the tensile strength also increased \cite{27}. This was due to good interaction between the hydroxyl groups of cellulose with the hydrophilic sites of mango starch. The interaction also improved the dispersion of the filler inside the matrix of the mango puree films, which resulted in a significant decrease in the water vapor permeability through the mango puree films. Chen et al. (2009) used cellulose whiskers to produce nanocomposites with pea starch matrix. They showed that cellulose whiskers with high aspect ratio produced transparent films with best tensile strength when compared to those with low aspect ratio \cite{41}. Several scientists showed that aspect ratio and orientation of the fillers in the films affect the mechanical properties of the film.

Cho et al. (2011) prepared Poly(Vinyl Alcohol) or PVA nanocomposites reinforced with
nano-sized cellulose obtained from acid hydrolysis of MCC. The films showed improved tensile strength and thermal properties for loadings up to 7% by weight of cellulose. Their results showed that nanocellulose has great potential to reinforce PVA polymers\[^{42}\]. Nadanathangam et al. (2011) used nanocellulose to reinforce starch films. They used GA to equally distribute the filler into the starch matrix. The resulting films showed improved tensile strength by 3.5 times and improved water barrier properties by 2 times than pure starch films. They also showed that the nano-sized cellulose improved the properties of starch films when compared to micro-sized cellulose in a past research work\[^{26}\].

Therefore, the improvement in mechanical properties of the nanocomposites is not only due to the intrinsic strength of the nanoparticles (which includes the geometry and the stiffness of the cellulose particles) but also could be due to the formation of a fibril network in the polymer matrix linked through hydrogen bonds which may provide additional support/strength to the nanocomposites\[^{41}\]. The improvement of barrier properties on the other hand is by increasing the tortuosity and in turn decreasing the water vapor permeability or water absorption capacity.

Thus, this study aims at using nano-sized cellulose as the filler to improve the mechanical and barrier properties of zein films. To the best of our knowledge, there has been no study in literature so far on the synthesis of zein nanocomposite films using cellulose as filler.

1.2.6. Potential Applications of Zein-Cellulose Nanocomposite Films

Use of zein, cellulose (as nanofiller) and TBC (as plasticizer) in this research, will help produce biodegradable films that are environment friendly when compared to conventional packaging (see figure 8). Thus, zein-cellulose nanocomposites will be 100%
biopolymer-based system having a potential demand in variety of eco-friendly applications.

Figure 8. Depicts the biodegradability of the bionanocomposite films making them environmentally friendly than traditional packaging.

Nanocomposites produced with better mechanical and barrier properties will help extend the shelf life of food, thereby enhancing food-quality. Along with reducing the solid packaging wastes, it also reduces the reliance on fossil fuel. Thus, the nanocomposites promises to expand the usage of biodegradable films in packaging and edible films/coating applications.

In addition, the package could be further designed for control or burst release applications of antimicrobials or antioxidants in active or functional food packaging technologies and encapsulation of value-adding food additives such as nutraceuticals. Although promising results can be obtained, the road to successfully eliminating conventional packaging systems is long.
This research work aims at using nanotechnology to develop zein nanocomposites, which promises to reduce the environmental impact along with having better mechanical, and barrier properties.

1.3. Hypothesis and Objectives

1.3.1. Hypothesis

We hypothesize that incorporation of nano-sized cellulose as filler will improve the mechanical and water barrier properties of zein films.

1.3.2. Objectives

Objective 1: Preparation of nano-sized cellulose for its application as filler in nanocomposites

Objective 2: Preparation and characterization of zein-cellulose nanocomposites films

1.3.3. Scope of Research

The objectives were further divided into 3 phases for ease of work:

(a) Preparation and characterization of nano-sized cellulose from Avicel PH 101

Wet media milling process produces physically stable nanometer-sized particles of poorly soluble compounds. Cellulose in water is one such example. In wet milling machine there is shearing of the media against small beads in the milling chamber, which brings about particle size reduction.

An important aspect of particle size reduction is that smaller the particle size, the greater the agglomeration/aggregation. The increased surface area of the nanoparticles creates a positive gain in free energy and therefore tends to
aggregate. Addition of stabilizer reduces the free energy associated with the particles and acts as an energy barrier to prevent agglomeration \[^{[33]}\]. GA and starch were used as stabilizers. Nano-cellulose was prepared from MCC or Avicel PH 101 (food-grade, FMC Biopolymer, PA) using Wet Mills MiniSeries (Netzsch Premier Technologies, LLC., PA, US).

(b) **Preparation of Zein Nanocomposite Films using different filler concentrations**

The second phase of the research involved the preparation of zein nanocomposite films. Zein, cellulose (nanofiller – naked and GA-stabilized) and plasticizer (TBC) were used to make films. 1%, 3%, 5% and 10% of filler concentration by weight was incorporated in the film by solvent-cast technique. The controls were pure zein films and zein films with only plasticizer and no filler.

(c) **Characterization of Zein - Cellulose Nanocomposite Films**

Zein - cellulose nanocomposite films were characterized using the following techniques:

1. **Mechanical properties measurement**: The strength and resistance of packaging materials against different applied loads is understood by conducting mechanical properties measurements. The tensile strength and elongation at break (ETB) of the films were measured using CT3 Texture Analyzer - 25kg (Brookfield Engineering Labs Inc., MA, US).

   The various parameters which were obtained were - Ultimate Tensile Strength (UTS), which is the maximum tensile stress; ETB, which is the strain at the moment the sample breaks, and toughness, which is the area under the stress strain curve before the break point, it represents the
energy that the material can absorb before it ruptures.

2. Water Vapor Permeability and Water absorption measurements: The barrier properties of the films were measured by performing WVP and water absorption experiments. WVP was determined according to the ASTM E96/E96M-05 method \(^{[43]} \). The data collected gives weight gain over time graph from which WVP is calculated. The water absorption values were measured using ASTM D870-90 standard method \(^{[44]} \) with a slight modification. The water gain over time graph was used to measure the rate of water uptake. The samples were immersed for up to two hours. The water absorption % vs. time was plotted to get the rate of water uptake and that is nothing but the slopes of the curves obtained.

3. Thermal properties measurement: Differential scanning calorimetry was performed using DSC Q2000 Series (TA Instruments, New Castle, DE). The changes in glass transition temperature \((T_g)\) were automatically analyzed using the software. DSC was used to analyze the change in \(T_g\) before and after the addition of filler.

4. FTIR Analysis: Thermal Nicolet Nexus 670 FT-IR spectrometer was used to study the IR absorption patterns for films with different cellulose loadings; this data would give us information about the various interaction/s that may exist in the films among various molecules in the zein-cellulose nanocomposites.

5. Tapping Mode - Atomic Force Microscopy: This was used to study surface morphology of the films. Surface roughness was another factor
that was measured using the AFM Nanoscope software.

1.3.3. Challenges

Preparation of nano-sized cellulose was significantly challenging. Even though the conditions to prepare cellulose were similar for every run, the particle size of each sample collected varied. Thus, nano-size obtained each time was different and was not repeatable. To overcome this variability, a large amount of sample was collected at each interval and the sample that showed the least particle size was used to make zein-cellulose nanocomposites for the research.
2. EXPERIMENTAL DETAILS

2.1. Materials

The α-zein of biochemical-grade purity was obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Tributyl citrate (TBC, ≥ 97%) was obtained from Sigma-Aldrich Chemical Company. Microcrystalline cellulose or Avicel™ type PH-101 was obtained from FMC Biopolymer, (Philadelphia, PA) and glacial acetic acid was obtained from Fisher Scientific, Inc. (Pittsburgh, PA).

2.2. Methods

2.2.1. Preparation of nano-sized cellulose (Filler)

Nano-sized cellulose was prepared using MCC (Avicel™; Average size 50µm) by wet milling media process using Wet Mills MiniSeries (Netzsch Premier Technologies, LLC., PA, US; figure 7). Initially, aqueous suspensions with 1%, 2%, 3%, 5% cellulose loadings were milled using the process parameters listed in Table 1. Later, the concentration, which gave the least particle size, was selected and milled again with GA and starch as stabilizers separately.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Volume</td>
<td>300 ml</td>
</tr>
<tr>
<td>Pump Speed</td>
<td>100 rpm</td>
</tr>
<tr>
<td>Mill Speed</td>
<td>3301 rpm</td>
</tr>
<tr>
<td>Mill Power</td>
<td>0.35 KW</td>
</tr>
<tr>
<td>Mill Run Time</td>
<td>1h</td>
</tr>
</tbody>
</table>
Dynamic Light Scattering (DLS) particle size analyzer (Brookhaven 90plus®, NY, US) was used to monitor the particle size of the milled sample.

1.2.3. Dynamic Light Scattering (DLS)

After every sample collection from the milling machine, 1% concentration of the same was prepared using DI water. DLS analyzer (Brookhaven 90plus®, NY, US) was used to measure the hydrodynamic radius of cellulose particles in the samples. Three measurements were taken over 9 minutes to obtain average results.

2.2.3. Preparation of Zein Nanocomposite Films

Zein-Cellulose nanocomposite films were prepared with 1, 3, 5 and 10 wt% cellulose (noted as 1%ZMT to 10%ZMT) and 1, 3, 5 and 10 wt% cellulose stabilized with GA (noted as 1%ZMGT to 10%ZMGT). TBC of concentrations 10 wt% was used in film preparation. Zein, cellulose (nanofiller) and TBC (plasticizer) solutions were prepared by dissolving their corresponding amount in 70 ⁰C glacial acetic acid to the concentration of 2g (zein + TBC + filler) / 10ml glacial acetic acid, the solutions were magnetically stirred at 70 ⁰C for 30 min, and then cast in Teflon Petri dishes and dried in vacuum oven at 40 ⁰C for 48 hours. There were two controls for the experiment:

- Zein films without the filler and plasticizer (noted as pure zein films)
- Zein films with only plasticizer and without the filler (noted as +10%TBC)

2.2.4. Mechanical Properties Measurement

Triplicate zein nanocomposite films with a dimensions of 40 × 10 × (0.5 -1.0) mm³ were cut from the central region of the films and were conditioned at 50% RH and 25 ⁰C for 1 month before tensile test. Thickness of films was measured by vernier caliper. The
mechanical properties were measured on a CT3 Texture Analyzer – 25 kg (Brookfield Engineering Labs Inc., MA, US) with an initial grip distance of 18 mm and a test speed of 1 mm/s. Ultimate Tensile Strength, Elongation at break, and Young’s modulus was compared for each set of triplicates.

2.2.5. Water Vapor Permeability (WVP) Measurement

Water Vapor Permeability (WVP) was determined according to the ASTM E96/E96M-05 method\textsuperscript{[43]} at 25 ± 2°C. Aluminum cups were filled with anhydrous calcium chloride and sealed with zein nanocomposite films. They were placed in desiccators containing saturated potassium sulfate solution (97% RH) at 25°C. Duplicate specimens for each concentration of nanofillers along with the controls were weighed twice everyday for 10 days. Weight gain vs. time graph was plotted, after linear regression, slope of the straight line was calculated by

\[
WVP = \frac{G/t}{A \cdot P_S (RH_1 - RH_2)} \times \text{thickness},
\]

where

\[
\begin{align*}
G/t & \quad \text{the slope of the linear regression} \\
\text{thickness} & \quad \text{average thickness of zein films} \\
A & \quad \text{permeation area} \\
P_S & \quad \text{the saturated water vapor pressure at 25°C} \\
RH_1 - RH_2 & \quad \text{difference of relative humidity between the two sides of zein films.}
\end{align*}
\]

2.2.6. Thermal properties Measurement

Differential Scanning Calorimeter was used to measure the thermal properties of the zein nanocomposite films. DSC Q2000 Series (TA Instruments, New Castle, DE) was used to measure the same. The DSC was supplied with compressed nitrogen gas. The sample
preparation involved weighing out approximately 5mg of sample into aluminum pans without lid. They were heated from -30°C to 210°C at a modulated rate of 5°C/min. The software connected to the instrument developed thermal curves with glass transition temperature \((T_g)\) values.

2.2.7. Water Absorption

Water absorption properties were measured by a modification of the ASTM D870-90 standard method \(^{[44]}\). Triplicate samples measuring 30 mm x 30 mm were preconditioned at 50% RH and 25 °C for two days. Glass beakers were pre-dried at 60 °C for 24 h and weighed. The conditioned specimens were submerged in distilled water in beakers at 25 °C for up to two hours and weighed after every 15 minutes. The surface water was wiped off before weighing the specimens. Water Absorption was calculated as a percentage ratio of water absorbed by the film to its initial dry weight. The water in the beaker was evaporated at 60 °C for 24 h and weighed to determine the loss of soluble matter from the sheets.

2.2.8. FTIR Analysis

FTIR spectra of the zein nanocomposite film samples at different concentration of nanofillers were analyzed using Thermal Nicolet Nexus 670 FT-IR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) at 25°C using the Smart MIRacle Accessory\(^{TM}\). Each spectrum was averaged over 256 scans with 4 cm\(^{-1}\) resolution.

2.2.9. Atomic Force Microscopy (AFM)

The tapping mode Atomic Force Microscopy (TP-AFM) using Nanoscope IIIA Multimode AFM (Veeco Instruments Inc., Santa Barbara, CA), was used to study the
surface topography of the zein nanocomposite films. Sample was prepared using Spin Casting technique where, pre-cleaned silicon wafers were used to form zein films, WS-400A-6NPP/LITE spin coater (Laurell Technologies Corp., North Wales, PA) was used to spin coat the zein films at 4000rpm for 80 seconds. This technique results in clear homogenous films on the Si wafers; this is further dried overnight to remove the residual solvent. The Nanoscope software calculated Root-Mean-Square Roughness (RMS) value. RMS is the standard deviation of all the points of the scanned area of films.
3. RESULTS AND DISCUSSION

3.1. Particle size analysis of nano-sized cellulose

Nano-sized cellulose was prepared from avicel PH-101 by wet milling machine. The initial particle size of avicel PH-101 is 50 µm. Samples without stabilizer, with starch and with GA were milled separately. Initially 1%, 2%, 3% and 5% of avicel solutions (without stabilizers) were prepared and milled for 60 min. Samples were collected after every 15 min and the particle size was measured using Dynamic Light Scattering (DLS). Figure 9 shows the visual difference between the milled and the un-milled samples. The milled sample is stable in suspension when compared to the un-milled sample where the particles are big and therefore settles down. The DLS results showed that 2% concentration by weight gave the smallest particle size, as shown in figure 10. The figure also shows that during the first 15 min there is significant (p < 0.05) reduction in particle size.

Unstabilized cellulose often increases in particle size over time; the increase in size may be due to the increased surface area, which causes particles to aggregate. Avicel was therefore combined with GA and starch as stabilizers to see its affect on the particle size reduction. Table 2 lists the approximate particle size of the milled samples after 1 h of milling. As seen, the particle size was reduced by a degree of ~100 nm when 2% cellulose was combined with GA as stabilizer but was not significantly different. Starch however did not turn out to be a good stabilizer in this case. Figure 11 shows that cellulose milled with GA as stabilizer gives significantly smaller particle sizes (p < 0.05) when compared to cellulose milled with modified starch, which gave larger particle sizes. Therefore, GA is a better stabilizer than starch for cellulose nano-suspensions.
Figure 9. Avicel PH 101, milled and un-milled samples

Table 2: Particle size of Avicel with and without stabilizers

<table>
<thead>
<tr>
<th>Type</th>
<th>Particle Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure 2% Avicel</td>
<td>801.7 ± 47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% Avicel with 20% GA</td>
<td>732.1 ± 26.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2% Avicel with 20% Starch</td>
<td>1110.8 ± 54.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values with no letters in common are significantly different p < 0.05
Figure 10: Particle size reduction after 1 h of milling in log scale. The figure shows smallest particle size was achieved after 60min of milling.

Figure 11. Particle size reduction of cellulose milled with GA and starch as stabilizers.
The cellulose nano-suspensions (with and without GA) were kept for 45 days and the size stability was measured after 1, 10, 25 and 45 days, respectively. Figure 12 shows the size stability over a period of 45 days. Although it is expected that the particle size of naked cellulose would increase with time, that was not the case, there is no significant difference between particle size over a 45 day period as seen in the figure. Both stabilized and naked cellulose seem to be stable over this time period.

![Figure 12. Particle size stability over 45 days of cellulose milled with GA and without GA](image)

3.2. Characterization of Zein-Cellulose Nanocomposite films

Zein-cellulose nanocomposites were obtained after blending the nano-sized cellulose with zein along with plasticizer concentration of 10%. The films obtained were translucent and yellow for films containing 0%, 1%, 3% and 5% concentration by weight. All films were of thickness ~ 0.5 - 1 mm. The nanocomposites with 10% filler concentration were slightly translucent with a whitish hue (due to increased amount of
cellulose) in appearance. Figure 13 displays the films obtained after solvent casting method.

![Figure 13. (a) Pure Zein films; (b) Zein films with naked cellulose as filler; (c) Zein films with GA-stabilized cellulose as filler; All films looked the same irrespective of the concentration of filler](image)

### 3.2.1. Mechanical Properties

Zein-cellulose nanocomposites are prepared using 10% TBC as plasticizer and cellulose as filler. The plasticizer helps to increase the flexibility and extensibility of the nanocomposites. Cellulose on the other hand acts as filler, which is known to increase mechanical properties of nanocomposites. Figure 14 and figure 15 shows the stress-strain curves of nanocomposites loaded with cellulose and cellulose stabilized with GA, respectively. The typical tensile curves of pure zein films and TBC plasticized zein films are also included.

Pure zein films without plasticizer or filler are brittle and the figure suggests a linear relationship between stress and strain corresponding to a small elastic deformation. The addition of plasticizer improves the flexibility of the film with lesser rigidity as indicated by decrease in Young’s modulus. The general trend seen for films with filler is that in
both the figures, as the concentration of the filler increases, the elongation % increases significantly but only up to 5% loading and then decreases at 10% loading. Overall the filler, which was GA-stabilized had greater elongation % (about 30 times greater than pure zein films) compared to films with only naked cellulose as filler (about 16 times greater than pure zein films) at 5% concentration of both fillers. This may be due to the plasticizing effect of the added GA\textsuperscript{[26]}.

The Ultimate Tensile Strength (UTS) can also be extracted from the stress-strain curves; the UTS remained constant for films with naked cellulose as filler whereas the films with stabilized cellulose were softer and more flexible with lesser UTS values as the concentration increased. Young’s modulus is a measure of rigidity of the films, in both cases the Young’s modulus decreased as the cellulose concentration increased, showing that the films were more flexible compared to the pure zein films which is known to be brittle.

Scientists in the past have found an opposite affect where, the inclusion of filler has improved the tensile strength with decreased elongation %. For example, Luecha et al. (2010) developed zein-MMT nanocomposites and found that as the MMT concentration increased the Young’s modulus with the elongation % decreasing for the films prepared by the solvent-cast technique\textsuperscript{[25]}. Teaca et al. (2012) also found similar results where cellulose increased the tensile strength of the starch films while decreasing their elongation at break \textsuperscript{[45]}.

Nadanathangam et al. (2011) developed starch-nanocellulose films with GA and found an increase in the tensile strength of the films by 2.5 times pure starch films, while the elongation at break was almost the same as the pure starch films. The inclusion of GA improved the properties by equally distributing the nanocellulose
and this in turn distributed the stress uniformly throughout the film. The plasticizing effect of the GA was also seen by the increase in elongation at break once the GA was added [26].

In this research, the decrease in modulus and no change in tensile strength could be due to no/reduced interaction between the zein and the cellulose in the nanocomposites. If there were an interaction then the tensile strength of the film would increase due to the formation of stronger bonds between the zein-cellulose chains along with cellulose-cellulose interaction. The stronger bond formation would restrict the motion of the zein chains and hence would reduce the elongation %. An opposite effect seen in our research explains that there was no interaction between the zein and the cellulose; this allowed the zein chains to move freely and hence greater elongation %. The reduced/no interaction between the zein and the cellulose is proven with the DSC and FTIR results below. Overall, it can be concluded that 5% concentration of filler is the optimal concentration at which the films are flexible and less brittle with the same tensile strength as that of pure zein films.
Figure 14. Typical Stress-Strain curves for plasticized zein films with Cellulose as filler (Above);

Effect of cellulose on the relative mechanical properties of zein-cellulose nanocomposite films (Below)
Figure 15. Typical Stress-Strain curves for plasticized zein films with Cellulose stabilized with GA as filler (Above); Effect of Stabilized Cellulose on the relative mechanical properties of zein-cellulose nanocomposite films (Below)
3.2.2. Moisture Barrier Properties

Water Vapor Permeability (WVP) was measured to determine the barrier properties of zein-cellulose nanocomposites (figure 16 and figure 17). The WVP usually depends on the hydrophobicity of the polymer used and the distribution of fillers in the polymer matrix. The nanocomposites kept at high relative humidity made the films softer and swollen. The moisture makes the zein molecules more flow-able thereby making the polymer film less dense and therefore the water molecule easily passes through the film. It is expected that inclusion of the filler in the polymer film matrix will introduce a tortuous path and thereby restricts the water vapor permeability or improves the barrier properties of the zein nanocomposites. Naked cellulose and stabilized cellulose were therefore used as filler in the plasticized zein films.

*Zein –Cellulose Nanocomposites with Naked Cellulose as filler:*

Zein-cellulose nanocomposites with naked cellulose as filler showed significant (p < 0.05) improvement in barrier properties at 1% loading of cellulose, and after 1% as the concentration of the cellulose increased, the water vapor permeability increased significantly (as shown in figure 16). This may be due to two reasons,

(a) Uneven distribution of cellulose in the nanocomposite films

(b) Increased hydrophilicity due to the presence of naked cellulose which is hygroscopic in nature
Figure 16. Effect of Naked Cellulose on the water barrier properties of zein-cellulose nanocomposites; WVP at 1% is significantly different as compared to control group (p < 0.05)

*Zein–Cellulose Nanocomposites with Stabilized Cellulose as filler:*

Zein-cellulose nanocomposite films with GA-stabilized cellulose as filler showed improved barrier properties to moisture at 97% RH with the increase in cellulose content (As shown in figure 17). 5% cellulose loading showed the best value for the barrier to moisture, although there was no significant difference between WVP values for 1% to 10% filler loading.

The difference in results with the type of cellulose used could be due to the presence of GA in the latter. Nadanathangam et al. (2011) used cellulose to reinforce starch films. They used GA to equally distribute the filler into the starch matrix. The resulting films showed improved water barrier properties by 2 times than pure starch films. Improved
barrier properties were because of the presence/addition of GA since it improved the distribution/dispersion of the filler in the matrix \[^{26}\]. Therefore the results obtained for the zein-cellulose nanocomposites were similar to the starch nanocomposites with GA developed by Nadanathangam et al. (2011).

It was also stated earlier that smaller the particle size, the greater the tortuosity factor and greater tortuosity factor reduces the gas/moisture permeability. In this case, GA-stabilized cellulose is smaller in particle size when compare to naked cellulose, therefore the increased resistance to WVP by stabilized cellulose could be due to the decreased particle size when compared to naked cellulose. However, further analysis is required, methods such as TEM would give further insight into the exact dimensions of the cellulose with and without GA as stabilizer. This would help determine the values for tortuosity factor and extent of WVP restriction.
3.2.3. Water Absorption

Zein-cellulose nanocomposites were submerged in deionized water for 2 hrs and the weight gain was measured after every 15 min. The soaking of zein nanocomposites in water made the films softer, opaque, and sticky in appearance. The water absorption % was calculated as a percentage ratio of weight gain divided by time, and based on the type of filler used (figures 18 and 19). Loss of soluble matter was less than 1% and therefore no significant loss was observed. The equation developed by Lai et al. (1997) was applied to fit the data points in the figures (shown as solid lines) \[^{[46]}\]. The equation describes the kinetics of water absorption by zein films which is given by:

\[
y(t) = M_S \left(1 - e^{-t/T}\right)
\]
Table 3 and table 4 list the values of $M_s$ (Water Absorption Capacity), $M_s/T$ (Initial rate of absorption) and R (Correlation Coefficient); the R-value is greater than 0.994 indicating a reasonable fit.

**Zein –Cellulose Nanocomposites with Naked Cellulose as filler:**

As shown in figure 18 and the $M_s$ values listed in table 3, the water absorption % decreases with increase in concentration of the filler (up to 5%) and then increases at 10% concentration of the filler. A possible reason for the increase in water absorption capacity for 10% concentration of filler could be because of the hygroscopic nature of the naked cellulose in the film. Another plausible reason could be the uneven distribution of the cellulose in the zein matrix.

![Figure 18. Effect of naked cellulose filler on the water absorption of zein nanocomposites](image)
Table 3. Kinetic parameters of water absorption of zein-cellulose nanocomposite films w. naked cellulose as filler

<table>
<thead>
<tr>
<th></th>
<th>$M_s$ (g)</th>
<th>$M_s/T$ (g/min)</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Zein</td>
<td>73.0766 ± 1.9263</td>
<td>1.7173</td>
<td>0.9943</td>
</tr>
<tr>
<td>+10% TBC</td>
<td>77.1512 ± 1.5575</td>
<td>1.1264</td>
<td>0.999</td>
</tr>
<tr>
<td>+1% ZMT</td>
<td>65.4977 ± 1.5438</td>
<td>1.1789</td>
<td>0.9976</td>
</tr>
<tr>
<td>+3% ZMT</td>
<td>58.8889 ± 0.7172</td>
<td>0.9893</td>
<td>0.9995</td>
</tr>
<tr>
<td>+5% ZMT</td>
<td>57.8106 ± 0.926</td>
<td>1.0521</td>
<td>0.9989</td>
</tr>
<tr>
<td>+10% ZMT</td>
<td>61.7776 ± 0.6368</td>
<td>1.0625</td>
<td>0.9996</td>
</tr>
</tbody>
</table>

Zein–Cellulose Nanocomposites with Stabilized Cellulose as filler

As shown in figure 19 and the $M_s$ values listed in table 4, the water absorption % decreases with increase in concentration of the filler. The initial water absorption rate was also reduced with increase in concentration of the stabilized cellulose. Thus, along with the slowing down of initial water absorption there is also a delay in attaining equilibrium.
Figure 19. Effect of GA stabilized cellulose filler on the water absorption of zein nanocomposites
Table 4. Kinetic parameters of water absorption of zein-cellulose nanocomposite films w. GA stabilized cellulose as filler

<table>
<thead>
<tr>
<th></th>
<th>$M_s$ (g)</th>
<th>$M_s/T$ (g/min)</th>
<th>$R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Zein</td>
<td>73.0766 ± 1.9263</td>
<td>1.7173</td>
<td>0.9943</td>
</tr>
<tr>
<td>+10% TBC</td>
<td>77.1512 ± 1.5575</td>
<td>1.1264</td>
<td>0.999</td>
</tr>
<tr>
<td>+1% ZMGT</td>
<td>65.4421 ± 0.7174</td>
<td>1.2303</td>
<td>0.9995</td>
</tr>
<tr>
<td>+3% ZMGT</td>
<td>62.7354 ± 0.5839</td>
<td>1.1104</td>
<td>0.9997</td>
</tr>
<tr>
<td>+5% ZMGT</td>
<td>58.4332 ± 0.9118</td>
<td>1.1160</td>
<td>0.9989</td>
</tr>
<tr>
<td>+10% ZMGT</td>
<td>57.8981 ± 1.1617</td>
<td>0.8569</td>
<td>0.999</td>
</tr>
</tbody>
</table>

In summary, statistical analysis suggests that the zein nanocomposites with stabilized cellulose seems to be having lower water absorption capacity when compared to naked cellulose, this could be due to the even distribution of the GA stabilized cellulose in the zein matrix (as suggested by water vapor permeability results as well). Also, in general, as the concentration of the filler increased, the tortuosity factor also increased which reduces the water uptake by the films. Therefore, the addition of stabilized cellulose as filler limits the water absorption capacity of the zein-cellulose nanocomposites.

3.2.4. Thermal Properties

Zein and tributyl citrate (TBC) seem to have certain degree of miscibility since the physical appearance of zein films with 10% TBC is transparent with no phase separation. This can further be confirmed by the DSC thermograms represented below (figure 20a...
and b), which is indicated as a shift in \( T_g \) (Glass Transition Temperature) for zein films with 10% TBC. The \( T_g \) for pure zein films is 162.05 °C and it is shifted to a lower \( T_g \) of 147.84 °C by adding 10% TBC. The interaction of TBC with zein could be hydrophobic interactions owing to their nature. Pure TBC has a first order transition peak seen at -20°C, but once the plasticizer was included in the zein films the peak was not observed. This suggests that the zein and TBC mix up homogenously during solvent - casting. Once the fillers were added into the zein films plasticized with TBC, there was no further change in \( T_g \), indicating little or no affect of filler on the thermal properties of zein films. Scientists have found similar results in the past; Lu et al. (2008) evaluated the thermal properties of PVA nanocomposites with microfibrillated cellulose as filler and did not find any change in \( T_g \) \[^{23, 47}\]. Grunert et al. (2002) during their work with cellulose acetate butyrate reinforced with cellulose nanocrystals found that the nanocrystals had no effect on the glass transition of the films \[^{23, 48}\]. Although no past work involving zein and nano-sized cellulose exists, this could be a starting point to further evaluate the arrangement of cellulose within the zein matrix. The DSC thermograms also support the results obtained for mechanical properties of these nanocomposites, due to no interaction between the zein and the cellulose there is no difference in the tensile strength of the nanocomposites when compared to pure zein films.
Figure 20. (a) DSC thermograms for nanocomposites with naked cellulose as filler; (b) DSC thermograms for nanocomposites with GA-stabilized cellulose as filler.

3.2.5. Fourier Transform Infrared Spectroscopy

FTIR analysis was conducted for zein-cellulose nanocomposite films with different cellulose loadings. The characteristic peaks for cellulose are the hydrogen-bonded stretch at 3299 cm\(^{-1}\), the OH bending of the adsorbed water at 1644 cm\(^{-1}\), the CH stretching at 2921 cm\(^{-1}\), the HCH and OCH in-plane bending vibrations at 1430 cm\(^{-1}\), the CH deformation vibration at 1373 cm\(^{-1}\), the COC, CCO, and CCH deformation modes and stretching vibrations in which the motions of the C-5 and C-6 atoms are at 850 cm\(^{-1}\), and the C–OH out-of-plane bending mode around 670 cm\(^{-1}\)\(^{[26]}\). The characteristic peaks for zein amide band I and II are located at 1649 cm\(^{-1}\) and 1536 cm\(^{-1}\)\(^{[26]}\).

TBC is an ester of citric acid and the characteristic peaks for an ester is CO stretch between 1200-1000 cm\(^{-1}\) and 1760-1670 cm\(^{-1}\). As seen from the figures 21a and b below,
it is clear that the characteristic peaks are seen but the bands are highly overlapping and the spectra is complex with exact band assignment difficult. However, no new peaks/new bond formation is seen after the incorporation of cellulose. The band absorption at 3425 cm\(^{-1}\) represents the O-H stretching which could be due to the intramolecular interaction/hydrogen bond formation at the C-3 position of the cellulose. There could also be possible intramolecular interaction between C-2 and C-6 positions of cellulose as well\(^{[45]}\). The intensity of the peaks increases with the cellulose load increase. These results correlate with the DSC data proving no interaction between the cellulose and zein in the nanocomposites.

Figure 21. FTIR spectra of (a) films with naked cellulose as filler; (b) films with GA-stabilized cellulose as filler
3.2.6. Atomic Force Microscopy

The tapping mode Atomic Force Microscopy (TP-AFM) was used to study the surface morphology of zein films. Figure 22 shows the typical morphology at different cellulose loadings. Irrespective of the type of cellulose and amount of loading, all films were flat, smooth, featureless and comparable to the controls with no significant difference between them. However, films with higher loadings of 5% and 10% were thicker compared to the control and the low loading films.

The NanoScope v5.30 software as provided by the AFM manufacturer measured the RMS value of all the films. Figure 23 shows the RMS values, which describes the roughness of the films. It is clear from the figure that as the concentration of the filler increased the roughness slightly increases from 2.561 nm (for 1%) to 5.5795 nm (for 10%), however the effect is small. For pure zein films the roughness value is 0.999 nm. The reason for this small value is due to the interaction between the positively charged glutamine residues on zein and the negatively charged silicon surface with SiO groups\textsuperscript{[21, 36]}. The AFM images of the zein films seems to be flat but the roughness of the films is slightly increased with increase in cellulose concentration, this could be due to the increased presence of cellulose which is hydrophilic in nature, the cellulose does not interact with zein (as proved by DSC and FTIR) and has low miscibility, due to these reasons minute specks of cellulose slightly increase the surface roughness of the zein films.

The presence of 10% loading of TBC also did not have any affect on the surface structure of the zein films. There was no phase separation seen due to the presence of the plasticizer. The presence of cellulose in the zein films could not be seen through the
AFM, the better method to analyze the structural arrangement of cellulose inside the zein nanocomposites would be the use of Transmission Electron Microscopy (TEM).

Figure 22. TP-AFM images of zein-cellulose nanocomposites with different cellulose loadings. A. Pure zein film (0% cellulose), B. Films with 10% TBC (0% cellulose), C. 1% cellulose, D. 3% cellulose, E. 5% cellulose, F. 10% cellulose. The scan size is fixed at 10\(\mu\)m X 10\(\mu\)m
Figure 23. Plot of average RMS values roughness (nm) of zein-cellulose nanocomposites at different loadings of nano-sized cellulose.
4. CONCLUSION

Zein-cellulose nanocomposites were developed by the solvent-cast technique. The effect of different cellulose loadings on the mechanical and barrier properties was investigated. The results indicate that the incorporation of cellulose along with TBC as plasticizer helped reduce the brittleness and rigidity of the zein films. The addition of cellulose and the plasticizer together helped decrease the intermolecular forces between the zein chains, which in turn resulted in mobility of the zein chains, decreasing the brittleness and the rigidity of the nanocomposites.

This study also shows that formation of zein-cellulose nanocomposites had better mechanical and water barrier properties when compared to pure zein films. 5% loading of cellulose increased the elongation at break from 4.07% to 63.55% in case of naked cellulose and 4.07% to 166.60% in case of GA-stabilized cellulose. The best tensile properties of the nanocomposites made them flexible and tough at the same time. Nano-cellulose is crystalline in nature and has high modulus, therefore acts as excellent reinforcing agent. 5% is the optimal concentration of nano-cellulose at which the zein-cellulose nanocomposites have better mechanical properties, and particularly, has improved elongation of the films. The increase in elongation but no change in tensile strength could be explained by the FTIR and DSC results, which showed that there was no interaction between the zein and cellulose in the nanocomposites. If there were an interaction then there would be a network formation between zein and cellulose, which would increase the tensile strength of the films. No interaction between them allowed the zein chains to move freely. Therefore, the filler helped in increasing the elongation %
with no effect on the tensile strength of the films. The presence of GA may have enhanced the elongation % of the films due to its plasticizing effect.

The zein-cellulose nanocomposites also had improved water barrier properties, 1% loading of naked cellulose and 5% loading of GA-stabilized cellulose reduced the water vapor permeability by 4-6 times the values for pure zein film. The concentration of naked cellulose greater than 1% did not seem to reduce the WVP; this may be either due to uneven dispersion of the filler or the hygroscopic nature of the filler or both.

The water uptake by the nanocomposites decreased linearly with increase in filler concentration. As expected, the increase in concentration of the filler increases the tortuosity factor, which leads to retardation of water molecules and reduced rate of water uptake. 10% loading of naked cellulose however shows an increased water uptake value when compared to 5% loading; this could be again because of uneven distribution of the cellulose or the intrinsic hygroscopic nature of cellulose. In case of nanocomposites with GA-stabilized filler – the presence of GA in the films may have helped achieve even distribution of the cellulose in the zein matrix. Nadanathangam et al. (2011) found similar results when GA was incorporated in starch-nanocellulose films. It not only helped in even distribution of cellulose in the starch matrix but also helped in improving its mechanical and barrier properties.[26]

Incorporation of tributyl citrate, a hydrophobic plasticizer not only improves the flexibility of the zein films but also helps in improving the water barrier properties of the zein films by filling the micro voids in the film. 10% concentration of the plasticizer has good miscibility in the zein matrix; its interaction with the zein was seen by the decrease in glass transition temperature of the zein-TBC films.
This study also showed that there is no interaction between the zein and the cellulose as indicated by the FTIR and DSC results. FTIR data also showed that the cellulose may have formed a fibril network in the zein matrix due to formation of hydrogen bonds between them. 5% could be the minimum concentration of nanocellulose that must be present in order to form the fibril network \[41\], and hence better elongation % and water barrier properties at that loading. The presence of GA further enhanced the properties at 5% loading.

The increased loading of cellulose increased the thickness of the films but kept the film surface flat. There was also no visual difference between pure zein films and films with cellulose, all the films were yellow and transparent.

The zein-cellulose nanocomposites forms a 100% bio-based system with potential applications in food packaging and as edible films. The incorporation of the cellulose in zein films is a novel approach and this study gives a better understanding of using cellulose as nanoreinforcement for zein films.
5. FUTURE WORK

In this research work zein-cellulose nanocomposites were developed, it has improved mechanical and barrier properties. The next steps of research on these films can be divided into the following categories:

1) **Structural Characterization of Zein-Cellulose Nanocomposite films:**

To get improved properties, dispersion of the nanofillers in the polymer matrix is a key aspect. Therefore, controlled arrangement and interaction of the cellulose with the hydrophobic domains of the zein molecule could help achieve desired properties. The final molecular conformation is defined by the polymer and nanofillers together with the plasticizer and processing \[^{39}\]. Therefore it is important to first find out about the structural conformation of the zein and the cellulose to further improve the properties of nanocomposites.

FTIR and the DSC results showed that the zein and cellulose did not interact with each other in the nanocomposites developed. However, further characterization of the structural arrangement of all the components in the zein–cellulose nanocomposites is required to give more information on the particle dispersion, changes in the polymer matrix, the nature of the particle-polymer interface, etc. Various techniques are used to do so, the most common ones are X-ray Diffraction (XRD), wide angle and small angle scanning electron microscopy (SEM), Transmission Electron Microscopy (TEM), etc. TEM is a preferred method to study the nanofiller dispersion. TEM would give all the information such as polymer structure, void size and shape, filler size/shape and distribution. XRD on the other hand gives information on the degree & type of arrangement in the polymer matrix \[^{16}\].
If there is still no interaction between the zein and the cellulose, surface modification of
the nano-sized cellulose can be carried out in order to increase its interaction with the
hydrophobic polymer matrix. This may further improve the properties of the zein-
cellulose nanocomposite films. Chemical modification of the surface of cellulose, for
example, by esterification is shown to improve the adhesion between the cellulose fibers
and the non-polar matrix; in turn it has resulted in enhanced mechanical properties and
thermal stability along with decreasing the water uptake capacity \[^{16}\]. Other methods to
increase interaction between the filler and the matrix would be surface modification of
zein, addition of surfactant, grafting between the hydrophobic matrix and the hydrophilic
fibers, etc \[^{49}\]. However, in this study some sort of interaction between the zein and
cellulose may have been enhanced by the addition of GA as stabilizer because it not only
improved the mechanical & barrier properties of the films but also helped in more
uniform distribution of the filler in the polymer matrix.

The orientation of the cellulose inside the zein matrix is also an important aspect; the
orientation depends on the type of method used to develop these films. Use of techniques
such as blown extrusion, etc. could change the properties of the films and perhaps may
further improve their properties. Sozer et al. (2010) showed that zein-MMT
nanocomposite films had better thermal resistance when the preparation method was
blown extrusion when compared to films prepared by solvent cast techniques. The latter
however had better tensile properties. Therefore, the preparation technique chosen
depends on their final desired properties \[^{25}\].

Different types of cellulose could be tested to improve the properties of zein films as
well. For example, Microfibrous Cellulose known as MFC produced by the company
CPKelco, GA. This cellulose is produced by fermentation of *Acetobacter xylinum*, which is grown on sugar substrate. It can be expected that if MFC is used in zein films it may produce a 3D network which may further improve the mechanical and barrier properties of zein films \[^{50}\]. Other types of cellulose like powdered cellulose from wood pulp manufactured by Organic Valley can also be used as filler for zein nanocomposites. This type of cellulose is usually used in food products to enhance its texture and taste \[^{51}\].

2) **Application of Zein-Cellulose Nanocomposites on food systems**

Zein-cellulose nanocomposites have potential applications in food packaging. The type of food systems used highly depends on the properties of the packaging films. These nanocomposites can be tested for its use as a part of multi-layer films so as to enhance the shelf life of products such as processed meats, cheese, confectionary, cereals, etc. It can also be used directly to wrap fruits and vegetables. Rakotonirainy et al. (2001) used zein films to wrap broccoli pieces; they concluded that zein films combined with polyethylene films had better barrier properties \[^{52}\]. To avoid combining other plastic packaging along with zein films, it is expected that zein-cellulose nanocomposites have similar effects on fresh fruits and vegetables. Therefore it is necessary to test its affects on different food systems. The use of zein-cellulose nanocomposites would make the packaging system a 100% bio-based system and hence has the potential to replace plastic packaging.

Oxygen barrier properties would be another characterization technique, which could be used to be measure its gas barrier properties. Based on the results, the films could also be used as an oxygen barrier layer for PET bottles and other films. This combination could be used to prevent oxidation in case of fruit juices, dairy foods, etc.
The zein-cellulose nanocomposites can also be tested for its properties at different humidity environments, this study would be useful to see if the films have the same properties irrespective of the type of environment they are exposed to. Effect of temperature on the nanocomposites can be measured, for example, freezing has affects on lot of plastics. If these nanocomposites perform well then they can also be used in frozen food packaging applications.

Other applications would include nano-encapsulation of value-adding food additive in these films. Zein-cellulose nanocomposites can also be tested for its control or burst-release applications of the bioactive food substances. Since zein-cellulose nanocomposites have good water resistance properties they may also find its use in thermoformed trays [17].

Zein-cellulose nanocomposites can also be used as an edible film. Edible film by definition is a thin layer of edible matter formed on food as a coating. As we already know, zein-cellulose nanocomposite is a 100% bio-based system and all the ingredients in the film is food grade and hence can be used as an edible film. Edible films can also carry active substances such as antioxidants, antimicrobials, etc. Similar to packaging, edible films are also used to extend the shelf life of a food product by limiting moisture and oxygen migration which in turn prevents spoilage, lipid oxidation of food, etc [24]. Also, if edible films are used for fresh fruits and vegetables then permeability of oxygen and carbon dioxide would be essential for them to breathe, therefore moderate barrier properties are required for the prolonged shelf life of the products. These nanocomposites can also be sprayed on sugar confections where moisture migration is a problem, these
edible films may help in moisture migration, thereby extending shelf life of sugar confections and reducing the need for other types of packaging.
6. REFERENCES


44. ASTM D870-09 testing water resistance of coatings using water immersion. *In Annual book of ASTM standards, West Conshohocken, PA*. 2010; 06.01.