

# Preparation and Characterization of Gelatin and Chitosan Films

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An Honors Thesis for the Department of  
Chemical and Biological Engineering

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## Abstract

This paper outlines the study of fabrication and characterization of gelatin and chitosan films with the goal of determining the optimal method of preparing these films in order to improve on their mechanical properties. Gelatin films were prepared by spin coating and casting on PDMS mold. Chitosan films were prepared by spin coating and drying known masses of samples in the fume hood or the oven. Films were stored in a vacuum chamber when not being tested. Each film was tensile tested for its Modulus of Elasticity, Tensile Strength, Yield Strength, and Elongation at Break. Tensile properties of films are affected by various innate properties of the film such as moisture content of the films, physicochemical properties of the films, humidity of the atmosphere during testing. The results of this project indicated that gelatin films prepared by casting were better suited for tensile testing. In order to minimize atmospheric effects, films must be stored in a vacuum box or dessicator until testing. Chitosan films that are washed with sodium hydroxide solution after drying were less susceptible to humidity; chitosan films prepared with acetic acid tend to be easier to handle during film preparation and testing.

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## 1. Introduction

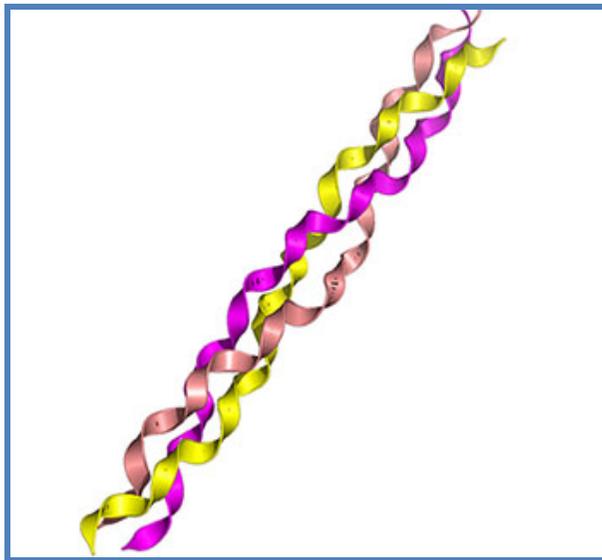
This project analyzes the optimal mode of fabrication for pure gelatin and chitosan films (i.e. with no cross linkers). These analyses involve the preparation and tensile testing of gelatin and chitosan films prepared under different conditions. The gelatin and chitosan films were defined by their Modulus of Elasticity (ME), Tensile Strength (TS), Yield Strength (YS), and Extension till Break (EB). The ME is a measure of the stiffness of the film and is calculated as the ratio of the applied stress to the strain. The TS is the stress at which the films undergo permanent deformation and break. The YS is the stress at which the deformation of the films changes from elastic to plastic. In the plastic deformation range, the film deformations are permanent i.e. the film does not return to its original state after extension; as is the case with elastic deformation. EB is the extension of the film at TS. Tensile tests were performed using the Instron 3360 Series Dual Column System. The figure below shows the Instron 3360 Series that is used for testing in this project.



**Figure 1: Instron 3360 Series Dual Column System (for Tensile Testing)**

## 1.1. Background: Gelatin

Collagen is a biopolymeric fibre and the most abundant mammalian and fish protein. It is the main protein in connective tissue; it serves as the main stress-bearing component of connective tissues and rich in hydroxyproline, proline, and glycine residues (Veis 1964). Each collagen fibre is made up of three tropocollagen (left-handed helix) units which form a right-handed triple helix (see Figure 1 below).



**Figure 2: Collagen Fibre made of 3 coiled tropocollagen units**

The predominant sequence of amino acids in collagen is Gly-X-Y, where X and Y could be any amino acids but are often proline and hydroxyproline respectively. This sequence allows for the feasible helix formation between the three tropocollagen units. There are different types of collagen; gelatin is produced from Collagen Type I.

Gelatin is an edible biodegradable and biocompatible (Krochta and De Mulder-Johnston n.d.) polymer that is produced by the thermal or physical and chemical degradation of collagen.

Gelatin is prepared when the quaternary structure of collagen is destroyed so that free

tropocollagen units are formed. There are two types of gelatin; Type A and Type B. The type of gelatin that is formed from collagen is dependent on whether the collagen is pre-treated with an alkaline or an acid. Type A is prepared through acid pre-treatment and has an isotonic point between 7 and 9 (pH between 3.8 and 5.5). Gelatin Type B is prepared by alkaline pre-treatment of collagen and has an isotonic point between 4.7 and 5.2 (pH between 5 and 7.5). Gelatin Type A was used for the experiments described in this paper. Figure 2 below shows a chain of the gelatin polymer.

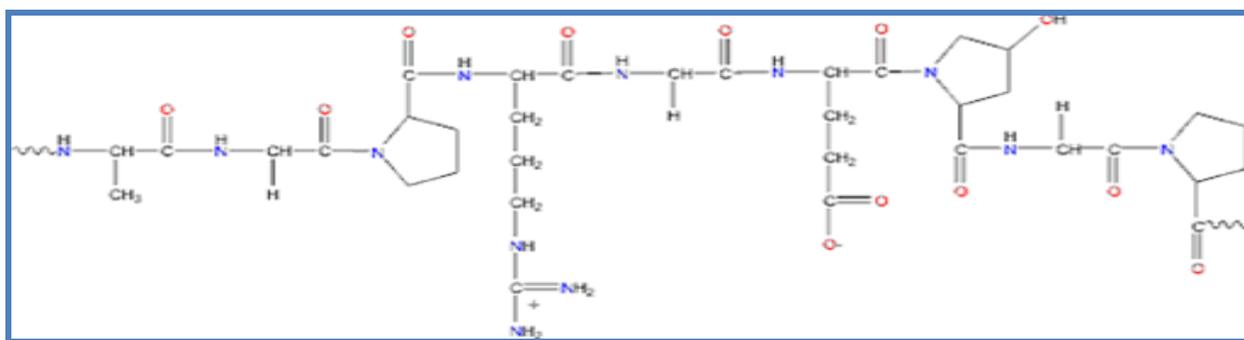


Figure 3: Gelatin chain displaying 8 peptide units

There are numerous uses of gelatin in the present day including drug delivery, food product coating(Antoniewski, et al. 2007), degradable packaging(Cuq, Gontrard and Guilbert 1998), and wound dressing(Vaz, et al. 2003). Most of the uses of gelatin are based on its ability to form biodegradable gels.

The biggest challenge in the use of gelatin lies in its poor mechanical properties. Even though the functional groups of peptides in gelatin do not allow for much derivatization and immobilization many research groups have worked on ways to improve the mechanical properties of gelatin by introducing molecules that will act as cross linking agents such as glutaraldehyde, D, L glyceradehyde (Vandelli, et al. 2001), genipin(Bigia, et al. 2002), and transglutaminase(Chambi

and Grosso 2006). In this project however, the focus is on how to fabricate gelatin films (without cross linkers) with maximized mechanical properties.

Whether a gel or a film is prepared is dependent on the temperature at which the films are cast relative to the gelation temperature of gelatin (which is dependent on the amount of gelatin present in solution). Films that are cast below the gelation temperature tend to be amorphous and gel-like and multiple form triple helix structures. These films are fragile and flexible. Films that are cast above the gelation temperature tend to be more crystalline and brittle (after dehydration); they form less triple helix structures. Films that are fairly dry but not completely dehydrated tend to be less brittle and well suited for tensile testing.

## 1.2. Background: Chitosan

Chitin is a non-starch polysaccharide biopolymer made up of N-acetyl-D-glucosamine repeat units. It is the main component of crustacean exoskeletons and fungi cell walls. It is the second most abundant biopolymer after cellulose. The N-acetyl-D-glucosamine units are bound to each other by  $\beta$ -1,4 linkages. Figure 3 below shows the chitin biopolymer.

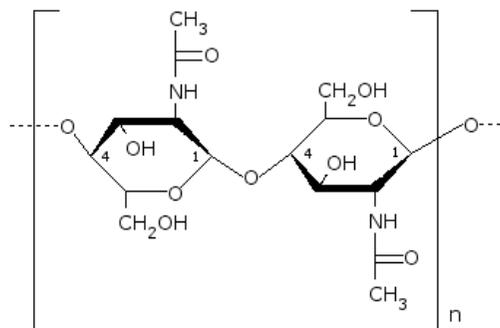


Figure 4: 2 N-acetyl-D-glucosamine residues in chitin

Chitosan is a non-toxic, biocompatible, and biodegradable polymer prepared by the alkali N-deacetylation of chitin. Deacetylation is however not a complete process i.e. different types of

chitosan have varying degrees of deacetylation(Wenling, et al. 2005). This also affects the molecular weights of chitosan chains; two chains with the same number of repeat units but different degrees of deacetylation will have different molecular weights. The degree of deacetylation of chitosan affects its mechanical properties(Wan, et al. 2003). Figure 4 below shows the conversion of chitin to chitosan.

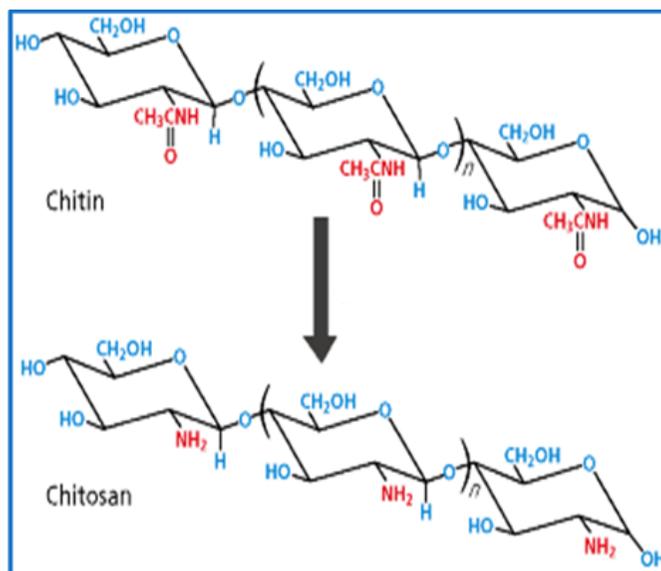


Figure 5: Preparation of Chitosan from Chitin through alkaline deacetylation

Chitosan films are extensively used in biomedical applications(Khor and Lim 2003) such as drug and gene delivery(Genta, Perugini and Pavanetto 1998) and wound dressing(Henriksen 1996). In all these applications of chitosan, some of the most important factors to consider include the physicochemical and mechanical properties of chitosan. The amino- and hydroxyl groups on chitosan chains allow for relatively easy derivatization and immobilization of chitosan as compared to gelatin. Chitosan chains tend to have inter- and intra- molecular hydrogen bonding. Currently, research groups are looking into the possibility of improving the mechanical properties of chitosan by addition of cross linkers such as glutaraldehyde(Chen, Shao and Huang

2000)and genipin(Jin and Song 2006). However, in this project the focus is on the fabrication of chitosan films (without cross linkers) in order to maximize mechanical properties.

## **2. Materials and Methods**

### **2.1. Materials**

Medium molecular weight Chitosan was purchased from Sigma-Aldrich. According to the vendor, the degree of deacetylation was between 75-85%. Type A Gelatin from porcine skin, ~175 g Bloom, was purchased from Sigma-Aldrich. Sodium Hydroxide, Hydrogen Chloride, and Acetic Acid were all of reagent grade.

### **2.2. Methods**

#### **2.2.1. Preparation of Gelatin Solution**

15% w/w gelatin solution was used for all the tests in this project. The solubility of gelatin in aqueous solutions is temperature dependent. The appropriate mass of gelatin was added to the appropriate volume of deionized water at 60 °C for three hours. Ultimately, the solution is prepared for immediate use. In the case where there is left over solution, it is stored in a refrigerator and heated before use.

#### **2.2.2. Preparation of Gelatin Films**

The first set of gelatin films was prepared by spin coating gelatin solution on a plastic slide (the slides were scratched on the surface which came in contact with the solution in order to prevent the formation of gelatin solution droplets). The Laurell Spin Processor WS-400-6NPP-LITE, as shown in Figure 5 below, was used for spin coating. The films were prepared using different amounts of gelatin solution, spinning speeds and spinning times. The samples were tested at the

following spinning speed: 300 rpm, 200 rpm, 100 rpm, and 50 rpm. At each spin speed, the spin time was varied: 60 sec, 30 sec, and 15 sec.

The subsequent sets of gelatin films were prepared by evenly distributing a layer of the gelation solution on Polydimethylsiloxane (PDMS) molds. The molds were bone-shaped, a suitable shape for tensile testing. The films were then air- or refrigerator-dried for at least 24 hours. The samples then underwent tensile testing



**Figure 6: Laurell Spin Processor WS-400-6NPP-LITE**

### **2.2.3. Preparation of Chitosan Solution**

~1% and ~2% Chitosan solutions were used for this project. The solubility of chitosan in aqueous solutions is pH dependent; as the acidity of the solution increases the solubility of Chitosan increases. Some solutions were prepared with pH ~3 and others with pH ~5. The pH levels were set by either HCl or acetic acid. The required mass of chitosan was added to the appropriate volume of deionized water and stirred for about 12 hours while enough hydrochloric acid or acetic acid was added to bring the solution to the desired pH level. The solution was then filtered to remove the insoluble clumps that were still in the solution.

### **2.2.4. Preparation of Chitosan Films**

The first set of chitosan films were prepared by spin coating chitosan solution on plastic and glass slides using the same parameters as that used for gelatin films prepared by spin coating. An attempt was made to prepare chitosan films by evenly distributing a layer of chitosan solution on the surface of the dog bone-shaped PDMS (as done for gelatin films). These films were prepared using a chitosan solution of pH ~3 with the pH level set by HCl.

The second set of chitosan films were prepared by pouring a known mass of chitosan into 90mm Petri-dishes and air drying the samples in a hood. These films were also prepared using a chitosan solution of pH ~3 with the pH level set by HCl.

The subsequent sets of chitosan films were prepared by oven drying known masses of the samples in 90mm Pyrex Petri-dishes. The drying times for these films were varied: 24hrs, 32hrs, and 48hrs. Films used for these tests were prepared from 1% chitosan solution (pH ~5 with pH level set by HCl). For drying-temperature variation, 1% solutions (pH ~5 with pH level set by HCl) were oven dried at 40 °C, 50 °C, and 60 °C. These temperatures were chosen because they had been used repeatedly by other groups. To determine the effect of NaOH concentration and type of acid on the films samples were prepared from two different ~1% solutions:

- a. pH ~5 with pH level set by HCl
- b. pH ~5 with pH level set by Acetic Acid

Samples prepared using these solutions were oven dried for 24hrs at 60 °C. After drying, the samples were soaked in 0 M, 1 M, and 5 M NaOH for 15 minutes. The samples were then washed with deionized water for 15 minutes to neutralize the NaOH.

### **2.3.Tensile Testing**

Prior to testing, the thickness of each film was measured with a Micrometer Gauge (with 0.001 precision). Using the Instron instrument, an axial tensile test was run on each film. A 10N load was used for each sample. Samples that had air bubbles and/or tears were not tested. All tests were performed under ambient conditions. The data was analyzed using the MATLAB code shown in Appendix A.

### 3. Results and Discussions

#### 3.1. Tensile Testing

The output data of the Instron instrument for tensile testing included data for the true stress and true strain recorded for each film during testing. The True Stress and True Strain data was used to produce a Stress-Strain graph (Figure 7) for each sample. Figure 7 also shows the data that can be obtained from a Stress-Strain graph.

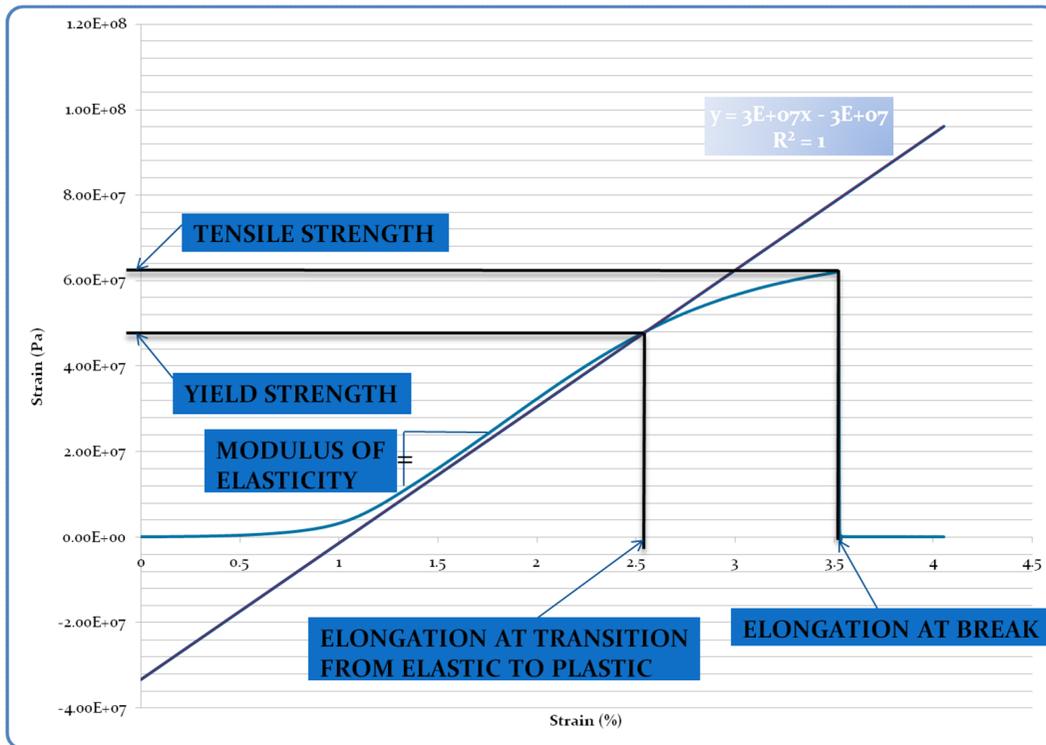


Figure 7: Stress-Strain graph for a biopolymeric film

The MATLAB Code shown in Appendix A was used to analyze the data for each film. There were at least 1000 data points for each sample. The Stress-Strain graph of each sample begins with a region with  $\sim 0$  slope. It then transitions to a constant slope region; the slope of this region is the Modulus of Elasticity of the film. The MATLAB Code regresses 10 data points at a time

and compares the slope of these points to the next 10 data points. It continues to search for data points with similar slopes that have an R-squared value greater than 0.999 in order to determine the Modulus of Elasticity. The Modulus of Elasticity is then used to produce a 0.05% offset line that intersects the actual Stress-Strain graph at the Yield Stress point. This intersection was determined by solving the equations of the offset line and a polynomial that is determined using a total of 7 data points around the point where the stress values of the offset line exceed the actual Stress-Strain graph.

### 3.2. Gelatin

#### 3.2.1. Gelatin Films prepared by Spin Coating

The first set of samples of gelatin films was prepared by spin coating 15% w/w gelatin solution onto plastic slides at different speeds in order to control the thickness of the films that were produced. The different spinning speeds used were 300 rpm, 200 rpm, 100 rpm, and 50 rpm. At each spin speed, the spin time was varied: 60 sec, 30 sec, and 15 sec. One set of films was prepared using ~0.5 mL of solution while the second set of films was prepared using ~1.0 mL of solution. Tables 1 and 2 below show the qualitative observations made for the films that were tested.

**Table 1: Conditions and Observations for Gelatin films Prepared by Spin-Coating from ~0.5 mL 15% w/w Gelatin Solution**

Spin Speed (rpm)	Spin Time (seconds)		
	60	30	15
50	Unable to peel film	1 $\mu$ m average thickness	1 $\mu$ m average thickness
100	Unable to peel film	0.5 $\mu$ m average thickness	0.5 $\mu$ m average thickness
200	Unable to peel film	Unable to peel film	Unable to peel film

<b>300</b>	Unable to peel film	Unable to peel film	Unable to peel film
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**Table 2: Conditions and Observations for Gelatin films Prepared by Spin-Coating from ~1.0 mL 15% w/w Gelatin Solution**

<b>Spin Speed (rpm)</b>	<b>Spin Time (seconds)</b>		
	<b>60</b>	<b>30</b>	<b>15</b>
<b>50</b>	Unable to peel film	1.5 $\mu\text{m}$ average thickness	1.7 $\mu\text{m}$ average thickness
<b>100</b>	Unable to peel film	0.6 $\mu\text{m}$ average thickness	0.7 $\mu\text{m}$ average thickness
<b>200</b>	Unable to peel film	Unable to peel film	Unable to peel film
<b>300</b>	Unable to peel film	Unable to peel film	Unable to peel film

As seen from Tables 1 and 2 above, it was not possible to peel the films that had been spun at 300 rpm or 200 rpm (regardless of the spin time) and at 50 and 100 rpm (for 60 seconds). The samples prepared with 50 and 100 rpm spin speed and 30 and 15 second spin times were the only films that could be peeled from the plastic slide.

These results indicate that at high spin speeds (200 and 300 rpm) the gelatin films that were formed were too thin to be peeled from the plastic slides without damaging the films. This observation can be explained by the fact that the thickness of the film is directly proportional to the angular speed of spinning (measured in rpm). As the angular speed of spinning increases the thickness of the film decreases. Also, the results show that as the spin time increases the film thickness decreases. This is also consistent with the theory that film thickness is inversely proportional to spin time and spin speed (Chang, Wu and Chen 2001). The tables also show that films that were spun at 50 and 100 rpm formed slightly thicker films i.e. films that could be removed from the slides without excessive damage. Also, by increasing the volume of gelatin solution on the slides the average thickness of the films was increased. This observation is

explained by the fact that the net amount of gelatin increases when the volume of gelatin solution increases. Thus it is imperative that the thicknesses of the films would also increase.

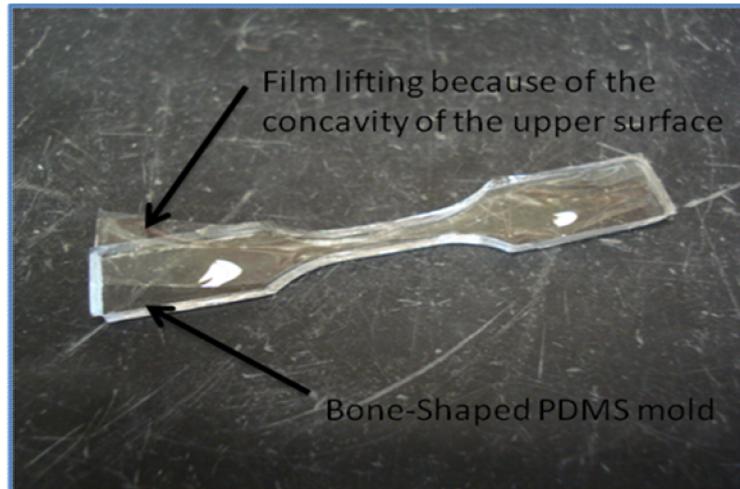
Even though the increase in volume of gelatin solution for samples prepared at 50 and 100 rpm resulted in an increase in the film thicknesses, further experimentation proved that increasing the volume of gelatin solution beyond this point results in samples that were not effectively distributed over the slides (when tested at low spin speeds). Samples with gelatin solution higher than 1 mL that were prepared with high spin speeds (200 rpm and above) resulted in films that were only slightly thicker than the films prepared with less gelatin solution. This observation is explained by the fact that at high spin speeds the excess volume of gelatin is lost during the spinning (i.e. it is scattered onto the wall of the spin coating instrument).

### **3.2.2. Gelatin Films prepared by Casting on PDMS Mold**

The second set of samples that was prepared by evenly spreading a layer of gelatin solution on the surface of a PDMS mold. The samples were either dried in the refrigerator or allowed to air dry in a well ventilated hood. The samples that had been air dried were completely dry after 24 hours; samples that had been dried in the refrigerator took an average of 10 days to dry. Ideally, gelatin solutions that are stored in the refrigerator tend to form gels and not films. This explains the disparity in drying times between samples dried in the refrigerator and those dried in the hood.

For all samples, whether dried in the refrigerator or air dried showed concavity in the films (as displayed in Figure 7 below). This observation is explained by the fact that the upper surface of the films dries at a faster rate than the lower surface. Thus the upper surface shrinks faster than the lower surface and causes the concave structure of the film. This concavity is expected to affect the mechanical properties of film; though this effect was not investigated in this project.

Films formed in this manner were on the average, 8 –15  $\mu\text{m}$  thick. The thicknesses of the films were measured as an average of the thicknesses measured at the two ends (shoulders) and the center (narrow section) of the film. These films were easy to handle during tensile testing.



**Figure 8: Film Concavity as Displayed by Film Prepared by Drying on a Bone-Shaped PDMS mold**

The observed concavity of the film can be explained by the uneven exposure of the different surfaces of the films to the atmosphere. The upper surface, which is directly in contact with the atmosphere dries up and ‘shrinks’ faster than the lower surface thus forcing the development of a concave structure. The only way this could be avoided would have been by drying the films while both ends are clamped and both surfaces are left open to the atmosphere. However, the gel nature of the samples does not allow for easy handling of the films while they are drying.

Table 3 below shows the average mechanical properties of the gelatin films prepared by casting on PDMS molds and dried in the hood for 24 hrs.

**Table 2: Mechanical Properties of Gelatin Films Prepared by PDMS mold casting and drying for 24 hrs in the fume hood.**

<b>Mechanical Property</b>	<b>Measure</b>
Modulus of Elasticity (MPa)	14.2
Tensile Strength (MPa)	37.0
Yield Strength (MPa)	13.3
Elongation at Break (%)	20.7

A thorough search through literature did not present any data for the mechanical properties of ~175 Bloom gelatin. However, data is present for gelatin films prepared from different Bloom values of gelatin. The Bloom value of the gelatin that is used in testing is indicative of the degree of renaturation (i.e. the degree of formation of triple helix strands). It is expected that the modulus of elasticity will increase as the Bloom value of the gelatin solution increases (Bigi, Panzavolta and Rubini 2004). As compared to the Modulus of Elasticity values presented by Bigi et al, the samples in this project seemed to have recorded higher values than expected. This difference might be as a result of the difference in the dryness of the films.

In summary, the results from these tests were not conclusive. In order to get a better idea of how this method of fabrication affects the mechanical properties of gelatin new experiments that take into consideration the degree of renaturation and film dryness must be carried out.

### **3.3.Chitosan**

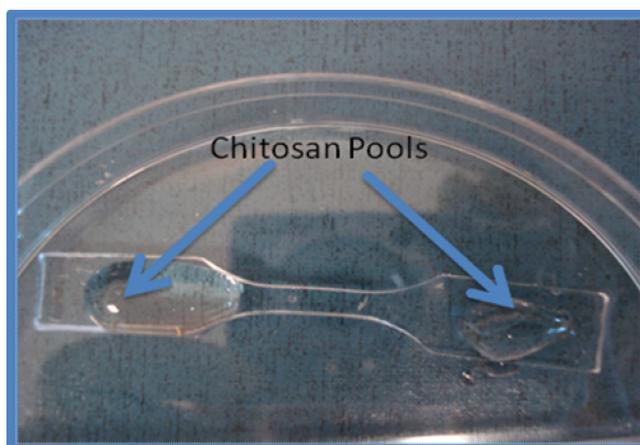
#### **3.3.1. Chitosan Films prepared by Spin Coating**

The first set of chitosan films was prepared using the same spin coating parameters and conditions as that used for the gelatin samples. The chitosan solutions used for these samples

were prepared by dissolving chitosan in an acidic media with pH ~3. The acidity was set by adding HCl. The same observations were made for chitosan (for both glass and plastic slides) as for gelatin films prepared using this method.

### 3.3.2. Chitosan Films prepared by Casting on PDMS Mold

An attempt was made to prepare chitosan films by evenly distributing ~1% and ~2% chitosan solution on PDMS molds. These samples were prepared using the same procedure as the gelatin samples prepared by casting onto PDMS molds. Figure 9 below shows the result of spreading chitosan solution on a PDMS mold.



**Figure 9: Formation of 'Chitosan Pools' on PDMS molds**

Figure 9 above shows a picture of the effect of evenly distributing a layer chitosan solutions on a PDMS mold; the layer of solution collects into droplets within 30 seconds of spreading the solution on the mold. This observation can be explained by the difference in hydrophobicity between PDMS and chitosan. The chitosan solution (prepared in an acidic medium) is hydrophilic whereas the surface of the PDMS mold is hydrophobic; thus making it difficult for the chitosan solution to wet and remain evenly distributed on the surface of the PDMS mold.

### 3.3.3. Chitosan Films prepared by Fume Hood Casting

Another set of films was prepared by pouring chitosan solution into Petri dishes and air drying the samples a fume hood for 24 hrs. Samples were prepared from 1% and 2% chitosan solutions. These films were cut into the dob-bone-shape and tested for their mechanical properties. Like the gelatin samples that were dried in the fume hood, these samples formed concave films. Tables 4 and 5 and Figure 10 below show the results of tensile testing for these samples.

**Table 3: Mechanical Properties of ~1% Chitosan (pH ~3, HCl) films prepared by 24 hr drying in fume hood (Samples 1, 2, 3, and 4 represent groups of samples with similar measures)**

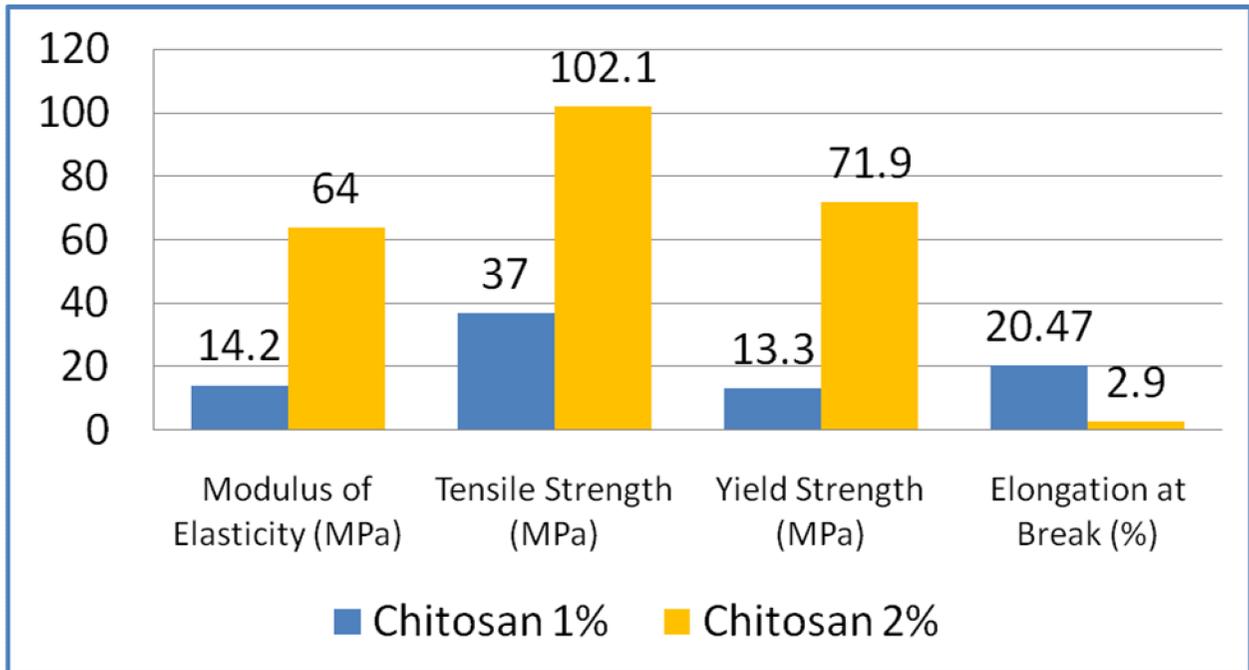
Mechanical Property	Sample Group					Standard Deviation
	1	2	3	4	Average	
Modulus of Elasticity (MPa)	15.1	15.2	12.8	13.8	14.2	1.14
Tensile Strength (MPa)	38.6	27.8	35.4	46.0	37.0	7.56
Yield Strength (MPa)	15.0	14.4	11.3	12.6	13.3	1.72
Elongation at Break (%)	18.7	22.6	18.3	22.2	20.47	2.26

**Table 4: Mechanical Properties of ~2% Chitosan (pH ~3, HCl) films prepared by 24 hr drying in fume hood (Samples 1, 2, 3, 4, and 5 represent groups of samples with similar measures)**

Mechanical Property	Sample Group					Standard Deviation	
	1	2	3	4	5		Average
Modulus of Elasticity (MPa)	61.6	65.2	65.6	57.7	69.9	64.0	4.58
Tensile Strength (MPa)	96.2	112.9	93.8	96.1	111.5	102.1	10.34
Yield Strength (MPa)	54.5	79.1	76.0	68.8	80.9	71.9	9.27
Elongation at Break (%)	3.16	3.43	2.16	2.79	2.97	2.90	0.48

Each group in Tables 4 and 5 represents groups of samples that had similar values of Modulus of Elasticity, Tensile Strength, Yield Strength and Elongation at Break. Tables 4 and 5 also show the standard deviations of each of the mechanical properties that were determined.

Figure 10 below shows the comparison between the average mechanical properties of ~1% Chitosan and ~2% Chitosan films.



**Figure 10: Comparison between samples prepared from ~1% Chitosan and ~2% Chitosan (both with pH ~3)**

The tables and figure show that the Modulus of Elasticity, Tensile Strength and Yield Strength of ~1% Chitosan are significantly less than that of ~2% Chitosan. The initial assumption was that, this observation was as a result of the difference in concentration of the Chitosan solutions.

However, an analysis of the process of drying shows that the difference in concentration should have no effect on the mechanical properties of the films. Another difference between the ~1% Chitosan and ~2% Chitosan films was the presence of un-dissolved particles of chitosan in the ~2% solution. These particles were very small and it was expected that the presence of such particles would introduce stress points to the film. If this were the case, the opposite effect would

have been seen; the ME, TS, and YS of the 2% solution would have been less than that of the 1% solution.

The only other difference between the two solutions was the moisture content. The 1% and 2% solutions were not prepared on the same day or tested within the same number of hours after preparation. All the ~1% Chitosan films were tested on the same day while all the ~2% Chitosan films were tested together on another day. Thus, it is expected that both sets of films might not have been prepared and/or tested under the same moisture conditions. Literature research shows that as moisture content of chitosan films increases, the ME, TS, and YS decrease considerably while increasing EB(Lazaridou and Biliaderis 2002). Based on this knowledge, it is assumed that the moisture level of samples prepared with ~1% Chitosan was higher than that of samples prepared with ~2% Chitosan. More testing is required in this regard to determine the dependence of ME, TS, and YS on the moisture content of the films.

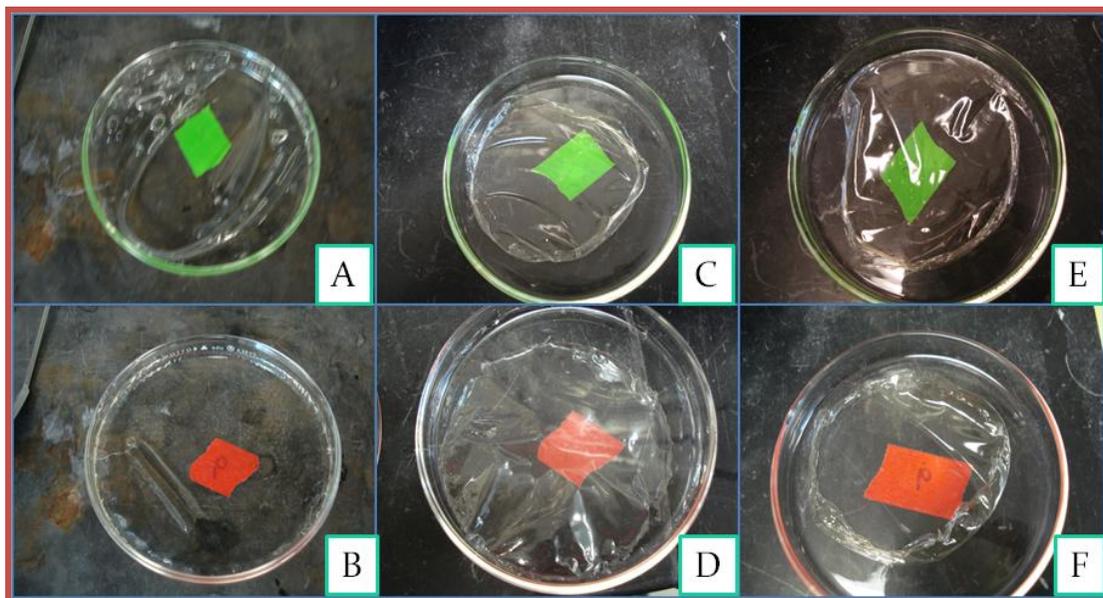
#### **3.3.4. Chitosan Films prepared by Oven Casting**

The last set of chitosan films was prepared by oven drying (at different drying temperatures) 1% Chitosan solutions poured in 90mm Pyrex Petri-dishes for different drying times. These films were dried in the oven in order to prepare films under consistent conditions and in order to set a system of equal moisture content for the films. The films were stored in a vacuum chamber until they were ready to be tested.

For drying-temperature variation, 1% solutions (pH ~5 with pH level set by HCl) were oven dried at 40 °C, 50 °C, and 60 °C. These temperatures were chosen because they had been used repeatedly by other groups. For the same amount of solution (~20g) samples dried at 60 °C dried in 24 hrs as opposed to 32 and 40 hrs for 50 °C and 40 °C respectively. All subsequent samples were dried at 60 °C for 24 hrs.

To determine the effect of NaOH concentration and type of acid on the films samples were prepared from two different ~1% solutions, one based on acetic acid and the other on HCl. The samples were oven dried for 24hrs at 60 °C, soaked in 0 M, 1 M, or 5 M NaOH for 15 minutes and then washed with deionized water for 15 minutes to neutralize the NaOH.

Figures 11 below show samples the results that were obtained



**Figure 11: Chitosan films oven dried and washed after drying.**

**A: 1% Chitosan solution based on Acetic acid and washed only with deionized water**

**B: 1% Chitosan solution based on Hydrochloric acid and washed only with deionized water**

**C: 1% Chitosan solution based on Acetic acid and washed with 1M NaOH and deionized water**

**D: 1% Chitosan solution based on Hydrochloric acid and washed with 1M NaOH and deionized water**

**E: 1% Chitosan solution based on Acetic acid and washed with 5M NaOH and deionized water**

**F: 1% Chitosan solution based on Hydrochloric acid and washed with 5M NaOH and deionized water**

As seen in Figure 11, the film prepared from an acetic acid based chitosan solution, picture A, stretched out radially and formed a tight roll when it was washed with deionized water; this happened ~10 minutes after the film was soaked in deionized water. The film however, did not dissolve even after being left in the deionized water for 5 hours. Picture B shows a film prepared from an HCl based chitosan solution 5 minutes after deionized water had been poured onto it. The film began to disintegrate and swell. Pictures C and D show dried films that were prepared from acetic acid and HCl solutions respectively. These samples were washed with 1M NaOH

and deionized water after they were oven dried. Pictures E and F show dried films that were prepared from acetic acid and HCl solutions respectively. These samples were washed with 5M NaOH and deionized water after they were oven dried.

A general observation that was made was that films prepared using the solution based on acetic acid were much easier to handle and did not tear easily whereas the films prepared using the solution based on HCl were more difficult to handle. Films prepared based on acetic acid were less crumpled than those based on HCl after washing and drying.

Films that were washed with higher concentration of NaOH had to be washed for more than 15 minutes in deionized water in order to reduce the degree of crumpling in the films.

By washing the films with NaOH, the surfaces of the films are neutralized and less likely to be affected by atmospheric moisture. The expectation is that such films, if kept in a vacuum chamber until testing, will measure consistent tensile testing data.

The average mass of solution used to prepare these films was 20 g; the average thickness of the films was 3  $\mu\text{m}$ . These films were too thin to undergo effective tensile testing.

## 4. Conclusions and Recommendations

The data and analysis outlined in this paper demonstrate the dependence of mechanical properties of biopolymeric films on the innate properties of the polymers themselves and the atmospheric conditions under which they are tested. The mechanical properties of gelatin are largely affected by the degree of renaturation (indicated by roughly by its Bloom index). The mechanical properties of chitosan are dependent on the acid that is used to set the pH of the solution, the degree of deacetylation, and the hydrophilic nature of the surfaces of the films. For subsequent work, the researcher may consider testing larger masses (~50 g) of chitosan solution in 90 mm Petri dishes in order to obtain film thickness of about 10  $\mu\text{m}$ . Considering the response of gelatin and chitosan films to humidity; it is recommended that films be stored in a vacuum chamber or under vacuum conditions until testing. Further work is required to understand the effect of NaOH on the mechanical properties of chitosan and how the hydrophilic nature of the surface of gelatin films affects its mechanical properties.

## Appendix

### MATLAB Code for Data Analysis

```
function dataanalysis(File,NoSheets)
%A=Stress
%B=Strain

NoSheets=input('Number of Excel Sheets in File:');

for o=1:NoSheets;
A=xlsread('Gelatin_15.xlsx', o, 'R1:R1000');
B=xlsread('Gelatin_15.xlsx', o, 'O1:O1000');
n=length(A);
if length(B)~=n, error('A and B must be same length');end

    %Determination of linear range of Stress-Strain Graph
    c=1;
    for i=1:10:(n-20);
        a=A(i:(i+10));
        b=B(i:(i+10));
        stats1=regstats(b,a,'linear',{'beta','rsquare'});

        d=A((i+(11)):(i+(20)));
        e=B((i+(11)):(i+(20)));
        stats2=regstats(e,d,'linear',{'beta','rsquare'});

        if stats1.rsquare>0.9999|stats2.rsquare>0.9999;
            index1(c)=i;
            c=c+1;
        end
    end

    end

    %To find 0.05% offset
    f=A((min(index1):(max(index1))));
    g=B((min(index1):(max(index1))));
    stats3=regstats(f,g,'linear',{'beta','rsquare'});
    int=(stats3.beta(1));
    slope=(stats3.beta(2));
    Aoffset=slope*(B-0.05)+int;
    r2=(stats3.rsquare);
    plot(B,Aoffset,'r')
    hold on
    plot(B,A,'g')

    for i=1:n;
        h=(A(i)-Aoffset(i));

        if h<=0;
```

```

        highoff=i
        break
    end
end

%To find Yield Strength
j=A((highoff-3):(highoff+3));
k=B((highoff-3):(highoff+3));
p=polyfit(k,j,3);
eqsm=[p(1) p(2) (p(3)-slope) (p(4)-int)];
pint=roots(eqsm);

for i=1:3;
    pintcomplex=pint(i);

    if imag(pint(i))==0;
        yext=real(pint(i));
        yield=slope*(yext)+int;
        break
    end

end

maxstress=max(A);
for i=1:n;
    t=(A(i));
    if t==maxstress;
        tensile=t;
        l=i;
        text=B(i);
        break
    end
end

disp('Film Number:')
disp(o)
disp('Modulus of Elasticity:')
disp(slope)
disp('Yield Strength:')
disp(yield)
disp('Tensile Strength:')
disp(tensile)
disp('Extension at Break:')
disp(text)
disp('Extension at Tensile Strength:')
disp(yext)
end
end

```

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